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A. Plenary Conferences

I

Cell Cycle Control by TGF-β Signals

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TGF-β and the related factors, activins and bone morphogenetic proteins (BMPs) are pivotal regulators of many cell functions during embryogenesis and in the adult. These factors are widely multifunctional, eliciting different gene responses and, in turn, different cellular responses depending on the type and status of the cell. Alterations of this signaling network underlie several inherited and somatic disorders. Work in our laboratory has led to the elucidation of a TGF-β signal transduction system involving: (i) a ligand-induced membrane receptor complex that phosphorylates SMAD proteins at the cell surface, and (ii) a SMAD-induced transcriptional complex that activates target genes in the nucleus. Distinct repertoires of receptors, SMAD proteins and their DNA-binding partners seemingly underlie, in a cell specific manner, the multifunctional nature of TGF-β and related factors. Based on the crystal structure of the TGF-β type I receptor (in collaboration with M. Huse and J. Kuriyan) and the crystal structure of the Smad N- and C-terminal domains (in collaboration with Y. Shi and N. Pavletich), we have identified structural elements that dictate the selective interactions of Smads with receptors and DNA, and how tumor-derived Smad mutations inactivate Smad signaling.

Some of the most important gene responses elicited by TGF-β in epithelial cells are devoted to arresting cell cycle progression. TGF-β plays a dual role in tumorigenesis. On one hand, TGF-β antimitogenic signaling is lost in some cancers by mutational inactivation of the TGF-β type II receptor (TβR-II) or its substrates and mediators, Smad2 and Smad4. On the other hand, TGF-β can exacerbate the malignant phenotype at later stages of tumorigenesis by fostering tumor invasion and the development of metastases . These effects become manifest in cells harboring Ras mutations. We found that oncogenic Ras inhibits TGF-β signaling by inhibiting TGF-β induced nuclear accumulation of Smads. Our results suggest a mechanism for the counterbalanced regulation of Smads by TGF-β and Ras signals in normal cells, and for the silencing of antimitogenic TGF-β functions by hyperactive Ras in cancer cells. Likewise, TGF-β and interferon-γ (IFN-γ) have opposite effects on diverse functions of hematopietic and immune systems. We found that IFN-γ acting via its Jak1/Stat1 pathway induces the pression of the antagonistic Smad, Smad7, which inhibits TGF-β signaling through the Smad pathway. These emerging principles of TGF-β signal transduction and its regulation may help in the search for agents to modify the activity of these pathways.

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II

SIGNALING BY MAP KINASES

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Mitogen-activated Protein Kinases (MAPKs) have been shown to be pivotal elements in the processes that govern cell destiny upon the reception of an external stimuli. Either promoting proliferation, switching on the genetic guidelines that will lead to terminal differentiation or directing cells into apoptosis. MAPKs activation is a key step in the conveyance of signals from surface receptors to the nucleus through signaling routes that include a small GTPase, a cascade of serine/threonine kinases (MAPKKKs), and a dual specificity kinase (MAPKK) as upstream components, and this pathway pattern is strictly conserved throughout the eucariotic evolutionary scale.

To date, more than sixteen different MAPKs have been described. These, however, are grouped into four main families: Extracellular-signal Regulated Kinases (ERKs), Stress-Activated Protein Kinases (SAPKs), Osmotic Stress-regulated kinases (p38 /HOG) and Big MAP Kinases (BMK / ERK5). Although these MAPKs are regulated through well defined pathways, far from being isolated and independent circuits, there is a constant cross-talk and interconnection among many of the components that constitute the different MAPKs pathways. As is the case of the Ras exchange factor Ras-GRF that, as we have shown, requires the participation of cdc42, a component of the SAPKs pathway, to activate ERK2. Another characteristic of the MAPKs pathways is its extreme redundancy. Most MAPKs and their upstream regulators have at least one isoform, and although these isoforms are almost identical in some cases, this does not imply that they are equally regulated and that they serve the same purposes, as we have found is the case with p38 and its splice isoform Mxi2. These two proteins show marked differences in their regulation and substrate specificity. It is now becoming apparent that these differences may be explained in part by the role played by a novel component: scaffold proteins. These proteins seem to serve as structural anchors that bring about the assembly of a signaling complex in a highly specific fashion.

As far as their role is concerned, one or another MAPK is activated by almost every stimuli know so far. On a very general basis, ERKs mediate an "all is well" signal and are mainly involved in proliferation and differentiation, while SAPKs and p38 mediate inflammatory and apoptotic responses. ERKs are also found to be constitutively activated in some human neoplasias, for this reason they have been recently viewed as potential antineoplastic therapy targets. However, this is not a general rule, as myeloid leukemia cell growth and differentiation is completely independent of ERK activation . So a careful scrutiny of the exact role played by the MAPKs on the different neoplasias is required prior to any therapeutic approach.

III

Mechanisms of Apoptosis

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Several members of a growing multigene family of bcl-2 homologs (Bcl-2, Bcl-X_L, etc.), as well as a whole series of additional oncogenes (e.g. *c-myc, raf-1*) and anti-oncogenes (e.g. *bax, p53*) intervene in cancer development at two different levels. At a first level, mutations in the expression level and/or primary structure of apoptosis-regulatory genes may intervene in oncogenesis *ab initio* by favoring the persistence of mutated cells that, in normal circumstances, would be eliminated by apoptosis. At a second level, the Darwinian selection of cancer cells by adverse intrinsic conditions (limited trophic supply, oxygen shortage) and/or therapeutic agents (chemotherapy, radiotherapy) favors the survival of proliferating cells which acquire an increasing resistance to apoptosis induction.

The process of apoptosis can be subdivided into three functionally distinct phases: (i) the initiation phase, during which a number of "private" signal transduction or damage pathways are activated in a stimulus-dependent fashion; (ii) the common effector/decision phase during which the cell "decides" to die and (iii) the degradation phase, beyond regulation, during which the cells acquire the morphological and biochemical hallmarks of apoptosis. Much of the available data are compatible with the notion that the disruption of mitochondrial membrane barrier function constitutes the decisive event of apoptosis. This suggests a scenario in which the initiation phase is mainly pre-mitochondrial, the effector/decision phase is essentially mitochondrial, and the degradation phase is post-mitochondrial.

Recent findings have molecularly identified a number of molecules which intervene at precise levels of the above scenario. Thus, a number of endogenous signal transducing molecules (e.g. reactive oxygen species, NO, calcium, amhipathic peptides, pro-apoptotic "BH3-only" members of the Bcl-2 family) and some experimental chemotherapeutic agents (e.g. lonidamine, betulinic acid, arsenite) directly act on mitochondria to increase their membrane permeability. The barrier function of mitochondrial membranes is regulated by the "permeability transition pore complex (PTPC)" which contains sessile outer and inner membrane proteins interacting with proteins from the Bcl-2/Bax family within the inner/outer membrane contact sites. The final result of PTPC-mediated membrane permeabilization is the non-specific release of soluble intermembrane proteins. Such proteins include cytochrome *c* (which interacts with Apaf-1 to activate caspases), mitochondrial pro-caspases-2, -3 and-9 (which are activated during the translocation into the cytosol), as well as "apoptosis inducing factor" (AIF, which translocates into the nucleus and cancauses caspase-independent cell death).

This scenario has important implications for the understanding of cancer-related dysregulations in apoptosis, as well as for the design of therapeutic strategies aimed at overcoming apoptosis resistance.

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B. Scientific Topics

1. Genetic Alterations and Tumoral Pathology

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INACTIVACION OF p16INK4A /Rb and p53/p21Waf1 PATHWAYS IN THE PROGRESSION OF HUMAN TUMORS

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Malignant transformation of cells is associated with progressive alterations in the regulatory mechanisms of cell cycle control. p16INK4A/Rb and p53/p21Waf1 pathways are considered to control important checkpoints in cell division. Alterations in different elements of these pathways are considered the most frequent genetic events in the development of human tumors. However, the cooperative or alternative effects of these alterations are not clear and may play different roles in different tumor models. In squamous cell carcinomas of Head and Neck, deregulation of p53 and gene mutations seem to occur in early steps of tumor development and they are independent of the stage of dissemination. p21Waf1 expression in these tumors is independent of the status of p53 gene and is associated with cell differentiation. These changes are not associated with the prognosis of the patients. Similarly, inactivation of p16INK4a by homozygous deletions, mutations and concomitant heterozygous deletions, and hypermethylation also seems to occur early in the development of the tumors and is not related to the stage of dissemination. However, amplification and overexpression of cyclin D1 is a late event in the progression of these tumors clearly associated with advances stages. Contrary to this model, inactivation of p16INK4A/Rb and p53/p21Waf1 pathways in human non-Hodgkin/s lymphomas seem to be late events associated with the development of primary aggressive tumors and the progression from indolent to high grade tumors. Furthermore, inactivation of INK4a locus and p53 occur alternatively in these neoplasms. Contrary to laryngeal carcinomas, oncogenic activation of cyclin D1 is an early event in the pathogenesis of a particular lymphoma of virgin B-cell lymphocytes. Deregulated overexpression of cyclin D1 may overcome the negative regulatory effect of Rb and p27 in these tumors. In conclusion, these observations suggest that genetic alterations in regulatory elements of p16INK4A/Rb and p53/p21Waf1 pathways may occur in different moments of the evolution of the tumors and they may have different significance depending on the lineage of the tumor cell.

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ENDOMETRIAL CARCINOMA: MOLECULAR ALTERATIONS RELATED WITH TUMOR PROGRESSION.

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Introduction: According to the dualistic model proposed for endometrial carcinogenesis, endometrioid carcinomas (EC) present molecular changes different from those of non-endometroid carcinomas (NEC).
Materials: Genomic DNA from tumor and normal tissues of 42 patients with endometrial carcinomas and 97 patients with endometrial hyperplasia (EH).
Methods: PCR amplification using dinucletide repeat markers in chromosomes 5, 10, 12, 17, and 18; SSCP-PCR and sequencing for BAT-26, BAX, TGF-bRII, IGF-IIR, hMSH6, and hMSH3.
Results: Microsatellite instability (MI)was more frequent in EC (11/33, 33%) than in NEC (1/9, 11%). MI was found in only two of the 97 EH; both of them were complex hyperplasias associated with EC. There were frameshift mutations in the polyG tract of BAX in 7 of the 13 MI+ EC, but in none of the MI- EC.
Conclusions: EC present MI more frequently than NEC. The finding of MI in EH adjacent to EC suggests that MI is an early event in endometrial carcinogenesis. The hight frequency of BAX polyG mutations in MI+ EC indicates that these alterations are important for tumor progression.

n

SELECTIVE USE OF CDKIs AND CYCLINS BY DIFFERENT CELL LINEAGES IN SPECIFIC TISSUE MICROENVIRONMENTS

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Cell cycle progression is regulated by the balance between complexes formed by Cyclins, Cyclin-dependent Kinases (CDKs), and CDK Inhibitors.
Most of the studies on these cell cycle regulators have been performed in cell lines or using knockout models, yielding some conflicting results about the role these proteins play in cell cycle control, both in normal and tumoral tissue.
Studies based on Immunohistochemical studies fail to confirm some of the predictions performed after in vitro experiments, and add relevant pieces of information to the overall scheme of cell cycle regulation.
This study has been performed trying to characterize the pattern of expression of some of these proteins in lymphoid tissue, using for comparison the distribution in a restricted array of epithelial tissues. Immunohistochemical data were confirmed by using FCM or WB, and expanded by using double staining with confocal microscopy.
Thus, most of the CDKIs and cyclins are expressed by different normal cell types.
p21/WAF1 appears to be strongly expressed by epithelial cells along a differentiation pathway, coinciding with cell cycle stop, and frequently in paralell with p53.
Lymphoid normal tissue express low or undetectable level of p21, with the exception of a relatively poorly characterized B-cell subpopulation, the monocytoid B-cells (MBCs), which are the only normal lymphoid subpopulation expressing p21. In contrast lymphocytes show high expression of p27/KIP1, always in a pattern opposed to cell proliferation. MBCs express only a weak level of p27/KIP1, unexpectedly taking into account the low level of proliferation.
p16/INK4A is expressed by most of normal lymphocytes, being the level of expression upregulated along cell cycle progression.
Cyclin E is just weakly expressed by lymphoid cells, contrasting with a stronger staining in trofoblastic placentary cells.
Cyclin D1 expression is not detected in normal lymphocytes, although some neoplastic types show high (MCL) or medium (HCL) level of CyclinD1 expression. Highests level of expresion of this protein are usually associated to 11;14 translocation.
Cyclin D2 is just weakly detected in normal and tumoral lymphocytes, with association to proliferation.
Cyclin D3 is expressed by proliferating lymphocytes, either benign or tumoral.
Cyclin A is only very weakly expressed, as fine nuclear dots, by the nuclei of normal lymphocytes, in contrast with a stronger expression by endothelial cells, macrophages and epithelial tissues. MBCs show a stronger staining by CyclinA, which parallels that seen for p21 in this cell subpopulation.
Cyclin B is detected as a cytoplasmic protein present in a subset of proliferating cells, consistent with cells in G2-M phases.
Some of the strongest deviations of this pattern were found in specific lymphoma types. Thus the relation between CyclinD and p27 protein appears to depend of ths specific type of CyclinD used, since CyclinD1 positive neoplasias (Mantle Cell Lymphoma) tend to show anomalous low level of p27/KIP1, while CyclinD3 overexpression (detected in a subset of Diffuse Large B-cell Lymphoma) associates with a stronger aberrant p27/KIP1 staining p21 high level of expression is found more characteristically in EBV posiitve neoplasms, such as Hodgkin's Disease (HD) and lymphomas in immunocompromised patients.
p16 level in tumours is inversely regulated with Rb expression. HD is a propotype of neoplasm with low or undetectable level of p16 expression (usually as consequence of gene methylation), which is paralelled by an increase in Rb staining.

As general conclusion, it seems that CDKIs and Cyclin use is dependent of specific cell types and tissue microenvironments, being at the same time regulated in a cell cycle dependent pattern.

A011

KEY ROLE OF THE RET GENE IN RADIATION-ASSOCIATED THYROID TUMORIGENESIS

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Introduction: In order to have a more conclusive data about the role played by different genes in radiation-associated thyroid tumorigenesis, we have studied 39 malignant and benign thyroid tumors from patients with a history of external radiation for benign or malignant conditions. The results obtained with these tumors concerning the *ras, gsp, ret and trk* oncogenes, were compared with data obtained by us screening 39 malignant and benign «spontaneous» thyroid tumors and by others, studying post-Chernobyl tumors.

Materials and methods: tumoral DNA or RNA prepared from paraffin included or fresh tissues, were screened using the RT-PCR, XL-PCR, hybridization with synthetic probes (mutated or not), Southern blot and direct sequencing techniques.

Results and conclusions: Our results showed that the overall frequencies of *ras, gsp, ret and trk* alterations in the radiation-associated tumors were 14%, 3%, 64% and 9% respectively, while in the «spontaneous» samples they were 25%, 7%, 8% and 6% respectively. All *Ret* alterations were RET/PTC rearrangements. Most RET/PTC rearrangements were due to an intra-chromosomal inversion (RET/PTC 1 and PTC 3). Although the overall frequency of RET/PTC rearrangements was similar in papillary carcinomas occurring after therapeutic irradiation and in the Chernobyl area, the predominant type of chimeric gene was different: RET/PTC 1 and PTC 3 respectively. In conclusion: 1) our results show and/or confirm the crucial role played by the *ret* proto-oncogene activating rearrangements in the development of radiation-associated thyroid tumors appearing after therapeutic or accidental radiation and 2) suggest that RET/PTC 1 may be associated with tumors with a long latency period (average 15 years in our patients) and a typical histology, while RET/PTC 3 may be associated with tumors with an agressive behaviour, a short latency period (average 3 years in the Chernobyl tumors) and a particular histology. The «break-points» of the different RET/PTC chimeric genes have been characterized.

A046

DCC AND *SMAD4* ALTERATIONS IN HUMAN COLORECTAL AND PANCREATIC TUMOR DISSEMINATION.

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Background: Chromosome 18q is lost a high proportion of colorectal and pancreatic cancers. Three candidate tumor suppressor genes, *DCC*, *Smad4* and *Smad2* have been cloned and identified from this chromosome region. DCC and *Smad4* aberrations have been previously identified in pancreatic and colorectal tumors. However, its biological significance remains controversial. **Aim:** To compare the presence of concurrent genetic aberrations in DCC and neighboring *Smad4* and *Smad2* genes during colorectal and pancreatic distal dissemination. **Material:** We have used a panel of orthotopically implanted colorectal (n=13) and pancreatic (n=8) xenografts and corresponding metastases (n=35). In some cases, corresponding primary tumors and synchronous adenomas (n=16) were also studied. The analysis of DCC aberrations were studied by intragenic and flanking LOH, RT-PCR, western blot and IHC and the analysis of Smad4 aberrations by STS markers, one microsatellite and RT-PCR.
Results: We have shown that while LOH at DCC locus occurred at a similar frequency (50%) in both tumors; 5/10 in colorectal and 2/4 in pancreatic, diminished DCC expression was exclusively present in colorectal tumors (5/13) harboring intragenic DCC LOH. In contrast, loss of DCC expression occurred more often in metastases of pancreatic xenografts (10/17) than in those of colorectal xenografts, even in the absence of concomitant LOH. *Smad4* gene aberrations were detected at a similar frequency in colorectal (4/13) and pancreatic (2/8) xenografts and were selected for during distal dissemination of both tumors. Acquisition of alterations in both genes occurred independently and may (3/35) coexist in the same tumor cell. **Conclusions:** Our results suggest that genetic heterogeneity at 18q21 is high in colorectal and pancreatic cancer cells. However, its molecular basis may differ between both tumors.

A057

Expression of a new isoform of tumor susceptibility protein, TSG101, lacking the transcriptional repressor domain, in Burkitt lymphomas

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The tumor susceptibility gene, *TSG101*, has been identified as a putative new class of tumor suppressor genes. We have studied its expression in twenty-two Burkitt Lymphoma (BL) cell lines. Several aberrant messages were detected in all cell lines. The aberrant splice donor sites are located within exon I at positions 132, 154 , 172 y 284 in the mature cDNA. Aberrant splice acceptor sites are located within exon 5 at positions 847 y 1054. All the aberrant messages are coexpressed together with a normal message, suggesting that the aberrant forms might result from further processing of the mature transcript. The normal transcript codes for a protein of 46 kDa (TSG101A). One of the aberrant messages maintains the open reading frame by joining nucleotides 283-1055 and codes for a protein of 17 kDa (TSG101B),. The coding potential of these two transcripts has been demonstrated by *in vitro* transcription-translation experiments. The TSG101B isoform has lost its leucine zipper that functions as a transcriptional repressor, and retains its N- terminus that appears to function as an inhibitor of the E2 family of ubiquitination enzymes, and also has homology to the CROC-1/UEV-1 gene product. The TSG101B isoform was detected in seventeen out of twenty-two (72 %) BL cell lines, but not in normal lymphoid populations. The detection of two isoforms of TSG101 with different dimerization properties opens up a new level of regulation of these proteins that possibly are implicated in cell cycle control.

A058

ALLELIC LOSS OF THE PTEN GENE REGION (10q23) AND POOR PROGNOSIS IN BREAST CARCINOMAS.

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Aim: Loss of heterozygosity (LOH) in loci of the 10q23 region, that harbor PTEN gene (phosphatase and tensin homologue deleted on chromosome 10), and mutations in the sequence of this gene, have been found in several primary human tumors including breast carcinomas, suggesting that this gene could be implicated in their pathogenesis.

Methods: We investigate allelic losses in the 10q23 region, and their correlations with age and eight pathologic parameters (tumor size, lymp node metastases, presence of oestrogen and progesterone receptors, histologic type, peritumoral vessel invasion pathological stage and histologic grade) in 105 breast carcinomas. The LOH analysis was performed amplifying DNA by PCR method, using five markers of the 10q23 region (D10S1687, D10S541, D10S2491, D10S583 and D10S571), the products were run in 12% nondenaturing polyacrilamide gels and stained with a commercially available silver method.

Results: After analyze tumor and normal tissue of all patients, LOH in at least one marker of the PTEN region was found in 29.5% of tumors. The statistical analysis between carcinomas with and without LOH respect to the pathologic parameters shows a significant diference with age (p=0.03), lymph node metastases (p=0.02) and higher histologic grade (p=0.02); a tendence to significance was found for progesterone receptors (p=0.05) and tumor size (p=0.06). LOH in an individual marker and relation statistically significant with tumor characteristics was observed in locus D10S541 for lymph node metastases (p=0.04), in D10S2491 (intragenic to PTEN gene) for lymph node metastases (p=0.02) and for tumor size (p=0.03), and in D10S583 for progesterone recetors (p=0.01) and for high grade (p=0.03).

Conclusions: These results suggest that PTEN gene, or other genes of 10q23 region, could be related functionally with breast cancer, and probably influencing the developing of poor tumor histologic characteristics that ussually predict a poor prognosis of the disease.

A066

GENETIC STUDY OF THE RET, GDNF AND GFRA1 GENES IN MEN2A FAMILIES

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Multiple Endocrine Neoplasia type 2 (MEN2A) is an autosomal dominant cancer syndrome characterized by medullary thyroid cancer (MTC), adrenal pheochromocytoma, and parathyroid hyperplasia. Physical and genetic mapping data and biological assays have involved *ret* gene (coding for Ret a receptor tyrosine kinase) in this syndrome. Ret ligands have been recently identified as Glial Derived Neurotrophic Factor (GDNF) and Neurturin. Ret activation seems to be mediated by different GPI-linked co-receptor proteins, named GFRA1, A2 and A3.

We have analyzed, by SSCP and direct DNA sequencing, the genes *ret* (exons 10,11,12,13,14,15 and 16), *gdnf* (exons 1 and 2) and *gfra1* (exons 4,5,6 and 7) from 25 individuals of five families with MEN2A. All cases of MEN2A showed the oncogenic mutation on the codon 634 of *ret* (exon 11): 50% of families displayed the mutation C-R and the other 50% C-Y. Furthermore, we detected a mutation of codon 691 (also in *ret*) changing G-S, with a similar frequency in unaffected individuals than in MEN2A patients. Several polymorphism were detected on different exons, including *gfra1* gene, and we are interested to compare the germline genetic data with the situation on tumor samples.

A123

INCREASED NUMBER OF CHROMOSOMAL IMBALANCES AND DNA AMPLIFICATIONS IN MANTLE CELL LYMPHOMAS

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Mantle cell lymphomas are characterized by 11q13 chromosomal translocations and cyclin D1 overexpression. The secondary genetic and molecular events involved in the progression of these tumors are not well known. In this study, we have analyzed 45 mantle cell lymphomas, 32 typical and 13 blastoid variants, by comparative genomic hybridization. To identify the possible genes included in the abnormal chromosome regions, selected cases were analyzed for *p53*, *P16*, *RB*, *C-MYC*, *N-MYC*, *BCL2*, *BCL6*, *CDK4* and *BMI-1* gene alterations. The most frequent imbalances detected by CGH were gains of chromosomes 3q, 7p, 8q, 12q, 18q, and 9q34 and losses of chromosomes 13, 6q, 1p, 11q14-q23, 10p14-p15, 17p, and 9p. High-level DNA amplifications were identified in 11 different regions of the genome. The CGH analysis allowed the identification of regional consensus areas in most of the frequently involved chromosomes. Chromosome gains, and losses and DNA amplifications were significantly higher in blastoid variants. The significant differences between blastoid and typical tumors were gains of 3q, 7p, and 12q, and losses of 17p. CGH losses of 17p correlated with *P53* gene deletions and mutations. Similarly, gains of 12q and high-level DNA amplifications of 10p12-p13 were associated with *CDK4* and *BMI-1* gene amplifications, respectively. One of 2 cases with 8q24 amplification showed *C-MYC* amplification by Southern blot. Alterations in 2p, 3q, 13, and 18q were not associated with *N-MYC*, *BCL6*, *RB* or *BCL2* alterations, respectively, suggesting that other genes may be the targets of these genetic abnormalities in MCLs. Increased number of gains, gains of 3q, gains of 12q and losses of 9p were significantly associated with a shorter survival of the patients. These results indicate that an increased number of chromosome imbalances are associated with blastoid variants of MCLs and may have prognostic significance.

A099

TUMOR SUPPRESSOR GENES IN 18q21: INVOLVEMENT IN GASTROINTESTINAL TUMORS.

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Background. The 18q21 region is frequently involved in gastrointestinal tumors. Two putative tumor suppressor genes have been identified in this region, DPC4 and DCC. The mechanisms involving the inactivation of these genes have not been completely elucidated.
Aim. The aim of the study was to analyze genetic losses at 18q21 and expression of DPC4 and DCC in colorectal tumors (n=10) and cell lines (n=22) and in pancreatic cell lines (n=17) using: 1) microsatellite analysis at 4 marker loci flanking these genes, 2) RT-PCR, and 3) direct sequencing in selected cases.
Results. Homozygous losses at 18q21 were not found in any of the colon cancer cell lines examined; by contrast a higher proportion of apparent homozygosity than expected was found, indicating common loss of one allele. DCC mRNA was detected in all but one cell line. In colorectal tumors, apparent homozygous losses were detected in 3 cases and DCC mRNA was detected in 10/10 cases.

Similarly, no homozygous losses at 18q21, and a high rate of apparent homozygosity, were detected in pancreatic cancer cell lines. DPC4 mRNA was detected in 12/17 cell lines. cDNA from 11 of these pancreas cancer cell lines was sequenced and 3 non-conservative mutations were identified.
Conclusions. These results indicate that homozygous deletions at 18q21 are infrequent in colorectal and pancreatic cancers while LOH is a common event. In pancreas cancer cell lines, the proportion of homozygous deletions and mutations is lower than that previously reported in the literature. It remains to be determined if other mechanisms of gene inactivation, such as methylation, may be involved.

A001

ALTERATIONS IN THE 1q31-1q32 REGION IN MAMMARY TUMORS

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Frequent amplification or alterations have been described in a wide range of tumors including breast cancer, in human chromosome region 1q31-32. Previously, a female-specific recombination hotspot within this region was shown to coincide with a minimal common region of allelic imbalance (AI) in mammary tumors, suggesting a possible relationship between these two observations.

We have analyzed with a pannel of 18 microsatellite markers the minimal common region of AI in the 33 mammary tumors and we have narrowed down the region to 2.5 cM. Within this region we have identified several genes that could be positional candidates to have a role in tumor development. STS markers of these genes have been used to detect their possible amplification or deletion within the genome. These studies that have been performed using a PCR multiplex technique together with Comparative Genomic Hybridization (CGH) have shown that the critical region appears to be amplified.

In addition, we have analyzed expression levels of the candidate genes and thus defined their possible implication in the tumor phenotype.

A003

GDP-L-FUCOSE:ASIALOFETUIN FUCOSYLTRANSFERASE ACTIVITY IN HUMAN COLON ADENOCARCINOMA

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Malignant transformation is frequently accompanied by a drastic alteration in surface oligosaccharide expression. Glycoconjugates on cell surfaces containing fucose exist in a wide variety of human cancers, specially in adenocarcinomas derived from gastrointestinal and respiratory epithelium. Carbohydrate determinants were suggested as cancer associated antigens and it has been proved to be ligands for adhesion molecules related to metastasis processes.

In this study we have determined GDP-fucose:asialofetuin fucosyltransferase activity using total cellular membranes obtained from human colon adenocarcinoma and normal adjacent tissue. The activity was assayed using conditions previously established in our work and results indicate a statistically significant increment of the activity in tumoral tissue with respect to the healthy tissue for fucosyltransferase activity acting on total acceptors in asialofetuin presence ($p<0.001$), on asialofetuin ($p<0.05$) or on endogenous acceptors ($p<0.001$) (according to Wilcoxon's test). Other reports have shown that, acting on asialofetuin, the majority of the transferase activity corresponds to $\alpha(1,3)$fucosyltransferase. This enhanced fucosyltransferase activity could give an explanation, at least partially, to the presence of altered glycoconjugates on cellular surface. On the other hand, fucose incorporation on endogenous acceptors was about 27% from the total. We haven't find correlation between enhanced activity and clinical stages of the tumours. In addition, the Km determination (for GDP-fucose) and Vmax of fucosyltransferase activity in presence or absence of asialofetuin was undertaken. Results point out no statistically significant differences between healthy tissue and the tumoral one.

This work was supported by a grant from Xunta de Galicia (XUGA-30106A97).

A040

DISTRIBUTION OF GENETIC ALTERATIONS OF TUMOR SUPRESSOR GENES IN DIFFERENT SUBTYPES OF NON HODGKIN LYMPHOMAS

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Non Hodgkin's lymphomas (NHL), as well as the rest of human neoplasias, arise after the acumulation of genetic lesions that deregulate cell division. It is known that the pathogenesis of human lymphomas is heterogeneous, and a great number of genes are involved in the development

We have analyzed in a group of 40 NHL, belonging to the main histologic subtypes, some of the most commonly tumor suppressor genes involved in different tumors, p53, p16, p15, and the recently described p73 y PTEN, to investigate their implication in NHL. We analyzed the presence of LOH in 9p21, using 6 polimorphic microsatellite markers, to probe the involvement of p16 and p15 genes. We have also studied 4 markers on 1p36, where the p73 gene is located; and 3 markers on 10q23 to detect LOH affecting PTEN gene. Mutations in p53 and p16 genes have been analyzed by SSCP technique. Abnormal methylation of 5' CpG island of p16 and p15 genes, as an alternative mechanism of inactivation, have also been studied.

The analysis of the distribution of these alterations showed that abnormal methylation of p16 gene is the most important alteration detected in low grade B-cell NHLs. P53 gene mutations, and LOH at p16, p73 and PTEN regions have been detected exclusively in high grade B lymphomas. Abnormal methylation of p16 gene in high grade B lymphomas was found in an important proportion of tumors. Multiples alterations of these genes only appeared in agressive lymphomas. Alterations of these genes were very rare in T-cell lymphomas, suggesting the implication of other genes in this type of tumors.

The knowledge of all the genetic lesions that appeared in lymphomas could help to the establishment of a model of development of these tumors.

A015

DIET AND GENETIC ALTERATIONS IN COLORECTAL CANCER. Interim Analysis of a Genetic Epidemiology Case-Control Study.

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Background: A hospital based case-control study of colorectal cancer is being conducted to assess the relationship between diet and genetic alterations found in the tumours.

Methods: Cases and controls are interviewed with a structured questionnaire on risk factors and dietary history. For operated cases, samples of tumour and normal tissue are collected and frozen. After DNA extraction, mutations in K-ras, codons 12 and 13, and p53, exons 5 to 9, were detected by SSCP and cyclic sequencing. Microsatellite instability was analysed by PCR amplification of 5 different loci. Only exploratory food group analysis has been done. Classification of cases according to genetic alterations allows different logistic regression modelling for risk estimation. Beyond classical analysis (all cases *vs* controls), a case-case comparison is done, and the risk of cancer related to food groups is estimated for tumours with and without mutations in K-ras and p53.

Results: Currently, 380 cases have been interviewed out of 446 identified and 362 controls out of 395. Tumour tissues of 201 cases have been analysed for mutations in K-ras (41% of them mutated). 178 cases have been analysed for p53 (59% mutated). High total caloric intake is a mayor risk factor for colorectal cancer (average of 49% increase in risk per 1000 kcal, 95%CI= 18% to 87%). Analyses of associations of food groups with colorectal cancer show expected results of a protective effect of vegetables, especially those consumed raw in salads (OR=0.86 per 100 grams daily, 95%CI= 0.76 to 0.98). Initial screening of differences in food group consumption between cases with mutations in K-ras or p53 and cases with wild type genes has not shown very strong associations.

Conclusions: The analyses done so far based on food groups show results consistent with previous work (Bautista et al. CEBP 1997;6:57-61). Detailed nutrient analysis is ongoing.

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A041

C-MYC ONCOGEN AMPLIFICATION IN HUMAN GLIOBLASTOMA CELL LINE XENOGRAFTS IN NUDE MICE

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The average survival of patients with multiform glioblastoma is approximately one year, registering a survival of just 5 % in a period of 5 years. The cell lines provide very important material to study the biology and new therapies of the glioblastomas.

The cell line R197 has been established from a human glioblastoma kept in vivo by means of xenografts in athimic nude mice. The original tumour belonged to a patient diagnosed as having a multiform glioblastoma and without medical treatment. The tumoral sample, obtained through surgery, was disintegrated using a solution of Tripsina/EDTA obtaining a cell suspension whose viability was determined with Tripan blue. In right front thorax of nude mice (Swiss, un/nu of 6 weeks) were inoculated 10^6 tumoral cells subcutaneously. The developed tumours were processed similarly to be reinoculated in nudes, which facilitates the survival and adjustment of the tumoral stem cells in the host. From xenografts n°41 cellular cultures, which present a stabilisation of their biological parameters, were established.

The phenotype of R197 has been studied according to morphological, inmunocytochemical, kinetic and cariological parameters. R197 behaves like an oncogen in athimic nude mice. In the developed xenografts the tumoral kinetics-curves and fraction of growth-as well as the histopathological pattern and the tumoral indicators have been distinguishing features.

The gene analysis of R197 (cellular culture/xenograft) has focused on the expression of the oncogenes erb B 1 and c-myc and the suppressor genes p53 and pRb. A case of c-myc amplification has only been described in a cell line derived from a human infantile glioblastoma. In R197 this amplification could suggest a selective advantage for the tumoral growth attributed to the environment of the host nude. The amplification of erb B1 has been correlated with the progression and invasive capacity of the oncogenes. Proteins p53 and pRb, involved in the control of the regular cycle, act as tumoral suppressors. The cell line R197 constitutes valuable material to get to know the genes involved in the development of these tumours and also to evaluate new therapies in vivo on an experimental basis.

A053

DNA AMPLIFICATION ON CHROMOSOME 6p12 IN NON-SMALL CELL LUNG CANCER

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INTRODUCTION: Gene amplification is crearly an important aspect of tumor growth and development, and has prognostic significance in certain tumors. In the present work, Arbitrarily primed-PCR (AP-PCR) has been applied to detect and characterise amplified DNA fragments in human non-small cell lung cancer (NSCLC).
PATIENTS AND METHODS: Genomic DNA from 65 NSCLC patients undergoing radically surgery was analysed by AP-PCR. Amplified tumor bands were isolated from the gel, cloned into a pGEM-T vector, sequenced and mapped to human chromosomes.
RESULTS: Our results revealed that gains of genomic sequences occur at high frequency (64% of all genomic changes analysed) in NSCLC tumors. Moreover, we succeed in detecting a genomic sequence which was highly amplified in one of the tumors analysed. In addition, intensity of this DNA fragment was also increased in 29 (45%) of the 65 patients from this study.The amplified DNA fragment was identified as a 600 bp sequence mapped to human chromosome locus 6p12. Close to this locus is located *pim*-1, an oncogene that can cooperate with *c-myc* in lymphomagenesis. When the amplified sequence was compared to the GenBank database, no significant homology with known human sequences was found. Interestingly, survival studies revealed that free disease interval of NSCLC patients was shorter in those patients bearing the amplified sequence (P=0.05 by the Breslow test).
CONCLUSION: Our results suggest the presence of an amplified sequence on chromosome 6p12 which is likely to be implicated in the pathogenesis of human non-small cell lung cancer.

A071

THE *PTEN* TUMOR SUPPRESSOR GENE IS FREQUENTLY ALTERED IN MOUSE THYMIC LYMPHOMAS INDUCED BY γ-RAYS

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PTEN, also named *MMAC1*, was recently identified as a tumor suppressor gene located at 10q23.3, a region which is frequently deleted in a wide variety of human tumors. Somatic mutations or homozygous deletions of this gene have been found in breast cancer, glioblastoma, prostatic cancer, endometrial cancer, skin cancer and kidney tumors. The protein encoded by *PTEN* is a dual-specificity phosphatase that can dephosphorylate serine, threonine and tyrosine residues. Loss of PTEN phosphatase activity results in the loss of its putative tumor-suppressive activity.

We have developed an animal model for the identification of tumor suppressor genes involved in T-cell lymphomagenesis by detecting significant loss of heterozygosity (LOH) for polymorphic markers in tumoral DNA. The LOH analysis of 83 primary T-cell lymphomas, induced by g-irradiation in (C57BL/6J x BALB/cJ) F1 hybrid mice, demonstrated frequent allelic losses in a region of mouse chromosome 19 syntenic to the human band 10q23.3. Structural and functional analyses revealed frequent altered expression and, at less extend, somatic mutations of *Pten* in tumoral samples.

These results clearly support the involvement of alterations of *Pten* in the progression of mouse primary T-cell linphomas

A080

VERY LOW RATE OF PTEN/MMAC1 MUTATIONS IN EPITHELIAL OVARIAN CANCER.

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INTRODUCTION: The PTEN/MMAC1 gene (10q23) has been recently isolated related to advanced stages of cancer with a potential role as tumor suppressor gene. Considerable differences in the frequency of PTEN/MMAC1 mutations have been described in different types of tumor from endometrial carcinoma with a high rate (50%) to prostate cancer with a very low incidence (2%).
AIMS: In this work we have studied the frequency and type of PTEN/MMAC1 mutations in epithelial ovarian carcinoma from different stages and histological types.
MATERIAL AND METHODS: Forty-four tumor samples have been analyzed: 28 were advanced stages (III and IV) and 16 early stages (Ia/Ib). Histological classification was: 24 serous papillary, 7 undifferentiated, 5 endometrioid, 4 mucinous, 3 mixed and 1 clear cell type. DNA isolation was performed by phenol-chlorophorm extraction. PCR amplification of the nine exons was done by using intronic primers and further sequencing analysis with dye terminators on an automated DNA sequencer ABI-377.
RESULTS: Only one PTEN/MMAC1 mutation (1/44, 2.27%) has been detected in an advanced ovarian carcinoma (stage IV) with endometrioid differentiation. This mutation has been found in one of the putative tyrosin phosphatase site producing a truncated protein lacking a significant number of potential functional sites located downstream exon 7.
CONCLUSIONS: These data suggest that the incidence of PTEN/MMAC1 in epithelial ovarian carcinoma is very low (2.27%) and in our series the results hint a greater relation with advanced stages of the disease.

A088

7q31-32 ALLELIC LOSS IS A FREQUENT FINDING IN SPLENIC MARGINAL ZONE LYMPHOMA

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Introduction: Splenic Marginal Zone Lymphomas (SMZL) has been recognised as an entity defined on the basis of its morphological, phenotypic and clinical characteristic features. Nevertheless, no characteristic genetic alterations have been described to date for this entity, thus making an exact diagnosis of SMZL difficult in some cases. As initial studies showed that chromosome region 7q22-32 is deleted in some of these cases, we analysed a larger group of SMZL and other lymphoproliferative disorders which may partially overlap with it.
Material and methods: To better define the frequency of 7q deletion in SMZL and further precise the deleted region, polymerase chain reaction (PCR) analysis of 13 microsatellite loci spanning from 7q21 to 7q36 was performed on 20 SMZL and 26 non-SMZL.
Results: The frequency of allelic loss in SMZL (8/20; 40%) was higher than that observed in other B-cell lymphoproliferative syndromes (2/26; 7.7%). This difference was statistically significant ($p<0.05$). The most frequently deleted microsatellite was D7S487 (5/11; 45% of the informative cases). Surrounding this microsatellite the smallest common deleted region (SCDR) of 5cM has been identified, defined between D7S685 and D7S514. By comparative multiplex PCR analysis, we detected an homozygous deletion in the D7S685 (7q31.3) marker in one case.
Conclusions: These results suggest that 7q31-q32 loss may be used as a genetic marker of this neoplasia, in conjunction with other morphologic, phenotypic and clinical features. A correlation between 7q allelic loss and tumoral progression (death secondary to the tumour or large cell transformation) in SMZL showed a borderline statistical significance. The observation of a homozygous deletion in this chromosomal region may indicate that there is a tumour suppressor gene involved in the pathogenesis of this lymphoproliferative neoplasia.

A089

P27/KIP1—CYCLIN D3 COLOCALIZATION IN A SUBSET OF AGGRESSIVE B-CELL LYMPHOMAS.

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p27 cyclin-dependent kinase inhibitor down-regulation is essential for transition to S-phase of cell cycle. Proliferating cells in reactive lymphoid tissue (RLT) show undetectable p27 expression. In contrast with this observation, an anomalous high p27 expression has been previously shown in a group of aggressive B-cell lymphomas (BCL) with high proliferation index and adverse clinical outcome, thus suggesting that the abnormally accumulated p27 protein has been rendered functionally inactive.

We have analyzed the causes of this anomalous presence of p27 in a group of aggressive BCL including 54 cases of diffuse large B-cell lymphomas and 20 Burkitt lymphomas, studying simultaneously p27, cyclin D3, cyclin D1 and cyclin E expression, since it has been described that high levels of expression of cyclin D1 or E leads to increase in p27 levels in some cell types. Double immunostaining and Laser Scanning Confocal Microscopy study was performed in 5 tonsils and 15 BCL.

An statistically significant association between p27 and cyclin D3 expression was found for the overall group. Additionally, it was observed that cases with stronger Cyclin D3 expression show also higher p27 expression. The relation between both proteins was also demonstrated at a subcellular level, by laser confocal studies, showing that in cases with high expression of both proteins there was a marked colocalization.
These results could support the existence of complexes Cyclin D3-p27 in a subset of aggressive B-cell lymphoma cases, where p27 lacks the inhibitory activity found when bound to cyclin E/CDK 2 complexes. This interaction between both proteins could lead to an abnormal nuclear accumulation, detectable by immunohistochemical techniques.

A110

DETECTION OF t(2;5)(p23;q35) AND ALK PROTEIN IN CD30+ LYMPHOMAS

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The t(2;5) (p23;q35) generates a chimeric transcript NPM-ALK encoded by the nucleophosmin (NPM) gene fused to the anaplastic lymphoma kinase gene (ALK). This chromosomal alteration is recurrently detected in Anaplastic Large Cell Lymphoma (ALCL CD30+) of T or null phenotype, though some authors describe the t(2;5) and/or ALK protein positivity in cases of Hodgkin's Disease (HD) and in other high grade lymphomas of different histologyc subtype that lack the activation marker CD30.

In this study the presence of t(2;5) is analyzed in a group of 25 lymphomas, which includes T, B and null ALCL, HD, ALCL-HD borderline entities and non- Hodgkin's T-cell lymphomas CD30+ , all of them with frozen material available.

The study was carried out using nested RT-PCR assay and performing hybridization with a NPM-ALK radiactive labeled junction-specific probe. The existence of chimeric transcripts was correlated with the expression and cellular distribution of ALK protein detected by immunohistochemistry using ALK-1 antibody.

The results show and increased sensitivity for nested RT-PCR compared with that of RT-PCR standard. This fact suggests that nested RT-PCR may be specially indicated to study paraffin-embedded specimens. A low percentage of positive cases for t(2;5) was observed in our series. These cases are mainly extranodal ALCL-T.

The present study contributes to clarify the function of t(2;5) in ALCL pathogenesis and its role as specific marker for this neoplasm. Furthermore, this work helps to determinate the utility of ALK-1 antibody in the analysis of ALK gene disregulation mechanisms and in the possible definition of new entities.

A122

c-myc mRNA EXPRESSION AND GENOMIC ALTERATIONS IN MANTLE CELL LYMPHOMAS AND OTHER NODAL NON-HODGKIN'S LYMPHOMAS

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Cyclin D1 is a weak oncogene that cooperates with *c-myc* activation in the development of B-cell lymphomas in transgenic animals. Cyclin D1 is constantly overexpressed in human mantle cell lymphomas (MCL). However, the status of *c-myc* gene in these tumors is not known. We have examined the *c-myc* mRNA expression and the gene configuration, including a mutational analysis of exon 1, intron 1, and exon 2 regulatory elements, in a series of 33 MCL, 22 typical and 11 blastoid variants. In addition, *c-myc* alterations were also examined in 56 additional nodal non-Hodgkin's lymphomas (NHL). *c-myc* mRNA overexpression was found in 38%(11/29) MCL with a slight higher frequency in blastoid variants (5/10, 50%) than in typical cases (6/19, 31%). This overexpression did not correlate to the proliferative activity of the tumors or the clinical characteristics and survival of the patients. Only one blastoid MCL showed *c-myc* gene amplification. No rearrangements or mutations in exon 1, intron 1, or exon 2 were detected in any case. In other nodal NHL *c-myc* overexpression was found in 24%(7/29) indolent tumors but in 70%(19/27) aggressive variants. Gene rearrangement was only found in one Burkitt's lymphoma and it was also associated with numerous mutations in regulatory regions of the gene. Point mutations in intron 1 or exon 2 were also detected in 8 of 35(23%) additional nodal NHL, 1/19(5%) indolent and 7/16(44%) aggressive variants. No gene amplifications or rearrangements were detected in any of these tumors. These results indicate that *c-myc* is overexpressed in a subset of MCL with a relative higher incidence in blastoid than typical variants. However, structural gene alterations are rare events in these tumors. *c-myc* is also overexpressed in a similar number of other nodal NHL. Point mutations in regulatory regions are more frequent in these tumors than in MCL and they are not associated with gene rearrangements

2. Apoptosis and Cancer

i

Functional interactions between oncogenes and tumor supressor genes: inhibition by c-Myc of apoptosis mediated by p53 in leukemia cells.

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Mutations and deletions of the *p53* tumor suppressor gene is the most common genetic alteration in human cancer and, as a general rule, tumors without wild-type p53 are more aggressive and have worse prognosis. p53 is a transcription factor whose target genes are involved in cell cycle arrest ($p21^{Waf1}$, *gadd45*) or apoptosis (*bax*, *IGF-BP3*). c-Myc is a nuclear protein of the helix-loop-helix/leucine zipper (bHLHLZ) family involved in the control of cell proliferation and differentiation. Paradoxically, c-Myc overexpression also mediates apoptosis in cells subjected to sub-optimal growth conditions, as deprivation of growth factors. The c-Myc-mediated apoptosis requires wild-type p53 in fibroblasts. The chronic myeloid leukemia (CML) is usually diagnosed in a benign chronic phase, characterized by a clonal granulocytosis. After 1-3 years, the disease progresses to a rapid fatal blast crisis. Alterations in the p53 gene are very rare during the chronic phase of the disease, but progression to the acute form of the disease (blast crisis) is accompanied by alterations in the *p53* tumor suppressor gene in a significant number of cases. Moreover, overexpression of c-*myc* is a common finding in blastic phase CML. We have studied the functional interaction between p53 and c-Myc in K562, a p53-null cell line derived from a chronic myeloid leukemia in blast crisis. We first constructed a K562 cell line transfected with the mutant $p53Val^{135}$, which encodes for a termosensitive protein that adopts a "mutated" conformation at 37°C and wild-type conformation at 32°C. These cells undergo apoptosis beginning after 24 h of exposure at 32°C. The shift to 32°C was also accompanied by up-regulation of $p21^{Waf1}$ and Bax (consistent with the wild-type conformation of p53). This apoptosis was inhibited by ectopic expression of Bcl-2 and by the caspases inhibitor Z-VAD.

To explore the relationship between p53 and c-Myc in K562 cells we constructed double transfected cell lines with conditional expression of both wild-type p53 and c-Myc. We used an expression vector with the c-*myc* driven by the metallothionein promoter, which is induced by zinc. We generated two cell lines with conditional expression of c-Myc and wild-type p53: by electroporation of the c-*myc* gene in *p53*-transfected cells and by retroviral transduction of the *p53* gene in c-*myc*-transfected cells. In both cell lines the coexpression of wild-type p53 and c-Myc was achieved by incubating the cells at 32°C in the presence of 50-100 μM $ZnCl_2$. Using these cell lines we found that: (i) c-Myc expression significantly inhibited the apoptosis mediated by p53, as assessed by different morphological and biochemical criteria, as DNA fragmentation, annexin V binding and fraction of apoptotic cells in the culture; (ii) c-Myc expression also abrogated the p53-mediated up-regulation of $p21^{Waf1}$ mRNA and protein, while no effect was detected on Bax expression; and (iii) c-Myc also reduced the transactivation of human $p21^{Waf1}$ promoter by wild-type p53 under conditions where the apoptosis inhibition was detected. The reducion on p21Waf1 promoter activity by zinc was about 60% in both double transfectant cell lines. In K562 this reduction was dependent on the amount of c-Myc expresion vector transfected. Taken together, these data suggest that c-Myc overexpression in some tumors may antagonize the pro-apoptotic function of wild-type p53. Thus the results contribute to explain why many tumors show overexpression of c-Myc despite the pro-apoptotic effect of this protein in some cell culture models.

j

Cytokines resistance contributes to metastatic lymph node specificity of breast cancer cells

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Metastasis is a highly complex process involving the survival of tumor cells, both in the blood stream and within specific organs. Cell-death and survival are determined by a number of gene products from an expanding family of the Bcl-2 gene, either promoting or preventing apoptosis.

To examine whether the prevention of cell-death influences the metastatic behavior, we transfected a human breast cancer cell line MDA-MB-435 with the Bcl-x_L cDNA and then studied metastatic ability of the selected clones *in vivo*, and followed tumors and metastasis from consecutive *in vitro-in vivo* implants. Our results show that Bcl-x_L-clones had a decreased tumor growth latency and an increased metastatic ability. After three *in vivo-in vitro* passes the metastatic behaviour of 435/Bcl-x_L exerted a new lymph node metastatic affinity. Apoptosis-resistance to cytokines was induced in 435 cells by Bcl-x_L-expression with minor modifications in their proliferation rates. These cells also showed diminished adhesion to extracellular matrix proteins and a survival advantage in suspension over 435/Neo cells. Moreover, to determine survival in blood stream and in cells lodged in the lungs, we injected 435/Bcl-x_L and 435/Neo cells at 1:3 proportion i.v., and animals were killed at intervals of 15' to 12h after injection. Tumor cells were recovered from the lungs and Southern-blot analysis revealed the presence of exogenous Bcl-x_L cDNA. These results showed that 435/Bcl-x_L cells had a survival advantage in circulation over 435/Neo cells. This advantage *in vivo* was attributable to Bcl-x_L expression.

We conclude that Bcl-x_L expression in breast cancer cells can increase metastatic activity and lymph node specificity. This advantage could be created by inducing resistance to apoptosis against cytokines, increasing cell survival in circulation, and enhancing anchorage-independent growth.

A024

PRO-APOPTOTIC THERAPY OF *In vivo* HUMAN MELANOMAS USING VITRONECTIN RECEPTOR ANTAGONISTS

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The αv integrin family has an important role in neovascularization processes and in the progression of several malignant cancers such as melanoma. In our lab some av integrin antagonists have been developed. One of them, the Mab 17E6 has a high activity in vitro as well as in vivo models. These preliminary results encouraged us to study the molecular mechanisms involved in the blockade of the vitronectin receptor (αvβ3) by the Mab 17E6.
With the aims of 1) investigating the molecules associated to the transignaling mediated by the av integrins and 2) to obtain new therapeutic drugs, we studied the activation of the pathways that trigger apoptosis in this model.
The Mab 17E6 was able to significantly block tumor progression in several models of human melanoma growth grafted on immunosuppressed mice. Dose-response studies showed a better efficacy of such monoclonal compared to the reference one (LM609).
Using the TUNEL staining technique we observed an increase in the apoptotic labeling in tumor sections from the 17E6 treated mice. In the models where a tumor regression was detected after the treatment with the antagonists, we also observed an increase in the bax levels and a decrease of the bcl-2 levels by means of western and northern blot. In vitro experiments of melanoma cell culture within 3D collagen gels also provided further evidence of the apoptosis triggering.
In conclusion, in the used therapeutic models the Mab 17E6 can inhibit the growth of pre-established tumors, probably by means of the activation of apoptotic mechanisms. The Mab 17E6 could have a therapeutic potential in the treatment of several tumors.

A037

PROLIFERATIVE DEFECTS IN MICE DEFICIENT FOR *E2F-1* TRANSCRIPTION FACTOR

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E2F transcription factors play an important role in cell cycle control regulating the expression of genes in G_1 phase, such as DHFR, TK, N-Myc, c-Myc. E2F activity is controlled by the pRB family members. The E2Fs can either stimulate or inhibit transcription depending on their association with pRB, which is regulated by phosphorylation. It has been suggested that the phosphorylation of pRB and the subsequent release of E2F triggers the progression through the cell cycle by activating and/or derepressing E2F-regulated genes. The E2F family consists of five closely related proteins (E2F-1 to E2F-5), of which E2F-1 is the best characterized member.

To analyze the role of E2F-1 in cell cycle progression we have used E2F-1 knockout mice previously generated by us (Field, SJ *et al* (1996) Cell, 85:549-561). We have found proliferative defects in mature T cells purified from lymph nodes and stimulated *in vitro* with the mitogens concanavalin A and interleukin-2 for several days. At several times during the treatment, cells were pulsed with BrdU, followed by staining with anti-BrdU and propidium iodide. The cell cycle distribution analysis showed a significant delay in S phase entry in E2F-1$^{-/-}$ T cells compared with E2F-1$^{+/+}$ T cells. This delay is acompanied by apoptosis at 24-48 hr. of cells unable to enter the cell cycle. These results show that E2F-1 is required for efficient proliferation, and could explain why E2F-1$^{-/-}$ mice are smaller than wild-type littermates. The analysis of the molecules involved in cell cycle control as well as in DNA replication in this experimental system will reveal E2F-1 gene targets that are critical for its function as a cell growth regulator.

A014

c-MYC INBIBITS APOPTOSIS AND p21^{waf1} TRANSACTIVATION MEDIATED BY WILD-TYPE p53 IN HUMAN MYELODIDE LEUKEMIA CELLS

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The progression from the chronic phase of chronic myeloid leukemia to the acute form of the disease (blast crisis) is accompained by alterations in the *p53* suppressor gene and *c-myc* overexpression in a significant number of cases. We have studied the functional interation between *p53* and *c-Myc* in K562, a *p53-null* cell line derived from a chronic myeloid leukemia in blast crisis. We first constructed a K562 cell line transfected with the mutant *p53 Val*^{135}, which encodes for a termosensitive protein that folds as a "mutated" conformation at 37°C and wild-type conformation at 32°C, as assessed by cell morphology, DAPI staining and genomic DNA fragmentation. The p53-mediated apoptosis was accompanied by up-regulation of *p21*^{waf1} and *Bax* and by down-regulation of *c-myc*. This apoptosis was inhibited by ectopic expression of *Bcl-2* and by the caspases inhibitor *Z-VAD*, indicanting that *p53* mediates a "clasical" apoptotic pathway in K562 cells.

To explore the relationship between *p53* and *c-Myc* in K562 cells we constructed double transfected cells lines with conditional expression of both wild-type *p53* and *c-Myc*. We used an expression vector with the *c-myc* driven by the metallothionein promoter, which is induced by zinc. We generated two types of double-transfectant cells lines: by electroporation of the *c-myc* gene in p53-transfected cells and by retroviral transduction of the *p53* gene in *c-myc* transfected cells. In both cell lines the coexpression of wild-type *p53* and *c-Myc* was achieved by incubating the cells a 32°C in the presence of 75 μM $ZnSO_4$. Using these cells lines we found that: (i) *c-Myc* expression significantly inhibited the apoptosis mediated by *p53*; (ii) *c-Myc* expression also abrogated the p53-mediated up-regulation of *p21*^{waf1} mRNA and protein, while no effect was detected on *Bax* expression; and (iii) *c-Myc* also reduced the transactivation of human *p21*^{waf1} promoter by wild-type *p53* under conditions where the apoptosis inhibition was detected. Taken together, these data suggest that *c-Myc* overexpression in tumors may antagonize the pro-apoptotic function of wild-type *p53*.

A021

BAX GENE MUTATIONS IN BREAST CANCER.

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Bcl-2 family proteins are implicated in regulation of cell death by apoptosis. Deregulate apoptosis could lead the accumulation of genetic alterations necessary for metastatic progression of breast cancer. We reported previously that lymph node metastasis in breast cancer are related with overexpression of Bcl-2 and loss of apoptosis in primary tumor.

The aim of this study was to explore Bax gene in the context of loss of apoptosis related to metastatic progression in breast cancer.

We studied 106 ductals breast carcinomas, at T_1 and T_2 (<5cm.) stage, 46 of them had lymph node metastasis. Frameshift mutations in a microsatellite in the third exon of Bax gene have been studied. Moreover, we analysed microsatellite instabillity with three sequences (D4S2948, D21S415 and Bat 26) in 87 specimens which we had tumor and normal breast.

We detected one mutation of Bax gene in a T_1N_0 tumor with loss of apoptosis and no expression of Bcl-2. 7%(n=6) of tumors shown instability at least for two of the analysed sequences, 3 of them had lymph node involvement.

The low frequency of Bax mutations and genomic instability of breast cancer argue that this mechanism might be unrelevant in metastatic progression of small ductal breast carcinomas. Bax mutations are not implicated in the loss of apoptosis observed in metastatic ductal breast carcinomas.

A022

Bcl-x_L promote breast cancer cells survival in circulation

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Metastatic cell behaviour reflects a combination of many gradually acquired phenotypes each contributing to progression. Thus deregulated expression of genes that prevent cell death could increase survival of tumor cells leading the accumulation of genetic alterations, and ultimately promote metastasis.

We have reported previously that Bcl-x_L overexpression in breast cancer cells promotes metastasis. Since programme cell death can be a factor of rapid elimination of tumor cells, the antiapoptotic protein Bcl-x_L could increase metastasis by increasing survival. In order to characterise the effect of Bcl-x_L in breast cancer, we study in MDA-MB-435/Bcl-x_L and MDA-MB-435/Neo cells the in vitro migration and adhesion, and the *in vivo* viability of blood circulating cells. To determine survival in circulation we injected i.v. 435/Bcl-x_L and 435/Neo cells at 1:3 proportion, injected animals were sacrificed at various time intervals after injection, 15' to 12h. DNA from cells recovered from lungs was analysed by Southern-blot.

The results of *in vivo* experiments showed that 435/Bcl-x_L cells had a survival advantage in circulation with regard to 435/Neo cells. Moreover, 435/Bcl-x_L cells had a diminished adhesion to Laminin and Collagen IV, without modification in cell migration.

In conclusion, in breast cancer cells suppression of programme cell death by overexpression of Bcl-x_L might facilitate metastasis by inducing a survival advantage in circulation and enhancing anchorage independent growth.

A023

RESISTANCE TO APOPTOSIS INDUCED BY BCL-2 AND/OR BCL-X_L FAVOURS METASTATIC PROGRESSION OF BREAST CANCER

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Bcl-2 and Bcl-x_L are cell death-related genes from Bcl-2 family that act as antidotes of apoptosis. Overexpression of these proteins could favour the accumulation of genetic alterations involved in metastatic progression.

To assess the role that Bcl-2 and Bcl-x_L can play in metastatic phenotype of human breast cancer we transfected MDA-MB 435 cells with Bcl-2 or Bcl-x_L gene. We studied the *in vitro* proliferation and apoptosis of selected clones: 435/Bcl-2, 435/Bcl-x_L and 435/Neo. Besides, we explored the *in vivo* tumorigenicity and metastatic behaviour of cells implanted orthotopicaly into the m.f.p. of Nude/Balb-c mice. To study the putative accumulation of genetic alterations we also followed tumor development and metastasis from consecutive *in vitro/in vivo* implants.

435/Bcl-2 and 435/Bcl-x_L clones had acquired *in vitro* resistance to apoptosis induced by growth factor without modification in proliferative index. The two clones had increased tumorigenicity and lung metastatic ability. After three *in vivo/in vitro* passes the metastatic behaviour of 435/Bcl-x_L exerted a new lymph node metastatic affinity.

These results suggest that Bcl-2 and Bcl-x_L in 435 breast cancer cells increase metastatic ability and induce tumorigenicity by loss of apoptosis. Moreover, anti-apoptotic genes may be implicated in organospecifity by acquired resistance to apoptosis induced by growth factors.

A035

APOPTOSIS AND MITOTIC ARREST ARE INDEPENDENTLY INDUCED BY THE PROTEIN PHOSPHATASES INHIBITOR OKADAIC ACID IN K562 LEUKEMIA CELLS

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Treatment of the human myeloid leukemia K562 cells with the serine/threonine protein phosphatases inhibitor okadaic acid induced mitotic arrest followed by apoptosis in a synchronized manner. The effect was observed at drug concentrations known to inhibit only the phosphatase type 2A in K562 cells. More than 50% of the cells showing metaphasic figures could be observed 16-24 hours after the addition of 15 nM okadaic acid. The drug induced a normal mitosis rather than premature chromosome condensation. After the peak of metaphasic cells an increasing number of cells with typical morphological and biochemical features of apoptosis was detected, reaching 80% of cells after 72 hours. We investigated whether apoptosis was a consequence of the preceding mitosis arrest or was independently induced by okadaic acid. We found that (i) okadaic acid-mediated apoptosis was significatively reduced in cells with high constitutive expression of Bcl-2 (obtained by retroviral transduction), but the drug still induced the mitotic arrest; (ii) pretreatment of cells with the DNA synthesis inhibitor hydroxyurea did not preclude the apoptosis mediated by okadaic acid; (iii) c-*myc* down-regulation was dependent on apoptosis and not on mitotic arrest; and (iv) while mitosis was abrogated by the protein synthesis inhibitor cycloheximide, the cells still undergo apoptosis. The results suggest that inhibition of protein phosphatase 2A by okadaic acid provokes mitotic arrest and apoptosis by independent mechanisms.

3. Advances in Cancer Treatment

A042

EFFECTS OF PCI (POTATO CARBOXYPEPTIDASE INHIBITOR) ON BREAST CARCINOMA.

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Epidermal growth factor receptor (EGFR) transduction pathway seems to play a prominent role in the development of carcinomas, and constitutes a target for antitumor therapy. We have previously described(1) that potato carboxypeptidase inhibitor (PCI), a 39-amino acid protease inhibitor, can block binding of EGF to its receptor and inhibit activation of receptor protein tyrosin kinase. The experiments presented here demonstrate that protracted treatment of tumor cells with PCI down-regulates EGFR expression (A431 and MDA-MB-453 cells). Our previous results involving cell cultures and nude mouse xenografts demonstrated the capacity of PCI to inhibit the growth of human pancreatic cell lines. To find more about the potential of PCI as a new therapeutic agent, we have tested if it has any effects on human breast carcinoma cell lines (MCF-7 and MDA-MB-453, EGFR expressing cells), finding that protracted treatment with the inhibitor reduces cell proliferation. All these results suggest that PCI has a potential for therapeutic application in the treatment of carcinomas.

(1) C.Blanco-Aparicio et al. Potato Carboxypeptidase Inhibitor, a T-knot Protein, is an epidermal growth factor antagonist that inhibits tumor cell growth. J.Biol.Chem. 1998, 273(20): 12370-77

A073

CULTURE OF DENDRITIC CELLS FROM CANCER PATIENTS FOR IMMUNOTHERAPY. JI Mayordomo, P. Lasierra, L Palomera, D Isla, L Larraz, MD García, A Tres. Oncology Medical, Inmunology and Hematology Divisions. Hospital Clínico Universitario. Zaragoza. Spain.

Background: Dendritic cells (DC) are the most potent antigen-presenting cells. The development of methods to generate large numbers of mouse DC from hematopoietic progenitors cultured in GM-CSF and interleukin-4 has made testing of in vivo immunization with DC pulsed with tumor peptides capable of being recognized by T lymphocytes possible. This strategy has been showhn to inducide regression of established tumors in murine models (Mayordomo et al, Nature Medicine 1995, 1: 1297) **Aims:** To check if large numbers of DC can be generated in cancer patients from peripheral blood mononuclear cells. **Patients and Methods:** Peripheral blood mononuclear cells have been harvested from 24 cancer patients undergoing leukapheresis after movilization with G-CSF=/- chemotherapy have been. After separation of mononucear cells, they were cultured in GM-CSF (1000 U/ml) plus interleukin-4 (100 U/ml) for 7 days before phenotypic assessment. **Results:** Patient diagnoses were: breast cancer (17), non Hodgkin's lymphoma (4), germ cell tumor (2) and rhabdomyosarcoma (1). The phenotype of cultured cells was consistent with dendritic cells: intense positivity for HLA-DR and DC86, plus negativity for markers of other lineages, including CD3, CD4, CD8 and CD14. The identity of dendritic cells was confirmed by their typical round morphology with cytoplasmic projections by light microscopy and their immunocytochemical positivity for HLA-DR. Although the number of cells generated varied from patient to patient, more than 10^9 DC cold be generated from a single leukapheresis in >50% of patients. **Conclusions:** It is possible to generate sufficient numbers of Dc for immunization trials from cancer patients through culture of peripheral blood mononuclear cells in GM-CSF plus interleukin-4.(FISS Project 98/0662 and DGA)

A060

THE ANTINEOPLASTIC ACTIVITY AND GENOME REPLICATION OF THE PARVOVIRUS MVM CORRELATE WITH A DIFFERENT PHOSPHORYLATION PATTERN OF THE VIRAL NS-1 PROTEIN

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Parvovirus multiplication is tightly regulated by functions expressed during cellular proliferation at specific differentiation stages, which determines that these viruses display selective cytotoxic and cytolitic activities against neoplastic growth, and that they are currently considered as potential anticancer agents. We have studied the molecular bases of the oncolitic activity of two strains of the parvovirus Minute Virus of Mice (MVMp and MVMi) interacting with the human glioblastoma cell lines U-87 MG and U-373 MG.

MATERIALS AND METHODS. The tumor cell lines were infected with MVMp and MVMi in the presence of [32P]orthophosphate. 32P-labeled NS-1p and NS-1i purified proteins were trypsin digested and their phosphopeptide maps analyzed. Recombinant vaccinia viruses with the NS-1 protein gene from each MVM strain were prepared and used to infect the two tumor cell lines. The vaccinia expressed NS-1 was similarly labeled and subjected to phosphopeptide analysis.

RESULTS AND CONCLUSIONS. Whereas the U-373 MG cells were completely susceptible
to MVMp but not the U-87 MG cells, both cell lines infected with MVMi tolerated high levels of viral RNA and protein synthesis, although no sign of DNA amplification was detected. We verified the conversion of the input virion single stranded DNA to a duplex replicative form, indicating that the replicative arrest occurred at a subsequent step. This suggests that the NS-1 phosphoprotein, a protein implicated in transactivation, viral DNA amplification and cytotoxicity, has altered some of its activities (nicking, binding and helicase) in these tumor cells. Phosphopeptide maps of NS-1 immune-purified from U-87 MG and U-373 MG cells infected with MVMi were similar, but differed from the NS-1 map obtained in the MVMp infected U-373 MG cells. This different phosphorylation correlated with the viral replication capacity. Interestingly, the NS-1p and NS-1i proteins purified from infections with recombinant vaccinia viruses displayed phosphorylation maps similar to the ones of the MVM infections. These recombinant vaccinia viruses are currently being used to test the biochemical activities of the NS-1 proteins purified by affinity chromatography.

A077

Antineoplastic effect of a farnesyl-transferase inhibitor in N-Ras overexpressing tumors. Ramon Mangues (1), Teresa Corral (2), Nancy E. Khol (3), Jackson B. Gibbs (3), Allen Oliff (3), Silvia Guerrero (1), Isolda Casanova (1), Lourdes Farré (1), Gabriel Capellà (4) y Angel Pellicer (2). *Lab. Investigació Gastrointestinal, Institut de Recerca, Hospital de Sant Pau (1), Department of Pathology, New York University Medical Center, New York (2), Department of Cancer Research, Merck Research Laboratories, West Point, Pensylvannia, USA (3), y Institut Català d'Oncologia (4).*

Introduction. Ras proteins should be farnesylated on their CAAX box at the carboxy terminus of the protein to be transformant. Peptidomimetics of the CAAX box are potent inhibitors of the farnesyl-transferase in vitro and inhibit transformation by Ras in cells in culture. **Objective:** To evaluate the antitumor effect of a farnesyl-transferasae inhibitor, L-744,832, in mammary carcinomas and lymphomas in transgenic mice overexpressing the N-ras proto-oncogene and to study the effect of the compound on the level of Ras protein farnesylation in cultured cells. **Methods:** Six 10 month old transgenic mice were injected with 40 mg/kg of L-744,832 for 5.5 weeks. Six non-trangenic littermates were injected with vehicle over the same period. Mammary tumor volume was monitored and the presence or absence of linfomas was recorded at the end of treatment. We measured the expression level of N-Ras and H-Ras proteins in tumor tissue and the inhibition of the Ras protein farnesylation in extracts of cells exposed to the compound by Western with N-Ras and H-Ras specific antibodies. **Results:** We observed a reduction in mammary tumor mean growth rate (-0.7 (experimental) vs. 28.2 (control) mm^3/día, $p<0.001$, t test). We observed lymphomas in 3/6 mice in the control group and in 0/6 mice in the experimental group ($p<0.05$, test Chi^2). The level of N-Ras expression in tumor tissue was about 20 fold the endogenous N-Ras protein level. L-744-832 inhibited the H-Ras farnesylation but did not significantly inhibit N-Ras farnesylation in cultured cells exposed to the compound. **Conclusions:** 1) The farnesyl-transferase inhibitor, L-744,832, is effective in treating N-Ras overexpressing mammary carcinomas and lymphomas in this transgenic model. 2) The antineoplastic effect is not associated with the inhibition of the N-Ras protein farnesylation; other pharmacological targets should exist that explain this activity.

A101

THERAPEUTICAL APPROACHES BASED ON ADENOVIRUS-MEDIATED SUPPRESSOR GENES(p53 AND pP16INK4A) INTRODUCTION IN HUMAN EXOCRINE PANCREATIC CANCER.

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Introduction: The development of new therapeutic approaches is particularly urgent in pancreatic tumours due to the scanty effectiveness of the ones presently in use. Gene therapy approaches based on the reintroduction of wt-genes of p53 and p16 appear as very promissing because of the key role of these proteins in the cell cycle control and the response to DNA damage together with its high incidence of mutations in pancreatic tumours.
Materials and methods: The reintroduction of wt-p53 and/or wt-p16 genes in cell lines derived from human pancreatic adenocarcinomas (NP-9, NP-18, NP-29 and NP-31) has been succesfully assayed by infection with defective recombinant adenoviruses (Ad5CMV-p53 and Ad5RSV-p16). Effects on cell proliferation, variations on cell cycle profiles and apoptosis induction have been determined.
Results: The reintroduction of wt-p53 gene in p53-mutant cells (NP-9, NP-18 and NP-31) elicited high growth inhibitions and marked increases in apoptosis. In contrast, the overexpression of p53 in endogenously-expressing wt-p53 (NP-29) does not induce apoptosis. The presence of wt-p16 contributes synergically to the apoptosis induction. This effect has been observed in the wt-p16 cell line (NP-18) and in mutant-p16 cells (NP-9, NP-31) when wt-p16 is overexpressed.
Conclusion: Our results point out that the effects provoked by overexpression of p53 and/or p16 are clearly dependent on tumour genetic alterations pattern. The knowledge of these alterations is neccessary to develop efficient therapies based on the reintroduction of tumour suppressor genes.

A097

CHARACTERIZATION OF BLEOMYCIN HYDROLASE , A CYSTEINE PROTEINASE INVOLVED IN CHEMOTHERAPY RESISTANCE.

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Bleomycins are glycopeptidic antibiotics used as chemotherapeutic agents in the treatment of human malignancies. Despite their antitumoral activity, the use of bleomycin in clinical practice is hampered by pulmonar toxicity and the development of tumoral resistances. These two complications have been related to the metabolic inactivation capacity of this drug by normal and tumoral tissues. In the present work molecular cloning and characterization of bleomycin hydrolase are shown. Bleomycin-hydrolase cDNA sequence isolated from a brain cDNA library contains an open reading frame coding for a peptide of 455 amino acids. This sequence resembles that of cysteine proteinases and is widely expressed in human tissues. Northern analysis of head and neck squamous cell carcinomas showed a slight overexpression in these tumors when compared with that of normal adjacent mucosa. Besides this, human bleomycin hydrolase shows variable levels of expression in lymphomas (low in Hodgkin's disease and higher in Burkitt's lymphoma). Bleomycin hydrolase gene contains 12 exons and its promotor sequence shares common features with those of other ubiquitously expressed genes. Moreover, this promotor sequence contains a polymorphic CCG tandem repetition sequence. Bleomycin hydrolase gene has been mapped by FISH in 17q11.2. Detailed analysis of this region and data from loss of heterozygosity studies in neurofibromatosis type 1 tumors place this gene in a position telomeric with respect to NF1, but in close vicinity with this tumor suppressor gene. The existance of loss of heterozygosity of this drug inactivating enzyme in neurofibromatosis type 1 tumors could be associated with a higher sensitivity of these malignancies to bleomycin chemotherapy.

A006

NEW ASSOCIATION SCHEDULES WITH OXALIPLATIN, TOPOTECAN AND 5-FU IN SENSITIVE AND 5-FU-RESISTANT CELL LINES.

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Topotecan (TPT) and Oxaliplatin (OXA) have shown a high therapeutic potential. **Objective:** to analyze induced cell toxicity by TPT, OXA and 5-FU combinations in sensitive and resistant 5-FU cell lines. The 5-FU resistant cell line, generated in our laboratory, showed a 5-FU resistence six times higher than the sensitive cell at IC 50. **Material and Methods:** we used 4 sequential models of administration: OXA-TPT, TPT-OXA, OXA-5-FU and 5-FU-OXA. The citotoxicity was analyzed by means of cholorimetric MTT-test and the response rate using the combination index CI (CI following the Chou and Talalay method). **Results:** The 4 sequential models demonstrated to be very sinergistic, being besides independent from the 5-FU resistance fenotipe. (See summarized in the chart below the results of CI for different survival fractions). In the OXA-5_FU combination we can also see that the inclusion of OXA circunvexes the resistance to 5-FU.

		OXA-TPT	TPT-OXA	OXA-5FU	5FU-OXA
IC70	HT29	0.86	0.64	0.92	0.50
	HT29R	*0.59*	*0.46*	*0.27*	*0.53*
IC50	HT29	0.77	0.79	0.58	0.40
	HT29R	*0.65*	*0.55*	*0.24*	*0.47*
IC30	HT29	0.73	0.96	0.37	0.31
	HT29R	*0.72*	*0.66*	*0.24*	*0.47*

Conclusion: The OXA/TPT and OXA/5-FU combination demonstrated to have a high therapeutic potential in sensitive lines as well as in 5-FU resistant lines. This feature should be on mind to design new schedules for advanced colorectal cancer treatment.

A027

KINETIC ANALYSIS OF A COMBINATION OF GEMCITABINE WITH VINORELBINE IN HUMAN BREAST ADENOCARCINOMA MCF-7 CELLS IN VITRO

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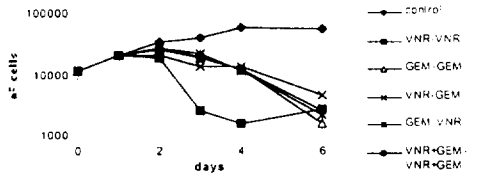
The new cytotoxics agents gemcitabine (GEM) and vinorelbine (VNR) are being tested clinically in breast cancer. We have evaluated the cytotoxic effect of GEM in combination with VNR in a simultaneous or a sequential treatment against MCF-7 human breast adenocarcinoma cell line. The cells were pre-cultured for 24 hr in 96 wells plates. In the first experiment they were treated with GEM and VNR in simultaneous exposure during 24 or 48 hr. In the next experiment, cells were examined on a sequential treatment with GEM for 24 hr followed by another 24 hr exposure to VNR or the inverse combination of drugs (VNR followed to GEM). After these treatments, the cultures were washed and replaced in drug-free medium. The analysis of growth-inhibitory activity was showed in plots with a steep slope between 1 to 6 days. The cells were counted the employing MTT assay. Our results show that the growth-inhibitory effect with simultaneous GEM/VNR exposure was similar to that obtained with GEM alone. We have studied also the effect of GEM/VNR or VNR/GEM in a sequential treatment and we showed that the two combinations had an equal growth-inhibitory activity.



Preliminary experiments were performed combining GEM/Taxol. When GEM was used simultaneously with taxol the growth-inhibitory effect was higher than obtained when the drugs were used individually. Also we have studied the effect of GEM/Taxol or Taxol/GEM in a sequential treatment and we showed that the two combinations had an equal growth-inhibitory effect. Our results have implications for clinical trials of GEM with VNR because of the potential for less than additive effect of the combination.

A028

EFFECT OF OLEIC ACID ON THE CHEMOSENSITIVITY OF BREAST CANCER CELLS

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Recent studies have postulated that fatty acids may increase the efficacy of anti-cancer drugs in cell culture. Thus, we used the MCF-7 human breast cancer cell line to investigate whether exogenous oleic acid (OA) could enhance the citotoxicity activity of navelbine (NVB), a semysinthetic vinca alkaloid that is a potent inhibitor of mitotic microtubule polymerization. First, to determine the cytotoxic effect of NVB, dose effect experiments were performed employing the MTT-test and the results were expressed in terms of the drug concentration required to inhibit the 24 hr. cell growth by 50% (ID50). Next, to test the effect of OA on the drug sensitivity, cells were co-incubated with 40 μM NVB (ID50) and various concentrations of OA (0.5, 5, 50 and 500 μg/ml) for 24 hr. Both 50 μg/ml and 500 μg/ml OA were most potent at enhancing the cytotoxic action of NVB. Cell viability decreased from 50±2% (NVB alone) to 30±6% (NVB+50 μg/ml OA) and to 21±3% (NVB+500 μg/ml OA). This trend was also aparent after a 48 h. co-exposure but was confined to the most significant interactions observed in the shorter co-exposure period. Interestingly, this interaction was not due to the toxicity of the fatty acid itself. OA alone at concentrations of 50 μg/ml and 500 μg/ml resulted in an increased proliferative response (7±2% and 23±4% higher than untreated cells, respectively). One of the mechanisms that may explain the enhanced chemosensitivity to NVB resulting from OA supplementation, is the possible modification of the fatty acid composition of cells leading to increased membrane fluidity and drug transport. To validate this hypothesis, MCF-7 cells were co-incubated with 500 μg/ml OA and Gemcitabine (GEM), a pyrimidine analogue, at concentration of 14 mM (ID20). There was, however, a weak increase in the cell viability from 80±3% (GEM alone) to 87±3% (GEM+500 μg/ml OA). Preliminary results employing OA and taxol, an anti-neoplastic agent that obstructs disasssembly of microtubules, have indicated a increased sensitivity to drug on MCF-7 cells. These observations would suggest that the effect of OA on the chemosensitivity in breast cancer may be related to the mechanism of action of the chemotherapy drugs.

A050

MITOCHONDRIAL GLUTATHIONE DEPLETION POTENTIATES TNF-INDUCED CYTOTOXICITY IN TUMOR CELLS *IN VIVO*.

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High intracellular glutathione (GSH) levels associate with tumor resistance to TNF. Reactive oxygen intermediates (ROIs) produced in the mitochondria mediate TNF cytotoxicity, and the mitochondrial GSH (mtGSH) may work as a possible scavenger. We used Ehrlich ascites-tumor (EAT) cells to study whether TNF-induced cytotoxicity depends on the mtGSH levels, and if these can be manipulated *in vivo* to improve the efficacy of the cytokine. Over a period of 2 weeks between inoculation of the cells and severe cachexia in the host, the mtGSH content remained constant. ATP (1 mmol/kg per day)-induced tumor growth inhibition was accompanied by a selective decrease (50%) in the cytosolic (cyt)content of GSH within the cancer cells *in vivo*. ATP adm., when combined with diethylmaleate (DEM; 0.2 mmol/kg), further depleted the cytGSH to 6% of controls and, in addition, depleted the mtGSH to 47% of controls. Adm. of sublethal doses of recombinant human TNF-α (10^6 U rhTNF-α/kg per day) or recombinant murine TNF-α (0.5 x 10^6 U rmTNF-α/kg per day) to EAT-bearing mice promoted oxidative stress (increases in intracellular peroxide levels, $O_2^{-\cdot}$ generation and mtGSSG) in the cancer cells, which associated with a decrease in the mtGSH content and the mitochondrial membrane potential. Adm. of ATP, DEM and TNF-α further increased these effects, induced tumor necrosis, resulted in a marked reduction of viable EAT cells (approx. 90% with rhTNF-α and 80% with rmTNF-α) and extended host-survival. We applied this strategy to two solid tumors with metastatic capacity, the B16 melanoma (B16M) and the Lewis lung carcinoma (LLC), and found that treatment with ATP, DEM and rhTNF-α promoted tumor necrosis and reduction in size, and lead to a complete cure in approx. 60 % of the B16M and 70% of the LLC. The % of cures for both tumors was much lower (10-15%) when rmTNF-α was used instead of rhTNF-α. We demonstrate that mtGSH depletion decreases tumor resistance to TNF and potentiates TNF-induced cancer regression *in vivo*.

4. Biology of the Metastasis/Invasion

A030

SUBCELLULAR ORGANIZATION OF β-CATENIN AND PLAKOGLOBIN AND ITS RELATION WITH E-CADHERIN EXPRESSION AND TUMOR PROGRESSION.

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INTRODUCTION. Cell adhesion mediated by the cadherin-catenin complexes is fundamental for the establishment and maintenance of cell-cell interactions of multicellular organisms with an essential role during embryogenesis. In mammals, the epithelial E-cadherin (E-CD) plays an essential role in the homeostasis and architecture of all epithelial tissues. Significant alterations in expression/function of E-CD occurs during tumor progression, especially relevant for tumor invasion. Apart from their role in adhesion complexes, β-catenin and plakoglobin are downstream effectors of the *Wnt* signaling pathway, an are endowed with transcriptional activation after their translocation to the nucleus.

MATERIAL AND METHODS. The relation between E-CD expression and signaling activity of β-catenin and plakoglobin has been analysed in a cell system based on the highly tumorigenic and metastatic HaCa4 cell line (E-CD-), derived from a squamous cell carinoma, and cell clones derived after transfection with E-CD cDNA (E62, E24). Additional cell lines were derived from E24 cells after blocking E-CD expression with anti-sense E-CD cDNA (P1-5), as well as from a metastatic foci of E62 (E62M). The expression and localization of the catenins in the various cell lines was analysed by immunoprecipitation, western blot of different cell extracts and immunofluorescence of cells fixed in methanol or 3.7% formaldehyde +/- permeabilization in NP-40, as well as in isolated nuclei.

RESULTS. The biochemical analysis performed in the different cell lines showed increased cytoplasmic levels of β-catenin and/or plakoglobin in cell lines deficient in E-CD and showing invasive and metastatic properties. On the other hand, the immunofluorescence analysis indicated a significant pennuclear accumulation of β-catenin and/or plakoglobin in E-CD- deficient cells whereas no such pennuclear staining was observed in E-CD+ cells.

CONCLUSIONS. Our results indicate that loss of E-CD during tumor progression and invasion induces cytoplasmic stabilization of β-catenin and plakoglobin, which can led to activation of the signaling activities of both molecules. In addition, our results suggest a potential repressor mechanism of β-catenin and plakoglobin signaling, apparently involved in their nuclear translocation.

A098

EXPRESSION AND REGULATION OF COLLAGENASE-3 IN HUMAN MALIGNANT TUMORS

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Collagenase-3 is a matrix metalloproteinase first identified in breast carcinomas and potentially involved in fetal ossification. Biochemical characterization of this proteinase has shown that it is a very potent enzyme with ability to degrade different fibrillar collagens as well as a wide range of extracellular matrix components like fibronectin, tenascin or type IV collagen. In this work we present a study of collagenase-3 expression in different types of human carcinomas and we analyze the mechanisms regulating its expression. First analyses performed in breast carcinomas showed that collagenase-3 gene is mainly expressed by stromal tissue sorrounding tumor cells, specially in fibroblastic cells immediately adjacent to islands of cells present at the invasive edge of the tumor, but not by the epithelial tumor cells. A series of coculture experiments using human fibroblasts and breast cancer cells led us to conclude that epithelial tumor cells produce difussible factors that up-regulate collagenase-3 transcripts in stromal fibroblasts. Additional studies have revealed that collagenase-3 is also overexpressed in other malignant tumors, like squamous cell carcinomas of the larynx, chondrosarcomas, basal cell carcinomas of the skin, some melanomas and ovarian carcinomas. Production of this proteinase is always associated with advanced local invasion of the tumors, suggesting that this protein may contribute to the progression of aggresive carcinomas, although the mechanisms controling its expression may vary depending of the tumor type. Thus, IL-1β and TGF-β induce expression of collagenase-3 in breast carcinomas, and bFGF is a mediator in the expression of this proteinase in chondrosarcomas, while TNF-α and TGF-α would be the inducers in larynx carcinomas. We have preliminarly analyzed the signal transduction pathways that could be involved in the expression of this gene in response to the different cytokines and growth factors and performed a structural and functional characterization of the promoter of collagenase-3 gene identifying sequences that could mediate its expression in different tumors.

A108

IDENTIFICATION BY RAP-PCR AND CHARACTERIZATION OF METASTASIS CANDIDATE GENES IN A MOUSE MAMMARY TUMOR MODEL

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The metastatic process is complex and many genes are thoght to be involved in it. The aim of our work was the identification of some of these genes. We used the RNA Arbitrarily Primed PCR (RAP-PCR) method to find gene expression differences between several cell clones of a mouse mammary tumor model (MXT). This is a suitable system for studying metastasis, as the different clones have different abilities to invade the basement membranes and also exhibit different metastatic ability and hormone-dependency, but are genetically related because they derive from a unique parental cell line. The mRNA fingerprints obtained by RAP-PCR from each clone were compared regarding specific bands which showed marked differences. After selection and cloning of several bands, the isolated cDNAs were used as probes in Northern blot to verify the differences in the corresponding anonymous mRNAs. Sequence analysis of the selected bands and the use of these probes to screen a cDNA library from MXT-S15 clone allowed us to identify several murine cDNAs. One of them was identified as ADAMTS-1, a recently described gene encoding a novel ADAM family protein with thrombospondin type I motifs. A higher expresion of this gene was observed in highly metastatic clones. We also identified a cDNA presenting a 100% homology with moesin, a protein that is thought to work as cross-linker between plasma membrane and actin-based cytoskeleton and involved in signal transduction. Differences in moesin expression were also detected among the clones with different metastatic ability. The whole cDNA of these genes was obtained by the same library screening and was cloned (sense and antisense) in the pZeoSV2 expression vector and their role in metastasis is under investigation in the transfectant cells. Moreover, the ADAMTS-1 gene promoter is being studied to determine putative regions involved in transcriptional activity.Our results support the hypothesis that metastasis candidate genes in breast cancer can be detected and isolated by RAP-PCR when their expression differs among the metastatic and non-metastatic clones.

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A119

MELANOMA-INDUCED ENDOTHELIAL PRODUCTION OF TNFα AND IL-1β INCREASES VCAM-1-DEPENDENT ADHESION AND METASTASIS IN LIVER.

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Non-IL-1β mRNA containing B16 melanoma (B16M) cells were used to study whether tumor cell capacity to upregulate host production of TNFa and IL-1β contributes to metastasis progression. Primary cultured mouse hepatic sinusoidal endothelium (HSE) cells were treated with B16M cell-conditioned medium (CM), and their secretion of IL-1β and TNFa and their adhesiveness for other B16M cells determined along treatment. *In vitro* B16M-CM-treated HSE cells significantly ($P<0.01$) increased their production of TNFa (4-fold) and IL-1β (3-fold) by the 2nd and 8th hour, respectively, and their adhesion (3-fold) for other B16M cells by the 8th hour. There was a significant ($P<0.01$) increase of B16M cell adhesion to HSE cells isolated on the 12nd hour from B16M cell intrasplenically-injected mice compared to HSE from saline-injected mice, which correlated with enhanced HSE expression of VCAM-1. Anti-VCAM-1 antibodies suppressed B16M-CM-induced HSE adhesiveness for B16M cells *in vitro*, and 70-80% reduced hepatic metastasis. p55 TNF soluble receptor (TNFbp) 50% reduced HSE cell adhesiveness for B16M cells ($P<0.01$) , without being affected HSE cell production of IL-1β. HSE cell treatment with IL-1 receptor antagonist (IL-1Ra) 75% decreased adhesion-stimulating activity of B16M-CM ($P<0.01$) , without affecting HSE production of IL-1β or TNFa. Moreover, complete abrogation of B16M-CM-dependent adhesion was found when HSE cells received TNFbp plus IL-1Ra together with B16M-CM. Production of TNFa- and IL-1β-stimulating factors, which subsequently increased VCAM-1-dependent adhesion in HSE, constitutes a key phenotypic property of non-IL-1-producing melanoma able to metastasize in liver by IL-1-dependent mechanism.

A029

LOCALIZATION AND FUNCTION OF ANGIOGENIN IN HUMAN ADENOCARCINOMA BREAST CANCER CELLS

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We have previously demonstrated that the expression of angiogenin in breast carcinoma extracts is significantly associated with a favorable patient prognosis. The results of our clinical study suggested that angiogenin might be a substrate for the adhesion of breast carcinoma cells. To test this hypothesis, we performed *in vitro* adhesion studies with angiogenin using MCF-7 and MDA-MB-231 breast cancer cell lines and fibronectin and collagen IV as adhesion controls. We found that angiogenin is an adhesion substrate for these cells. Cell adherence correlated with the concentration of angiogenin used to coat the plastic surface. Additional experiments showed that the adhesion of MDA-MB-231 and MCF-7 cells to angiogenin-coated wells was comparable to that obtained with fibronectin or collagen IV coating. The specificity of the angiogenin-dependent adhesion was tested using an anti-angiogenin antibody (50 μg/ml). Angiogenin (10 μg/ml) induced a 60% to 90% increase in MDA-MB-231 and MCF-7 cell adhesion, which was completely blocked by the anti-angiogenin antibody. We have also studied the tissue expression of angiogenin. Tissue sections were obtained from some of the breast tumors in which cytosol angiogenin had been studied. These sections were subjected to immunohistochemical studies using a polyclonal anti-angiogenin antibody and the avidin-biotin complex method. An intense staining was observed in most of the tumor cells. The subcellular analysis revealed a cytoplasmic staining pattern. In addition to tumor cells we found a weak number of stained stromal cells (fibroblasts and inflammatory cells) in the vicinity of the malignant cells. Our studies show in breast carcinoma that angiogenin is produced by the cancer cells, and that angiogenin plays an important role in the *in vitro* adhesion of breast carcinoma cells, which is consistent with its favorable *in vivo* prognostic value in patients with breast cancer.

A051

GLUTATHIONE CONTENT OF CANCER CELLS REGULATES THE POTENTIAL FOR INVASION AND PROGRESSION OF LIVER METASTATIC MELANOMA.

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Malignant melanoma is highly resistant to radiotherapy and chemotherapeutic drugs. Glutathione (GSH) regulates the sensitivity of tumor cells to drug and radiation-induced cytotoxicity. We used B16 melanoma (B16M) to study reg. of intracel. GSH levels in malignant cells with metastatic capacity. GSH increases in B16M cells during the initial period of exponential growth *in vitro*, to reach a max. of 37±3 nmol/10^6 cells 12 h after plating, and then gradually decreases to control values (10±2 nmol/10^6 cells) when cultures approach confluency (72h). On the contrary, intracel. GSSG levels (0.5±0.2 nmol/10^6 cells) and the rate of GSH+GSSG efflux (2.5±0.4 nmol/10^6 cells x h) remain constant as B16M grows. Changes in enzyme activities involved in GSH synthesis or the glutathione redox cycle do not explain shifts in the glutathione status. Two facts can explain changes in GSH levels: a) high levels of GSH induce a feed-back inhibition of its own synthesis in cells from cultures with low cell density (LD); b) transport of Cys, whose availability is the rate-limiting step for GSH synthesis, is limited by cell-cell contact in cultures with high cell density (HD). Buthionine sulfoximine (BSO)-induced GSH synthesis inhibition deplets GSH and inhibits B16M cell proliferation *in vitro*; however, a BSO concentration of a 100 μM or lower results in a transient GSH depletion followed by a rebound effect of GSH recovery. After intrasplenical inoculation, B16M cells with high GSH content (LD cells cultured for 12 h) show higher metastatic activity in the liver than cells with low GSH content (HD). BSO administration to B16M-bearing mice can provoke rebound effects in GSH synthesis within the tumor cells, enhance their survival and increase metastatic invasion of the liver. Our results demonstrate how changes of GSH levels in malignant melanoma cells can regulate their metastatic behaviour.

A069

HLA CLASS II ANTIGEN EXPRESSION IS INVERSELY CORRELATED WITH METASTATIC POTENTIAL IN MDA-MB-435 HUMAN BREAST CANCER CELL LINES

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The MDA-MB-435 human breast cancer cells, designated as Parent cell line, and its two variants 435-Lung2 and 435-Brain1 showed different metastasis abilities in tumor bearing nude mouse. The ability to produce lung and lymph node metastases were the highest in 435-Lung2, median in 435-Parent and the lowest in 435-Brain1 cell bearing nude mice. To search genes involved in tumor metastasis processes, we use mRNA arbitrarily primered PCR method (RAP-PCR) to find out genes showing differential expressions among three MDA-MB-435 cell lines. A single band enhanced markedly in 435-Brain1 from RAP-PCR fingerprint was cut out and cloned. The sequence confirmed that this cDNA is HLA class II DP-β. Northern blot using human HLA class II DP-β as a probe exhibited a strong signal in 435-Brain1, very weak signal in 435-Parent and almost no signal in 435-Lung2. The expressions of the cell surface antigens were examined by flow cytometry and indirect immunofluorescence using monoclonal antibodies against HLA class II DP and DR. The percentages of 435-Parent, Lung2 and Brain1 cells expressing HLA class II DP and DR antigens were 7.8%, 2.0%, 38.7% and 20.5%, 4.0%, 57.5% respectively. Thus, the antigen expressions were consistent with their mRNA expression levels. However, an equal signal among the three MDA-MB-435 cell lines was detected by Southern blot and this suggests that up or down regulation of HLA class II DP expression is controlled at the transcription level in these cell lines. Histocompatibility antigen II plays an important role in the immunoresponses. Our results showed that HLA class II antigen expressions among three MDA-MB-435 breast cancer cell lines were inversely related to their metastatic potential. HLA class II antigen might present tumor peptides to and activate immune effector cells and subsequently resulted in low metastasis in lung and lymph nodes in 435-Brain1 bearing mice. Supported by a grand SAF 98-049 to A. Fabra and a grand to B Shi from Agencia Española De Coopración Internacion.

A107

SPARC'S mRNA IS OVEREXPRESSED IN METASTATIC CELL LINES OF MOUSE MAMMARY ADENOCARCINOMA.

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In order to identify genes involved in metastasis, we used a mouse mammary tumor model. We isolated different clones with different cellular properties, metastatic ability and hormone-dependency, but genetically related because they come from the parenteral cell line MXT-S. To investigate the genes differentially expressed by MXT derived clones, we used the RNA-Arbritally Primed PCR (RAP-PCR). mRNA fingerprints from each clone were obtained, compared and we found some differences in specific bands. After selection and cloning of a number of bands, the isolated DNAs were used as probes in Nothern blot analysis to verify differences in messenger RNAs levels between the clones. The sequence analysis of a selected band was identified as SPARC (Secreted Protein Acidic and Rich in Cysteine), a glycoprotein that has been associated with cell-matrix interactions during remodelling, morphogenesis, migration and proliferative processes. This gene was found overexpressed in the highly metastatic cell line MXT-C1.1 respect to the parenteral cell line MXT-S, which exhibited a high proliferative but low metastatic properties. The full length mRNA has been obtained using a MXT-S15 cDNA library and SPARC cDNA was cloned (sense and antisense orientation) in pZeoSV2 expression vector to know its role in metastasis in the transfectant cells. In other way we have cloned the promoter region of SPARC into Luciferase expression reporter vector reporter in order to study it's transcription regulation in MXT's clones.
Our results suport the hypothesis that genes differentially expressed in metastasic cells that could be detected and identified by RAP-PCR.
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A112

TGF-β1 AS A MODULATOR OF EPITHELIAL DIFFERENTION AND MALIGNANCY IN MOUSE SQUAMOUS CARCINOMA CELLS.

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TGF-β1 is a negative regulator of basal keratinocyte proliferation. Immortalized keratinocytes respond to TGF-β1 by inhibiting cell proliferation, while carcinoma cell lines are insensitive or less responsive to the factor, a phenomenon is associated with later stages of tumor progression.

We have studied the effects of TGF-β1 on proliferation and differentiation of immortalized keratinocytes, benign papilloma derived cells and carcinomas cell lines, representative of different stages of mouse skin carcinogenesis. TGF-β1 blocks cell growth and induces cell death on immortalized keratinocytes and papilloma derived cells. However, on carcinoma cells that scape the growth arrest, TGF-β1 induces an epithelial-mesenchymal transdifferentiation (EMT). This phenotypic change is associated in vitro with a vast cytoskeletal dissorganization, loss of cell adhesion mediated by E cadherin and expression/secretion of extracelular matrix proteases (MMP-9 and u-PA). In vivo , TGF-β1-induced EMT is associated with a transition to a more invasive and metastatic undifferentiated carcinomas (Frontelo et al, 1998).

These results confirm our previous hipothesis (Caulín et al, 1995), suggesting that TGF-β1 has a dual role in epidermal carcinogenesis, acting as a tumor suppressor at first stages and also as a promoter of malignancy at late stages of carcinogenesis.

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Caulin C, Scholl FG, Frontelo P, Gamallo C, Quintanilla M. 1995. Chronic exposure of cultured transformed mouse epidermal cells to transforming growth factor-beta 1 induces an epithelial-mesenchymal transdifferentiation and a spindle tumoral phenotype. Cell Growth Differ 6(8):1027-35

A115

ENDOTOXIN INCREASES LIVER METASTASIZING POTENTIAL OF MURINE COLON CANCER CELLS.

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Normal and neoplastic colonic cells produce proinflammatory cytokines in response to lipopolysaccharide. Some steps in the metastatic process are facilitated by proinflammatory cytokines. Since augmentation of LPS frequently occurs in systemic and portal blood of colon cancer patients following surgery, the role of LPS in the metastatic progression of colon cancer was studied in an animal model of colon cancer metastasis. Highly-metastatic murine 51BLiM5 cells were intrasplenically injected and hepatic metastasis studied in both normal mice receiving untreated and LPS-pretreated cancer cells, and LPS-treated mice receiving untreated cancer cells. The effect of LPS on colon cancer cell proliferation, adhesion to syngenic hepatic sinusoidal endothelium, and migration *in vitro* was also assessed. Intravenous LPS injection prior to inoculation of 51BLiM5 cells produced a significant increase metastasis that was unaffected by interleukin-1 receptor antagonist. Pretreatment of 51BLiM5 cells with LPS for 24 h prior to injection also significantly enhanced hepatic metastasis in untreated mice by a mechanism involving tyrosine phosphorilation, since genistein —a tyrosine kinase inhibitor— abrogated this effect. In addition, LPS treatment of 51BLiM5 cells significantly enhanced their proliferation, adherence to endothelium and migration. Autologous mouse serum increased LPS binding to 51BLiM5 cancer cells 2-fold. LPS increased the hepatic metastasizing potential of 51BLiM5 colon cancer cells by a mechanism which depended upon tumor cell response to LPS, suggesting that increased levels of LPS in serum may promote progression of hepatic metastasis during dissemination of colon cancer cells.

A126

Enhanced expression of α(1,3)-Fucosyltransferase genes is related to E-selectin-mediated adhesion and metastatic potential of human lung adenocarcinoma cells

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α(1,3)- and α(1,4)-fucosylated oligosaccharides such as sialyl-Lewis^x (sialyl-Le^x) and sialyl-Lewis^a (sialyl-Le^a) have been reported to participate in tumor cell adhesion to activated endothelium. We examined by cytofluorometry the expression of Le^x, sialyl-Le^x, sialyl-Le^x dimeric, Le^a and sialyl-Le^a on the surface of two human lung adenocarcinoma cell lines with different lung colonization potential. Metastatic HAL-8Luc cells expressed higher levels of all of these antigens than the closely related nonmetastatic HAL-24Luc cells. Synthesis of these side chain oligosaccharides is dependent on the activity of one or more specific α(1,3)-fucosyltransferases (α(1,3)-Fuc-Ts) and, to date, five *α(1,3)-Fuc-T* genes have been cloned: *Fuc-TIII*, *Fuc-TIV*, *Fuc-TV*, *Fuc-TVI* and *Fuc-TVII*. The expression of these five genes was also higher in the metastatic cells than in the nonmetastatic counterparts, as was shown by Northern blot analysis.

In vitro static adhesion assays showed that only the metastatic cell line adheres significantly to interleukin-1β-stimulated human endothelial cells. From adhesion inhibition experiments using mAbs we concluded that the main molecules implicated in this binding are: sialyl-Le^x expressed on tumor cells and E-selectin present on endothelial cells.

To analyze the role of each α(1,3)-Fuc-T in the synthesis of sialyl-Le^x determinant and their implication in the E-selectin adhesion capacity of these cells, we have stably transfected HAL-24Luc cells with the cDNAs for the five known α(1,3)-Fuc-T enzymes. We have performed cytofluorometric analysis, endothelial cell adhesion experiments and experimental metastasis assays with these stably transfected clones.

A032

DIAGNOSTIC EFFICACY OF THE HEPATIC ECOGRAPHIC GUIDED FINE NEEDLE ASPIRATION (FNA). CLINICAL-CYTOHYSTOLOGICAL RELATIONSHIP IN A SECONDARY HOSPITAL REVIEW.

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INTRODUCTION. For the diagnosis of hepatic lesions (HL), FNA is a fast and effective diagnosis method with a low morbidity (0.1%). We review the cytological diagnosis and the clinical and hystological relationship using the FNA under ecographic control in patients (p) with a clinical suspicious of HL malignance in a secondary hospital with 180 beds.
MATERIAL AND METHOD. A total number of 40 patients were studied. The mean age was 65.1 (range 49-87) and 60% of them were male. The clinical orientation was hepatic metastasis in 20 p, previously neoplasm in 8 p and in the remaining 12 p was an unexpected finding. The mean of stains per case was 6 (2 Giemsa, 2 HE and 2 Pap.). A cellular block was performed in the cases with enough material. A total number of 49 cytologic studies were performed.
RESULTS. 1. In 17/20 of the patients with the clinical orientation of metastasis, the cytologic study was positive and in 12 (70%) of them the primary neoplasm could be diagnosed (2 rectum, 2 pancreas, 2 lung, 2 bile duct system and 4 digestive non localized) and in 3 cases the material was insufficient (clinic diagnosis of pancreatic source). 2. In the patients without neoplasm previously diagnosed, in 10 clinical hepatocarcinoma the cytological diagnosis was performed in 9, and 2 more cases were benign. 3. In the cases with previously known neoplasm, in 7 of 8 cases (87,5%) the diagnosis of metastasis was performed and in 1 case the diagnosis was no-malignance.
COMMENTS. We obtained a good correlation among the cytological diagnosis by FNA and the final diagnosis. No false-positive were obtained, although in 10% of the cases the material was insufficient. The survival was low in the cases of metastasis without no previously known neoplasm and no yatrogenic complications were observed.

A048

A MODEL STUDY OF PEDIATRIC TUMOR CELL PROLIFERATION: SPECIFIC CYTOTOXIC ACTION AGAINST NEOPLASTIC CELLS AND PROTEOLYTIC BALANCE MODULATION.

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INTRODUCTION: Two complementary strategies in cancer treatment are the direct actions against tumor cell proliferation and indirect actions to avoid invasiveness and metastasis. The search for specific cytotoxic compounds belongs to the first strategy and the use of antiangiogenic agents belongs to the second one.
In this work, with diverse pediatric tumor cell lines cultures, oxidative damage by ascorbic acid, as well as cytotoxicity of the antiangiogenic compounds genistein and 2-methoxyestradiol have been studied in several pediatric tumor cell lines. In addition, the modulation of the proteolytic balance by these two antiangiogenic compounds has also been studied.
METHODS: Neuroblastoma, osteosarcoma, rhabdomyosarcoma and retinoblastoma cell lines have been used. Cytotoxicity of the compounds was evaluated by cell counting and/or by MTT assays. MMPs, TIMPs, uPA and PAI activities were determined by using zymography.
RESULTS AND CONCLUSIONS: The three assayed compounds are markedly cytotoxic for the pediatric tumor cell lines studied. Genistein induced a shift towards antiproteolysis. On the other hand, 2-metoxyestradiol did not produce any clear net shift of the proteolytic balance.

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A114

LEUKOCYTE RECRUITMENT IN AN EXPERIMENTAL MODEL OF COLORECTAL CANCER. X. Bessa, J.I. Elizalde, A. Castells, F. Mitjans[a], A Salas, A. Soriano, J. Panés, J. Piulats[a], J.M. Piqué. Department of Gastroenterology, Institut de Malalties Digestives, Hospital Clínic i Provincial, y [a]Bioinvestigation laboratory Merck Farma y Química. Barcelona.

Background: The infiltration of tumoral tissue by the immune cells, depend on their ability to interact with the endothelium of angiogenic blood vessels. It has been suggested a deficient leukocyte-endothelium interaction in differents models of experimental cancer, but the etiology hasn't been studied.
Aims: 1) Characterize the leukocyte-endothelium interactions and its molecular determinants in the angiogenic blood vessels in this model of colorectal cancer 2) Study if the alteration of leukocyte recruitment can be related with a deficient expression of certain cell adhesion.
Methods: A subcutaneous injection of 2 x10^6 cells of the cellular line HT29 (human colon adenocarcinoma) in Swiss nude mice produced after 4 weeks a macroscopically tumor with evident vascularization. Intravital microscopy was used to characterize the leukocyte-endothelium interactions in the angiogenic blood vessels respect to the non-tumoral vessels, in control animals and after the administration of LPS. The participation of ICAM-1 and VCAM were established with the administration of the corresponding monoclonal antibodies at blocking doses. The endothelial expression of ICAM-1 and VCAM were assessed using the dual-radiolabeled mAb technique and were corrected for the endothelial surface.
Results: In control animals , the leukocyte *rolling* in the angiogenic blood vessels were significantly lower than the nontumoral vessels (1,2±2 vs 30,8±14 cells/30 seg.; p<0,05), without adhesion phenomens in any localization. LPS estimulation produced a less adhesion in the tumoral vessels respect to the nontumoral vessels (4,2±2 vs 16,3±3 cells/50 mm; p<0,05). A monoclonal antibody against ICAM-1 completely reverted the increment of adhesion in the tumoral and nontumoral vessels (0,1±0,2 and 0.4±0.9 cells/50 mm, respectibly), but the immunoneutralization with VCAM-1 didn't produce any significant effect. However, no significant differences were observed between the endothelial expression of ICAM in the tumoral and nontumoral vessels, in control animals (0.35±0.01 vs 0.34±0.01, respectibly) and after LPS estimulation (0.64±0.05 vs 0.63±0.01 respectibly)
Conclusions: The leukocyte adhesion after the administration of LPS is markedly reduced in the colorectal tumor vessels. Although ICAM-1 is the principal molecular determinant of leukocyte adhesion, the less recruitment in the tumoral vessels isn't a consequence of a minor expression of ICAM-1.

A116

EXPRESSION OF CD44 V3 ISOFORMS RESPONDS TO EXON V3-SPECIFIC CIS-ACTING ELEMENTS IN BREAST TISSUE

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The repertoire of distinct CD44 protein isoforms is generated by means of alternative pre-mRNA splicing of ten variable exons (from exon v1 to v10) located in the central region of the CD44 gene. We have identified two alternative splicing pathways of the CD44 pre-mRNA variable region which account for the generation of all the CD44 isoforms described in breast tissue. An alternative splicing pathway that reflects inclusion of variable exons in a gradual 3' to 5' fashion is evidenced in breast ductal carcinoma and its lymph node metastases. This pathway is compatible with a mechanism that generates the standard form of CD44 (devoid of v-exons) and is distinguishable from an alternative splicing pathway that involves exclusively v-exon 3 and is observable in both normal and carcinoma breast tissue. Cis-acting elements of CD44 v-exon 3 have been mapped in the context of exogenous constitutive exons using a transfection assay in human breast cell lines. A functional map of the splicing control of CD44 v-exon 3 in these cell lines is provided.

A120

HYDROGEN PEROXIDE ENHANCES METASTATIC POTENTIAL OF MURINE MELANOMA IN LIVER.

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Background: In previous reports, we have shown that hepatic metastasizing ability of B16 melanoma (B16M) cells decreases when catalase is administered 10 min before their intrasplenic injection, suggesting that hydrogen peroxide released during melanoma cell lodgement into the liver has pro-metastatic effects. In addition, factors enhancing reactive oxigen intermediate (ROI) production in liver, such as endotoxins and pro-inflammatory cytokines, also increase melanoma metastasis in liver. *Purpose:* In the present work, we test the effect of H_2O_2 -at different incubation times and concentrations- on the hepatic metastasizing ability and *in vitro* adhesiveness to hepatic sinusoidal endothelium. *Results*: A proliferation assay was used to first analyze *in vitro* the B16M cell response to H_2O_2. We found that 10μM for 24h significantly ($p<0.05$) increased proliferation when compared to untreated cells, while 50μM or higher concentrations decreased proliferation. Intrasplenic injection of H_2O_2 treated B16M cells into C57BL/6J mice significantly ($p<0.05$) increased the hepatic metastatis development with respect to untreated B16M cells. This effect was time- and concentration-dependent, and the maximum effect was reached when B16M cells were treated with H_2O_2 10μM for 8h. In vitro, B16M cell treatment with 10μM H_2O_2 for 8h significantly ($p<0.01$) enhanced (30%) B16 melanoma cell adhesion to HSE compared to untreated B16M. Anti-VCAM-1 antibody (10 μg/ml) did not significantly alter untreated B16M cell adhesion to HSE, while it completely abrogated ($p<0.01$) H_2O_2 (10μM 8h)-mediated adherence to HSE. *Conclusion:* Our results reveal that H_2O_2 increases hepatic metastasis potential of B16M cells and suggest the possible involvement of B16M cell activation of very late antigen-1 (VLA-4), which mediate their adhesion to hepatic endothelium via vascular cell adhesion molecule-1(VCAM-1).

A125

TRAFFIC OF PROSTATE ADENOCARCINOMA TUMOR CELLS TO LYMPH NODES VISUALIZED USING THE LUCIFERASE GENE AS A LINEAGE MARKER

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Tumor cell traffic between intramuscular tumors experimentally induced in nude mice and lymph nodes was studied using PC-3.luc prostate adenocarcinoma cells permanently transfected with the luciferase gene as a tumor cell marker.

This sensitive approach allowed the detection of 1 luminescent tumor cell mixed with $1 \cdot 10^7$ unlabeled PC-3 cells and of 1 tumor cell/lymph node. PC-3.luc cells inoculated in nude mice showed a 1000 fold expansion, accompanied by a 4.5 fold increase in tumor cell density (tumor cell number/gram of tumor), during the first 90 days of primary tumor growth. No macroscopic secondary tumors were found in organs. other than lymph nodes, by the end of the experiment.

Tumor cell spread to lymph nodes was detected at Day 21, when there were $2 \cdot 10^5$ tumor cells at the inoculation sites, before discrete primary tumors could be identified. The total tumor cell burden in the tested lymph nodes was modeled by a power function of primary tumor cell number (determination coefficient $R^2 = 0.9472$). By the end of the experiment, on Day 110, there were 1.8 metastatic cells in the studied lymph nodes for every 1000 primary-tumor cells.

These results suggest that empirically obtained tumor specific indexes could be used to characterize the invasion of lymph nodes by tumor cells.

The path of spread for PC-3.luc cells from intramuscular sites appears to follow the lymphatic system and at no time during the experiment were tumor cells found in blood. An upper limit of no more than 16 blood circulating tumor cells was established for these experiments.

The observation of tumor cells that were invading the lymphatic system from the onset of tumor growth but unable to establish secondary tumors in other organs emphasizes the potential of this procedure in studying the multi-step nature of metastasis.

5. Hereditary Cancer

A017

IDENTIFICATION OF THE 185delAG BRCA1 MUTATION IN SPANISH GYPSY POPULATION

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Inherited mutations in the BRCA1 gene confer a predisposition to breast and ovarian cancers. A single BRCA1 mutation, 185delAG, has been detected in approximately 1% of the Ashkenazi Jewish individuals. In an extensive study carried out in Spain (90 at risk families and 160 women with early-onset breast cancer without family history), the BRCA1 gene was screened by SSCP. This mutation was found in two women with breast cancer associated with the haplotype (four polymorphic markers) described in Ashkenazim. Surprisingly, we identified this mutation in three unrelated Spanish Gypsy chromosomes. In the first case, it was found in a high risk family of breast/ovarian cancer. The mutation also appeared in a young woman with breast and ovarian cancer whose family history was not available. In the third case, it was detected in a woman without cancer belonging to a control group of Spanish Gypsies (n=25). To our knowledge, this is the first time that the 185delAG mutation has been identified in a non-Jewish, well defined ethnic population.

A095

FOUNDER MUTATION IN FAMILIAL ADENOMATOUS POLYPOSIS (FAP) IN THE BALEARIC ISLANDS.

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Background. Incidence of Familial Adenomatous Polyposis (FAP) is approximately 2.29 x10^{-5} habitants. *APC* gene mutations have been found in 60-70% of all FAP families being codons 1309 (20%) and 1061 (8%) known hotspots. No founder effects of *APC* gene mutations have been reported so far. **Aim.** To search for mutations in the *APC* gene in a population-based registry of FAP. **Patients.** Fifty four Spanish patients of 11 FAP families registered in the Balearic Registry for FAP were studied. One of the cases analyzed occurred in the absence of family history. **Methods.** Genomic DNA was prepared from PBL Mutations at exons 8, 11 and fragments A to K of the exon 15 of the *APC* gene were analyzed by SSCP/PCR and sequencing using an ALFexpress DNA Sequencer, Pharmacia Biotech. To establish the haplotype, five intragenic polymorphisms (nt 1458, nt 4479, nt 5037, nt 5880, SspI) and 4 microsatellite markers (D5S299, D5S82, D5S346, D5S318) were also studied. **Results.** Mutations in the APC gene were detected in 7 out of 11 (64%) families analyzed. Six families shared the same mutation, a 5bp deletion (ACAAA) at codon 1061. The remaining one, the case without family history, harbored a 5bp deletion at codon 1309. Five of the families containing the 5bp deletion at codon 1061 shared the same haplotype. Moreover, they originated in the same geographic area within the Majorica Island. **Conclusion.** Our results are consistent with a founder effect of the 5bp deletion at codon 1061 in FAP families of the Balearic Islands.

A038

CLINICAL AND MOLECULAR ANALYSIS OF SPANISH FAMILIES WITH SUSPECT OF MULTIPLE ENDOCRINE NEOPLASIA TYPE 1.

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Introduction: Multiple endocrine neoplasia type 1 (MEN 1) is characterized by the combined presence of tumors of the parathyroid, pancreatic and pituitary glands. The gene responsible of this disease has been recently isolated and mutations have been observed in aproximately 90% of MEN 1 patients analyzed. We present the study of 14 patients with clinic characteristics suggestive for MENI and one patient with primary familial hyperparathyroidism (FPHPT).
Material and Methods: We analyzed 15 patients with clinic suspect MEN 1 or FPHPT by automatic sequencing of the entire coding sequence of the gene. In the cases in which a mutations was detected and there was DNA available from other family members, we performed a segregation analysis to confirm thar the change was responsible of the disease.
Results: We detected germline mutations in the gene in 9/14 (64,3%) of the patients with suspect of MEN 1, 5 of them have not been previously described. Most of the alterations consisted on deletions and insertions which caused a STOP codon and were more frequently localized in exons 2 and 10. The segregation analysis in 4 of the families, allowed us to detect 9 carrier individuals, of which 33% were asymptomatic at the moment of the study. As in previous studies, we could not establish a genotype-phenotype correlation for any of the mutations detected. We did not detect any mutation in the FPHPT case.
Conclusions: 1.- The localization of the mutations allow us to define two "hot" regions in the MEN 1 gene. 2.- For the moment, it is not possible to establish a genotype-phenotype correlation. 3.- This study, in adittion to previous studies, suggests that other gene, different from MEN 1 must be implicated in the development of FPHPT.

A009

MUTATIONAL ANALYSIS OF THE BRCA2 GENE IN SPANISH WOMEN WITH EARLY ONSET BREAST CANCER .

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Inherited mutations in the BRCA1 and BRCA2 genes are responsible for about 5-10% of ovarian and breast cancer. These mutations confer up to 80-90% lifetime risk of breast cancer. We have screened 57 out of a sample of 228 women with early-onset breast cancer for BRCA2 mutations. This sample was unselected for a family history of breast or ovarian cancer. This study can help to estimate the prevalence and penetrance of the BRCA2 mutations in the Spanish population.

Screening for BRCA2 mutations was performed by using PCR-SSCP analysis of multiplexes and DNA fragments resulting from digestion of 1500-bp long PCR products with restriction endonucleases. Sequencing of abnormal bands was used to identify mutations.

We have detected 8 abnormal SSCP patterns. Four of them were due to polymorphisms. Two were missense mutations which change Proline to Arginine in exon 11 and Valine to Isoleucine in exon 18. Finally, two were localised in the splicing consensus sequence of intron 2. One of these splicing mutations makes an exon skipping of exon 2 in the mature RNA. Since this exon contains the translation start signals, synthesis of BRCA2 should be blocked in the mutant allele. The second splicing mutant found in our study makes an exon skipping of exons 3,4 and 6,7 and presumably produces a truncated protein.

A018

MUTATION SCREENING OF THE BRCA1 GENE IN 90 SPANISH BREAST CANCER FAMILIES

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Inherited mutations in the BRCA1 gene confer a predisposition to breast and ovarian cancers. DNA testing for BRCA1 germline mutations was carried out, by SSCP and PTT, in 60 "high risk" families (three or more cases) and 23 "small" families (two cases) and in 7 families with males affected. Ovarian cancer cases were diagnosed in 20 families. We detected BRCA1 germline mutations in 7 (8 %) families and 12 polymorphisms and rare variants. Five mutations were frameshift and three were missense (two mutations were present in one family in the same patient). Evaluation of the family cancer histories shows that 6 of the mutations appeared among the high risk breast or breast/ovarian families. The results show that BRCA1 is implicated in a small fraction of Spanish breast/ovarian cancer families, suggesting the involvement of another susceptibility gene(s).

A019

BRCA2 GERM-LINE MUTATION SCREENING IN MALE BREAST CANCER PATIENTS

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Breast cancer is a rare disease in men, affecting less than 0.1% of the male population. The breast/ovarian cancer genes BRCA1 and BRCA2 are responsible of the majority of multiple-case breast cancer families. Unlike BRCA1, mutations in the BRCA2 gene have been associated with a predisposition to male breast cancer development. In this study, the entire coding regions of BRCA2 were screened by SSCP and PTT for mutations in 16 male breast cancer patients (8 of them without family history). Two different truncating mutations were identified in two families, with male and female members affected: 3374delA in exon 11, and 9254delATCAT in exon 23. The low frequency of the mutations detected (2/16= 12.5%) agrees with other studies, and could be due to the existence of somatic or germ-line mutations in other genes, responsible for male breast cancer.

A020

MUTATIONS IN BRCA1 AND BRCA2: A STUDY OF FAMILIAL BREAST CANCER IN THE REGION OF CASTILLA Y LEON

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BRCA1 and BRCA2 are two major genes involved in hereditary breast and ovarian cancer. The goal of this study has been, first, to identify carriers of mutations in these genes and, secondly, to characterize the most frequent mutations in the geographical environment of Castilla y León. Forty patients fulfiiling the high risk criteria for familial breast cancer are being analysed for mutations. Methods employed are the Protein Truncated Test (PTT) for exons 11 (BRCA1) and 10 and 11 (BRCA2), and heteroduplex analysis of PCR multiplex by CSGE for the remaining exons. Mutations are confirmed by direct sequentiation. Fifty-per-cent of patients have been studied up to now, covering 60% of BRCA2 and the most frequent mutations in BRCA1 in exons 2, 20 and 11. We found two identical mutations in BRCA2 (1536del14) in two patients with no familial relationship. The presentation will show the complete analysis of the population selected. We will illustrate also the use of PTT + CSGE multiplex for rapid screening of new mutations occurring in a given population. Identification of mutations will allow to help local health authorities to develop preventive programs on carriers and to develop faster mutational analysis in high risk populations, once the most frequent mutations are described.

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A039

MOLECULAR ANALYSIS OF THE BRCA1 AND BRCA 2 GENES IN 32 BREAST/OVARIAN CANCER SPANISH FAMILIES

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Introduction: Mutations in the BRCA1 gene are responsible of about 45% of familial breast cancer cases and most of the breast/ovarian cancer families. BRCA2 is responsible of the other 45% of the cases and is implicated in the families with male breast cancer. Direct genetic studies show that these percentages may be different among populations and that the proportion of carrier families is not as high as linkage studies suggested. In the present study we try to estimate the percentage of cases due to each gene in Spanish population.
Material and Methods: We selected a total of 32 families containing three of more cases of women affected with breast and or ovarian cancer (at least one of them diagnosed before 50 years) or at least a case of male breast cancer diagnosed at any age. We performed a complete analysis of the BRCA1 and BRCA2 genes using SSCP, PTT and sequencing methods.
Results: Until now, we have analyzed the entire sequence of the BRCA2 gene and part of the BRCA1 gene. We have found 5 distinct mutations in the BRCA2 gene in 6 families, three of them with male breast cancer. We have detected only one mutation in the BRCA1 gene, in one family with three women affected with ovarian cancer, one with breast and ovarian and one with breast cancer.
Conclusions: The percentage of mutations is low, suggesting that a third susceptibility gene could be responsible of a high proportion of our familial breast cancer cases. 60% (3/5) of the families with male breast cancer showed mutations in the BRCA2 gene, which supports the relationship between this type of cancer and the gene. 20% (1/5) of the families with breast and ovarian cancer showed mutations in the BRCA1 gene. It seems that the proportion of mutations is low in families with only breast cancer, which is in agreement with the results reported in recent studies.

A049

IDENTIFICATION OF THE SAME BRCA1 MUTATION IN A LARGE FAMILY AND ITS IMPLICATION IN BREAST AND OVARY CANCER. Javier Román García[1], Carmen Montoriol Sabate[3], Inmaculada Morer[3], Josep Maria Hilari Serra[3], Jesús García Foncillas[2], Fernando de Cuevillas Matozzi[3]. 1 Clínica Ruber Internacional; 2 Clínica Universitaria de Navarra; 3 Laboratorio Echevarne.

INTRODUCTION.

BRCA1 is located in the chromosome 17q21. This gene is most associated with familial breast and ovary cancer syndromes. Women with the BRCA1 mutation have an 80 to 90% lifetime risk of developing breast cancer and a 40 to 50% risk of ovary cancer. A deletion or alteration in just one copy of the BRCA1 gene predisposes to breast and ovary cancer, and potentially colon and prostate cancer.

MATERIAL AND METHODS.

We have analyzed DNA samples of four very well studied patients. Three sisters had breast cancer and one ovary cancer. At the moment only one is alive. In addition, we are studying another sister, three brothers and eight sons and daughters. We are sequencing the entire gene by an ABI-377.

RESULTS.

In the four samples analyzed we have found the same mutation at the 1294 codon in the exon 11.

CONCLUSION.

The mutation found originate and STOP codon and an alterated protein which is related with an early ovary cancer. We are studying the rest of the family in order to asses the risk of breast and ovary cancer.

A109

USE OF TGGE FOR STUDYING OF GERM-LINE MUTATIONS IN BRCA1 GENE

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Apart from DNA sequencing, the only method with theoretical predicted sensibility of virtually 100% is denaturating gradient gel electroforesis (DGGE/TGGE).

A comprehensive mutational scaning test for the BRCA1 coding sequence and exon/intron boundaries was designed and evaluated.

In a two-step PCR, the BRCA1 region encompasing exons 1-24 were amplified as seven fragments of : 9899 bp (exons 1-3), 9730 bp (exons 5-9), 4753 bp (exons 10-11), 8934 bp (exons 12-13), 10674 bp (exons 14-17), 7194 bp (exons18-20) and 11395 bp (exons 21-24) in step one. These fragmont served as templates for the subsequents amplifications of individual exons, exon 11 was divided in 16 overlaping fragments in step two.

All target fragments, designed to have optimal melting charactoristics were prepared by PCR, using specific primers, followed by heteroduplexing. The resulting PCR products were resolved in a 10% polyacrylamide (37.5:1) gel with an incresing (1°C/h) temperature gradient (TGGE). Melting of DNA occurs in the " melting domains ", mutational diferences among different fragments are revealed as migrational differences in the gel. The resulting of TGGE test was evaluated by screening (in a blinded fashion) of 23 DNA samples from three families with breast cancer history (with previously identified germline mutations).

Samples with a pattern of heteroduplex were sequenced. All mutations were correctly detected by the two methods TGGE and sequencing.

This assay provides an accurate cos-effective and non-radiactive method for mutational scaning of all BRCA1 coding exons.

6. Cell Cycle

b

Anti-tumoral responses mediated by the INK4a-ARF locus.

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Normal, healthy, cells possess mechanisms that protect them from becoming neoplasically transformed by the simple action of oncogenes. Another characteristic of normal cells is their inability to grow indefinitely in culture. Indeed, upon accumulation of a certain number of doublings, normal cells enter into a permanent arrest known as senescence.

The INK4a-ARF locus encodes two tumor suppressor, p16INK4a and p19ARF, that regulate two different tumor suppressor pathways: p16INK4a activates Rb by inhibiting the CDK4 and CDK6 kinases; and p19ARF activates p53 by inhibiting the destabilizing effects of MDM2. The cell-cycle inhibitor p21 mediates some of the effects of p53 in arresting proliferation.

We have reported that oncogenic Ras triggers an anti-proliferative response mediated by both p16INK4a and p19ARF, thus resulting in hypophosphorylation of Rb and in stabilization of p53. We regard this response as an anti-tumoral mechanism. Indeed, rodent cells genetically deficient in either p16INK4a or p19ARF are efficiently transformed by oncogenic Ras.

We have also compared senescence in fibroblasts derived from mice deficient in p16INK4a, p53 or p21. We have confirmed previous reports indicating that p16INK4a and p53 are essential for senescence in murine fibroblasts, and we have found that fibroblasts derived from p21-deficient mice senesce in a manner indistinguishable from wild-type cells. Moreover, p21-deficient cells are refractory to transformation by oncogenic Ras, which is in contrast to p53-deficient cells that are efficiently transformed by Ras. We conclude that the p16INK4a/Rb and p19ARF/p53 pathways are essential for senescence and for resistance to transformation, but p21 is dispensable for both processes. These data correlate well with the tumor phenotype of the corresponding knock-out mice.

Our data are compatible with a model in which p16INK4a and p19ARF sense the aberrant effects of some oncogenes and the accumulation of an excessive number of cell divisions. This model would be consistent with the fact that p16INK4a and p19ARF are inactivated during the late stages of tumorigenesis in many tumors.

A070

EVIDENCE FOR TWO NEW TUMOR SUPPRESSOR *LOCI* ON MOUSE CHROMOSOME 4 AND INVOLVEMENT OF *P19/ARF* GENE IN THE DEVELOPMENT OF g-RADIATION-INDUCED THYMIC LYMPHOMAS

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Recent studies in our laboratory reported frequent loss of heterozygosity (LOH) on mouse chromosome 4 in g-radiation-induced thymic lymphomas of F1 hybrid mice. These studies allowed to identify three candidate tumor suppressor gene regions: TLSR1 (located between D4Wsm1 and D4Mit9), TLSR2 (centered at D4Mit205b), and TLSR3 (encompassing Mom-1 and D4Mit68).

To determine the possible existence of other tumor suppressor gene loci on the proximal-mid part of chromosome 4, and to clarify whether the $p16^{INK4a}$ (a and b) and $p15^{INK4b}$ are the inactivation targets of deletion at TLSR1, we have performed additional analyses in 83 primary T-cell lymphomas. We identified two distinct regions of significant allelic losses in the proximal-mid part of chromosome 4, defined by the markers D4Mit116 (TLSR4) and D4Mit21 (TLSR5), and confirmed that the *INK4a* and *INK4b* loci are the candidates for TLSR1. Furthermore, LOH analysis and expression studies with $p19^{ARF}$ indicate that this gene is frequently altered in these tumors.

Taken together, this evidence and previous data demonstrate the existence of at least five different candidate sites for tumor suppressor genes on chromosome 4, thus revealing a main role for this chromosome in the development of mouse tumors.

c

Regulation of cell cycle entry and exit

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p130, a protein structurally and functionally related to the product of the retinoblastoma susceptibility gene (pRB) and to p107, is a nuclear phosphoprotein, which exhibits growth suppressor capabilities (1-4). We have shown that p130 exists in at least three phosphorylated forms (p130 forms 1, 2, and 3) and an unphosphorylated form in mammalian cells that can be resolved by SDS/PAGE. Moreover, we have shown that the phosphorylation status of p130 is regulated in a cell cycle-dependent manner and that phosphorylation of p130 to specific forms regulates p130/E2F-4 interaction (5). This and the data from others indicating that p130 is the main pRB-related protein in E2F complexes in quiescent cells, suggested that p130 plays a critical role at specific transitions of the cell cycle by negatively controlling E2F transcription factors. Our results have demonstrated that specific phosphorylated forms of p130 (forms 1 and 2) accumulate when cells exit the cell cycle resulting in accumulation of p130/E2F-4 complexes (6). p130 forms 1 and 2 are the forms of p130 that associate with E1A, a viral oncoprotein that targets pocket proteins to force quiescent cells to enter the cell cycle and replicate DNA, suggesting that p130 forms 1 and 2 are the p130 forms with growth suppressor capabilities. We have also investigated the relation between the protein levels and the phosphorylation status of the pRB family of proteins. In this regard, pRB and p107 changes in protein levels are independent of their state of phosphorylation. However, while p130 phosphorylation to forms characteristic of quiescent/differentiated cells results in accumulation of p130 protein, phosphorylation of p130 to form 3 (characteristic of cycling cells) is accompanied by down-regulation of its protein levels (6-7). We have also shown that p130 and p107 phosphorylation status and protein levels are regulated in *in vivo* models of cellular proliferation as in cultured cells. Moreover, regulation of p130 forms during differentiation is in striking contrast with the changes observed for p107 (7). The modulation of p130 phosphorylation and protein levels during skeletal muscle differentiation as well as during granulocytic differentiation of 32Dcl3 cells is consistent with our previous analysis of p130 regulation in cells exiting the cell cycle to quiescence (6-7). In both differentiation models, the accumulation of p130 forms 1 and 2 correlates with growth arrest and initiation of terminal differentiation. High levels of these forms are maintained in terminally differentiated myotubes and adult mouse tissues, consistent with a role for p130 in differentiated cells.

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A087

P27/KIP1–CYCLIN D3 COLOCALIZATION IN A SUBSET OF AGGRESSIVE B-CELL LYMPHOMAS.

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p27 cyclin-dependent kinase inhibitor down-regulation is essential for transition to S-phase of cell cycle. Proliferating cells in reactive lymphoid tissue (RLT) show undetectable p27 expression. In contrast with this observation, an anomalous high p27 expression has been previously shown in a group of aggressive B-cell lymphomas (BCL) with high proliferation index and adverse clinical outcome, thus suggesting that the abnormally accumulated p27 protein has been rendered functionally inactive.

We have analyzed the causes of this anomalous presence of p27 in a group of aggressive BCL including 54 cases of diffuse large B-cell lymphomas and 20 Burkitt lymphomas, studying simultaneously p27, cyclin D3, cyclin D1 and cyclin E expression, since it has been described that high levels of expression of cyclin D1 or E leads to increase in p27 levels in some cell types. Double immunostaining and Laser Scanning Confocal Microscopy study was performed in 5 tonsils and 15 BCL.

An statistically significant association between p27 and cyclin D3 expression was found for the overall group. Additionally, it was observed that cases with stronger Cyclin D3 expression show also higher p27 expression. The relation between both proteins was also demonstrated at a subcellular level, by laser confocal studies, showing that in cases with high expression of both proteins there was a marked colocalization.

These results could support the existence of complexes Cyclin D3-p27 in a subset of aggressive B-cell lymphoma cases, where p27 lacks the inhibitory activity found when bound to cyclin E/CDK 2 complexes. This interaction between both proteins could lead to an abnormal nuclear accumulation, detectable by immunohistochemical techniques.

A121

CDC25A AND CDC25B2 CELL CYCLE ACTIVATING PHOSPHATASES ARE OVEREXPRESSED IN HIGH GRADE NON-HODGKIN'S LYMPHOMAS.

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The cdc25 cell cycle activating phosphatases trigger the CDK-cyclin complexes by removing the thr14 and tyr15 phosphates. cdc25A and B but not cdc25C are overexpressed in some human tumors and have been shown to have tranforming potential in cooperation with other oncogenic factors. Three different splicing variants of the cdc25B have been identified with the resulting cdc25B2 being more active *in vivo* than cdc25B3 and B1. To determinate the role of cdc25 genes in the pathogenesis of NHLs we have analyzed the expression of cdc25A, B1, B2, B3 and C mRNA in peripheral blood lymphocytes from 3 normal blood donors, 8 non-neoplastic lymphoid tissues and 89 non-Hodgkin's lymphomas (NHLs) (19 SLL/CLL, 3 HCL, 12 follicular, 19 typical MCL, 10 blastic MCL, 22 large cell lymphomas, 1 lymphoblastic, 2 Burkitt's and 1 ALCL), by semiquantitative RT-PCR. No expression of these phosphatases was observed in the normal peripheral blood lymphocytes. In reactive tissues, cdc25B1 and B3 expression was observed in all cases. However, cdc25B2 and C were negative and only very low levels of cdc25A were detected. Overexpression of cdc25A and cdc25B2 was found in the 35% and 38% of the tumors, respectively, being more frequently found in aggressive (67% and 64% respectively) than in indolent lymphomas. Only the 13% and the 21% of the indolent tumors overexpressed cdc25A and cdc25B2, respectively. cdc25B1 and B3 splice variants were detected in virtually all tumors and no differences were found between high and low grade lymphomas. cdc25C was not detected in any of the tumors. In conclusion, these findings suggest that cdc25A and B2, but not cdc25B1, B3 and C, are overexpressed in a relatively large number of NHLs and may participate in the pathogenesis of aggressive variants.

A124

Upregulation of cyclin T1/CDK9 complexes during T cell activation.

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Cyclin T1 was identified as a regulatory subunit for CDK9 and as a component of the transcription elongation factor P-TEFb that phosphorylates the C-terminus domain (CTD) of RNA polymerase II (RNAP II) in vitro. P-TEFb positively regulates transcriptional elongation and it is >believed to regulate the expression of a number of genes. In this regard, a number of regulatory genes involved in the genesis of human cancer, including the oncogenes c-myc, L-myc, N-myc, c-myb and c-fos among others, are known to be regulated, at least in part, by transcriptional elongation-dependent mechanisms.
p-TEFb has also been implicated in the regulation of productive elongation of HIV-1 transcripts, which is mediated by the HIV-1 protein Tat. Recently, it has been reported that a CTD-kinase activity that associates with GST-Tat in pull down assays from protein extracts of peripheral blood >lymphocytes (PBLs) is upregulated during T-cell activation. Since CDK9 and cyclin T1 have been found to associate with Tat in vitro and in transient transfection experiments, it is likely that induction of the Tat associated kinase (TAK) upon activation of PBLs might result from activation of the cyclin T1/CDK9 pair. However, the exact mechanism for TAK activation remains unknown.
Here we report that the levels of cyclin T1 are dramatically upregulated by two independent signaling pathways triggered respectively by PMA and PHA in PBLs. Upregulation of cyclin T1 in stimulated PBLs results in induction of the CTD kinase activity of the cyclin T1/CDK9 complex, which >in turn correlates directly with phosphorylation of RNAP II in vivo. These results provide a mechanism to explain induction of the GST-Tat associated kinase activity during T cell activation. Interestingly, stimulation of a single mitogenic pathway, not sufficient for PBLs to progress through S phase, is sufficient for activation of the cyclin T1/CDK9 complex. However, the expression of cyclin T1 is not growth and/or cell cycle regulated in other human cell types, suggesting that regulation of cyclin T1 expression is dependent on tissue-specific signaling pathways. In addition, we report here that endogenous CDK9 and cyclin T1 complexes associate with HIV-1 generated Tat in T cells, demonstrating that Tat recruits the cyclin T1/CDK9 pair in relevant cells and under physiological conditions (HIV-1 infected T cells). This, together with our results showing that HIV-1 replication in stimulated PBLs correlates with the levels of cyclin T1 protein and associated CTD kinase activity, suggests that the cyclin T1/CDK9 pair is one of the HIV-1-required host cellular cofactors generated during T cell activation. Studies on the consequences of disrupting cyclinT1/CDK9 function in cells are underway.

A059

CELL CYCLE REGULATES THE NUCLEAR TRANSLOCATION OF MVM PARVOVIRUS MVM CAPSID PROTEINS.

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Parvoviruses are icosahedric viruses with ssDNA that replicate and mature in the nucleus. Parvoviruses infection requires cellular functions transiently expressed during the S phase of the cell cycle. These requirements may explain the oncosupression ability of these viruses, reflected by the specific killing of transformed cells infected in vitro and by the supression of tumours in inoculated experimentation animals. It,s not known which steps in the viral cycle depend on the S phase. We have analyzed whether the subcellular location of VP1 and VP2 capsid proteins of MVM is cell cycle dependent.

MATERIALS AND METHODS. MVM VP gene was transient or stably transfected in a stablished mouse fibroblast cell line (A9) or in human transformed fibroblast cells (NB324K), both susceptible to MVM infection. Cells received different syncronization treatments, and the subcellular location of VP proteins was analyzed by indirect immunofluorescence with anti-MVM antibodies.

RESULTS AND CONCLUSIONS. VP proteins from confluence arrested A9 or NB324K cells (G1 phase) were exclusively localized in the cytoplasm. Similar results were obtained when syncronizing by serum starvation (quiescence Go) in A9, or by isoleucine starvation (G1/S transition) in A9 and NB324K cells. As the cells were released from the arrest (respectively: subculture, serum adition or inhibitors removal), VP proteins progressively translocated to the nucleus correlating with the entry in the S phase (monitorized by PCNA expression), and independently of the syncronization method followed. However, a double block with timidine keeps the VPs in the cytoplasm, despite of a normal PCNA expression. Therefore, the nuclear translocation of MVM structural proteins proceeds simultaneously with cellular DNA synthesis.
When independently expressed, VP2 but no VP1 is translocated into the nucleus in a cell cycle dependent manner, corresponding with the presence in this protein of a nuclear location motif (NLM) dependent on conformation. This cell-cycle dependent signal can contribute to the dependence of MVM multiplication for the S phase of cell cycle, and perhaps to the mechanisms underlying the oncosupressive phenomenon mediated by the parvoviruses.

A074

P15/INK4B/MTS1, A CDK INHIBITOR PROMOTER, METHYLATION STUDY IN METASTATIC CUTANEOUS MELANOMAS.

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INTRODUCTION: p15 is 44% identical to p16 over the first 50 aminoacids (aa) and 97% identical over the next 81 aa and is located 35 Kb centromeric to the p16 gene on the 9p21. Deletion of this locus inactivates almost always p15 together with p16, providing greater selective advantages, than only p16 deleted. When activated, p15 inhibits CDK4/CDK6 complex, thus releasing p27 to inhibit other CDKs, promoting cell cycle arrest. p15 mutations seem to be a late event in melanoma natural history, suggesting this gene role rather for melanoma progression than initiation.
Methylation of cytosines located 5' to guanosine is known to have a profound effect on the expression of genes. Aberrant methylation of CpG islands is a frequent event in malignant cells and is associated with inactivation of suppressor genes in different types of cancer.
Methylation of p16 promoter region, that is also a melanoma predisposition locus, as has previously been reported is not a common mechanism of this cell cycle regulator gene silencing.
AIMS: In the present study we have studied the p15 promoter methylation, using a methylation specific PCR.
MATERIAL AND METHODS: As a material for the present study we employed immediately frozen cutaneous and lymph-node biopsies of patients with sporadic metastatic cutaneous melanomas and 3 melanoma cell lines and normal melanocytes, obtained from healthy donors as an internal negative control. Previously modified in bisulfite reaction DNA is "hot-start" amplified with specific sets of primers, allowing distinguishing methylated from unmethylated DNA.
RESULTS: We report that none of 20 tumor samples have presented p15 promoter region methylation, neither have done melanoma cell lines examined.
CONCLUSIONS: Thus our findings permit to conclude that this type p15 gene inactivation is not a common event in sporadic melanomas.

A075

INACTIVATION OF P16 IN PROSTATE CANCER: MUTATIONAL AND METHYLATION ANALYSIS.

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BACKGROUND: p16 plays a role in the control of cell cycle. It was observed that deletions of the locus 9p21 determine both p15 an p16 loss conferring a greater selective advantage for tumor cells than only p16 inactivation. Aberrant methylation of CpG islands is a frequent event in human tumor cells and it is associated with tumor suppressor genes inactivation; methylation of p16 promoter region has been described yet in same types of cancers. Inactivation of p16 has been described by different causes (mutations, methylation or homozygous deletion). The role of p16 in prostate cancer remains unclear.
AIMS: In this study we have analyzed the different ways of p16 inactivation and its role in prostate cancer.
MATERIAL AND METHODS: Tumor samples from 50 patients diagnosed of prostate cancer have been evaluated. DNA isolation has been performed by using phenol-chlorophorm extraction. PCR amplification of exons 1 and 2 of p16 was done with further cycle sequencing analysis with dye terminators. Bisulfite DNA modification was performed and specific primers for top-methylated and unmethylated sequences were used.
RESULTS: From 50 tumor samples only one sample with early stage showed p16 mutation at codon 84 (GAC, Asp->TAC, Tyr). This low frequency confirms previous reports (1-2%). The incidence of p16 methylation detected in our series is very similar to the literature (13%) showing that these ways of inactivation are uncommon.
CONCLUSIONS: These data suggest that p16 inactivation by mutation or methylation is very unfrequent event in prostate carcinoma.

A083

CELL CYCLE EXIT AND *IN VITRO* DIFFERENTIATION OF HUMAN COLON CANCER CELL LINES

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INTRODUCTION: HT-29 colon cancer cultures contain >95% undifferentiated cells; by contrast, HT-29-derived subpopulations designated M6 and M3 display, after confluence, mucus-secreting and absorptive phenotypes, respectively. Our aim is to analyze cell cycle exit mechanisms in cells with variable capacity of differentiation and to evaluate the putative involvement of cell cycle exit pathways in this process. MATERIALS AND METHODS: The process of cell cycle exit and differentiation was studied by cell proliferation analysis, expression of cell cycle proteins, and presence of morphological and biochemical markers of differentiation.
RESULTS: The kinetics of cell cycle exit were similar, regardless of the ability to differentiate. Cell growth arrest by contact inhibition ocurred early, as cells reached confluence, and was associated with the induction of CKIs and acquisition of a pattern of pocket protein phosphorylation characteristic of cell quiescence. Upon cell cycle arrest, cells gradually formed either undifferentiated multilayers or differentiated monolayers. Both cell cycle arrest and differentiation were reversible if the contact inhibition constraint was relieved. Moreover, intercellular contacts were calcium-dependent and low calcium medium concentration blocked differentiation.
CONCLUSIONS: 1) HT-29 cell differentiation involves prior negative signalling in G1 progression pathways, 2) at least in part, common mechanisms are involved in cell cycle arrest and differentiation, and 3) unlike normal colonic epithelium, HT-29 cells do not undergo terminal differentiation and apoptosis.

7. Cancer and Cell Differentiation

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INTERACTION OF THE NUCLEAR STEROID/THYROID HORMONE RECEPTORS WITH THE MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) PATHWAYS.

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Lipophilic hormones such as steroids, retinoic acid, thyroid hormone or vitamin D regulate many aspects of vertebrate development, cell differentiation and homeostasis by binding to intracellular receptors which are members of the nuclear hormone receptor (HR) superfamily. HR are ligand-activated transcription factors, which regulate gene expression by binding to specific DNA-response elements or by interfering with the activity of other transcriptional activators, such as AP-1 (Jun/Fos). The latter mode of transcriptional regulation is known as transrepression and, among other, mutual competition for coactivators such as CREB-binding protein (CBP) have been proposed as a mechanism to explain it at molecular level. Recently, we have shown that HR inhibit the induction of the Jun N-terminal kinase (JNK), a MAPK which is the major mediator of c-Jun N-terminal phosphorylation. Since N-terminal phosphorylation of c-Jun is a requisite for AP-1 to interact with CBP, interference with the JNK pathway represents a novel mechanism by which HR antagonize AP-1. Inhibition of the JNK cascade by dexamethasone takes place shortly after hormone addition and even in presence of actinomycin D. In addition, dexamethasone induces accumulation of the JNK inhibitor $p21^{waf-1}$ by activating *waf-1* transcription. Therefore, interference with the JNK pathway by dexamethasone is likely to occur by alternative modes involving both transcriptional-dependent and -independent mechanisms. HR may also negative regulate activation of other MAPKs pathways such as the Extracellular-regulated kinase (ERK) pathway and, in consequence, antagonize ERK-targeted transcriptional activators such as Elk-1. Regulation of the activity of different MAPK pathways constitutes an alternative mode whereby hormones such as steroids and retinoids may control cell fate and conduct their immunosupressive, anti-inflammatory and antineoplastic actions.

e

Gli proteins in development and cancer

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The first part of the presentation will focus on the role of Gli proteins as mediators of Hedgehog signals. in neural patterning in the early embryo and in follicular development. Specifically, the role of Shh signaling and Gli gene function will be discussed in the context of basal cell carcinoma development. In the second part of the presentation, the role of Shh signaling in the development of the cerebellum will be presented. We have investigated a possible endogenous role of SHH in cerebellar development. We find that SHH is produced by Purkinje neurons. The results treating chick cerebellar explants or purified mouse cells with SHH or a blocking anti-SHH antibody show the requirement of SHH in the proliferation of granule neuron precursors. In addition, glial differentiation is induced by SHH. Blocking SHH signaling in vivo leads to the development of hypoplastic cerebella with abnormal foliation, in which Purkinje neurons are abnormally positioned and Bergmann glia and differentiated granule neurons are abnormal or absent. Together, these in vivo and in vitro results demonstrate previously unknown functions of SHH in the elaboration of pattern in the maturing central nervous system and provide a molecular model for the coordinate regulation of cortical development in the cerebellum. The implications of our work to an understnading of medulloblastomas will be discussed.

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A010

THYROID HORMONE INHIBITS THE PROLIFERATION AND AFFECTS THE PHENOTYPE OF MAMMARY EPITHELIAL CELLS.

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INTRODUCCION

Multiple data suggest a relationship between thyroid hormones (triiodothyronine, T3 and thyroxine, T4) and carcinogenesis. Studies on breast cancer are inconclusive, suggesting contradictory effects of thyroid status and diseases on tumor progression. However, and despite that alterations in the *erb*A loci have been detected in human tumors, very little is known about the molecular and cellular basis for the proposed effects of T3 on mammary epithelial cells. To define this interaction, we have examined the effect of T3 on the growth capacity and phenotype of normal, non-tumorigenic EpH4 mammary epithelial cells.

MATERIALS AND METHODS

EpH4, EpH4 + TRα-1 (over-expressing thyroid hormone receptors (TR-α1), and EpH4 + v-*erb*A (over-expressing the v-*erb*A oncogene encoding a mutated receptor unable to bind hormone) were used.

^{3}H-thymidine incorporation, FACScan, Immunofluorescence, northern and western blot analysis were performed.

RESULTS

T3 inhibits in a dose-dependent manner the proliferation of EpH4 cells expressing appropriate receptors (EpH4 + TRα-1), whereas had no effect on cells which express the v-*erb*A oncogene (EpH4 + v-*erb*A). In addition, T3 potentiated the growth inhibitory activity of transforming growth factor β (TGFβ). We have found that T3 inhibits cyclin D1 expression. In addition, T3 cause phenotypic changes in EpH4 + TRα-1 cell monolayers grown on porous filters leading to a rapid decrease in the transepithelial electrical resistance. This indicates that T3 affects intercellular adhesiveness. In line with this, hormone treatment caused a partial internalization and/or reduction of proteins involved in cell adhesion such as fodrin, vinculin and E-cadherin.

CONCLUSIONS

Our data demonstrate that T3 modulates the proliferation and phenotype of EpH4 cells suggesting that it may play a role in the biology of mammary epithelial cells.

A012

IDENTIFICATION AND CHARACTERIZATION OF PROTEOGLYCANS FROM NEUROECTODERMAL TUMOR CELLS

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Little is known about proteoglycans in the nervous system, although an increasing number of them are being identified and cloned. Thus, for example, we can find the neurocan/brevican family, which is related to the large proteoglycan (versican/aggrecan), the phosphacan, which is the soluble form of a tyrosine phosphatase, or the NG2/MCSP family of integral membrane proteoglycans. These molecules are involved in modulating cell interactions by interacting specifically with several ECM components, as hyaluronan or tenascin-C o by binding growth factors. Their distribution suggests that they act as an extracellular network that contributes to the heterogeneity of the neuronal microenvironment and can be altered in tumors, contributing to the abnormal behavior of tumor cells.

In this work, we have identified a high molecular weight proteoglycan in several cell lines of neuroectodermal origin, such as melanoma, astrocytoma and neuroblastoma. This proteoglycan, which we have named PG-L, is produced into the extracellular medium. It has a molecular weight higher than 1000 kDa, bears chondroitin sulfate chains and shows a protein core composed of two bands of 350 and 400 kDa. This proteoglycan is different from the well-known melanoma-specific proteoglycan (MCSP) since it does not react with monoclonal antibodies against MCSP and they can be separated after gel chromatography in dissociative conditions. We have purified PG-L from U251 human astrocytoma conditioned medium after concentration, ion exchange chromatography and gel chromatography. The characterization of PG-L shows that the CS chains have an average molecular weight of 12 kDa and that the protein core is a glycoprotein as shown after digestion with glycosidases. PG-L can be related to some of the isoforms of versican, a chondroitin sulfate proteoglycan originally isolated from fibroblasts. Using the purified preparation, we have raised polyclonal antibodies which react specifically against PG-L in western blot and immunocytochemistry. The immunolocalization of PG-L shows that it can be found in the membrane as well as in Golgi-related structures.

A079

HLH TRANSCRIPTION FACTORS IMPLICATED IN THE DEVELOPMENT AND DIFERENTIATION OF EXOCRINE PANCREAS.

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The exocrine pancreas is composed of acinar cells, involved in enzyme secretion, and ductal cells which produce mucus and bicarbonate. Ductal cells are involved in the most common pancreatic pathologies, i.e. chronic pancreatitis and more than 90% of exocrine tumors. The expression of acinar enzymes is controlled by PTF-1, a transcriptional complex composed of three HLH (helix-loop-helix) factors : E2A and HEB, which are ubiquituous, and p48 being exclrine pancreas specific.

We have previously shown that, in normal human exocrine pancreas cultures (NPC), a switch from acinar to ductal phenotype occurs in the first days of culture. Based on their ultrastructural features and marker expression, NPC are undistinguishable from ductular complexes in chronic pancreatitis. In this work, we have examined the mechanisms involved in this phenotypical switch and their relationship to the phenotype of pancreas cancers.

We have raised a p48-specific antiserum which recognizes exclusively normal acinar cells. Using EMSA, RT-PCR, and western blotting we have shown that, in NPC, loss of the acinar phenotype is associated with loss of expression of p48 and disappearance of the PTF1 complex. Similarly, p48 expression is absent from ductal pancreatic adenocarcinomas and is absent from ductular complexes in chronic pancreatitis. Similar results were obtained using an azaserine-induced rat pancreas cancer model from which both acinar and ductal variants are available : p48 is present in acinar tumors but not in ductal tumors. Transfection of p48 cDNA into cells with a ductal phenotype does not induce an acinar phenotype nor the expression of a reporter cDNA (hGH) under the control of the PTF1-binding sequence. By contrast, transfection into amylase-expressing AR42J cells induces an increase in the levels of p48, amylase, and the reporter construct.

These results indicate that p48 expression is necessary, but not sufficient, for the acquisition of the acinar cell phenotype. Furthermore, p48 down-regulation may play a role in chronic pancreatitis and in pancreas cancer.

A118

Phosphorylation of Notch2 by Casein Kinase I (CKI): Possible implications in Hematopoietic differentiation.

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Several mammalian Notch molecules and ligands are coexpressed in many differentiating tissues including hematopoietic cells. However, it is still unclear the unique role that each homolog may have in the process of controlling cell differentiation in the different contexts.

We have previously demonstrated that truncated Notch1 and Notch2 specifically inhibit myeloid differentiation in response to G-CSF and GM-CSF respectively, indicating a connection between Notch function and cytokine network in Hematopoietic differentiation.

We formerly described the Notch Cytokine Regulatory (NCR) domain as responsible for the specific response of these molecules to G-CSF and GM-CSF.

We now have studied proliferation and differentiation of 32D cells expressing Notch2 NCR mutant molecules. Deletions in the NCR region eliminate Notch2 specificity, resulting in inhibition of differentiation by G-CSF and GM-CSF; and enhanced proliferation in the presence of IL-3. This deletion contains 2 putative CKI phosphorylation sites (SXXS) which are not present in any other Notch homolog.

The truncated Notch2 molecule is highly phosphorylated when expressed in 32D cells. In addition, recombinant CKI is able to phosphorylate truncated Notch2-GST fusion protein in vitro. We are currently investigating further connections between Notch2 regulation and CKI phosphorylation. These results suggest that regulation of Notch2 function in hematopoietic differentiation may involve phosphorylation by CKI.

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A036

STOCHASTIC SIMULATION OF LOGISTIC GROWTH OF A COLON ADENOCARCINOMA CELL LINE

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Introduction.- Simulation models (deterministic and stochastic) can show the cell growth characteristics by predicting temporal changes happened in the population. Deterministic model does not consider environmental factors, whereas the stochastic one shows the cause-effect relationships and the adaptive phenomenon.

Materials and Methods.- *Cell culture:* Colon adenocarcinoma cells were cultured in DME/F12-HAM medium (Ca^{++} and Mg^{++} free, with L-Gln and hepes) and supplemented with NaHCO3 7.5 % (28 ml/L), 10 % FBS and 1 % PSF 100X; at 37 °C in a 5 % CO_2/air atmosphere. It was obtained a growth curve by daily cellular re-count. *Simulation:* "K" value (maximum cellular density) was obtained from the experimental growth curve, and "r" value (renovation ratio) was calculated using the lining of the logistic deterministic model: Ln(K-N/N)=c-rt. Stochastic simulation was performed by the Pielou simulation, where time between events (cellular birth and death) is also stochastic. It has been developed a computer software that performs the simulations.

Results and Conclusions.- The deterministic model showed a cellular growth similar to experimental one, reaching the "K" threshold without oscillations. Different surviving curves were obtained after stochastic simulations, where it can be observed an oscillating growth of the cell number, due to the influence of random on cellular birth and death. The growth interval up to "K" threshold depends on both values, birth and death. The stochastic model makes a better prediction of the cellular growth, due to the incorporation of so birth, growth and densodependence rates, as the time and kind of event. This model can be used to study the growth of in vitro cultured cell lines under different conditions. In the same way it could be used to predict the effects of citostatic drugs and radiation on cellular growth.

A043

ADENOCARCINOMA PANCREATIC CELLS PRODUCE ANOMALOUS GLYCOSYLATION IN HUMANPANCREATIC RIBONUCLEASE

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Our group is studying human pancreatic ribonuclease as a possible tumor marker of pancreatic adenocarcinoma. Firstly, we described that adenocarcinoma pancreatic tumoral cells express and produce this enzyme and, using immunohystochemical and in situ hybridization techniques, we have also detected ribonuclease expression in pancreatic adenocarcinoma tissues. The protein produced by tumoral cells has the same aminoacid sequence and enzymatic parameters as the produced in normal situation. However, monosacharide analysis of its glycosydic chains shows a different pattern in the tumoral ribonuclease.

The main goal of this work is the characterization of the different glycosylation pattern between the ribonuclease produced by cultured pancreatic adenocarcinoma cells and the produced by normal pancreas, by using monoclonal antibodies against specific glucidic antigens.

We used cultured adenocarcinoma cells of different degrees of differentiation: Capan-1, MDA-Panc 3, IBF-CP3 and Panc-1. The cultured media was stored and concentrated by tangential filtration. On the other hand, normal pancreas from a healthy donor was homogeneized and precipitated with acetone to isolate the protein fraction. By sandwich ELISA with anti-ribonuclease polyclonal antibodies and monoclonal antibodies against glucidic antigens we analyzed the different pattern of glycosylation among them.

The monoclonal antibodies used are against blood group related antigens, in particular, sialyl lewis X and sialyl lewis a, which are usually expressed in tumoral cells from the digestive system.

Our preliminary results show that ribonuclease from Capan-1 and IBF CP3 presents sialyl lewis X antigen and ribonuclease from MDA-Panc 3 presents sialyl lewis a. In pancreatic ribonuclease both antigens are not detectable.

These results open the possibility to differentiate the tumoral from the normal ribonuclease, which could allow the use of this enzyme as a tumoral marker for pancreatic cancer.

A047

ROLE OF DCC (*DELETED IN COLORECTAL CANCER*) IN ADHESION OF INTESTINAL EPITHELIAL CELLS.

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The *Deleted in Colorectal Cancer* (*DCC*) gene was identified as tumour suppressor gene in human colorectal carcinomas (Fearon et al., 1990), encoding a 170-190kDa protein from the Immunoglobulin superfamily. However, regarding to its function, DCC was shown to be implicated in the development of nervous system as receptor of Netrin-1 (Chan et al., 1996; Keino-Masu et al., 1996; Kolodziej et al., 1996). Furthermore, studies on *Dcc* knocked-out mice (Fazeli et al., 1997) seriously questioned its implication in mucosecretory differentiation, as it was previously proposed (Hedrick et al., 1994).

Our results from the analysis of: 1) DCC mRNA expression by RT-PCR in 14 colon cancer cell lines presenting various phenotypes, 2) DCC protein expression by immunohistochemistry on frozen sections of human normal colon, and 3) full length DCC-transfected HT-29 cells, indicated that DCC was not selectively involved in the mucosecretory differentiation pathway, and that it was neither sufficient nor essential for normal intestinal differentiation. However, functional studies on our transfectants demonstrated that DCC was implicated in cell-to-substrate interaction. Indeed, DCC/HT-29 cells presented a decreased adhesiveness to the extracellular matrix comparing to control transfectants. Moreover, when inoculated subcutaneously in nude mice, DCC/HT-29 cells were less tumorigenic than the control ones, thus confirming its role in tumour suppression. Nonetheless, the *in vitro* analysis of their proliferative properties did not show any effect of DCC expression. This was suggesting that our cellular model was lacking *in vitro* of one necessary component, such as a ligand, to generate a cellular response. In order to investigate this possibility, we analysed the effect of 293/EBNA-Net-1 cells (secreting Netrin-1, DCC ligand in nervous system), as well as mesenchymal cells isolated from human intestine biopsies (C9 and C20 clones), on our DCC/HT-29 cells. To this aim, either conditioned media from these cells were used, or co-culture experiments were performed. The effect observed on HT-29 morphology and proliferation, as well as mobility and tumorigenicity, was apparently not specific. However, in coculture experiments, DCC expression decreased specifically the interaction between HT-29 and C9/C20 mesenchymal cells. Moreover, conditioned media from C9/C20 cells increased specifically the adhesion of HT-29 cells to the extracellular matrix in presence of DCC.

In conclusion, our studies indicate that DCC is implicated in cellular adhesion and tumour suppression, but not differentiation, of intestinal epithelial cells. Finally, the analysis of the effect of 293/EBNA-Net-1 and C9/C20 mesenchymal cells on DCC/HT-29 transfectants demonstrated to be specific when related to their adhesive properties towards the extracellular matrix.

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A078

In vitro DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO PANCREATIC CELLS

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Mouse embryonic stem cells (ES cells) are continuously growing cells derived from the inner cell mass of the 3.5day blastocyst. ES cells can be cultured in an undifferentiated state *in vitro* for extended periods of time. In some *in vitro* conditions they retain the ability to generate differentiated progeny. For instance, they can form three-dimentional aggregates called embryoid bodies (EB) from which they differentiate spontaneously into a large variety of cell types. The pancreas develops from an evagination of the foregut of the embryo, early in gestation. In adults it is composed by an exocrine and an endocrine compartment that originate from the endoderm of the primitive gut, as evidenced by the expression of the genes responsible for the production of the acinar enzymes and pancreatic hormones, respectively. However, the early pancreatic cell precursors are poorly characterized and have still not been isolated. The aim of this study was to establish an early pancreatic differentiation model using ES cells as an experimental system. For this, ENS-26 cells (P.Savatier, Lyon, France) were differentiated into EB by culturing them in suspension during 21 days. Putative precursors of pancreatic cells were clearly identified as early as 4 days. 1) Transcripts of p48, a transcription factor of the HLH family that is exclusively expressed in the exocrine pancreas, were detected by RT-PCR, showing a maximal expression at 21 days. Immunocytochemical analysis indicated that less than 5% of cells were positive for p48 staining and the location of the protein was mainly nuclear. 2) some of these cells were positive for insulin and glucagon staining, two markers of the endocrine pancreas that are firstly detected in the pancreatic diverticulum during mouse development. This pattern of expression was in accordance with the correspondant results obtained by RT-PCR analysis.3) HNF-3β, a winged helix transcription factor, expressed in the exocrine pancreas during mouse development was also detected after 4 days. In conclusion, because of the restrictive expression of p48 in the pancreas, these data indicate that the system of ES cells provides a suitable model to study early steps of the pancreatic differentiation.

A084

THE INTESTINAL DIFFERENTIATION PROGRAM ACTIVATED DURING GASTRIC CARCINOGENESIS DOES NOT FULLY RECAPITULATE A DEVELOPMENTAL PHENOTYPE.

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Gastric apomucins, MUC5AC and MUC6, and Lewis antigens show a characteristic pattern of expression in the epithelium of the normal stomach: MUC5AC is detected in the superficial epithelium associated to type 1 Lewis antigens whereas MUC6 is expressed in the deep glands associated to type 2 Lewis antigens. The appearance of cells with intestinal phenotype and mucins with different histological features during gastric carcinogenesis may implicate alterations in the mucin gene expression and in their pattern of glycosylation.

We have analysed the expression of gastric, MUC5AC and MUC6, and intestinal, MUC2 and MUC4 mucin genes, and association with Lewis antigens during gastric carcinogenesis, and to relate them to the developmental expression patterns. Indirect and double labelling immunoassays with specific antibodies, and in situ hybridization techniques have been used.

Our results show that in incomplete (n=13) and complete (n=4) intestinal metaplasia, gastric mucin expression is down-regulated whereas intestinal mucin expression is activated. All these mucin genes are detected associated to both type 1 and type 2 Lewis antigens. In gastric tumours (n=35), there is an up-regulation of intestinal mucin expression compared to the decreasing expression of gastric mucins, MUC5AC and MUC6, which are coexpressed at the single cell level. In foetal stomach (n=30), MUC2, MUC5AC and MUC6 but not MUC4, are detected. MUC5AC and MUC6 are coexpressed and associated with both type 1 and type 2 Lewis antigens.

Conclusions: 1) The alterations in mucin gene expression and the loss of association with Lewis antigens are early events in gastric carcinogenesis. 2) The pattern of mucin gene and Lewis antigen expression in gastric tumours does not fully recapitulate a foetal phenotype.

A0127

UV Irradiation Induces the Murine Urokinase-type Plasminogen Activator (uPA) Gene Via the cJun N-terminal Kinase (JNK) Signaling Pathway. Requirement of an AP1-enhancer Element.

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Ultraviolet light (UV) irradiation leads to severe damage like cutaneous inflammation, immunopression and cancer, but also results in a gene induction protective response termed the UV response. The signal triggering the UV response was thought to originate from DNA damage; recent findings, however, have evidenced that it is initiated at or near the cell membrane and transmitted via cytoplasmic kinase cascades to induce gene transcription. Urokinase-type plasminogen activator (uPA) was the first protein shown to be UV inducible in xeroderma pigmentosum DNA repair-deficient human cells. However, the underlying molecular mechanisms responsible for the induction were not elucidated. We have found that the endogenous murine uPA gene product is transcriptionally up-regulated by UV in NIH3T3 fibroblast and F9 teratocarcinoma cells. This induction required an AP1-enhancer element located at -2.4 kb, since deletion of this site abrogated the induction. We analyzed the contribution of the three different types of UV-inducible mitogen-activated protein (MAP) kinases (ERK, JNK/SAPK and p38) to the activation of the murine uPA promoter by UV. MEKK1, a specific JNK activator, induced transcription from the uPA promoter in the absence of UV treatment, whereas coexpression of catalytically inactive MEKK1(K432M) and of cytoplasmic JNK-inhibitor JIP-1 inhibited UV-induced uPA transcriptional activity. In contrast, neither dominant negative MKK6 (or SB203580) nor PD98059, which specifically inhibit p38 and ERK MAP kinase pathways, respectively, could abrogate the UV-induced effect. Moreover, our results indicated that wild type N-terminal cJun, but not mutated cJun (Ala63/73), was able to mediate UV-induced uPA transcriptional activity. Taken together, we show for the first time that kinases of the JNK family can activate the uPA promoter. This activation links external UV stimulation and AP1-dependent uPA transcription, providing a transcription-coupled signal transduction pathway for the induction of the murine uPA gene by UV.

8. Cancer Epidemiology

A026

EPIDEMIOLOGY OF BREAST CANCER IN THE HEALTH REGION OF GIRONA.

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AIM. To analyse the incidence, mortality, prevalence and survival of breast cancer in the Health region of Girona.

MATERIAL AND METHODS. The information was obtained from the population-based cancer Registry of Girona. The incidence and mortality corresponding to the new cases of invasive breast cancer diagnosed in the period 1994-1995. Cases detected by death certificate were included. IARC recommendations were used in the case of multiple tumours. Survival was calculated from the patients diagnosticated between 1980-89. The average time of follow up was 10 years. Observed survival was calculated by Kaplan Meier method and relative survival was calculated using a procedure described by Ederer.

RESULTS. During the period 1994-1995 were diagnosticated 539 cases in Girona Health Region.

The crude incidence rate was 102.9, the age standardised incidence rate was 65.4 new cases per 100.000 women/year.

One of each 3-4 cancers diagnosed in women was breast cancer, one of each 13 women will developed breast cancer before the age of 75. The number of death for breast cancer was 180 cases. The crude rate of mortality was 34.4 and age standardised mortality rate was 18.9. The average years of life lost because of breast cancer was 13.3. The estimate number of prevalents cases is about 5.000.

The observed survival of the 1406 cases corresponding to the period 1980-1989 at 5 and 10 years was 60.5% and 40.7%, the relative survival at 5 and 10 years was of 65% and 48.9%.

CONCLUSIONS. The incidence of breast cancer in Girona is high in relation to the incidence in Spain although is situated in an intermediate level when is compared with international registries. The standardised rate has increased from 43.1 cases 100.000/year for the period 80-84 to 65.4. At the present time the survival is similar to the European average.

A090

FOLLOW-UP OF A COHORT OF HBsAg-POSITIVE BLOOD DONORS IN CATALONIA

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Background: In Catalonia, Spain, the prevalence of HBsAg in a random sample of the general population is 1.7% among males and 1.6% among females.

Objective: i) to quantify the risk of death among HBV carriers, and ii) to determine risk factors for progression to liver cancer.

Methods: In the period 1970-1985, a cohort of 2228 HBsAg carriers (1772 males, 742 females) was identified among voluntary blood donors. Passive follow-up has been conducted by a record linkage with the mortality files for the period 1985-1996. An active follow-up of the cohort is being conducted (medical interviews).

Results: For males, an excess mortality due to liver cirrhosis (SMR= 281.5, CI95%: 172.5-459.5) and to liver cancer (SMR=431.8, CI95%: 206.1-904.7) has been demonstrated. The estimated attributable fraction for liver cirrhosis is 3% and 5.3% for liver cancer. For females, two cases of liver cirrhosis (SMR=195.4, CI95%: 49.0-778.9) and no cases of liver cancer have been found.

Conclusions: Among males, HBsAg positivity increases three-fold the risk of death due to liver cirrhosis and four-fold due to liver cancer. HBV accounts for a minor fraction (<5%) of the liver cancer cases observed.

Implications: In Catalonia, the low HBV attributable risk estimated for cirrhosis and liver cancer suggests a non significative long-terme mortality decrease for these causes after the hepatitis B, vaccination programme.

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A093

SMOKING AND DRINKING HABITS' CHARACTERISTICS AND RISK OF ESOPHAGEAL CANCER IN MEN AND WOMEN

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Background: The knowledge of which aspects of alcohol and cigarette consumption most strongly influence esophageal cancer risk has important public health implications for a tumor that depends on preventive strategies for its control. In particular, the assessment of these exposures among women and the impact of cessation taking into account the exposure history of the individual have been poorly assessed. **Methods**: Data from a series of 5 case-control studies of squamous-cell carcinoma of the esophagus conducted in high risk areas in South America, were combined and analyzed using multivariate logistic regression procedures. The studies were designed and coordinated by the IARC using the same research protocol and data collection procedures. A total of 830 cases and 1779 control subjects were included in the analysis. **Results and conclusions**: All exposure measures of alcohol and cigarette consumption and cessation were strongly related to esophageal cancer risk in both men and women. Women had the same exposure profile than men, but the magnitudes of the associations were somewhat lower than were those among men. The specific exposure characteristics that most strongly influenced the risk of esophageal cancer were: the average amount of alcohol consumed per day, the lifetime duration of cigarette smoking, the type of tobacco smoked and the time since quitting either habit. The relative risk for black tobacco smoking was about two times higher than was that for blond tobacco smoking and a synergistic interaction was identified with amount of cigarettes smoked. Strong inverse dose-response relationships were identified with time since quitting either habit. The risk reduction after cessation was consistently observed regardless of the previous duration and intensity of the habit and the type of tobacco or alcoholic drink consumed.

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A094

Prevalence of cancer in relatives of young women with breast cancer

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Of all cases of breast cancer, 4-5% are attributable to the inheritance of a / several gene/ genes. Ovarian cancer, Colon cancer, and, probably, pancreatic and prostatic cancer are associated with breast cancer. The data presented are part of a study whose purpose it is to estimate the prevalence of BRCA1 and BRCA2 mutations in women with breast cancer.
Objectives: To investigate whether there is an elevated number of cancers for different types in relatives of women with breast cancer younger than 45 years as compared to that observed in the general population of Catalunya.
Methods: Pedigrees were obtained from 147 women younger than 45 consecutively diagnosed with breast cancer in the population of Tarragona and Girona. Population-based cancer registries cover these two areas. For every first and second-degree relative of these 147 women with breast cancer the cumulative risk of developing cancer is calculated. The sum over all probabilities gives the expected number of cancers, which is done for each cancer type. These numbers are compared with the observed numbers in the study population by means of the Standard Mortality Ratio (SMR). Cumulative risks for developing cancer of Tarragona and Catalunya are used.
Results: 270 cases of cancer are observed including 59 breast cancers, 30 stomach cancers and 22 liver cancers. The global SMR for the whole population is less than 1, but differences are observed for different generations, e.g. 1,523 for sisters, 1,553 for mothers and 0,565 for grandfathers. The SMR for breast cancer in the female population amounts to 1,373. It is 2,488 for sisters, 2,373 for mothers, 1,563 for aunts and 0,788 for grandmothers.
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A025

CANCER INCIDENCE AND MORTALITY IN THE HEALTH REGION OF GIRONA, 1994-1995.

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AIM. To calculate cancer incidence and mortality at the Health Region of Girona.
MATERIAL AND METHODS. The data were obtained from the population-based cancer registry of Girona. The area covered by the registry is the Health Region of Girona with a population of 509.458 inhabitants. (Census of 1991)
The data corresponding to the registered cases during the period 1994-95. The parameters of quality were a percentage of histological verification of 88.7% in men and 88.9 % for women and the cases accepted as death certificate only, were 4.1% for men and 4.5% for women.

RESULTS. During the period 1994-1995, 4.383 cases of cancer (excluded skin no melanoma and cancer in situ), were registered.
The distribution according to sex was 2.543 cases in men with a crude incidence rate of 495.3 cases per 100.000/year, and an age standardised incidence rate of 291.0.
The number of cases in women were 1840 with a crude incidence rate of 351.3 and an age standardised incidence rate of 197.5.
Lung, colon-rectum, bladder, larynx and stomach were the 5 more frequents sites in men in the age range of 15 to 65 years. In the women the 5 more frequents sites were breast, colon-rectum, uterus, cervix uteri and ovary.
In men older than 65 years the 5 sites more frequents were, prostate, lung, colon-rectum, bladder and stomach.
For women older than 65 years the 5 sites more frequents were, breast, colon-rectum, unknown primary site, stomach and corpus uteri.
Mortality. During the period 1994-95, the number of cancer deaths were 2.426. That supposed a crude mortality rate for men of 292.4 and an age standardised mortality rate of 161.1. For women mortality rates were 176.6 and 78.8 respectively.

Lung cancer has been the first cause of mortality in men, and breast cancer was in women.

CONCLUSIONS. The cancer incidence in the Health Region stands at an intermediate level when we compare with the rest of cancer registries in Spain and Europe. The cancer more frequent in men is lung cancer and in women is breast cancer. These tumors are also the first causes of mortality for cancer, but in men older than 65 years old the tumor more frequent is prostate cancer and in women the breast cancer.

Cancer mortality in the Health Region of Girona is placed in an intermediate-low level compared with the rest of European registries.

A044

TITTLE: ANTIOXIDANT MICRONUTRIENTS AND BREAST CANCER: A STUDY OF THE PLASMA CONCENTRATION-TIME CURVE

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INTRODUCTION: Experimental studies show the defensive role of β-carotene, vitamin E and vitamin C from oxidative damage caused by free radicals with mutagenic properties. **SUBJECTS AND METHODS:** Transversal epidemiological study that includes an intervention (administration of a single oral dose of 45 mg of β-carotene, 225 mg of tocopheryl acetate and 450 mg of ascorbic acid) to compare the plasma concentration-time curve in two groups: breast cancer patients whit local o regional extension of the neoplasia (cases) and patients never been diagnosed of cancer (controls). Plasma levels of β-carotene, α-tocopherol and ascorbic acid were determined by a high performance liquid chromatografy (HPLC) method. Ascorbic acid was measured using a fluorometric technique. Samples were collected inmediatly before capsules ingestion and 2, 6, 8, 24 and 48 hours after dosing. The statistic analysis of data is based on repeated measures analysis of covariance. **RESULTS:** Plasmatic levels curve for β-carotene and for vitamin C shows differences ($p=0.036$ and $p=0.007$ respectively) between cases and controls, being the mean levels of cases lower than controls. Plasmatic response for α-tocopherol is analogous in both groups. **CONCLUSION:** There are differences in plasmatic levels curve for β-carotene and for vitamin C. It is also observed for these two vitamins a probably greater absortive efficiency. It is considered important to study the metabolic rutes of antioxidants micronutrients in organism's defense antioxidant system.

A052

SPANISH MULTICENTRIC STUDY ON BLADDER CANCER: GENETIC AND ENVIRONMENTAL FACTORS (EPICURO STUDY)

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Introduction: Bladder cancer is one of the most frequent cancers in Spain among men. Tobacco and occupational exposures are the main risk factors. The role of other exposures such as chlorinated water consumption, passive smoking, air pollution and diet is unclear. Interaction of genetic polymorphisms (e.g. NAT1, NAT2, GSTM1) with environmental factors has been scarcely examined. The importance of p53 as an independent prognostic factors has been suggested but evidence is still inconclusive. There is little information on the diagnostic or prognostic importance of other molecular alterations (p21, RB) particularly for superficial tumors. We present the first Spanish multicentric study which evaluates environmental exposures, genetic factors, clinico-pathologic and molecular characteristics of the tumor using valid and state-of-the-art epidemiological and laboratory methods.

Subjects and Methods. Multicentric hospital- based case-control and survival study. Approximately 1500 incident bladder cancer cases diagnosed between 1998-2000 in 20 hospitals of Alicante, Asturias, Cataluña and Tenerife and a similar number of controls are included in the study. Patients are interviewed for approximately 90 minutes using a computerized questionnaire with detailed questions on occupational and residential history, smoking habits, coffee consumption, medical and family history, diet, quality of life and disease symptoms. Information on urinary pH are obtained from all subjects as well as paraffin blocks from cases. Blood samples (40 ml) are shipped daily and processed at a central laboratory. Genetic polymorphisms, DNA repair assays and tumor markers are evaluated. Treatment is recorded and cases are followed for recurrence, progression and mortality.

Results and conclusions. This is the first multicentric study on bladder cancer carried out in Spain using state-of-the-art methodology in both the epidemiological and the molecular biology part. Due to its large size, it will be possible to evaluate gene-environment interactions. Participation rate is between 90-95% for questionnaire and blood samples. Results of this study will bring innovative information on the etiology, diagnosis, natural history and prognosis of one of the most frequent cancers in Spain.

A082

EPIDEMIOLOGICAL STUDY ON P53 AND K-RAS MUTATIONS IN DIVERSE HUMAN CANCERS IN THE BALEARIC ISLANDS

INTRODUCTION

The cancer mortality rate in the Balearic Islands is one of the highest in Spain. Nevertheless, at a genetic level, there is no study of molecular alterations in this pathology in the Balearic population. Mutations in suppressor genes such as the p53, and oncogenes such as the K-ras, play an important role in the onset and development of human cancers, so their study would be useful from both epidemiological and clinical points of view.

MATERIAL AND METHODS

The presence of mutations at exons 5-9 of the p53 gene and at codon 12 of the K-ras oncogene was studied in 72 samples of tumoral tissue of diverse origin (38.9% colon, 29.2% bladder, 13.9% breast, 18.0% other origins) proceeding from patients resident in the Balearic Islands. The PCR-SSCP method was used to screen p53 for mutations. Nested PCR-HphI digestion-gel was the strategy used to detect K-ras mutations.

RESULTS

11 samples (15.3%) were identified with mutations in 1 or 2 of the p53 exons. No case was observed of simultaneous mutation in 3 or more exons. Exon 6 showed a higher percentage of alteration, 42.9%, whereas only 7.1% of the mutations were observed on exons 7 or 8 . Broken down by pathologies, molecular alteration was seen in colon and bladder tumours of 17.9% and 9.5% respectively. K-ras codon 12 mutation was detected in 10 samples (13.9%), half of which were homozygous. Mutation was more frequent in colon tumours, 9 out of 28 samples (32.1%). Mutations were present simultaneously in p53 and K-ras in 5.6% of all cases, 75% of which were adenocarcinomas of the colon.

CONCLUSIONS

The study highlights the low frequency of mutations in the p53 gene in contrast with the results of other studies. At the same time, the low frequency of simultaneous mutations in p53 and K-ras would appear to suggest that these changes occur independently in the majority of tumorigenesis.

A092

SIMULTANEOUS EXPOSURE TO TOBACCO AND ALCOHOL CONSUMPTION AND ESOPHAGEAL CANCER RISK

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Background: Because many individuals are simultaneously exposed to cigarette and alcohol consumption, estimation of the joint effects of these habits' characteristics on esophageal cancer risk is important for health promotion and disease prevention strategies. **Methods**: Data from a series of 5 case-control studies of squamous-cell carcinoma of the esophagus, conducted by the IARC in high risk areas in South America, were combined and analyzed using multivariate logistic regression procedures. A total of 830 cases and 1779 control subjects were included. **Results and conclusions**: A history of simultaneous exposure to cigarette smoking and alcohol intake had a strong, dose-response, multiplicative effect on esophageal cancer risk. Among women, a synergistic interaction was found between smoking and alcohol. Among men, the synergistic interaction was only detected among moderately exposed subjects. Alcohol and tobacco alone were strongly related to the risk of esophageal cancer, even in the absence of exposure to the other risk factor. Moderate cigarette smoking without drinking and moderate alcohol drinking without smoking had little or no effect on esophageal cancer risk. However, the simultaneous exposure to the same moderate amounts increased the risk 13- to 19-fold in men and women, respectively. Combined exposure tc heavy alcohol drinking and black tobacco smoking was associated with a 107-fold increased risk, identifying thus the subgroup with the highest risk for esophageal cancer. Highest risk reduction was observed when both habits were stopped. Quitting cigarette smoking was associated with a subsequent risk reduction for both current and ex-alcohol drinkers. The risk reduction effects associated with alcohol consumption cessation were modest and long term unless there was a few years of concomitant cigarette smoking cessation. **Implications**: Priority should be given to smoking prevention and cessation programs.

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9. Genomic Instability in Cancer

K

Overall deregulation in gene expression as a novel indicator of tumor aggressiveness in colorectal cancer

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Malignant transformation of the cell is accompanied and characterized by disruption of genetic material and aberrant expression of multiple genes. Systematic analysis of differential gene expression in human tumor samples may provide with an estimate of the degree of genetic and epigenetic deregulation in neoplastic cells. We have assessed, by means of a RNA differential display technique, the overall gene expression deregulation in a prospectively collected series of 68 human colorectal carcinomas. An index of differential expression has been calculated for each case. A similar proportion of the displayed sequences (23%) was under- and over-represented in the tumor respect the normal tissue. An increased variation in the expression profile was observed in advanced Dukes' stages ($P<0.02$) and correlated with lymph node invasion ($P<0.05$). Furthermore, a diminished overall survival was associated to increased rates of deregulation (Log-rank, $P<0.02$) and specially down-regulation ($P<0.001$). When Cox multivariate analysis was performed in front of Dukes' stage, both indexes of differential expression were independent indicators of a worse outcome ($P=0.05$ and $P<0.01$ respectively). We conclude that estimation of the fraction of differentially displayed tags by RNA fingerprinting may have relevant applications in the prognostic assessment of colorectal cancer.

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L

TRANSCRIPTION-ASSOCIATED GENETIC INSTABILITY IN THE EUKARYOTE *S. cerevisiae*

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One common characteristic of many tumor cells is their high levels of genetic instability. This is observed as increased mutation rates, structural alterations of microsatellite DNA, genomic rearrangements (translocations, deletions or inversions) and changes in chromosome number (aneuploidy or poliploidy). Important clues to understand the molecular basis of genetic instability in humans have been provided by studies in simple eukaryotic organisms such as the yeast *S. cerevisiae*. This is the case of microsatellite instability associated to some HNPCC tumors. The identification and genetic analysis of yeast genes involved in DNA mismatch repair has been essential to establish an association between such tumors and defective mismatch repair.

Our interest is to understand genetic instability associated to long dispersed DNA repeats in eukaryotes. These repeats may be a source of chromosomal rearrangements such as large deletions, inversions and translocations. We have devised genetic systems to identify genes and molecular mechanisms involved in the instability of large DNA repeats. Our study clearly shows that the impairment of RNA pol II-dependent transcriptional elongation may be a new source of genetic instability, as measured by DNA repeat recombination and plasmid instability (1,2). We have identified two eukaryotic genes, *HPR1* and *THO2*, whose null mutations confer a strong increase of recombination between DNA repeats. In contrast to known mutations leading to increased genetic instability, these new genes seem not to be affected in either DNA replication or repair. Instead, the hyper-recombination phenotype of the mutants is absolutely dependent on transcription. The inability of the mutant cells to elongate transcription is linked to the plasmid instability phenotype and to the induction of recombination at the region where elongation is impaired (2,3).

One of the genes identified, *THO2*, has homologs in *S. pombe*, *C. elegans*, mice and humans. Therefore, it is likely that transcription impairments could be a general source of genetic rearrangements in eukaryotes.

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A016

MUTATIONS AT K-ras AND p53 AND MICROSATELLITE INSTABILITY, ALTERNATIVE GENETIC PATHWAYS IN COLORECTAL CANCER

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Introduction. Mutations in K-ras oncogene and p53 tumor supressor gene as well as microsatellite instability (MIN) are frequent genetic alterations in colorectal cancer and represent 3 different mechanisms in carcinogenic process.
Aim. To analyze the relationship between these genetic alterations and different clinical and pathological parameters.
Methods. Two hundred and ten patients who have undergone surgical resection were prospectively included in this study. Mutations in K-ras, codons 12 and 13, and p53, exons 5 to 9, were detected by SSCP and cyclic sequencing. Microsatellite instability was analyzed by PCR amplification of 5 different loci, 4 (CA)n and 1 An, and electrophoresis in sequencing gels. Also we have analyzed the microsatellite sequence (G)8, present in the coding sequence of BAX, an apoptotic gene.
Results. 43% of the tumors (89/205) displayed K-ras mutations, 50% (79/157) had mutations at p53 and 9% (18/206) were MIN+, with two or more unstable microsatellites. Among the MIN+ tumors 55% (10/18) had mutation in BAX but none of the 60 MIN- tumors analyzed had BAX mutated. None of the tumors had the 3 alterations simultaneously. Tumors with K-ras mutated were frequently MIN-, had less mutations at p53, usually were Dukes A o B and were more frequent in individuals under 73 years. Tumors with mutations in p53 were higly invasive (most of them were pT3), also displayed a low percentage of mutations in K-ras and almost all were MIN-. MIN+ tumors were right sided, poorly differentiated and early onset (under 50 years), they rarely had mutations in K-ras or p53 and had a high percent of mutations in BAX.
Conclusions. The 3 genetic alterations analyzed in this study represent alternative pathways in colorectal cancer and tumors harvoring each of them have different clinical and pathological characteristics.
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A061

INACTIVATION OF DNA REPAIR GENES BY PROMOTER HYPERMETHYLATION IN HUMAN CANCER.

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The main function of DNA methylation is the organization of the chromatin in regions with and without transcriptional activity. In cancer, gene silencing induced by hypermethylation affecting the CpG islands located in the promoters is observed. The epigenetic event causes abrogation of gene function like a classical genetic mutation. In contrast, we can reverse in vitro the lesion by the use of demethylating drugs. The target genes are usually key elements in cancer, including tumor and metastasis suppressor genes (p16, p15, Rb, VHL, E-cadherin). Our recent data provide three new targets:

-hMLH1: gene involved in the DNA mismatch repair pathway. hMLH1 germ line mutations (in addition to hMSH2) cause the Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC). The tumors (colorectal, gastric and endometrial) observed in these patients display the microsatellite instability phenotype (MSI). MSI is also observed in the corresponding sporadic tumors, but somatic mutations in hMLH1 or hMSH2 are found only in less than 10%. We strongly suggest that the mechanism accounting for the MSI in the vast majority of sporadic tumors is hypermethylation-associated inactivation of hMLH1. Thus, hMLH1 promoter hypermethylation observed in approximately 20% of colorectal, gastric and endometrial tumors, leads to MSI and to genetic defects in targets genes like BAX, TGFβ1RII or IGFIIR.
-MGMT: O6-Methylguanine DNA Methyltransferase. MGMT is a DNA repair gene involved in removing alkyl groups in the base Guanine. This lesion is promutagenic and should be corrected to avoid mutations in oncogenes and tumor suppressor genes. It had been reported loss of MGMT activity in cancer cells, but now our results demonstrate that MGMT is inactivated by promoter hypermethylation in across several tumor types. Brain tumors and colorectal carcinomas are the most common targets.
-GSTP1: Glutathione-S-transferase P1. The GSTP1 gene is involved in the detoxification of several carcinogens. GSTP1 binds electrophilic compounds to the glutathione allowing their easy excretion. Overexpressed in several tumor types, it is loss in others. GSTP1 inactivation may lead to DNA alterations induced by hormone metabolites. We report that GSTP1 is inactivated by promoter hypermethylation in prostate, breast and renal carcinoma, where carcinogenic products derived from estrogen and testosterone would cause DNA adducts.

A076

TGF-ß RECEPTOR (RII) MUTATIONS AND MICROSATELLITE INSTABILITY (MI) IN SPORADIC COLORECTAL CANCERS.

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INTRODUCTION: TGF-β is a multifunctional polypeptide that regulates different cellular processes such as cell proliferation, cell migration, differentiation and extracellular matrix deposition. It exerts its effects by binding to specific cellular receptors. Three major types of receptors have been identified: type I (RI), type II (RII) and type III (RIII).
It has been demonstrated that RII gene contains a (A_{10}) repeat where mutations are detected and that RII receptor was frequently inactivated in human colon cancer cell line that exihibited microsatellite instability but not in colon cancer cells that did not display microsatellite instability.
AIMS: We studied sporadic colorectal cancers to analyze RII receptor of TGF-β and its potential correlation to microsatellite instability.
MATERIAL AND METHODS: Tumor biopsies from 22 patients diagnosed of colon carcinoma were collected and genomic DNA extracted by conventional method. PCR of poly(A) tract of RII gene was performed and amplified products sequenced on an automated DNA sequencer ABI-377 (Perkin Elmer, Foster City, USA). Sequences were evaluated by using BLAST software. MSI was analyzed by using the following loci: D1S2883, D2S123, D3S1611, D5S346, D7S501, D8S254, TP53-Di, TP53-Penta, NM23 and D18S35. Fluorescent multiplex PCR was performed to asses microsatellite instability in these loci.
RESULTS: Mutations of TGF-β RII gene have been detected in 5/22 (22,7%) tumor samples analyzed. We identified 3/5 (60%) insertions and 2/5 (40%) deletions. In positive patients cancer localization was at rectum in 3/5 (60%) and at sigma in 2/5 (40%). Tumors were classified according to Duke's system as stage A in 1/5 (20%) and stage C in 4/5 (80%). Microsatellite instability (MSI) was detected in 4/22 (18,18%) biopsies analyzed. Between all patients studied 3/4 (75%) biopsies presented MSI at two loci and 1/4 (25%) at three loci. MSI was detected in 3/5(60%) biopsies with TGF-β mutations and in 1/17 (5,8%) biopsies without TGF-β alteration and it was not detectable in 16/17 (94,2%) biopsies without TGF-β mutations.
CONCLUSIONS: Our findings reveal that the presence of RII mutations is clearly related with a high incidence of microsatellite instability determining a potential role of RII in the integrity of genome. On the other hand these data suggest that microsatellite instability is more frequent in rectum and sigma (distal or left-side colon) in advanced stages. It could be a late event in the colorectal carcinogenesis.

A031

DETERMINATION OF TUMORAL HETEROGENEITY IN HUMAN COLON ADENOCARCINOMA

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Introduction: There are more and more evidences that suggest that, in the moment of the diagnosis, most of cancers present a diverse biologic potential because of the tumoral heterogeneity (tumour subpopulations that present differences in growth rates, DNA content, antigenic expression...); which have deep clinical implications. It is believed that tumour heterogeneity is partially responsible for treatment failure and cancer recurrence. Tumoral heterogeneity in a biopsy of colon adenocarcinoma and in metastases from larynx carcinoma, are presented in this study, using flow cytometry.
Material and method: *Cell culture*: cells of both biopsies were grown in DME/F12-HAM medium (Ca^{++} and Mg^{++} free, with L-Gln and hepes), supplemented with sodium bicarbonate 7,5 % (28 ml/L), 10 % fetal bovine serum and 1 % antibiotic-antimycotic solution 100X (PSF, Gibco); at 37°C in a 5 % CO2/air atmosphere. *Clones isolation*: Cells were seeded in an increasing dilutions and posterior colonies isolation. The most different clones were chosen. *Flow cytometry*: DNA index was measured by flow cytometry (FACScan Becton Dickinson), using propidium iodide (125 µg/ml).
Results: Several clones were obtained from both biopsies according to the biggest difference in DNA content (HCA-2 and HCA-3 in colon adenocarcinoma; MCCEL-1 and MCCEL-2 in the metastases). Both clones from colon adenocarcinoma were aneuploid, with a DNA index of 1,40 and 1,24; regarding to the diploid control used. The metastases clones were hiperdiploid, with a DNA index of 2,47 and 2,43.
Conclusions: These results demonstrate the evidence of tumour population heterogeneity, and this heterogeneity is more significant in the metastases.

A045

EFFECT OF RAS AND P53 MUTATIONS ON THE GENOMIC INSTABILITY OF COLORECTAL CANCER.

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Background: Genomic instability is a fundamental characteristic of tumor cells related to tumor progression. In colorectal cancer two types of genomic instability have been described: microsatellite and chromosomal instability. The nature and causes of chromosomal instability has not been yet defined. **Aim:** To assess the influence of *ras* and *p53* mutations on genomic instability. **Material and methods**: Genomic instability of 119 colorectal tumors was assessed by two techniques: quantitation of allelic gains and losses, determined by comparison of tumor and normal AP-PCR (Arbitrarily Primed- PCR) DNA fingerprints, and aneuploidy (DNA content) analyzed by flow cytometry. Allelic gains and losses quantitated for each case were referred to the total number of visualized bands and this value was called "genomic damage fraction"(GDF). DNA index (expression of aneuploidy) and GDF were correlated with alterations in *ras* and *p53* genes. **Results:** GDF and DNA indexes reflect distinct types of genomic instability (lineal regression analysis p=NS). Aneuploid tumors (DNA content ≥ 1.2) showed higher GDF [aneuploid tumors GDF:0.187 vs diploid tumors GDF:0.150; p=0.02]. p53 mutations correlate with high GDF (p53(+):0.197 vs p53(-) :0.156; p=0.007). Aneuploidy was more frequently observed in p53 positive tumors (40/55 p53(+) vs 31/59 p53(-); p=0.01). While ras mutations did not influence GDF values they were more often detected in aneuploid tumors [29/40 ras(+) vs 30/58 ras(-); p=0.03]. ras(+)p53(+) tumors showed higher GDF values (GDF:0.226, p=0.004) and most of them were aneuploid (15 of 18; 83% p=0.01). **Conclusion:** Aneuploidy (DNA content) and allelic imbalances (measured by AP-PCR) are product of at least two independent types of genomic instability. *p53* could play a role in the control of both types of instability, while *ras* mutations would lead mainly to an aneuploidization of the genome.

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A055

PROGNOSTIC SIGNIFICANCE OF LOSS OF HETEROZYGOSITY AND MICROSATELLITE INSTABILITY IN COLORECTAL CANCER

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INTRODUCTION: Loss of Heterozygosity (LOH) and Microsatellite Instability (MSI), also known as Replication Errors Phenotype (RER), have been identified in a wide variety of human tumors, both familial and sporadic. In this study, we attempted to correlate these genomic alterations with other biological parameters to assess its significance in sporadic colorectal cancer prognosis.

MATERIAL AND METHODS: Eighty-six tumor and paired normal mucosa samples were included in the study. A PCR-based technique was performed to analyze twelve (CA)n dinucleotide repeats located near or within regions containing mismatch repair genes, or thought to contain some tumor-suppressor genes. PCR products were electrophoresed on urea/polyacrylamide gels. MSI appeared as a change in the length of microsatellite sequences (in tumor DNA compared with constitutional DNA), and LOH was observed when the complete loss of one or both alleles of the repeated locus appeared.

RESULTS: Overall, LOH frequency was significantly higher in RER^+ tumors as compared to those RER^-. Concerning prognostic implications, patients with RER^+ tumors were found to have an improved prognosis, meanwhile survival of patients whose tumors were LOH^+ was significantly shorter compared to those without.

CONCLUSION: Tumors displaying RER^+ and LOH^+ phenotype, as established by microsatellite analysis, show a differential prognosis. These data may provide an useful tool in order to set up therapeutic protocols in patiens affected by sporadic colorectal cancer.

A072

***hMSH2 variant sequence* associates with an increased colorectal cancer risk in the Spanish population.**

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Introduction. The mismatch repair gene *hMSH2* is mutated in a high proportion of HNPCC patients and in 3% of sporadic colorectal (CRC) tumours. Recently, the presence of a polymorphic T→C transition in the splicing donor of exon 13 of *hMSH2* has been associated with an increased susceptibility to develop CRC. While these observations have been made in sporadic tumours, the frequency of the variant sequence in familial CRC remains unknown. Finally, this polymorphism could associate with a worse prognosis. **Aims:** 1) To study whether the variant sequences increases CRC susceptibility; 2) whether it is a prognostic maker in sporadic CCR; 3) to analyze if it affects *hMSH2* exon 13 splicing and correlates with microsatellite mutator phenotype (MMP). **Patients and Methods:** The presence of the germline substitution was analysed by means of SSCP and sequencing pf a) 149 consecutive sporadic CCR patients; b) 77 members of HNPCC or HNPCC-like families; and c) 75 healthy donors. Exon 13 splicing was analysed by means of RT-PCR. **Results:** Variant sequence is more often detected in CRC patients when compared with healthy donors [23% (34/149) vs 11% (8/75); (P=0.02)]. The polymorphism was more prevalent in the more advanced stages of the disease [A/B=16% vs C/D=31%; P=0.03]. No correlation was observed with the MMP phenotype [MMP^+=14% (3/21) vs MMP^-=23%(28/121); P=NS]. No survival differences were observed according the presence of the polymorphism (P=NS). In HNPCC members polymorphism prevalence did not vary. The prevalence of the variant sequence did not vary between sporadic and familial CRC. The germline substitution did not affect the splicing process. **Conclusion:** The polymorphism in hMSH2 gene is a genetic risk factor for CRC in the Spanish population. No differences were observed between sporadic and familial CRC and it is not a poor prognosis marker.

A102

CLONAL DIVERGENCE IN TUMOR CELLS: A SYMPTOM OF GENOMIC INSTABILITY

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Genomic instability is characteristic of tumor cells and is responsible for the development of tumor cell heterogeneity. The emergent cell subclones in the tumor are subsequently selected according to their biological behaviour. **Aim**: To identify the mechanisms underlying genomic instability in established human colorectal cancer cell lines. **Matherial and methods**: We have analyzed the genetic clonal divergence in three cell lines: SW480 (mutated p53, hypotriploid), LoVo (wild-type p53, hyperdiploid, microsatellite instability) and HCT116 (wild-type p53, diploid, microsatellite instability), which are representative of different pathways of tumor progression in colorectal cancer. We have obtained a minimum of six clones of each cell line and grown them for approximately 50 cell divisions. Genomic divergence has been investigated by Arbitrarily Primed PCR (alellic imbalances), flow cytometry (ploidy analysis), Comparative Genomic Hybridization (chromosome alterations analysis) and classic karyotyping analyses. **Results**: A higher degree of genetic divergence was observed by Arbitrarily Primed PCR in the clones of the LoVo and SW480 cell lines in comparison with the HCT116. At the chromosome level, the clones of the LoVo cells maintained a stable karyotype, the HCT116 and most of its clones displayed some heterogeneity, and the SW480 showed a high level of chromosomal instability in all clones, with every one having different chromosome markers and reorganizations. At the ploidy level none of the lines showed heterogeneity. **Conclusions:** Our results indicate that different mechanisms of genomic instability are involved in the disruption of the tumor cell genome in these three cell lines. Characterization of the different mutator phenotypes present in neoplasms may contribute to understand the molecular progression pathways responsible for the malignant transformation of the cell.

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10. Molecular and Biological Markers in Cancer

f

BIOMARKERS IN NUTRITIONAL EPIDEMIOLOGY

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We are in the era of molecular and biochemical research and cancer epidemiology is now experiencing very important changes in the methods of assessment of exposure and disease. Analysis of biological materials is becoming widely used in the study of the relationships between nutrition and cancer. In nutritional epidemiology, biomarkers can be used as biochemical markers of dietary intake levels, as indicators of individual susceptibility to cancer risk, and as a measure of early (pre-cancer) states.

There are two types of markers of dietary intake: markers of absolute quantitative intake based on a time-related balance between intake and output, such as urinary nitrogen for proteins, and markers of concentration of a specific substance in biological material (plasma, urine, tissues, etc.) such as concentrations of fatty acids in erythrocyte membranes. These markers do not have time dimension and can provide only a correlate of habitual dietary intake levels. The main advantages of biomarkers of diet intake are that they may improve the measurement of exposure , they are objective measurements (not affected by lack of memory, incapacity of the subject to describe accurately several types of foods consumed or subject's motivation) , and they can provide estimate of intake for some compounds despite a lack of data in food composition tables.

For these reasons biomarkers are widely used as a gold standard in valididation and calibration studies and are seen as new methods that may overcome limitations and measurement errors of traditional methods like dietary questionnaires and food records. However there are several other sources of variation in the measured levels of markers apart from dietary intake, that should be taken into account like absortion, metabolism, distribution in the body, endogenous formation, and excretion. These sources are also influenced by the simultaneous exposure to other environmental factors, genetic polymorphisms, etc. This means that biomarkers are also affected by problems of reproducibility, validity, and reliability of measures . It has been stated that caution is needed against overenthusiasm in favour of biomarkers as the magical solution to the problems of dietary intake measurement.

g

TRANSLATIONAL RESEARCH IN LUNG CANCER

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Oncologists are often puzzled by the accumulation of genetic abnormalities in dominant and recessive oncogenes occurring in tumors. They need to be aware of how these abnormalities can be useful in early diagnosis, in detection of sub-clinical recurrence, in selection of treatment, and ultimately in prognosis. We have developed a comprehensive molecular lung cancer program to examine several steps of lung carcinogenesis. LOH in three main *loci* (3p14, 9p21 and 17p13) has been observed in normal lung tissue of smokers, while K-ras mutations represent a relatively late phenomenon observed in CIS. Recently, persistent 9p21 LOH has been observed in pre-invasive head and neck lesions in spite of complete histological remission. The assessment of HRAS minisatellite variations in a large group of patients has shown that the presence of these rare alleles has been correlated with poor prognosis in non-Hodgkin's lymphoma (JNCI 1998;90:1095). Microsatellite instability in resectable NSCLC has been found to be a predictor of poor survival. Further research, using tetranucleotide markers, is still needed in this area. We also detected the presence of LOH in serum tumor DNA in 28% of patients with resected tumors. In addition, hypermethylation of p16 and DAPK was observed in the serum of the same patients and MLH1 in endometrial tumors. A novel anti-apoptosis mechanism, the re-expression of *survivin* messenger RNA, was detected in 85% of tumor samples analyzed; *survivin* transcript is a useful diagnostic marker and a source of prognostic information. In much the same fashion, the *Sonic hedgehog* gene is involved in the morphogenesis of the embryonic lung. We also identified β-tubulin missense mutations in 33% of NSCLC patients. None of the patients with mutations had an objective response to paclitaxel treatment, leading us to conclude that tubulin mutations, clustered in GTP binding sites of exon 4, may be a novel mechanism of resistance to anti-tubulin drugs in NSCLC. We are planning to validate these findings in the setting of a multi-center clinical trial in stage I NSCLC; 600 patients will be randomized to receive either neoadjuvant chemotherapy with paclitaxel/carboplatin or surgery alone or surgery plus adjuvant chemotherapy with the same drugs. At the same time, we will be looking at several genetic abnormalities in the primary tumors as well as in the serum (LOH at 3p14, 9p21, 17p13; methylation of p16, DAPK, MLH1; K-ras mutations; HRAS rare alleles; and β-tubulin mutations). In summary, a comprehensive molecular lung cancer program within the framework of the Spanish Lung Cancer Group should help bridge the existing gap between genetic evidence and clinical relevance.

h

DIAGNOSTIC AND PROGNOSTIC MARKERS IN BLADDER CANCER

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The most important identified factors associated with the survival of bladder cancer patients are the delay in diagnosis, clinicopathological factors, treatment, general factors such as geographical region and social class and, probably, molecular alterations of the tumour. A long list of diagnostic and prognostic markers have been proposed but very few seem to have had any clinical application. The central items in evaluating diagnostic and prognostic markers in bladder cancer are their usefulness in reducing the number of cystoscopies routinely done in following up a patient and the identification of subgroups of high risk patients.

p53 appears as the only molecular marker having a prognostic value independent of grade and stage. More than 100 published studies have evaluated the prognostic role of p53 in bladder cancer. Most are relatively small studies using archival material, not always using standard lab techniques, and many provide a poor description of the selected patients. The most cited article on this issue examined patients doing cystectomy.

Most diagnostic and prognostic markers in bladder cancer do not seem to have higher positive or negative predictive values than the widely used cystopathological criteria. Although tens of studies have been published referring to the predictive value of p53, their still exist various controversial issues. These can be explained by the wide variation in study design and laboratory techniques applied, the frequently small size of the studies, and the opportunistic analysis of subgroups of patients. In the future, the predicted high increase in information on molecular markers will require the evaluation of well characterised large series of patients and the combination of advanced laboratory techniques with adequate epidemiological designs.

p

Uses of Molecular Markers: Biomarkers in Cancer Epidemiology, lessons from rodents and humans.

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If cancer can not be prevented by removing or neutralizing the etiologic agent that causes the malignant tumor the alternative preventative strategy of interfering with the process of carcinogenesis merits careful consideration. In order to exploit this avenue we need to know as much as possible about how cancers come to be and understand the forces driving the conversion of normal tissues in to malignant tumors. I will summarize recent molecular data acquired from the analysis of human tissues and from the study of preclinical models suggesting that a detailed understanding and interpretation of molecular markers requires the consideration of selection during carcinogenesis. The identification of the selective forces responsible for the micro-evolution of pre-neoplastic lesions and the development of technologies that will allow the measure of mutational load in somatic cells are pre-requisites for the accurate assessment of acquired risk. Once this is accomplished the monitoring of preventive interventions, such as chemoprevention, will become feasible.
In the last five years we have begun to identify possible selective agents determining the genotype of tumors. Molecular epidemiology studies in the human suggest that specific nutrients can modify the risk for colonic carcinoma of a specific genotype. The current hypothesis is that the nutrients differentially promote or inhibit the expansion of clones depending on their acquired genotype. Experiments in the rat demonstrate that nutrients are powerful selective forces that can favor the expansion of specific micro-clones, or conversely can inhibit the expansion of clones driven by specific mutated alleles. Specifically diets high in calcium content interdict the expansion of clones initiated by a mutation in codon 34 of the beta catenin gene, but still allow the expansion of cells harboring other abnormal beta catenin alleles.

A054

EVALUATION OF TELOMERASE ACTIVITY IN HUMAN TUMORS. CLINICAL CORRELATIONS IN NON-SMALL CELL LUNG CANCER

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INTRODUCTION: Telomerase activation is required for cellular immortalization and is found in most malignant tumors. In this study we investigated Telomerase activity, as well as hTR and hTRT expression, in human tumors, with the aim of evaluating possible clinical implications of these genetic markers.
MATERIAL AND METHODS: We analyzed 65 tumors, and paired normal samples, from different origins. Telomerase activity was evaluated by the TRAP (Telomeric Repeat Amplification Protocol) method; hTR expression by Northern-blot, and hTRT by RT-PCR. Moreover, statistical correlations were established with clinico-pathological features, in the group of non-small cell lung carcinomas (NSCLC) assayed.
RESULTS: Overall, 80% of the tumor samples showed Telomerase activity. In most of the cases, this parameter was associated with hTR and hTRT expression. In NSCLC, we have found significant correlations between Telomerase activity and the age of patients. The higher levels of Telomerase were significantly associated with the group of adenocarcinomas. Finally, a trend toward poor prognosis has been detected in the Telomerase-positive NSCLC patients.
CONCLUSION: These findings suggest that Telomerase activity may be useful as a diagnostic marker to detect the existence of immortal cells in clinical materials.

A062

DETECTION OF K-*ras* MUTATIONS IN FAECAL SAMPLES. A PROSPECTIVE STUDY USING TWO DIFFERENT TECHNIQUES.

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K-*ras* mutations are present in a significant proportion of colon adenomas and carcinomas. In this study we attempt to evaluate the diagnostic utility of K-*ras* mutations detection in samples, obtained before and/or during colonoscopy, from patients with clinical suspicion of colorectal lesions. We prospectively evaluated 75 nonselected patients undergoing diagnostic colonoscopy. Final diagnosis (by endoscopy and/or histologically) were: 18 colorectal carcinomas, 28 adenomas, 12 inflammatory bowel disease, 8 diverticulosis, 5 normal mucosa and 4 other benign diseases. We analyzed a total of 61 samples obtained before colonoscopy (pre), 6 during colonoscopy (col) and 8 with both samples (pre + col) . In 35 cases, mutations were also determined in biopsy samples (12 tumors and 23 polyps). K-*ras* mutations were detected by means of RFLP/PCR methods. We performed two *Bst*NI techniques: standard *Bst*NI (s) with a detection limit of 1 mutant in 10^2 normal cells and enriched *Bst*NI (e) with a detection limit of 10^{-3} . Amplification was possible in 65/75 faecal samples using the standard method, in 60/75 with the enriched method and in all 35 tissue biopsies by both methods. No differences were observed between (pre) and (col) samples. The incidence of K-*ras* mutations in the carcinoma group were 18% using standard *Bst*NI (s) method and 40% by enriched *Bst*NI (e) method. In the adenoma group, K-*ras* mutations were detected in 20% of the samples using (s) technique and in 22% with (e) method. No mutations were detected in the 29 samples from benign group using both methods. When tissue samples were studied, K-*ras* mutations were detected in 50% of tumor biopsies using both methods and in 48% of polyps biopsies. We concluded that molecular analysis in colonic epithelial cells exfoliated in faecal material may offer an early non invasive diagnosis of colorectal neoplasia.

A103

ANALYSIS OF β-CATENIN EXPRESSION IN SMALL CELL LUNG CARCINOMAS

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Introduction: β-catenin is a multifunctional protein involved in essential functions in normal cells: cadherin cell- cell adhesion system, in Wnt signalling system by its bind to Tcf/Lef transcriptional factors, in the control of cellular motility, and others β-catenin abnormalities are involved in carcinogenesis and tumor progression in many neoplasms.

Methods and Material : 1.-Analysis of β-catenin expression by immunohistochemistry in paraffin-embedded tissue of 50 patients with small cell lung carcinomas (SCLC) 2.- Correlation of the results of the analysis with the clinical and evolutive features

Results: 1.- Expression of β-catenin located in the cytoplasm without nuclear staining was observed in all SCLC 2.-In normal respiratory epithelium β-catenin was expressed in cellular membrane 3.-Comparing β-catenin expression in neoplastic tissue and normal epithelium we observed reduced expression in 21 cases, conserved expression in 15 cases, increased cytoplasmic expression in 14 cases. 4.- No correlation with clinical fetures (age, sex, performance status, metastases or stage) was found. 5.-An important statistical correlation of β-catenin expression and time to relapse and overall survival was observed. If β-catenin was abnormally accumulated in cellular cytoplasms time to relapse was significantly lower (p=0.0253) and overall survival was also lower (p= 0.0437) than if β-catenin expression was not increased.

Conclusions: β-catenin cytoplasmic accumulation could be the consequence of abnormal tyrosin-phosphorilation of the protein that produces a disruption of cell-cell adhesion complex. It also causes a loss of contact inhibition, proliferation and an increased cellular motility. β-catenin expression is a prognostic factor of survival and time to relapse. A systematic analysis of this protein couls be useful in clinical practice to select those patients in which we could apply different therapeutic approach.

A104

EXPRESSION OF ß-CATENIN IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA (ESCC).

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ß-catenin (ß-cat) is a multifunctional protein that acts in the cell adhesion and motility systems and signaling ways. The aim of the study is to determine its expression in ESCC, not reported yet, and to analyse its prognostic role in the evolution and survival.

MATERIALS AND METHODS : there were obtained clinico-pathological parameters of age, performance status, tobacco and alcohol consumption, localization, length, stage, differentiation grade and survival in 39 patients with histological diagnosis of ESCC. Immunocytochemistry (ICC) was used to study expression of ß-catenin in paraffin sections blocks of tumour samples. Results were correlated with clinico-pathological parameters and (ICC) expression of the proteins p53, E-cadherin, bcl-2, c-erbB-2 and Ki-67. Statistical study was performed (X^2, Fisher, Kaplan-Meier and Log-rank).

RESULTS :12/39 (31%) patients had conserved expression of ß-catenin in the cell surface, reduced staining was observed in 21 patients (51%) and nuclear expression in 7 (18%). The study of this 7 patients with nuclear expression has showed: all the cases were localized in upper (3/6) or middle third (4/20) but none in the lower; the 100% had intense expression of bcl-2 staining versus the 0% left; 1/7 patients had expression of p53 (14%) versus the 21/32 (65%) left; 6/7 cases had reduced E-cadherin staining (85%) versus the 23/30 (77%) left; 5/7 patients showed Ki-67% expression >30% (71%) versus the 15/32 (47%) left; finally, 5/7 patients achieved objetive response to chemotherapy (71%) versus the 18/31 (58%) left and median survival ß-catenin nuclear expression patients was 18 months versus 10 months left. No differences were observed in the other studied parameters. Patients with conserved expression of ß-catenin didn't showed differences with regard to reduced expression group.

CONCLUSIONS : It's the first description of nuclear expression of ß-catenin in ESCC. This subgroup of patients with nuclear ß-catenin expression has specific characteristics could determine a significative clinical evolution. Nowdays, we are studying the exon 3 of the human ß-catenin gene (CTNNB1) in this group of patients with nuclear expression.

A113

PROGNOSTIC VALUE OF E-CADHERIN AND BETA-CATENIN LOSS IN THE TRANSITIONAL CELL CARCINOMA (TCC) OF THE URINARY BLADDER.

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One of the manin problems in TCC of the urinary bladder is the absence of a specific tumoral marker associated with the clinical outcome of the patients. Thus, it is necessary to evaluate the primary TCC with different variables for knowing the progression risk.

Overexpression/mutation of p53 and loss of E-cadherin and beta-catenin has been associated with a worse prognosis in many carcinomas.

We studied the immunodetection of p53, E-cadherin and Beta-catenin in a cohort of patients (n= 40) treated and follow-up in the CSUB between 1992 and 1994 (16 superficially and low grade TCC and 24 of high grade of the whom 6 were superficial and 18 infiltrating tumors). TCC tumors were also analysed for p53 mutations using frozen samples by SSCP and direct sequencing analysis of exons 4 througth 9.

Overall, 38% of the TCC were positive for p53. Loss of E-cadherin and Beta-catenin was seen in 33% and 35% of patients, respectively.

The statistical analysis showed that mutation/overexpression and loss of E-cadherin and beta-catenin correlates significantly with the clinicopatological variables such as grade, stage and limph node metastases.

In the survival test (long rank test), loss of E-cadherin and Beta-catenin were associated significantly with survival, confering a higher mortality risk to the patients. Also, it could observe that when E-cadherin or Beta-catenin was detectable by immunohistochemistry, the immunodetection or not of the second of them allows to better adjust the mortality risk.

loss of E-cadherin and beta-catenin expression could be of prognostic value in TCC of the urinary bladder.

A002

SELENIUM TISSUE AND BREAST CANCER

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BACKGROUND. Selenium have been implicated in multiple physiological and patological processes. A large number of animal studies and ecologic studies suggest an inverse association between low dietary selenium intake and risk of various types of cancer. Actually, a hypothesis describing the effects of selenium deficiency on the serum of patients with breast cancer (BC).

OBJECTIVES. In the present study we investigated the differences concentration between the selenium in tumoral and normal tissue.

MATERIALS AND METHODS. This included 80 patients with BC, 40 women with chronic diseases and 40 healthy subjects. Selenium levels were measured on all blood samples. The required tissues were removed rapidly from each tumor and normal tissue. And them were fixation in formol. The selenium concentration in serum or other tissues has been compared between cancer patients and non cancer subjects. Selenium concentration was determined by the graphite furnace atomic absorption spectrometric method after a simple dilution procedure.

RESULTS. The mean global value of Se were lower in the BC patients than the control group (heathly and with chronic diseases women) ($p < 0.001$). Women with cancer in progression showed lower levels. The mean concentration of selenium was 0.2607 mg/kg (CI 0.2180-0.3034) in normal tissue and the tumoral tissue was 1.0188 (CI 0.8732-1.1624). In the tumoral tissue the selenium levels was 3.8 fold highest than normal tissue ($p < 0.001$). The cut-off point of serum selenium was 93.06 gr/dL.

CONCLUSIONS. The most important result from this study is the demostration that the selenium abandom the blood and intake in the tumoral tissue. Our data suggest that low serum selenium is a consequence but cannot be cause of breast cancer.

A004

CANDIDATE MUCIN GENES FOR THE DETECTION OF BLADDER CANCER CELLS IN BIOLOGICAL SAMPLES.

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Introduction. Mucin glycoproteins (MUC1-MUC8) are differentially expressed in normal epithelial tissues. The altered expression of mucin genes in tumors provides the basis for their study as novel diagnostic/prognostic markers. Preliminary data indicate that MUC2-specific antibodies can be used for the early diagnosis of bladder cancer recurrence.
Materials and methods. Immunohistochemical techniques, using apomucin-specific antibodies, and RT-PCR have been used in a pilot study to analyze mucin gene expression in a small panel of superficial and infiltrating bladder cancers obtained through TUR or cystectomy, respectively.
Results. We focus on those mucins which are absent from normal bladder epithelium (MUC2, MUC4, MUC5AC, MUC6) or whose expression in normal and neoplastic bladder tissues has not yet been described (MUC8). Results from immunohistochemical studies in bladder tumors are summarized below:

	MUC2	MUC4	MUC5AC	MUC6	MUC8
Superficial	N.D.	6/10	1/10	3/10	9/10
Infiltrating	6/10	4/10	0/10	0/10	7/10

Conclusions. In addition to MUC2, MUC4 appears a promising candidate for the identification of bladder cancer cells. Further work is necessary to establish if the combined study of these mucins has an additive value in bladder cancer recurrence.

A005

CLINICAL IMPLICATIONS OF TELOMERASE ACTIVITY IN NON-SMALL CELL LUNG CANCER (NSCLC)

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Introduction: Telomerase is a ribonucleoprotein that is necessary for the stabilization of telomere length in the human germline and stem cells and for cell immortality. Telomerase has been detected in tissues from many human cancers, including more than 80% of human NSCLCs, but not in most normal tissues.
Methods: To better understand the usefulness of telomerase as a diagnostic and prognostic factor, we have analyzed in our preliminary study 35 frozen tumor and 39 normal tissues from NSCLC patients using TRAP assay to determine the levels of telomerase activity in these specimens as related to clinicopathological characteristics.
Results: Our preliminary results show that telomerae activity can easily be detected in these clinical specimes. Telomerase activity was positive for 30 of 35 (85.7%) tumors and negative for 5 tumors, while it was detected in 9 of 39 (23.1%) normal tissues. There was no correlation between telomerase activity with any other clinicopathological features, inluding age, sex, tumor size, histology, and stage nor did telomerase activity correlate with smoking habit or prognostic implications.
Conclusions: Reactivations of telomerase is frequent in NSCLC but is not associated with aggressive tumor behavior. Telomerase activity may indicate an acumulation of other genetic alterations wich promote uncontrolled cell division through clonal selections.

A063

PROGNOSTIC VALUE OF QUANTITATIVELY DETERMINED p185$^{HER-2/neu}$ IN NODE POSITIVE BREAST CANCER PATIENTS

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Despite the numerous studies, the prognostic significance of HER-2/neu in breast cancer patients remains somewhat unclear. In lymph node positive patients, most of the studies have shown that p185$^{HER-2/neu}$ (p185) overexpression or HER-2/neu gene amplification is associated with a worse clinical outcome in the univariate survival analysis; however, this association remains in only a few studies in the multivariate analysis. In most of these studies, p185 expression was assessed by immunohistochemistry. In a recent work, in which p185 was determined by a quantitative procedure, low levels of p185 were associated with a worse prognosis. To clarify this situation, we analyzed quantitatively the tissue content of p185 by a commercial ELISA in 217 consecutive stages II and III node positive breast cancer patients. In the same samples we measured estrogen (ER) and progesterone (PR) receptors by radioligand binding assay, and pS2 and cathepsin D (CD) by an immunoradiometric assay. The patients were followed by a median of 50 months (range: 9 to 90 months). p185 was overexpressed in 17,5% of cases and was not associated with any of clinicopathological or biological variables, except with PR and CD. In the univariate survival analysis, p185 status, RE status, tumor size, histological grade, and number of positive lymph nodes were significantly associated with disease-free survival (DFS). In the multivariate analysis, p185 overexpression, ER negativity, size larger than 5 cm, and 10 or more positive nodes predicted a shorter DFS. In conclusion, overexpression of quantitatively estimated p185 is associated with a worse clinical outcome in node positive breast cancer patients.

A068

THE ROLE OF TELOMERE ADDITION IN THE STABILIZATION OF RADIATION INDUCED DNA BREAKS.

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Telomeres are specialized structures located at the end of linear eukaryotic chromosomes. These structures are essential for chromosome stability and thus to maintain chromosomal integrity. Telomerase can synthesize a new telomere to heal the end of a chromosome exposed to agents that produce double-strand breaks, such as ionizing radiations. By capping the breaks on chromosomes, telomerase stabilizes deleted and acentric fragments. To discover whether telomerase activity is due to an increased synthesis of the enzyme or to an activaction of telomerase due to radiation exposure, we have compared telomerase activity in two extracts of mouse cells: mature oocytes and sperm in control and radiation groups. The ovaries of the groups of females were irradiated for each oocyte stage and each group of four males were killed at days 1, 20, 30 and 40 post-radiation, to achieve spermatozoa that had been irradiated at the stages of mature spermatozoa, spermatid, spermatocyte and spermatogonia respectively. Testing the samples, we have measured telomerase activiy by the TRAP assay in the cell extracts. PCR products were loaded in 10% polyacrilamide gels. After electrophoresis, the gel was fixed and exposed to an X-ray film. After obtaining the results, a densitometric analysis was carried out in order to measure relative telomerase activity. Low levels of telomerase activity were detected in both control and irrradiated sperm. On contrast, different levels of telomerase activity were detected in control and irradiated oocytes at stages I and II of the meiosis. More activity was detected in irradiated oocytes than in the control group especially in oocytes 1 irradiated at 3 Gy and in oocytes II irradiated at 3 and 4 Gy. Some basal levels of telomerase activity in control mature sperm have been found by using the "Swim up" technique, that improves the isolation of mature sperm. Overall, we see that the irradiated oocytes and spermatozoa show a higher telomerase activity because the enzyme adds telomere sequences to the free DNA end as a way of capping the broken chromosomes that are a result of the radiation.

A085

DETECTION OF NUCLEAR β-CATENIN IN COLORECTAL TUMORS: FROZEN OR PARAFFIN-EMBEDDED SECTIONS?

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Introduction: β-catenin mediates the interaction of E-cadherin with α-catenin and the actin cytoskeleton. Recent evidences indicate that when APC is inactivated, β-catenin can translocate to the nucleus where it acts as a transcriptional regulator. In the course of a study of adhesion molecule expression in frozen colorectal cancer tissues, we were surprised by the lack of nuclear β-catenin detection.

Material and Methods: Two different antibodies recognizing the COOH-terminus of β-catenin were used to examine its distribution in SW480 colon cancer cells (harbouring mutated APC) and in frozen and paraffin-embedded samples of colon cancer tissues (n=11). The streptavidin-biotin-alkaline phosphatase technique was used.

Results: β-catenin was never detected in the nucleus of normal or tumor cells in frozen sections. By contrast, in 8/11 cases it was detected in the nucleus of tumor, but not of normal cells, in paraffin tissue sections. Similar results were obtained with both antibodies in SW480 cell pellets.

Conclusions: The different immunoreactivity of antibodies recognizing the COOH-terminus of β-catenin with sections of frozen and paraffin-embedded tissues suggests that this domain is cryptic in the former. These findings are relevant because of the increasing interest in the study of β-catenin in tumors, based on its dual role in cell adhesion and transcriptional regulation.

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A105

EXPRESSION OF P53, C-ERBB-2, BCL-2, KI-67 AND E-CADHERIN IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA.

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The esophageal squamous cell carcinoma (ESCC) (CE) has a big geographic variability and high mortality, there is no effective treatment and its biological behaviour is not stablished. The aim of the study is to determine the expression of oncogenic proteins that could act in ESCC carcinogenesis of our environment such as p53, E-cadherin, c-erbB-2, bcl-2 and Ki-67 and to analyse its prognostic role in the evolution and survival.

MATERIALS AND METHODS : there were obtained clinico-pathological parameters of age, performance status, tobacco and alcohol consumption, localization, length, stage, differentiation grade and survival in 40 patients with histological diagnosis of ESCC. Immunocytochemistry (ICC) was used to study expression of p53, cadherina-E, bcl-2, c-erbB-2 and Ki-67 in paraffin sections blocks of tumour samples. Results were correlated with clinico-pathological parameters. Statistical study was performed (X^2, Fisher, Kaplan-Meier and Log-rank).

RESULTS : The 63% of the patients present reduced expression of E-cadherin and the 16% have not expression. In some cases positive expression in tumoral intravascular emboli has been observed, whose significance is unknown. The 58% of samples have expression of p53. In the 25% of this cases there is p53 expression in the normal mucosa next to the tumor. Only 5/40 patients express positive staining for c-erbB-2 and in all of this 5 cases was not achieved chemotherapy response. 14/40 patients have expression of bcl-2. The cellular proliferation grade (Ki-67) was low in only 4/40 patients (10%), being elevated (16-30%) in 16/40 and very high (>30%) in 20/40 patients. The expresión of these proteins did not have predictive value statiscally significative with regard to performance status, stage, tumor localization, survival free progression, chemotherapy response, differentiation grade and survival.

CONCLUSIONS : The expression of p53, c-erbB-2, bcl-2, E-cadherin and Ki-67% in the ESCC studied in our environment is similar to that described in other studies, without evidence of having a prognostic value.

A086

BTA-trak EVALUATION AS TUMOR MARKER IN BLADDER CANCER

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INTRODUCTION, PATIENTS AND METHOD

The aim of this work is to validate a new tumor marker of bladder cancer, as a diagnostic test as well as to asses its relationship with STAGE and tumoral GRADE. We have analysed the urine concentrations of BTA-trak (DIA-SORIN®) (Basal membrane protein complex related with human complement factor H, hCFHrp) in 33 patients with bladder cancer (6 women and 27 men) aged between 57 and 85 years (mean:68.7 years) and 36 patients with benign pathologies of the urinary tract (16 women and 20 men) with ages comprised between 30 and 73years (mean: 56 years).

RESULTS

The BTA-trak concentration values ranged between 0.1 and 10000 U/ml (median: 52.9U/ml) in the first group and between 0.1 and 3527U/ml (median: 8.24U/ml) in the last group, with a statistical significant difference between them (p=0.00394).
The bladder pathologies in STAGE I (n=21) had a mean concentration of 1076±2987U/ml (median: 20.9U/ml) and of 799±1286U/ml (median: 489U/ml) in STAGE II (n=11) with a significant statistical difference between both groups (p=0.00416). In relation with tumoral grade we obtained: GRADE I (n=15) 837±2567U/ml (median: 26.2U/ml); GRADE II (n=7) 45.7±91.7U/ml (median: 9.1U/ml) and GRADE III (n=10) 1852±3148U/ml (median: 711U/ml), we found statistical diferences between GRADE I and GRADE III (p<0.05). The cutoff values were of 10U/ml (sensitivity: 72.7%; specificity: 57.1%) and of 50U/ml (sensitivity: 51.5% and specificity: 68.5).

CONCLUSIONS

1- The BTA-trak is a cuantitative analysis and therefore objective that increase the quality of other tests for the detection of bladder cancer.
2- It allows the diagnosis of bladder tumor not only in superficial stages and grades but also in infiltrating tumors.
3- We propose a cutoff value of 10U/ml in the first step and of 50U/ml for clinical alarm.

11. Signal Transduction

LL

Affinity of E-cadherin β–catenin complex is controlled by phosphorylation ofβ-catenin residue Tyr 655

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Although a conclusive relationship has not been established, alteration of cadherin-mediated cell-cell adhesion is frequently associated to phosphorylation of β-catenin. We have examined the role of phosphorylation in the control of β-catenin- E-cadherin binding using in vitro assays with recombinant proteins. β-catenin can be phosphorylated by recombinantp60c-src tyrosine protein kinase with a high stochiometry (2 mols P/mol of protein)This modification has functional relevance since decreases affinity of β-catenin by the cytosolic domain of E-cadherin by a factor of four. Phosphorylation of differentβ-catenindeletion mutants indicates that one of the phosphorylated Tyr is located in the N-terminus and another in the last armadillo repeat of this molecule (Tyr 655). Site-directed mutagenesis of Tyr 655 to Glu reduces significantly the amount of phosphate incorporated in in vitro phosphorylation reactions as well as in vivo, after transfection and incubation with phosphotyrosine phosphatase inhibitors. This mutant also shows a decreased affinity for the E-cadherin cytosolic domain. Analysis of transient transfections indicates that MDCK cells expressing 655Y-Dβ-catenin show an alterated morphology with fewer cell to cell contacts. This results indicate that phosphorylation of Tyr 655 controls the interaction of this protein with E-cadherin.

A056

Activation of the N-terminal kinase of c-jun (JNK) by ligation of the tetraspanin CD53 antigen in lymphoid cells

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The human CD53 antigen is a prototype of the Tetraspanin family of membrane proteins. These proteins have four transmembrane domains and the N and C termini are short and cytosolic. The surface level of these proteins have been correlated with the metastatic potential of tumor cells, possibly because of their effects on cell adhesion and motility. The mechanism by which these molecules contribute to intracellular signaling processes is unknown. Experiments in our laboratory suggest that the signal generated by CD53 ligation affects cell adhesion by a mechanism that requires *de novo* gene expression and translation. In this work we have studied the possible transduction pathway by which this molecule initiates gene expression. For this purpose we have we have studied the effect of CD53 antigen ligation on the activation of the N-terminal kinase of c-jun (JNK). For this purpose we used as substrate a GST-jun fusion protein. We have performed antibody dose and time course experiments to identify the optimal stimulation conditions. Ligation of CD53 antigen with the MEM53 mAb induces a fast phosphorylation of GST-jun which reaches its peak between 3 and 5 min to fall rapidly, this activation is followed by a weaker activation at 3 hours which probably indicates a second pathway of activation. The antibody used to induce c-jun phosphorylation coincides with that required to induce other effects such as nitric oxide production or homotypic adhesion. The effect on JNK activation has been detected in IR938 cells, a rat B-cell lymphoma, and in Jurkat cells, a human T-cell lymphoma. These results that part of the signal initiated in the membrane by the CD53 antigen is mediated by phosphorylation of c-jun

O

DUAL INVOLVEMENT OF GTPases OF THE RAS SUPERFAMILY IN TRANSFORMATION AND APOPTOSIS: MECHANISM OF ACTION AND DESIGN OF NOVEL ANTITUMORAL DRUGS.

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Mammalian cells talk to each other by means of growth factors that are usually secreted to the extracellular medium. These factors recognize and activate specific receptors, usually located at the cell surface, that modulate different pathways of intracellular signals. Activation of specific effectors and their respective signaling pathways is the basis of a broad repertoire of physiologic responses. Signal transduction systems are responsible for the regulation of cell proliferation, cell differentiation and the response to stress and DNA damage with the subsequent activation of the cell death program or apoptosis. The same structural components of the signaling cascade are frequently shared by different factors. The specific cell response depends upon the proper combination of stimuli as well as the activation of the corresponding required signaling systems.

Our group investigates the alterations on signal transduction pathways induced by different members of the ras superfamily after oncogenic transformation. Under different experimental conditions, Ras and Rho proteins, members of the same family of monomeric GTPases, are capable of modulating the generation of second messengers whose balance affect cell responses such as cell transformation and apoptosis. Thus, these proteins are important components of the decision making machinery that controls cell growth and cell death. In addition, Ras and Rho proteins may play a critical role in the response to stress, in the generation of tumor cells, and the appearance of resistance to chemotherapy. Our studies demonstrate that Ras and Rho proteins are involved in the regulation of multiple intracellular signals that are key elements for the generation of second messengers and regulation of gene transcription. We will discuss the importance of the generation of second messengers such as those produced by phospholipase D/Choline kinase pathway and sphingomyelinase, as well as the activation of specific transcription factors such as NF-kB and SRF. These studies constitute the rational for the design of novel antitumoral drugs based on the identification of the enzymes constitutively activated as a consequence of cell transformation. We will present results that envision a promising future for the design of new antitumor drugs affecting those key enzymes altered in the process of malignant transformation by oncogenes.

A065

FUNCTIONAL STUDY OF THE AMINUS TERMINUS REGION OF hSos1

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The Ras exchange factor hSos1 is involved in the coupling of growth factor receptors to Ras-dependent mitogenic signaling pathways. The amino terminus region of hSos1 is approximately 600 amino acids long and contains domains of homology to Dbl (DH) and pleckstrin (PH). The PH and DH domains are frequently found in signal transduction proteins, and several data indicate that these domains are critical for their biological activity. The PH of hSos1 have been implicated in the regulation of their guanine nucleotide exchange activity and ligand-dependent membrane targeting. Recently DH of hSos1 have been related with the activation of Rac1. However, the function of the amino terminus region of hSos1 is controversial about the positive or negative modulation effect on the Ras exchange activity.

We have analyzed this hSos1 region and we found that truncation mutants lacking the amino terminus of hSos1 display lower activity compared with that of the full-length hSos1 protein. Moreover, the expression of this region alone, analyzed by transient transfections in Cos1 cells, showed an inhibitory effect on MAPK activation. We are looking for protein targets of this region, by using the yeast-two hybrid system, and we found different putative positive clones that we are trying to confirm. Finally, we obtained several truncated forms (N-terminus, DH, PH and DHPH) that we are analyzing about the capacity to activate JNK, p38 and AKT and, we are looking about their distribution (S100/P100) in starving and mitogenic conditions.

A067

H-Ras ACTIVATION PROMOTES CYTOPLASMIC ACCUMULATION AND PHOSPHOINOSITIDE 3-OH KINASE ASSOCIATION OF β-CATENIN IN EPIDERMAL KERATINOCYTES.

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E-cadherin/catenin complexes are frequently altered in carcinomas, but the mechanisms underlying downregulation of those complexes during tumor progression are not fully understood. In addition, the cadherin-associated β-catenin also participates in the transduction of the Wnt signal. Activation of H-ras is an early event during tumor progression in mouse skin carcinogenesis. The effect of oncogenic H-Ras in E-cadherin/catenin complexes has been analysed in mouse epidermal keratinocytes using a combination of V12Ras microinjection and biochemical analysis in keratinocytes stably expressing V12Ras. Microinjection of V12Ras in keratinocytes downregulates E-cadherin/catenin complexes, leading to the loss of E-cadherin and α-catenin and to the cytoplasmic relocalization of β-catenin. This effect was suppressed by preincubation of the cells with the PI3K (phosphoinositide 3-OH kinase) specific inhibitor, wortmannin. Stable expression of V12Ras in murine keratinocytes induces a decrease of E-cadherin and α-catenin, solubilization of the complexes and cytoplasmic stabilization of β-catenin. In addition, in V12Ras transformants the interaction of β-catenin with the Adenomatous Polyposis Coli (APC) protein is lost, but interaction of β-catenin with the effectors of the Wnt signaling pathway GSK-3β (glycogen synthase kinase-3 β) or Lef-1 is not induced. Interestingly, the regulatory subunit of PI3K, p85α, interacts with β-catenin in keratinocytes and this interaction is strongly increased in the V12Ras transformants. These results indicate that E-cadherin downregulation and cytoplasmic relocalization of β-catenin are induced in epidermal keratinocytes by H-Ras activation, suggesting that β-catenin cytoplasmic stabilization and further signaling can be stimulated by pathways independent of Wnt.

A096

IDENTIFICATION OF TWO NOVEL PROTEASES POTENTIALLY INVOLVED IN THE PROTEOLYTIC PROCCESING OF FARNESYLATED PROTEINS

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Ras superfamily proteins undergo several post-traductional modifications which lead to their association with the internal side of the plama membrane. These modifications include the adition of farnesyl- or geranyl-geranyl- group to the cysteine residue from the C-A-A-X domain located at the carboxy terminus. Then, the tripeptide A-A-X is proteolyticaly removed and the prenyl-cysteine residue is methylated. In this work we describe the identification of two human proteins with a high degree of similarity to the yeast proteases responsible for the proteolytic maturation of prenylated proteins. The cDNAs encoding these novel human enzymes have been cloned from a ovary cDNA library, and encode polipeptides 475 and 329-residues long, which we have named Face-1 and Face-2 (farnesylated-proteins converting enzymes-1 and -2), respectivelyd. The amino acid sequences of these proteins include several transmembrane domains, as well as several motifs characteristic of distinct groups of metalloproteases. Thus, Face-1 would belong to the gluzincin family, characterized by the presence of a HEXXH motif, Face-2 belonging to a family of metalloproteases characterized by the HXXE motif. Fluorescent *in situ* hybridization (FISH) experiments demonstrated that the FACE-1 gene is located in chromosome 1p34, while FACE-2 maps to chromosome 11q13, in a region frequently amplified in several carcinomas and linfomas. Northern blot analysis of mRNA samples from a wide variety of tissues an tumor cell lines showed that both genes are expressed in all anlyzed human tissues and cell lines. Preliminary results indicate that Face-1 and Face-2 proteins are required for the function of ras superfamily members. On the basis of these findings we propose that the inhibition of Face-1 and/or Face-2 could be a part of new therapeutic strategies directed to block the activity of these oncogenes in tumoral procceses.

A111

SIGNAL TRANSDUCCION PATHWAYS ACTIVATED BY ANTINEOPLASIC DRUGS AND THEIR ROLE IN APOPTOSIS.

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Antineoplasic agents such as cisplatin and adriamycin execute their pharmacological role by eliciting the apoptotic program. We have studied the mechanism of apoptosis induction by cisplatin. While transplatin activates JNK and the transcription factor NFkB in a transient manner, cisplatin kinetic of induction is slower and persistent. This difference seems to be involved in the citotoxic effect of cisplatin. Transcriptional activation of c-jun by the MEKK1/SEK-1/JNK cascade is necessary for apoptosis induction by cisplatin but not to other drugs such as adriamycin. On the other hand overexpression of some genes from the MAP kinase phosphatase family, is able to induce cisplatin resistance suggesting that these enzymes are key regulators of the death pathway by cisplatin.

A013

ROLE OF PKC IN THE DEVELOPMENT AND PROGRESSION OF COLORECTAL CANCER

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Colorectal cancer is perhaps one of the tumours best characterised at the molecular level. During the last few years, several genetic alterations have been associated to both the development and to the progression of this type of cancer. However, there is evidence that epigenetic factors (factors not directly associated with DNA alterations) might contribute to both tumour initiation and tumour progression of colon cancer. Amongst these, tumour promoters – substances that could be present in the gastrointestinal tract, associated to the diet - are of special interest. The best-characterised tumour promoter is the phorbol ester TPA, a diacylglycerol analogue which causes a sustained activation of Protein Kinase C (PKC). In various colon cancer cell lines, PKC activation by phorbol esters leads to the loss of cell to cell contacts, to a stronger adhesion to the extracellular matrix, to a blockage in the differentiation process and finally, to the loss of the epithelial phenotype towards the acquisition of a fibroblast-like phenotype. PKC is not a single molecule, but rather a family of enzymes comprising at least 11 isoforms, 7 of which are susceptible of activation by phorbol esters. The specificity of each isoform has not been well characterised and to date it has not been possible to ascribe a specific role to one or other isoform. We have described that PKCα activation is sufficient to emulate all the effects of phorbol esters on intestinal epithelial cells. Overexpression of a constitutive activated mutant of PKCα in intestinal epithelial HT-29 M6 colon cancer cells leads to the down-regulation of E-cadherin, to the overexpression of u-PA, and to the acquisition of an invasive phenotype in tumorigenic assays. Associated to these changes, transfected cells exhibit a lower proliferation rate. One possible explanation to these results could be that tumour promotion is interfering, or participating, of one of the key pathways that control colon cancer development. The exposure of intestinal epithelial cell lines to TPA causes the traslocation of β-catenin to the nucleus, allowing its interaction with transcription factors from the TCF family. Such translocation is independent of the APC protein, and thus positions PKC as an important effector in the initial progression of colorectal cancer.

A033

EXPRESSION OF MYC NETWORK PROTEINS IN MYELOID LEUKEMIA

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C-myc is involved in the patogenecity of linphomas and leukemias. Mad proteins (*Mad1*, *Mxi1*) inhibits *c-myc* transforming activity.We compared the expression of *c-myc* with that of *mad* genes in myeloid leukemia.

Total RNA was obtained from normal granulocytes of peripheral blood, chronic myeloid leukemia in blastic crisis (CML-BC), chronic myielod leukemia in chronic phase (CML-CP) and acute myeloid leukemia (AML) patients (5-15 samples).The detection of *c-myc*, *mad1* and *mxi1* genes was carried out by reverse transcription and polymerase chain reaction (RT-PCR).The amount of cDNA synthesized was calibrated by using the relative expression level of the ribosomal protein RSP14.The PCR products were analyzed by agarose electrophoresis.

We found variable expression by semiquantitative RT-PCR.The highest expression of *mxi1* was in CML-CP patients (91%), followed by 86% in AML patients, 66% in CML-BC patients and 31% in normal granulocytes.The expression of *mad1* was 71% in CML-CP patients, followed by 86% in AML patients, 33% in CML-BC patients and 58% in normal granulocytes.The expression of *c-myc* was 16% in CML-CP patients, 71% in AML patients, 33% in CML-BC patients and 15% in normal granulocytes.

C-myc expression is increased in a majority of the AML and in some of the blast crisis CML.These data confirm that regulation of *c-myc* expression is deregulated in many AML and CML-BC specimens but not in CML-CP.On the other hand, *mad1* and *mxi1* expression is increased in CML-CP and AML patients while is decreased in blastic crisis CML.The opposite expression pattern of mad genes and c-myc in CML-CP versus CML-BC support the hypothesis that Mad proteins are physiological antagonists of c-Myc transforming or proliferative function.

A034

P38 AND MXI2. TWO ISOFORMS THAT DIFFER IN ACTIVITY AND SUBSTRATE SPECIFICITY

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Mitogen-activated protein kinases regulate pathways that are involved in multiple cellular processes. To date, three distinct kinase-modules have been fully characterized in mammalian cells:
The ERK, the JNK (SAPK) and the P38 pathways. P38 mitogen-activated protein kinases are a family of Serine/Threonine kinases that are activated by cellular stress and inflammatory cytokines. These group includes the isoforms P38α, P38β, P38γ, P38δ and Mxi2, which is an alternatively spliced form of the human P38α protein kinase.

Recently, Mxi2 has been reported to phosphorylate Max protein . Proteins of the Myc family function in the proliferation, differentiation and oncogenic transformation of higher cells. c-Myc, the prototype, requires for its action Max, so it is conceivable that Mxi2 could play an important role in controlling the activity of the Myc/Max complex.

Using transient expression in 293T cells as a model, we have done a comparative study of the regulation of the kinases P38α and Mxi2. By means of HA-epitope tagged versions of both kinases and performing in vitro inmunocomplex kinase assays, we found that:i) Mxi2 and P38α have different activation kinetics; ii) both kinases respond in a different way to stress stimuli; Mxi2 is very strongly activated by hydrogen peroxide but P38α responds to anisomycin and sorbitol the highest ; iii) they share some homologies and differences in their upstream activators, MKKs and MKKKs; as an example, both kinases showed an increase of activity when they were cotrasfected with MKK3, but MKK6 only activated P38α; iv) they are equally modulated by small GTP binding proteins , and we observed that both of them were highly activated by Rho and Rac, but not by Cdc42; v) Mxi2 activity was unaffected by the inhibitor SB 203580 and by the CL100 phosphatase that are both known to block P38α activity; vi) their substrate specificity using bacterially expressed GST-ATF2, GST-JUN, GST-ELK1, GST-SAP1 and their ability to induce transcription factors ELK, SAP, MYC, JUN, CHOP-regulated reporters was also studied.

A064

THE ISOFORM-SPECIFIC INSERTION OF hSos1 DEFINES A NEW GRB2-BINDING DOMAIN

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Sos guanine nucleotide exchange proteins are known to mediate Ras activation induced by various tyrosine kinase receptors. Previously, we identified two distinct human Sos1 isoforms (hSos1-Isf I and hSos1-Isf II) with different Grb2 binding affinity. These isoforms differ only by the presence in Isf II of a 15 amino acid stretch located close the first proline-rich motif required for Grb2 binding. Some human tissues express only one isoform (fetal brain, and adult skeletal muscle, liver, lung and pancreas) whereas others express different proportions of both in fetal and adult stages.

In this work, we examined the C-terminal region of hSos1 without the four canonical SH3 binding domain. *In vitro* binding assays (by GST-fusion proteins and yeast two-hybrid system) and *in vivo* functional studies (by transient and stable transfections in Cos1, 293T and NIH3T3 cells) showed that both hSos1 isoforms, without four SH3 binding motifs, retains the functional activity and the capacity to bind Grb2. Furthermore, the results suggest that the 15 amino acid stretch defines a new functional Grb2 binding domain that could explain the higher Grb2 binding affinity of hSos Isf II.

A117

REGULATION OF CADHERIN-CATENIN COMPLEX BY TYROSINE PHOSPHORYLATION.

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β-catenin is required for the stablishment of adherens junctions, acting as a link between E-cadherin and α-catenin. Disassembly of these junctions has been correlated with increased tyrosine phosphorylation of β- and p120 catenins. Here we report a direct evidence of this hypothesis. Recombinant β- and p120 catenins are phosphorylated by p60c-src tyrosine protein kinase in *in vitro* experiments. Binding assays show that tyrosine phosphorylation decreases affinity of β-catenin by the cytosolic domain of E-cadherin causing a change in K_a from $10.1 \cdot 10^4\ M^{-1}$ to $1.8 \cdot 10^4\ M^{-1}$. Two sites are phosphorylated and as indicated by phosphorylation of different β-catenin deletion mutants, one is located near the N-terminus (Tyr-87) and the other in the last armadillo repeat of β-catenin (Tyr-655). Site directed mutagenesis of Tyr-87→Glu does not affect the binding of β-catenin to E-cadherin. However, the mutant Tyr-655→Glu shows a ten fold decreased affinity for the E-cadherin cytosolic domain compared to the wild type form. P120-catenin has a very low affinity for E-cadherin, although tyrosine phosphorylation increases this interaction by a factor of three. Ternary binding experiments using these two catenins and E-cadherin are currently under progress. These results evidence that binding of E-cadherin to β- and p120 catenins is controlled by tyrosine phosphorylation.

I Joint Meeting SEOM-ASEICA

A. Plenary Symposia

1. Telomerase and Cancer
2. Angiogenesis and Cancer
3. Hereditary and Familial Cancer

1

TELOMERE FUNCTION: ANALYSIS OF CELLS AND MICE LACKING THE MOUSE TELOMERASE RNA

Eloisa Herrera1, Enrique Samper1, Prakash Hande2, Luis Martín-Rivera1, Fermín Goytisolo1, Eva González1, Paola Jurado1, Peter Lansdorp2 and María A. Blasco1.

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The characterisation of a strain of mice genetically deficient for the mouse telomerase RNA and that lacks telomerase activity has provided a wealth of information on the role of mammalian telomeres and telomerase. Telomerase activity is not required for the viability of the first generations of mTR-/- mice, however, as telomeres shorten in later generations, highly proliferative tissues such as the hematopoietic system and the germ line, show defects in their proliferative capacity. We will show that, in later mTR-/- mouse generations, an increasing number of embryos show developmental defects that consist in a failure to close the neural tube. Importantly, cells derived from mTR-/- embryos showing the neural tube open have significantly shorter telomeres, decreased cell viability and increased apoptosis than their mTR-/- littermates with a completely closed neural tube, suggesting that the neural tube defect is a consequence of the loss of telomere function. Interestingly, in wild-type mice, mTR is abundantly expressed in the neural folds that will form the future neural tube. Neural tube defects, including spina bifida, anencephaly and congenital hydrocephalus are among the major causes of infant mortality. The study of mTR-/- mice has been also crucial to test the role of telomerase in mouse senescence and tumorogenesis. In this regard, cells that lack telomerase activity are able to be neoplastically transformed with oncogenes and to form tumours in nude mice. We will present data suggesting that after an initial telomere shortening accompanied by an increase in chromosome end-to-end fusions, mTR-/- cells are able to stabilise their telomeres. This observation unequivocally proves the existence of telomerase-independent mechanisms capable of maintaining telomeres in immortal mammalian cells.

2

Extension of cellular lifespan: Implications for Aging and Cancer. Woodring E. Wright, M.D., Ph.D., Department of Cell Biology and Neuroscience, U.T. Southwestern Medical School, Dallas, TX.

Chromosomes are capped by structures called telomeres. DNA polymerase is unable to replicate the ends of linear DNA molecules, and the ribonucleoprotein telomerase compensates for this by adding telomeric repeats to the ends of the chromosome. Telomerase is turned off in most somatic tissues during development, and in its absence telomeres shorten. This ultimately limits the number of times normal human cells are able to divide. A signal that induces the M1 (Mortality Stage 1) mechanism is produced when there are still several kb of telomeric repeats remaining on most chromosomes. This signal may reflect the activation of a DNA-damage pathway produced from a rare telomere lacking protective repeats, or the activation of regulatory factors located in the subtelomeric DNA and previously silenced by trapping in telomere-induced heterochromatin. The key antiproliferative components of the M1 mechanism (both p53 and a pRB-like protein) are then induced to be in a constitutively active form that prevents cell division. If the M1 mechanism is blocked, cells continue to divide and telomeres continue to shorten until the M2 mechanism causes crisis. M2 probably represents the direct consequences of terminally shortened telomeres. Immortalization occurs when cells escape M2, usually following the derepression of telomerase but sometimes via a telomerase-negative pathway that may involve recombination of telomeres. 85% of all cancer cells express telomerase activity, and its activity is being examined for screening, diagnostic and prognostic purposes in many different tumor types. The recent ability to prevent telomere shortening by constitutively expressing the catalytic subunit of telomerase in normal diploid cells and the demonstration that this greatly extends cellular replicative lifespan profoundly effects our approaches for the treatments of genetic defects, diseases of aging and cancer.

3

CRITICAL DETERMINANTS OF NEOPLASTIC ANGIOGENESIS

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Angiogenesis is essential for progressive growth and spread of neoplasms. The induction of angiogenesis is mediated by multiple molecules that are released by some tumor cells and host cells. Among these molecules are members of the fibroblast growth factor (FGF) family, vascular endothelial cell growth factor/vascular permeability factor (VEGF/VPF), and interleukin-8 (IL-8). The extent of angiogenesis is determined by the balance between factors that stimulate and those that inhibit the process. In quiescent normal tissues, the inhibitory factors predominate. Both stimulating and inhibiting molecules of angiogenesis can also be produced by leukocytes, and the organ microenvironment can directly contribute to the induction and maintenance of the angiogenic factors bFGF, VEGF/VPF, and IL-8.

Several factors that downregulate or inhibit angiogenesis have already been incorporated into clinical trials, the most widely studied being interferon-alpha (IFN-α). We tested the ability of IFNs to downregulate bFGF mRNA expression and protein production in human carcinoma cell lines. We found that IFN-α or IFN-β (but not IFN-γ) downregulated the steady-state mRNA expression and protein production of bFGF and collagenase type IV in human renal, bladder, colon, and prostate cancer cells by mechanisms independent of antiproliferation. We next analyzed the expression of angiogenic molecules in surgical specimens of human carcinomas. Proliferation was associated with production of bFGF and VEGF and absence of IFN-β. Further immunohistochemical analysis revealed that IFN-β is produced by differentiated epithelial cells of the skin, aerodigestive tract, GI and GU tract and not by normal dividing cells nor by carcinomas. These data suggest that cell division which is associated with production of positive angiogenic molecules is inversely correlated with production of IFN-β, a negative regulator of angiogenesis.

The IFN-β genes have been cloned. Transduction with viral vectors containing the IFN-β cDNA produced downregulation of collagenase type IV and bFGF in tumor cells and activation of inducible nitric oxide in infiltrating macrophages. The inhibition of angiogenesis coupled with activation of tumoricidal properties in macrophages produced regression of human colon, prostate, ovarian, and bladder carcinomas implanted into orthotopic sites in nude mice.

Collectively, these data indicate that cytokines produced by different organ environments can regulate the process of angiogenesis which is an integral part of the metastatic cascade.

4

Family Studies and Hereditary Susceptibility to Bowel Cancer

D. Timothy Bishop
ICRF Genetic Epidemiology Laboratory, Leeds

Studies of families with numbers of cases of bowel cancer have identified two major forms of hereditary predisposition. The first, termed ÒFamilial Adenomatous PolyposisÓ (FAP) is recognised by the presence of large numbers of adenomatous polyps distributed throughout the bowel and beginning to develop in the teenage years. The second, termed ÒHereditary Non-polyposis Colorectal CancerÓ (HNPCC) was only recognised, until recently, by a striking dominant pattern of inheritance and predisposition to cancers at a variety of anatomic sites but primarily of the bowel and endometrium plus ovary, stomach, bladder and pancreas. Some of the genes responsible for these predispositions have been cloned. The APC gene (on chromosome 5) is the gene responsible for all families with FAP identified to date while predisposition to HNPCC arises because of mutations in genes which normally are involved in DNA mismatch repair. Failure of this system leads apparently to an inability to repair critical genes and the natural rate of accumuluation of such mutations plus selection are sufficient to produce this predisposition. Families with HNPCC can now be identified either through identifying germline mutations or by particular characteristics of the tumours in such families.

Evidence suggests that the majority but certainly not all, of families with a family history consustent with HNPCC are due to these mismartch repair genes. Other genes which either involve different genetic systems or perhaps do not produce the same tumour phenotype may also be important. Identification of high risk families without apparent mismatch repair defects suggest that there are other high risk predisposition genes, as does segregation analysis of population-based series of cases. There are currently no clues to the location of such genes.

There are several improtant unresolved clinical issues in these families, such as how to screen HNPCC families and the feasibility of chemoprevention. Studies of resistant starch and aspirin are currently in progress in both FAP and HNPCC families (for more information, contact Prof. John Burn, Dept. Of Human Genetics, University of Newcastle, UK).

5

FAMILY BREAST CANCER

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Breast cancer is the most common neoplasme among women in the western world. Although it usually appears as a sporadic manner, there is a small percentage of cases (5-10%) which is considered familial and attributed to hereditary mutations in various susceptibility genes that follow and autosomic dominant tract. Till now, two of them have been isolated; BRCA1, located in the long arm of chromosome 17 (17q21), and BRCA2 of the long arm of chromosome 13 (13q21). It is considered that women who are carriers of any mutation in any of these two genes have a risk of approximately 80% of developing breast cancer along their life time. The BRCA1 gene is also associated with an increased risk of suffering from ovary cancer, while BRCA2 gene is associated with breast cancer in males. In both cases there is an increased risk of other types of cancers, mainly of prostate, colon and pancreas.

Both genes present long codifying sequencies and a similar complex genetic structure. The proteins which codify have low homology with others except for one highly preserved domain of "Zinc fingers" type in the N terminal extremity involved in the protein-protein interaction and in the DNA recognition. At present it is known that both are associated with RAD51, a DNA repairing protein in S.cerevisae, which suggests that BRCA genes are actively involved in the DNA repair.

Many mutations described till now (more than 400 in BRCA1 and more than 100 in BRCA2) do not only impede the analysis of the gene but, also the establishment of a phenotype-genotype correlation. Furthermore, the mutations in BRCA1 occur in 45% of the high risk families while around 35% in BRCA2. Approximately 20% of the families do not present mutations which suggest the existence of at least a third gene of susceptibility (BRCA3) still unidentified.

Despite this heterogeneity, recurrent mutations very frequently appear in certain populations. These are "founder" mutations responsible for the majority of these specific populations. So, the Ashkenazi jews have two mutations in BRCA1 (185delAG) and BRCA2 (6174delT) which are almost specifics for the jewish population; in Island a mutation in BRCA2 (999del5) causes almost 100% of familial cancers. On the contrary, in our country no majoritary mutation has been identified.

Although there is not preventive measures in carriers of the disease, it is obvious that it is necessary to identify this group predisposed to cancer as a first step towards prevention and treatment. This approach, among others, is focused on the follow-up of the person trying to know the specific risk of each mutation, the influence of the exogenous factors on the development or not of a cancer within a carrier family of the mutation or on the role of those considered polymorphisms in the modification or modulation of the phenotype.

VII Congress of the SEOM

A. Plenary Conferences

I

NEW DRUGS

S B Kaye, CRC Department of Medical Oncology, University of Glasgow

Drug discovery and development in the field of cancer is approaching unprecedented levels of interest and productivity. Much of the activity currently involves new agents with conventional cellular targets, e.g. taxoids and topoisomerase I inhibitors, and these have already made a significant impact. New analogues and formulations for these agents are looking very promising in the near future, and a particularly fruitful avenue for exploration is the development of oral pro-drugs, which allow for prolonged tumour exposure in a practical and convenient form.

In addition, a great deal of attention is directed towards novel compounds whose targets are based on sound biological and molecular mechanisms and positive preclinical data, but for which proof of clinical utility is not yet available. These include signal transduction inhibitors, angiogenesis inhibitors, telomerase inhibitors, etc.

An extraordinary spectrum of compounds is under investigation, ranging from small low molecular weight molecules, through novel peptides, to monoclonal antibodies against specific membrane receptors and antisense oligonucleotides targeting intracellular genetic sequences.

For the next 5-10 years, conventional cytotoxic agents will continue to play a major part in cancer treatment, but it is entirely conceivable that these newer compounds will find an important role in combination therapy programmes for many common cancers.

II

New trends in hormone resistant prostate cancer by Ian F. Tannock, Princess Margaret Hospital, Toronto (Currently at Centre Léon Bérard, Lyon, France).

About 80% of patients with metastatic prostate cancer will respond to initial androgen withdrawal therapy for a mean duration of about one year. There may be brief responses to secondary hormone therapy, but the disease progresses inevitably to hormone-resistance. The main symptoms are pain due to bone metastases, and general fatigue. There are no treatments which have increased the survival of patients with Hormone Resistant Prostate Cancer (HRPC) and the aim is to palliate symptoms and to improve quality of life.

The basics of management of patients with HRPC consist of the appropriate use of narcotic analgesics, together with measures to prevent constipation, and use of radiotherapy to treat localized pain. However, pain is often generalized, and there may be poor tolerance of narcotics. Thus, there is a need for effective systemic treatments. In using these treatments, the main goal is relief of symptoms and the use of pain and other quality of life end-points will be described. Fall in serum PSA is a valuable adjunct to show activity of a treatment, but is not proof of benefit to patients (reducing the PSA is little help if it reduces the patient !).

Our approach is to evaluate gentle treatments and the Canadian randomized trial comparing mitoxantrone plus prednisone with prednisone alone illustrates this approach. The trial showed palliative benefit from using this well-tolerated chemotherapy (and PSA response in 30-40% of patients), and this treatment has been approved by the American FDA for treatment of HRPC. Treatment with strontium ^{89} and with suramin have also been shown to have palliative value in randomized clinical trials. Several other anticancer drugs including estramustine used with vinblastine or eposide have biological activity as shown by a fall in serum PSA. Further gains in palliation will require optimization of the use of available 'gentle' drugs. The current Canadian trial compares mitoxantrone plus prednisone plus the bisphosphonate clodronate with mitoxantrone and prednisone alone, using similar palliative end-points to the earlier trial.

Several ongoing trials are studying biological approaches including anti-angiogenesis and immunotherapy. Biological activity of these approaches should first be shown using PSA reduction, and if they are relatively non-toxic it is appropriate to test them at an earlier stage of disease. However their evaluation should be stringent, and when these agents are effective in animal models they lead to tumor shrinkage and not just stabilization of disease.

III

High dose chemotherapy and solid tumors by Ian Tannock, Princess Margaret Hospital, Toronto (Currently at Centre Léon Bérard, Lyon, France)

The principles of high dose chemotherapy followed by stem-cell transplantation are straight forward. For cancers that often respond to conventional dose chemotherapy, augmentation of dose might eradicate residual cancer cells and lead to cure. Unfortunately, this goal has not been achieved for most solid tumors.

For breast cancer, single arm series have shown an increase in the probability of complete response in patients with metastatic disease, and a suggestion of better survival in patients with locally aggressive cancers who receive high dose adjuvant chemotherapy. Neither of these results provide substantive support for use of the procedure. In metastatic disease, patients are selected on the basis of response to conventional-dose chemotherapy, and for adjuvant therapy they are selected by more intensive screening. When these factors are taken into account, outcome is not substantially different than after conventional chemotherapy.

Determination of the benefit of this procedure requires large randomized clinical trials and several are ongoing. Two small trials have been reported for metastatic breast cancer : the South African trial showed a survival advantage, and although criticized because of non standard chemotherapy in the control arm, it is modestly supportive of high dose chemotherapy. The second trial compared immediate versus delayed transplantation following achievement of a complete response with conventional chemotherapy. This trial showed poorer survival in the immediate transplantation group, which most would interpret as suggesting lack of benefit. If there is a beneficial effect from high dose chemotherapy, it is more likely to come from adjuvant therapy, and it may occur in patients with intermediate levels of risk (eg. 1-4 nodes and poor histology) where a modest increase in cell kill could lead to an increased rate of cure.

The limited value of high dose chemotherapy may be due to the shallow slope of the dose response curves for many anti cancer drugs. Also Withers et al have shown that a standard course of adjuvant chemotherapy for breast cancer probably reduces surviving fraction only by about 10^{-2}. Patients with microscopic disease may have a body burden up to 10^{9} cells, so that a relatively small increase in the level of cell kill (eg. by one or two logs) still has a relatively small effect on the probability of cure.

High dose chemotherapy with stem-cell transplantation is not appropriate for patients with breast cancer and most other solid tumors except as part of a large phase III clinical trial. Small randomized trials are of little value, and further phase II trials will not provide information about the value of the procedure.

IV

TRIUMPHS AND TRAGEDIES IN CANCER THERAPEUTICS: GERM CELL TUMORS AND SMALL CELL LUNG CANCER

Twenty-five years ago, we simultaneously began innovative combination chemotherapy studies in both small cell lung cancer and germ cell tumors. At that early time, both were known to be chemosensitive, in small cell lung cancer, also radiosensitive. It appeared that with the availability of new agents at that time such as cisplatin for small cell lung cancer and doxorubicin for small cell lung cancer, that a significant improvement in the cure rate would be inevitable. Furthermore, twenty years ago, etoposide became available for clinical trials with significant activity in both of these diseases.

Germ cell tumors have become a model for a curable neoplasm. As we approach the millennium, over 90% of patients are cured of their disease, and 80% of patients with disseminated germ cell tumors will survive their cancer. This is due to the remarkable chemosensitivity with cisplatin. Germ cell tumors have become a unique disease and during the lecture, I will discuss the possible and probable reasons for why testicular cancer enjoys such a high cure rate with chemotherapy compared to other solid tumors.

There has been a significant improvement in the quality of life and quantity of survival for small cell lung cancer. However, for the two-thirds of the patients who present with extensive disease, there has been only modest improvement in survival and only anecdotal cures. Modern chemotherapy with cisplatin + etoposide for extensive small cell lung cancer results in a high remission rate including 10-20% complete remissions. However, median survival time is only 6-10 months, 2 year survival is 5% and 5 year survival is 1-2%. By contrast, limited small cell lung cancer is now treated with cisplatin + etoposide combined with thoracic radiation (with or without prophylactic CNS irradiation). The median survival time is 20 months, 2 year survival 40%, and 5 year survival 20%.

During the past 25 years, both of these diseases have enjoyed high response rates, but a marked difference in cure rates. It is felt unlikely that any currently available cytolytic agents are capable of improving the cure rate in small cell lung cancer other than very minimally. Hopefully, new biologic targets including angiogenesis inhibitors and other mechanistic targets would be more beneficial than further alterations of classical chemotherapy agents.

B. Presidential Papers

1

HORMONAL RECEPTORS AS PROGNOSTIC FACTORS IN BREAST CANCER (BC): A RETROSPECTIVE STUDY OF 1474 PATIENTS (PTS) IN A SINGL INSTITUTION.

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The role of prognostic factors in optimizing treatment for BC pts has clearly changed with the trend towards general use of systemic therapy. These factors are used to identify pts with excellent prognosis or worse prognosis, or pts with a potential benefit with specific therapies. To assess the prognostic value of several factors (Hormona receptors (HR), S-Phase fraction (SPF), cathepsin D (CD), DNA-content (DNA) and pS protein), 1474 pts with operable BC were included in this study from February-82 to October-96. Determination of prognostic factors were done in the University Hospital of Valencia. All patients had periodic control in the same institution. **Characteristics** Lymph node involvement: 0: 649 pts (44%), Positive: 791 (56%), with 151 pts with 10 or more involved nodes, 256 (18%) with 4-9 nodes, and 384 (27%) with 1-3 nodes. Mea age was 57 years (29-99). Tumor size was ≤2 cm in 424 pts (29%), between 2-5 cm in 80 pts (55%), ≥5: 122 (8%). Conservative surgery was done in 173 pts (12%) and radica mastectomy in the rest. Adjuvant treatment was administered in 1318 pts (89%), 363 (24%) received chemotherapy alone, mostly with anthracyclin-containing regimens (241pts) and 58 pts wre included in a high-dose chemotherapy and PBSC transplantation program; 640 pts received hormonal therapy and only 156 pts (11% received no treatment. **Results:** With a median follow-up of 70 months (5-169), DFS at and 10 years were 68% and 55 % respectively, and 82 y 65% in OS. Univariate analysis showed significant differences in DFS and OS for HR, including fenotipic variants. Age also was a discrminative factor in prognosis, with worse results in survival for pts younger than 40 years. Other prognostic factors related with tumoral involvement (nodal involvement, tumor size) also showed strong prognostic value. New factors analyzed as CD, pS2 protein, DNA-content and SPF did not sowh an important prognostic value in th whole group, including stratification by nodal involvement. Multivariate analysis was done in terms of DFS and OS in the whole group and in node-negative and positive-group. PgR and nodal involvement were independent prognostic factors in terms of DFS and OS. .and age was an independent factor in DFS in all groups. **Conclusions:** HR are independent prognostic factors in DFS and OS, although their prognostic value is weak Lymph node involvement is the strongest prognostic factor in terms of survival, beside of tumor extension realted factors as tumor size and stage.

2

NEOADJUVANT (NEO) AND CONCOMITANT (CON) CHEMOTHERAPY (CT) PLUS RADIOTHERAPY (RT) IN ADVANCED HEAD AND NECK CARCINOMA (CHN). A Rueda, N Ribelles, J Solano, I Sevilla, J Contreras[1], A Márquez, L Alonso, A Sacchetti[2], R Urquiza, E Alba. University Hospital. Regional Hospital[1]. CROASA[2]. Málaga. Spain.

In patients (pts) with locally advanced CHN treated with RT, NeoCT reduces distant metastasis and ConCT improves local control. We examined the toxicity, tumor response and survival in pts with advanced CHN receiving RT combined with both NeoCT and ConCT. *Eligibility*: pts with stage III or IV CHN with normal renal function, performance status < 3 (ECOG) and no previous treatment. *Radiochemotherapy*: NeoCT (continous infusion), cisplatin 25 mg/m^2 x 5 days + Leucovorin 500 mg/m^2 x 5 days + 5-FU 800 mg/m^2 x 4 days q 3 wks x 2-3 cycles; then ConCT, carboplatin 300 mg/m^2 q 3 wks x 3 + UFT 600 mg daily p.o. during standard RT (65-72 Gy to the primary and nodes and 50 Gy to areas at risk). *Results*: between 12/93 and 12/97 46 pts were included. Median age 56 (36-70); stage III/IV, 8/38; N_{2-3}, 26; T_4, 28; unresectable, 38; non-laryngeal, 44. Toxicity (% of patients with grade 3+4) with NeoCT/ConCT: hematological 25/12, nausea 10/8, mucositis 41/62, diarrhoea 7/0; there was 1 toxic death (tumoral bleeding). Responses (R); post-NeoCT: complete (C)R 14(30%), partial (P) R 25 (54%), no R 7 (15%); post-ConCT+RT: CR 31 (67%), PR 8 (17%), no R 7 (15%). With a median follow-up of 36 months (6-60), 19 pts are alive and disease free. Three year overall and progression-free survival (Kaplan-Meier) are 40 and 46% respectively. These promising data suggest that randomized trials should be undertaken to compare this treatment approach to standard therapy.

3

LIVER RESECTION FOR COLORECTAL METASTASES. UTILITY OF SYSTEMIC ADJUVANT CHEMOTHERAPY.

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Introduction. **This study was performed to determine if liver resection for colorectal metastases is safe and effective and to evaluate predictors of outcome.**

Material and Methods. **Data of 130 consecutive resections performed in 119 patients, between january 1991 and october 1998 were collected prospectively. This data was analyzed retrospectively.**

Results. **The perioperative mortality was 4.6% (6 patients). The median hospital stay was 10 days. The 5-year survival rate was 43%. By univariate analysis, multiple liver tumors (≥ 4 nodes) was predictive of poorer outcome. Presence of bilobar disease, sincronic diagnostic of metastases, large tumors (> 5 cm), extrahepatic disease (if resectable), perioperative transfusion, re-resection of recurent metastases and simultaneous resection of liver metastases and of the colorectal tumor were not predictive of outcome. Postoperative systemic adjuvant chemotherapy was performed in 49 patients (57%), survival in these cases was much better (75%at 3 years p=.001). By multivariate analysis multiple tumors (≥ 4) was independant predictor of poorer outcome (aRR 5.3, CI 1.8-15.6). Postoperative systemic chemotherapy was independant predictor of better survival (aRR 0.17, CI 0.06-0.45).**

Conclusions. **Liver resection is safe and effective and should be considered the standard therapy for patients with colorectal metastases. A randomized trial to confirm the efficacy of postoperative adjuvant systemic chemotherapy is necessary.**

4

PACLITAXEL AND CISPLATIN AS NEOADJUVANT CHEMOTHERAPY IN BLADDER CANCER (STAGE II -III).

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AIM: Recent studies indicate that paclitaxel is an active single-agent in the treatment of urothelial cancer. In this study paclitaxel was combined with cisplatin. The aims of the study were to assess efficacy and toxicity of this combination to evaluate the results of neoadjuvant chemotherapy in bladder cancer (stage II and III) and discuss the possibility of avoiding radical cystectomy in case of pathologic complete response (pCR).

METHODS: Paclitaxel was administered at a dose of 175 mg/m^2 over 3 hours followed by Cisplatin 75 mg/m^2 . Cycles were repeated every 3 weeks (3 cycles) and then the patients were restaged by computerized tomography, cytoscopy and multiple biopsies. Cystectomy wasn't performed in case of pCR. Patients with a partial response (PR), stable disease (SD), or progressive disease (PD) underwent radical cystectomy. Patients with a pCR underwent radiation therapy after chemotherapy and avoiding radical cystectomy.

RESULTS: From January1996 to October 1998, 33 patients with stage II (6 pts) or stage III (27 pts) were entered into the study. Median age was 69 years, all pts had PS ≤ 2, adequate hematologic, renal and hepatic function. 33 pts and 95 courses were evaluable for toxicity. Neutropenia was the main hematological toxicity:GIII: 12%, GIV (1 pat). Neither death due to chemotherapy has ocurred. Other toxicities consisted mainly of WHO GII alopecia (60%) and nauseas/vomiting (10%). Polyneurophaty GI/II (18%). Peripheral polyneurophaty G3 yielding cessation of therapy in 1 pt. The full doses of paclitaxel and cisplatin were administered. Doses reductions were not needed. There were no treatment delays due to toxicity. Of 27 pts evaluable for response: 15 pCR(55,6%) and 8 PR. 2 EE, and 2 PD. (Overall response rate= 85%). Patients in pCR didn't undergo cystectomy and underwent radiation therapy after chemotherapy. Actually, CR is confirmed in12 pts.

CONCLUSION: These results suggest that the combination paclitaxel and cisplatin appears to be highly active as neoadjuvant chemotherapy (85% overall responses) in the treatment of bladder cancer (stage II-III). In 55,6% pts in pCR is possible to avoid cystectomy.

5

ECONOMIC ANALYSIS OF INTERFERON FOR HIGH-RISK (STAGE III) MELANOMA PATIENTS.

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Introduction: In the clinical trial E1684 *(Kirkwood, 1996)* adjuvant high-dose interferon alfa-2b (IFN) treatment of patients with surgically resected stage III melanoma (AJCC) was associated with an increase in disease-free survival and overall survival. The objective of this study was to determine lifetime health benefits, lifetime cost and cost-effectiveness (C/E) relationship in these patients in comparison with a control group.

Material and methods: Disease progression was studied using a Markov model *(Hillner, 1997)* comparing two hypothetical cohorts of 10000 patients, according to the results of E1684. The cost of each clinical state from the Health Authorities perspective (drugs, administration, hospitalization, medical visits, lab and diagnostic tests) was determined according to routine clinical practice in Spain by an expert panel.

Results: For a patient with a mean age of 50 years, lifetime incremental cost (IC) with a 6% discount rate, life years saved (LYS) and incremental C/E relationship (6% discount cost per LYS) are shown below:

Cost (in million ptas)	IC	LYS	IC/LYS
IFN vs. Control	2.9	1.9	1.5

According to the projection generated by the model, IFN treatment produces an increased survival of 1.9 years with an incremental cost per LYS of 1.5 million pesetas. This result was compared with other procedures considered as essential for survival and accepted by our National Health System (NHS), such as hemodyalisis for a patient with renal failure (3,4 million pesetas/year).

Conclusion: This study shows that IFN administration in high-risk (stage III) melanoma, a disease in which clinical efficacy has been demonstrated, is within the cost range acceptable by the Spanish Health System for routine medical interventions.

6

TRANSFUSIONS IN ONCOLOGY

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Introduction: blood transfusions are one of the main supportive therapies in cancer patients. As we lack specific guidelines for their use, it would be useful to know more about their use and common indications.

Objective: to assess the use of blood transfusions in a service of oncology.

Material and methods: We retrospectively revised all the transfusions performed at our service in an 18-month period (Jan-96 to June-97), both in hospitalised and ambulatory patients. The following items were registered: age, sex, diagnosis, time of transfusion (at diagnosis or afterwards), cause of the anemia, hemoglobin level, units of blood transfused, use of chemotherapy (with or without cisplatin), previous transfusions and current clinical situation.

Results: 167 transfusions were administered, 87 in hospitalised patients and 80 in the outpatient area. There were 96 men and 71 women, with a median age of 60 years (range 15-92), The primaries were lung in 33 patients (20%), gastric in 21 (12.5%), esophagus in 16 (9.5%), breast in 15, colon in 15, soft tissue sarcoma in 11, high-grade lymphoma in 11, low-grade lymphoma in 7, myeloma in 7 and other in 31. The transfusion was indicated at diagnosis in 11 cases (7%) and during the evolution of the disease in the remaining 156 patients. Previuos transfusions had been administered in 55 cases (33%). The hemoglobin level ranged between 10.4 and 4.6 g/dL, with a median of 7.4. A median of 3 units were transfused (range 2-6). Anemia was attributed to chemotherapy in 80 patients (48%) and to the tumor in 69 (41%), whereas it was due to digestive bleeding in 13 patients. One-hundred twenty patients (72%) were under chemotherapy by the time of transfusion, and chemotherapy included cisplatin in half of them.

Conclusions: 1) Patients with lung cancer, digestive tumors or hematologic malignancies received most of the transfusions. 2) Most transfusions were given in the evolution of the disease. 3) One third of our patients had received previous transfusions. 4) Most transfusions were atributable to chemotherapy, although over 40% were given as a palliative treatment for the underlying tumor.

C. Scientific Topics

1. Advanced Breast Cancer

8

METASTATIC BREAST CANCER. LONG-TERM SURVIVAL

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Metastatic breast cancer is a fatal disease in which survival may vary depending on the affected organs. We review the experience of the Oncology Service at the Puerta de Hierro Clinic on stage IV breast cancer as to the clinical features, response to treatment and survival.

MATERIAL & METHODS 157 patients (pts) diagnosed of and treated for breast cancer stage IV in our service from January 1983 to December 1997 were reviewed. The following clinical variables were analyzed: Metastasis (MTS) location, hormone receptors, and menstrual status. The disease-free interval, response duration and overall survival were estimated by the Kaplan-Meier method. To compare survival curves, a Log-Rank test was applied. Frequency data statistical analysis was based on the χ square test with either the Yates or the Fisher correction.

RESULTS. MTS location at diagnosis is shown in the Table below. In 53 pts (33%) they presented as single MTS and in 104 (67%) as multiple ones. 102 pts underwent hormone therapy, 27, chemotherapy and 5 patients were given both. A complete response was reached in 42 pts. Out of which 15 pts suffered from a relapse. In 48 pts, the relapse occurred within 2 years and 25/48 were dead. In 98 pts their relapse occurred after 2 years and 38/98 were dead ($p<0,001$). Overall survival rate (median) was 5.6 years with a disease-free interval of 2.9 months Differences between regional, bone and pleural with liver and lung MTS were found to be significant ($p<0,05$).

Location	Patients(%)	5-yr. Survival
Regional	69(44)	79%
Bone	44(28)	72%
Pleura	12(7.6)	75%
Lung	19(12.4)	56%
Liver	10(6.4)	45%
CNS	2(1.3)	nv
>1 Organ	37(24)	
<1 Organ	120(76)	

CONCLUSIONS. 1) Survival median in breast Cancer, stage IV is >5 years with currently available treatments. 2) When the disease-free interval is greater than 2 years the disease-free interval is < 2 yr. ($p<0,001$). 3) Regional, bone and pleural lesions show a statistically significant higher survival than liver and lung locations.

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CYTOXAN, EPIRUBICIN AND VINORELBINE (CEN) IN METASTATIC BREAST CANCER (MBC): A PHASE II STUDY OF THE GRUPO ONCOLOGICO DEL NORTE (GON). *AJ Lacave [1], E. Esteban [1], Gonçález de Sande JM[2], Puertas J[1], Muñiz I[1], Fra J[1], Palacio I[1], JM. Vieitez[1], E. Estrada[3] y Fernández JL[3]*, Medical Oncology Hospitals of Oviedo [1], León [*], Ponferrada [3].

In a previous phase III study performed by our group the CEF combination demonstrated a 61% response rate in patients (pts) with MBC. (Ann Oncol, 1994; 5:26). Vinorelbine (N) (an active drug in the treatment of BC) was introduced as a substitution for 5 fluorouracil (F) with CE. On this bases, we began a phase II study with this new 3-drug combination CEN. Criteria inclusion were the following; females ≤70 years old , measurable or evaluable disease, Karnofsky (K) ≥ 50%, adequate haematology, hepatic, cardiac and renal functions, no prior chemotherapy (CT) except for adjuvant CT anthracyclines or vinorelbine. From April till March 98, 50 pts were included in the study with the following characteristics median (M) age 53, M K 70%, adjuvant chemotherapy 17 pts (34%); M number of metastasic sites 2 (1-5); lung 20, liver 21, bone 21, and soft tissue 24. The treatment schedule used was (mg/m^2 i.v. day 1 and 8): C 400, E 30 and N 25. Cycles were repeated every 4 weeks.

Results: M number of cycles administered up till now was 5 (1-14). The most common haematological toxicity was neutropenia: 40% (20) of the pts had Grade (G) 3 or 4, 4 of them were febril. Trombopenia or anaemia G 3-4 were seen in 2 pts. Other non-haematological toxicities G 2-3 were: N/V 23 pts (46%), alopecia 25 (50%) and stomatitis 7 (14%). Cardiotoxicity G 2 in 5 pts. activity: 12% CR; 33% PR, 51% NC and 4% PD. The M duration of response an time to progression 34 weeks (95% CI: 23-45) and 31 weeks (12-51) respectively.

In conclusion the CEN combination appear to have an acceptable tolerance but moderate activity when compared to other regimens used recently in MBC. The study is still ongoing.

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DOCETAXEL-VINORELBINE IN ANTHRACYCLINE RESISTANT DISSEMINATED BREAST CARCINOMA

AN ONCOPAZ COOPERATIVE GROUP STUDY

Background and objective: docetaxel and vinorelbine are two of the most active drugs in breast carcinoma. We studied their combination in patients with disseminated disease who had previously been treated with an anthracycline.

Patients and method: 96 patients with metastatic breast carcinoma were included. They could have received adjuvant or neoadjuvant chemotherapy or first line chemotherapy for advanced disease: an anthracycline had been used in any of these cases. Their age ranged between 26-77 years, with a median of 54. ECOG performance status was 0 in 33 patients, 1 in 51 and 2 in 10. Docetaxel was administered on day 1 at 85 mg/m2; this dose was reduced at 75 mg/m2 after the appearance of severe neutropenia in the first patients. Vinorelbine was given on days 1 and 5 at 20 mg/m2. Courses were repeated every 21 days. Prohylactic G-CSF was allowed if the patient had had grade 4 neutropenia or febrile neutropenia in previous courses. Reevaluation was undertaken after 3 courses and, in the absence of progression, up to 6 courses were given.

Results: 511 courses were given, with a median of 5'3 per patient (range 1 to 6). Seventy-two percent of patients received 6 courses. There were 13 complete responses (14%) and 51 partial responses (56%), for an overall response rate of 64% (IC 95%: 60-79%). It is too soon to evaluate survival. Grade 3-4 toxicities were: alopecia in 60% of patients, neutropenia in 24% (only grade 4), febrile neutropenia in 16%, stomatitis in 16% and asthenia in 12%.

Conclusion: the combination of docetaxel and vinorelbine is very active and moderadately toxic with this schedule in patients with metastatic breast carcinoma previously treated with anthracyclines.

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EFFICACY OF HIGH-DOSE PACLITAXEL IN COMBINATION WITH CYCLOPHOSPHAMIDE, THIOTEPA, CARBOPLATIN AND STEM CELL RESCUE FOR METASTATIC BREAST CANCER. A PHASE II TRIAL.

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Aims: A phase I-II trial with escalating dose of paclitaxel (PTX) in combination with cyclophosphamide, thiotepa and carboplatin (CTCb) plus stem cell rescue for patients (pts) with metastatic breast cancer (MBC) and ovarian cancer was performed. **Patients and Methods:** From October 1995 to November 1996, 16 pts with MBC (16), ovarian(1) or unknown primary (1) cancer failing to enter complete response (CR) to conventional-dose chemotherapy (CHT) were treated in the phase I portion of the trial (Proc ASCO 1997. A-358). Dose levels of PTX (mg/m^2) were 500 (3 pts), 600 (3), 700 (6), 800 (3) and 650 (3). Dose-limiting toxicity at 700 mg/m^2 was grade 4 mucositis (3/6 pts) with sepsis and lethal adult respiratory distress in 2/6 pts. The recommended PTX dose for the phase II portion of the study, with 16 pts enrolled to date, was 650 mg/m^2. **Results:** All 25 pts with MBC with measurable disease, treated with PTX+CTCb while in partial response (PR) or disease stabilization to induction CHT (FEC) had objective responses (CR 18 pts; PR 7 pts; response rate = 100%). According to prior CHT, there were 17 CR + 4 PR in 21 pts with no prior CHT for MBC (9 had prior adjuvant CHT) and 1 CR + 3 PR in 4 pts with prior CHT for metastasis. Median time to progression was 19 months (mo) (range 4-27+) for all pts (19 mo for pts with no prior CHT for MBC and 6 mo for those with prior CHT). Median survival for all pts has not been reached (54% 2-year survival). For pts with no prior CHT for MBC, 2-year survival is 59%, and median survival was 15 mo for pts with prior CHT. **Conclusions:** The antitumor activity of PTX+CTCb with stem cell rescue in this small cohort of pts with MBC not in CR is encouraging, as is the duration of response. PTX+CTCb deserves comparison with non-PTX containing high-dose CHT in a randomized trial.

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PHASE II STUDY BIWEEKLY PACLITAXEL, DOXORUBICIN AND GEMCITABINE AS FIRST LINE CHEMOTHERAPY FOR METASTATIC BREAST CANCER (MBC).

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Introduction: Combination Paclitaxel and Doxorubicin have both demonstrated important activity in MBC (50-94%). Gemcitabine has shown a response rate of 30-40% as first line chemotherapy in MBC. The aim of our study was to determine the efficacy and toxicity of a combination of paclitaxel, doxorubicin and gemcitabine as a first line chemotherapy in MBC as a schedule dose density biweekly.

Material and methods: 21 patients were included diagnosed with MBC, 15 had prior adjuvant with antraciclines- containing chemotherapy. Median age was 53 years (40-67). PS was 0-1. Sites of disease were single (10 pts) or multiple (11 pts), including: bone (13), lung (11), lymph nodes (5) and liver (2). In case of patients required a delay in dose after 1st cycle, because of haematological toxicity, support with GCSF was allowed. LVEF was performed at the beginning, the 3th cycle, the end of study and every six months.

Dose escalation and toxicity.

Level Nº pts	D Mg/m2	T mg/m2	G mg/m2	G3-4 T.Haemat	G3-4 T.Non haem
1 (3)	20	135	2500	-	-
2 (6)	25	135	2500	4	-
3 (10)	30	135	2500	6	1
4 (2)	35	135	2500	2	1

Results: A total of 105 cycles were administered. 21 patients were assessable for toxicity: Neutropenia (G3 in 28,5% of pts, G4 in 23,8% of pts), thrombocytopenia (G3 in 1 pt), mucositis G3 in 2 pts, peripheral neurotoxicity G2 in 42% of pts; other toxicities were low. There was a death due to febrile neutropenia. 20 patients were evaluable for response. 6CR, 10PR, 2SD and 2PD with an overall response rate of 84%. With a median follow-up of 11 months the overall survival has not already reached.

Conclusions: All together dates indicate that this combination of doxorubicin, taxol and gemcitabine is highly active in first line chemotherapy in patients with MBC in spite of mild-high haematological toxicity and low cardiac toxicity.

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THE IDEAL DOSE OF TAXOTERE (T) IN PATIENTS WITH METASTATIC BREAST CANCER (MBC) PREVIOUSLY TREATED WITH ANTRACYCLINES (A): A DOSE FINDING STUDY OF THE GON ("GRUPO ONCOLOGICO DEL NORTE")

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T was approved in Europe in Jan. 96 for the treatment of MBC patients (pts) previously treated with A and the recommended dose was 100 mg/m^2. After having treated some patients with dose we found excessive toxicity. We therefore designed a tribal in order to know the ideal dose (ID) of T in this group of pts. Starting with 60/70 mg/m^2 of T every 3 weeks, (depending on the cumulated dose of A), the dose was tailored in order to obtain a neutropenia nadir of G.3 (boold count on 10th day) avoiding febril neutropenia and non haematologycal toxicity G. 3-4 (except alopecia). All pts received premedication with corticoids. From Jan. 97 until March 98 we entered 49 pts into the study. Pts characteristics were: median age 60 (34-71); Karnofsky I 70% (60-100); predominant disease sites (soft tissue 4, bone 9, visceral 36); measurable disease 21, evaluable 28; refractory to A 27; potentially sensitive 22; median number of cycles previously received 12 (2-24). The median number of cycles received of T was 5 (1-17). The median highest dose received of T was 60 mg/m^2 (50-100). The median dose intensity of T (6 cycles analyzed was 21 mg/m^2/week. It was possible to find the ID in 42 pts and the median ID was 60 mg/m^2 (50-100). The overall response rate was 30% (8/22) in the potentially sensitive and 26% (7/27) in the refractory group. Median nadir of neutrophils was 500 (0-3000). There were 2 febril neutropenias. There were no toxic deaths. W.H.O. grade 3-4 non haematological toxicity; vomiting 1, diarrhoea 1, skin 1; neurologic 2. In conclusion in unselected pts with MBC previously treated with A the dose of 100 mg/m^2 of T is very toxicity ID to start with in pts heavely pretreated (that is what usually happen in our daily clinic) is 60 mg/m^2.

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"PRELIMINARY DATA OF RANDOMIZED STUDY OF INTENSIVE CONSOLIDATION CHEMOTHERAPY VS OBSERVATION IN PATIENS WITH METASTATIC BREAST CANCER WITH LOW TUMOR BURDEN SENSIBLE TO INDUCTION CHEMOTERAPY WITH PACLITAXEL AND EPIRUBICIN"

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High-dose chemotherapy in advanced breast cancer obtains the best disease free survival results in those patients with one metastatic disease location achieving a complete or partial response. A study was developed to find out the real contribution of the intensive chemotherapy to those patients. Patients will receive 6 cycles of Paclitaxel 200 mg/m^2 in 1h-infusion and Epirubicin (90 mg/m^2, 10-20 min-infusion) every three weeks. Those patients achieving CR or PR not suitable for radiation are randomized in two different arms: observation and consolidation chemotherapy (DICEP type: etoposide 150 mg/m^2/12 h days 1, 2 and 3; Cisplatin 75 mg/m^2 days 1 and 2 and ciclophosphamide 2.25 g/m^2 days 4 and 5 every 6-8 weeks). Inclusion criteria: Metastatic breast cancer involving one disease site; Performance status ECOG $\leq$ 2, age between 18 and 60 years, normal hepatic and renal functions and normal blood count. Prior anthracyclines treatment is allowed whether disease free survival is > 1 year. The objective of this study is to compare the disease free survival. The study started in Jan 1997 and to date 35 patients have been registered. The first 11 patients included have achieved 4 CR, 2 PR, 1 PD and 4 are non-evaluables. To date 2 patients have been randomized to the consolidation chemotherapy group and 1 patient to the observation arm.

The preliminary haematological and non-haematological toxicity data in the induction chemotherapy shows palmoplantar pruritus in 4% of cycles. Recruitment is ongoing until a total of 96 patients.

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EFFICACY OF FORMESTANE (LENTARON®) IN ADVANCED BREAST CANCER PATIENTS. J. M. Baena, M. J. Gómez , A. Rueda, A. Mateos, J. A. Contreras, A. Senra. Medical Oncology Department, Puerta del Mar U.H. Cádiz.

Background: In postmenopausal advanced breast cancer patients, hormone therapy is widely accepted as the treatment of choice, because of its efficacy and good tolerability compared to chemotherapy. Formestane is an effective and competitive inhibitor of aromatase, the enzyme responsible for the conversion of androgens to estrone and estradiol. The aim of this study was to evaluate the tolerance and clinical activity of formestane after tamoxifen failure.

Methods : Eighteen postmenopausal patients with advanced breast cancer resistant to first-line endocrine therapy and PS (ECOG) 0-2, were treated with formestane at doses of 250 mg i.m. every 2 weeks. Patients were evaluable for tolerance and clinical activity after 8 weeks of treatment. These patients were not enrolled in a formal research protocol and rigorous criteria could not be utilized. Improvement was defined as some objective regression of disease, stable disease was defined as no change in disease for >8 weeks on therapy without progression and progressive disease is defined as worsening of disease.

Results : Seventeen females and 1 man (postcastration) had a median age of 57 years (47-74) ; 13 patients were estrogen receptor-positive, 1 negative and 4 unknown ; the sites of metastatic disease were soft tissue in 15 patients, bone in 8 and viscera in 10 (8 with only 1 site and 10 with several sites) ; previous tamoxifen treatment for metastatic disease had been given to 17 patients, only 1 case had received LHRH-A plus tamoxifen and 11 chemotherapy. Responses : 4 (22%. 95% C.I. 6-43%) had improvement, 8 (44%. 95% C.I. 19-63%) had stable disease and 6 (33%. 95% C.I. 12-54%) had progressive disease. Four responses were observed on soft tissue (3 cases) and pulmonary nodes (1 case). The median response duration was 9 months (3-15) and the median survival was 11 months (3-23). Side effect were mild and transient. Only 3 patients complained of nausea, 2 of local side effects, 1 of headache and 1 of itch.

Conclusions : Formestane is an effective and well-tolerated approach in the management of advanced breast cancer after tamoxifen failure.

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SHORT-COURSE INTRAVENOUS PROPHYLAXIS PRIOR TO THERAPY WITH PACLITAXEL: STUDY OF TOXICITY IN 60 PATIENTS.

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INTRODUCTION:A 30% of patients treated with paclitaxel presents hypersensitivity reactions. "Standard" premedication has included 20 mg dexamethasone administered 12 and 6 hours prior to treatment with paclitaxel, diphenhydramine (50 mg) and cimetidine (300 mg) or ranitidine (50 mg). The aim of this study was to evaluate a short-course intravenous prophylactic regimen for prevention of hypersensitivity reactions.

PATIENTS AND METHODS: A total of 60 patients were evaluated. Eligible outpatients received paclitaxel (breast cancer: 37 pats; ovarian cancer 18 pats; head and neck cancer 3 pats; colangiocarcinoma 1 pat). A total of 322 courses were evaluated. The drugs and doses administered were: Breast cancer(4 Epi 75 mg/m2 + Paclitaxel 135-175 mg/m2); Ovarian cancer (CDDP 60-80 mg/m2 + Paclitaxel 135-175 mg/m2 or Paclitaxel 175 mg/m2 or Carboplatin 350 mg/m2 and Paclitaxel 135 mg/m2); Head and neck carcinoma (Paclitaxel 175 mg/m2). All patients received a short course intravenous propnylactic regimen and standard course prophylactic anti-emesis (anti-5HT3). Intravenous prophylaxis for hipersensivity reactions was administered 30 minutes prior to paclitaxel and consisted of dexamethasone (20 mg/iv), dexclorphenhydramine maleato (5 mg/iv) and cimetidine (300 mg/iv).

RESULTS:Neither severe nor low hipersensitivity reactions were observed. There were two cases of hypotension (sickness of short duration and not associated with the interruption of treatment).

CONCLUSION:This single-dose intravenous prophylaxis used to prevent paclitaxel-associated hypersensitivity reactions, is as effective as the standard regimen. And this regimen is not associated with an increase of hipersensitivity reactions. Additionally, the modified regimen is more comfortable(the standard regimen requieres to take dexamethasone both the night before and the morning of treatment)and reduces the level of stress prior to therapy with paclitaxel.

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FINAL REPORT OF A PHASE II TRIAL WITH DOCETAXEL + VINORELBINE FOR ANTRACYCLIN-REFRACTORY METASTASIC BREAST CANCER.

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Preclinical data support antitumor sinergy between taxoids and Vinca alkaloids, as might be expected from mechanism of action: taxoids, two groups of anticancer drugs acting through interaction with tubulin. We implemented a phase II trial of docetaxel (TXT) plus vinorelbine (VRB) in patients (pts) with antracyclin-refractory breast cancer. Treatment incluyed TXT 75 mg/m^2 and VRB 30 mg/m^2, both on day 1 q. 3 weeks (wks) up to progression or 6 courses. The schedule is different from previously reported trials in that there is no day 8 dose of VRB. After 14 pts had been enrolled with 10 cases of febrile neutropenia resulting in 1 death, and taking into account that this is a palliative treatment, TXT and VRB doses were reduced to 60 and 24 mg/m2 respectively.. In 36 pts treated (all but one had previous antracyclin-containing chemotherapy and 12 had prior high-dose chemotherapy with stem cell rescue), aged 30-73 years (median 52), 137 courses were given (1-6 per pt, median 5). Grade 3-4 toxicities: febrile neutropenia (16 courses, 1 lethal), stomatitis (9). There were dose reductions (mostly 20 % decrease in docetaxel and vinorelbine dose) in 50% at initial doses and 17% at 60/24 mg/m2. In the present study, the predominant toxicity of the combination which has limited dose intensity has been grade 3-4 neutropenia and stomatitis. Response rate in 32 evaluable pts (4 pts received 1 course only) was 71,8 % (Complete Response, 3 pts, Partial Response, 20, Stable Disease, 3 and Progressive Disease, 6). With median follow-up of 12 months or until death, median time to failure was 4 months (range 0-15+). Median survival was 7 months (0-23+). TXT plus VRB is a very active second line regimen in patientns with metastatic breast cancer, even after progression to high dose chemotherapy, although most responses are not durable.

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TAXOL + 4-EPIRUBICIN AS 1ª LINE TREATMENT OF ADVANCED BREAST CANCER

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Introduction and aim: Determination of antitumoral activity of the combination of Taxol plus 4-Epirubicin in patients with metastatic breast cancer, evaluating the responses obtained and their duration, as well as the toxicity of this association.

Material and Method: Up to date 9 patients were enrolled with diagnostic histology of breast cancer and normal hematologic, renal and hepatic functions, who hadn't previously received any chemotherapy for advanced disease. Treatment was 4-Epirubicin 75 mg/m^2 and Taxol 200 mg/m^2 given as a 3 hours infusion. Median age was 54 years, ranging from 40 to 64 years. Median of ECOG was 1. 5 patients were premenopausal and 4 postmenopausal. Only 2 patients were estrogen receptors +. Two patients didn't receive any previous adjuvant chemotherapy, all the rest received CMF. 4 patients received hormonotherapy previously and 3 radiotherapy. The total number of sites of disease was 23 with mean per patient of 2,5: 3 patients had 1, 2 had 2, 1 with 3 sites of disease, 2 had 4 and 1 had 5. Sites of disease in relation to organs involved: in 7 patients, lung in 7 patients, pleural in 6, bone in 2, lymph nodes in 2, chest wall in 2, suprarenal in 2, mediastinum in 1 and breast in 1.

Results: With a mean of 4,4 (2-6) administered cycles with the present schedule, the responses obtained have been 4 partial reponse, 3 stable disease and 2 progression. Response rate obtained was 44,4%. For the patients who obtained partial response the mean of sites of disease was 1,7, for the patients with stabilization 3 sites and to 3,5 sites in patients with progression. The toxicity /cycles observed over a total of 40 cycles administered was; 1 grade III neutropenia, 3 grade III nausea-vomiting , 1 grade II peripheral neuropaty, one case of neutropenic fever and 1 patient with LVEF decreased to 44% who stopped treatment . One patient died for progression disease

Conclusion: Though the sample size of patients of this study is too small, toxicity was moderate and response rate obtained in this group of patients, with multiple metastatic sites show promising efficacy.

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WHICH IS THE ADEQUATE TREATMENT FOR BREAST CANCER IN THE ELDERLY?. **R Lasso, R Bernabé, J Salvador, E Garcia-Fdez, JJ Reina, ML Delgado. Sección Oncología Médica. Hospital Juan Ramón Jiménez. Huelva. Spain.**

INTRODUCTION: Currently, the initial and adyuvant treatment of breast cancer in elderly (>70 years) is not dearly defined. The probability of benefit with an aggressive treatment in this patients group is being assumed without paying attention to other questions such as quality of life, the effectiveness vs morbility in older individuals, an the aggressiveness of the tumor in these patients. We have studied the features and outcome in a group of patients over 70 years with breast cancer.

PATIENTS AND METHODS: Thirty-nine patients, 37 women and 2 men, with breast cancer and age over 70 years were studied. The mean age was 77 years (R:70-85). The stage distribution of the patients was as follows: 1(2,5%); IIA:15(37,5%), IIB 6(15%), IIIA:3(7,5%), IIIB:12(30%) y IV:3(7,5%). The type of tumours were invasive ductal carcinoma: 32, inflammatory breast: 2, invasive lobular carcinoma: 1, mucoid carcinoma: 1, colloid carcinoma: 2 and undifferentiated carcinoma 1 pt. Receptors were positive in 15 patients (37,5%), negative in 5 (12,5%) and unknown in the rest.

RESULTS: In 20 of the patients a local treatment with radical intention was performed (mastectomy or quadrantectomy plus radiotherapy), in 4 patients no surgery was performed (3 pt. stage IIIB and 1 pt. stage IV) and in 15 patients the local treatment performed was non-radical. Regarding the systemic treatment, 32 patients received only hormonal therapy with tamoxifen, 6 patients (the two males and the 4 patients with negative receptors) had chemotherapy plus tamoxifen and two patients were treated only with chemoterapy. The chemoterapy not include anthracyclines except in the inflammatory carcinoma. Out of the 33 patients were assessed for free-interval disease, a median value of 19 months (R:3-75) and with a follow-up median value of 20 months (R: 3-111), 37 out of 39 patients remained alive.

CONCLUSIONS: There is little information available on the histologic and clinical characteristic of breast cancer in the elderly. The decision of wich is the most adecuate treatment modality needs further data to establish the subgroups of patients with higher and lower risk and to determinate a well-balanced decision, taking account into the features of both the patient and the tumour.

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CARBOPLATIN-UTEFOS-LEUCOVORIN IN THE TREATMENT OF ADVANCED BREAST CANCER

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OBJECTIVES

Asses the efficacy and security of the Carboplatin-utefos-Leucovorin regimen as second line treatment in advanced breast cancer.

PATIENTS AND METHODS

A number of cycles were administrated every four weeks according to the following schedule:
Carboplatin i.v. 100 mg/m²/week
Lederfolin i.v. 500 mg/m² day one in 2 hours.
Utefos 400 mg/12 h continuos orally administration.
Lederfolin 15 mg/12 h continuos orally administration.

19 patients were treated (ages between 34 and 76 years old) with this regimen, all of them had metastasis, mainly bone and pulmonary disease.

11 patients had received a first line chemotherapy, 5 patients two lines, and 3 patients, three or more lines. 16 patients had already been treated with anthracyclines.

Previous chemotherapy regimens were: FAC/FEC in 8 patients, CMF in 6 and hormonotherapy in all of them.

RESULTS

Of a total of 19 patients, 18 were evaluable for response, and 17 for toxicity.

A range from 1 to 12 cycles by patient were administrated(median 5). Delays for toxicity were required in 11 patients, and dose reductions in 6 of them.

Grades 3-4 toxicity were: neutropenia in 5 patients (8 cycles), anemia in 2 patients (2 cycles), thrombocytopenia 1 patient (grade 2 thrombocytopenia in two patients).Grade 2 nausea and vomits in 2 patients. There were no toxic deaths.

One complete response, and four partial responses were achieved (26.3%).

CONCLUSION

The schedule Carboplatine-utefos-leucovorin has a moderate activity as second line treatment in anthracyclin resistant advanced breast cancer. Toxicity, mainly hematologic, is low.

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SUSTAINED REMISSION OF ULCERATIVE COLITIS FOLLOWING HIGH-DOSE CHEMOTHERAPY WITH STEM CELL SUPPORT FOR HIGH RISK BREAST CANCER.

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High-dose chemotherapy with autologous peripheral blood stem cell rescue is a promising treatment with high-risk breast cancer. The profound immune consequences of myeloablative chemotherapy are poorly understood. However, there is a suggestion from single-case reports of patients with autoimmune disorders being treated with high-dose chemotherapy for solid tumors that such immunosupressive therapy may well improve the course of severe autoimmune diseases. We report a case of ulcerative colitis entering sustained remission after high-dose chemotherapy with stem cell rescue for high-risk breast cancer.

A 57-year old female with an 8-year history of ulcerative colitis, including 3 admissions for parenteral therapy, and currently with mild symptoms in spite of maintenance therapy with salazopirine, was diagnosed with invasive ductal breast carcinoma and treated with modified radical mastectomy and axillary lymph node dissection. Postoperative staging was T2 N1(49 of 49 nodes with tumor)M0. There was extracapsular invasion in 5 nodes, and massive lymph vessel invasion in the primary tumor. The patient received 3 courses of chemotherapy (FAC) followed by high-dose chemotherapy (carboplatin, cyclophosphamide, thiotepa) with stem cell rescue, and subsequent locoregional radiation therapy and tamoxifen. During admission for high-dose chemotherapy, the patient experienced grade 2 diarrhoea. Upon discharge, the patient had no digestive symptoms. She remains tumor-free and colitis-free (including barium enema) 32 months after high-dose chemotherapy.

The profound immunosupression induced by high-dose chemotherapy with stem cell rescue may well influence the course of severe autoimmune diseases such as ulcerative colitis and multiple sclerosis. Clinical trials to test this hypothesis are ongoing.

2. Locoregional Breast Cancer

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LOCALLY ADVANCED BREAST CANCER (LABC): PROGNOSTIC VALUE OF p53, HER-2/neu, EGFR AND Ki 67 EXPRESSION AND OTHER CLINICO-PATHOLOGICAL PARAMETERS.

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Background: p53, HER-2/neu, Ki 67 and EGFR are prognostic factors that have been tested in node positive and negative breast cancer, but their significance in LABC has been rarely assessed. **Design:** One hundred and sixty-eight patients with LABC, with formalin embedded tissue were studied (median follow-up 8 years). In each case, HE slides were reviewed and conventional pathological parameters were evaluated and correlated with immunohistochemical overexpression of p53 (P Ab 1801), HER-2/neu and EGFR, and Ki67 Index. Distant disease-free and overall survivals were analyzed for each parameter. **Results:** Median age of patients was 61 yr, 74% of patients were postmenopausal, 73% of tumors were locally advanced without inflammatory characteristics, 27% had inflammatory characteristics and 39% were multicentric. 59% of tumors were grade III and 52% were ER+. Patients with grade III tumors had a shorter survival ($p<0.02$) while those with ER+ tumors had a longer survival ($p=0.01$). Grade and other conventional parameters were significantly worse in these cases than in more frequent breast cancer subsets (59% of LABC tumors and 28% of stages I & II were grade III). Percentage of immunohistochemical overexpression was of 67% for p53, 52% for HER-2/neu, and 45% for EGFR. Percentage of Ki67 positive cells was less than 5 in 33%, between 5 and 10 in 19%, between 11 and 19 in 18%, and >20 in 30%. No correlation was found between new and conventional parameters. p53 and EGFR overexpression and high Ki67 index did not show any prognostic value for survival. On the contrary, patients with HER-2/neu overexpression had a shorter survival ($p=0.02$).
Conclusions: Beside histological grade, and hormonal receptor status, only HER-2/neu overexpression offers aditional prognostic information in LABC.

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PROGNOSTIC FACTORS IN INFLAMMATORY BREAST CANCER.

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Introduction: Inflammatory breast cancer (IBC) is a relatively uncommon presentation of breast cancer with a extremely poor prognosis.
Methods: 5589 breast cancer patients (pts) were treated in our institution from May-77 to May-93. 163 were clinically diagnosed as IBC (2.9%). 133 pts were treated with preoperative chemotherapy followed by surgery (119 pts) followed by chemotherapy with or without radiotherapy. Clinical and pathologic prognostic factors for disease-free (DFS) and overall survival (OS) are analyzed.
Results: The 5-year DFS and OS for the whole group were 27.5% and 38.8%. Univariate analysis for DFS and OS showed as significative favourable prognostic factors: preoperative chemotherapy with anthracyclines, objective clinical response to preoperative chemotherapy, pathological axilar node involvement, DFS: 46% N0; 38.6% N1-3; 21.6% N4-10; 13.9% N>10, and OS: 47,4% N0; 42,7% N3; 24,7% n4-10; 18,7% N> 10. Positive estrogen receptor (49,6% vs 22.6%; p=0.004). Multivariate analysis for DFS and OS showed as significative independent favourable prognostic factors: pathological lymph node involvement after preoperative chemotherapy, positive estrogen receptors, and objective clinical response to preoperative chemotherapy. DFS and OS was better in pts with pahologic response (38.5% vs 28% for DFS and 43.3% vs 30.8% for OS) but significative difference is not reached probably due to scarce number of pathological responses obtained.
Conclusion: Clinical response to preopertive chemotherapy, positive estrogen receptors, pathological axillar node involvement after preoperative chemotherapy are significative prognostic factors in IBC.

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TUMOR CELL CONTAMIATION OF BONE MARROW AND PERIPHERAL BLOOD STEM CELLS IN BREAST CANCER PATIENTS. PROGNOSTIC SIGNIFICANCE.

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The incidence and prognosis of minimal residual disease (MRD) in autologous stem cells transplants still unclear.
We estudy the incidence of CTC (contaminating tumor cells) in BM and PBSC (peripheral blood stem cells) in 26 pts.(patients) with breast cancer (BRCA): High-risk (H-R) (N=13); inflamatory (5) and metastasic (N=8), BM post-CT (chemotherapy) and PBSC (mobilization G-CSF 5 μg/Kg/día).
In the study of the prognosis: 38 pts. BRCA H-R ≥10G+, PBSCT: between April-93 and January-96. Treatment pre-PBSCT(peripheral blood stem cell transplantation): locoregional surgery + 6 cycles CT type FEC.
We use an inmunocitochemical method with the monoclonal antibody (MoAb) A45-B/B3 -FA (Epimet®,Baxter). Sensibiity $1/10^5$ and studing 2×10^6 cels. Results: 14/26 (54%) pts shows BM with CTC, 9 of them present CTC in the PBCS too (9/14, 75%). Of 14 patients without CTC in BM 3 had CTC in PBSC (3/14, 21%). There is significant correlation between the incidence of CTC on PBSC and the presence of CTC in BM (0,53 kappa, p = 0,006). In the retrospective analisys 12/38 (33%) had CTC in PBSC, 8 of them relapse (67%) and 26/38 (68%) had PBSC without CTC, relapse 6/26 (23%). Follow-up medium is 41m. And the relative risk of relapse (RR) is 2,7 (95% CI 0,1-7,8) times greater in pts with PBSC+ respect the pts without CTC in PBSC.
This retrospective study suggest that the infusion of PBSC with CTC increases the risk of relapse after intensive chemotherapy in pts with breast cancer H-R >10G+ (p=0,1).

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RANDOMIZED TRIAL OF ADJUVANT TAMOXIFEN FOUR YEARS VS TWO YEARS IN NODE POSITIVE BREAST CANCER WOMEN.

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Introduction:
The benefits of adjuvant treatment of breast cancer using tamoxifene (TMX) has been clearly proven. Most of the trials have compared treatment schedules using TMX over a one to two-year period with an untreated group. On the other hand, experimental data suggest that a greater antineoplasic effect can be expected with prolonged exposure to TMX.
Patients and methods:
We initiated a trial in 1996 to show that, administering TMX over a four-year period to node positive postmenopausal breast cancer women actually increased disease free survival (DFS)than administering TMX over a two-year period. We included 60 to 75 year old patients who we know had had a mastectomy and five or more axiliary nodes. The 20 mg/day dosis of tamoxifine was iniciated in the first two months after surgery. No other adjuvant treatments were allowed. Two hundred and eight patients, who after 2 years were disease free, were randomly chosen to terminate treatment with TMX (142 patients) o prolong treatment for two more years (146 patients).
Results:
The variables (age, size of tumour, number of positive ganglia and state of hormonal receptors in the tumour) were well balanced, and no significant differences were observed between the two groups. Over a mean follow-up period of 5 years, 113 (39.2%) patients relapsed. DFSL after 5 years was 55% in the two-year TMX group, and 66% in the four-year group (p=0.036). In a Cox proportional hazard model which included the size of the tumour (p=0.025) and the number of positive ganglia (p<0.001), the role of this treatment was even more significant (p=006). Specific survival after 5 years was 71% in the two-year TMX group nad 80% in the four-year TMX group (p=0.139).
Conclusions:
In terms of disease free survival, treatment with adjuvant TMX over a 4-tear period is better than the same treatment over a 2-year period.

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CLINICAL AND PATHOLOGIC CHARACTERISTICS IN PATIENTS WITH BRCA1/2-MUTATION ASSOCIATED TO BREAST CANCER (BC) WITH A LONG CLINICAL FOLLOW-UP.

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Introduction: It is not known if the behaviour of hereditary breast cancer (HBC) differs from that of sporadic BC. **Objectives:** To analyse clinico-pathological characteristics in patients with BRCA1/2-mutation associated to BC. These data could be useful in the manegement of HBC. **Patients and Methods:** This study includes 17 patients with BC whom a germline BRCA1/2-mutation was diagnosed. The patients proceeded from 14 different families, and four patients had no family history of BC or ovarian cancer (OC). The study of the mutations was made with SSCP (exons 2, 5, 11, 13, 18, 20, 21, 24 for BRCA1, exon 11 for BRCA2), and PTT (exon 11 for BRCA1, exons 10 and 11 for BRCA2). The mutations found in the gen BRCA1 are: exon 2 185delAG, exon 2 188delGT, exon 2 189insTGTc, exon 5 330A'G, exon 18 5263G'A, exon 24 5625G'T; in the gen BRCA2 are: exon 11 6857delAA. **Results:** The mean age at diagnosis was 37 years (28-53).The mammogram was inconclusive (3 patients with microcalcifications). Only 3/17 patients presented with involvement of axillary nodes; no patient was diagnosed with metastatic disease. All cases were infiltrating ductal carcinoma; two of them were medullar carcinoma. Histological grade was available in 13/17 cases, with grade III being the most frequent (12/13). Hormonal status was negative in 8/10 patients. The mean of follow-up is 129 months (24-224). There were three local recurrences at 17, 108 and 151 months; and two distant relapses at 15 months (complete remision) and at 92 months. There were diagnosed three contralateral BC. **Conclusions:** Hereditary breast cancer has a malignant pathological features, but the clinical behaviour could be lesser agressive than it counterpart sporadic breast cancer in the same age group.

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EARLY RELAPSE PROGNOSTIC FACTORS IN LOCALLY ADVANCED HIGH RISK BREAST CARCINOMA (HRBC) PATIENTS TREATED WITH HIGH DOSE CHEMOTHERAPY (HDC) AND PERIPHERAL BLOOD STEM CELL SUPPORT (PSCT).

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Treatment with HDC and PSCT in HRBC is a current therapy in medical oncology practice in clinical trials or out of them. Despite of it, there is no clear evidence of its benefit. A prospective phase II trail was performed with HDC and PBCT in patients (p) with HRBC in order to assess the benefit in this poor-risk population.
PATIENTS and METHODS: From may 1995 to august 1997, 32 p with HRBC (stages IIIA-IIIB) ≥ 3 metastatic lymph nodes post induction chemo, were treated with HDC: 29p with STAMP V (cyclophosphamide 6.000 mg/m2, tiothepa 500 mg/m2 and carboplatin 800 mg/m2) plus PSCT support. 4 p were treated with CEP (CDDP 150 mg/m2, VP-16 1600 mg/m2, cyclophosphamide 6.000 mg/m2) without PSCT. 21p were stage IIIB and 11p IIIA; 28/32p (87%) had received induction chemo antracyclines. All patients underwent surgery and, after HDC received RT plus tamoxifen when hormonal positive receptors. The following variables were analysed: clinical stage, tumour size, clinical response to induction chemo, axilar N2 involvement, number of metastatised axilar nodes after induction chemo and breast residual disease.
RESULTS: Median follow-up is 26 m. (12-48) and overall survival (surv) at 2.5 years is 54% (median 33m. (CI: 28-39)). There were 3 toxic deaths. Mean number of involved nodes after induction chemo was 11 (3-30) 3/28p (10%) showing only residual microscopic disease. 13/28p (43%) had early relapse with median survival time since HDC of 13m (2-21). All of these patients had stage IIIB and presented systemic progression. Median survival after systemic relapse was 5 m (1-18m). At univariate analysis only the number of metastatic axilar lymph nodes after induction chemo predicted short term outcome: median survival of patients with (≤ 5 involved nodes, mean 4 (3-5)) was 43m, 2.5 years survival 91%. In patients with > 5 involved nodes (M 16 (9-32) median survival was 30m. and 2.5 years surv 38% (p=0.05).
CONCLUSIONS: Patients with HRBC and poor response to induction chemotherapy (more than 5 involved axilar lymph nodes) had a dismal short-term prognosis despite consolidation with HDC.

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BRCA-1 Mutations in early-onset breast cancer.

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The aim of this study is to determine the frequency of BRCA1 mutations in a population-based sample of young women with breast cancer (BC), who were not selected on the basis of family history, and the correlation with epidemiological, clinical and histopathological features.

Methods: We studied 102 women in whom BC was diagnosed younger than age 40 and who lived in our area. After informed consent, we obtained family history and epidemiological information in an individual interview. Clinical and histopathological characteristics was retrieved from a clinical history. Genomic DNA was extracted from blood samples and germ-line BRCA1 mutations were studied by polymerase chain reaction (PCR) and single-strand conformation polymorphism analysis (SSCP) in exon 2, exon 11.26 and exon 20.

Results: Fourteen mutations (13.7%) were identified. Six in exon 2, four in exon 11 and four in exon 20 (now undergoing sequencing).Four of the mutations were found among 38 women with a positive family history (10.5%) and ten of them belong to 64 women who reported no family history of BC (15.6%).Median age was 36 years (range 22-40).

Conclusion: The distribution of BRCA1 mutations among our study population was not related with a positive family history.These results suggest that other genetic or non genetic factors could be implicated in the familial clustering of breast cancer.

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HIGH-DOSE CYCLOPHOSPHAMIDE, CARBOPLATIN AND ETOPOSIDE (CCbE) WITH AUTOLOGOUS STEM CELL RESCUE FOR BREAST CANCER. FEASIBILITY AND TOXICITY STUDY IN 90 PATIENTS.

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Purpose : We evaluated the feasibility and toxicity of CCbE together with peripheral blood stem cell (PBSC) rescue in 90 breast cancer (BC) patients (p).

Methods : Sixty one p had a stage II-III with ≥6 nodes, 8 p had a stage III with ≥4 nodes after neoadjuvant chemotherapy (CT) and other 21 p had a metastatic disease with low tumoral burden (one or two sites) and in response after the induction chemotherapy. Apheresis were begun on the 5th day after receiving daily G-CSF (after the 3rd or the 4th cycles of adjuvant CT) or after a priming dose of cyclophosphamide and G-CSF, aiming at to minimum number of 2,5 x 10^6/kg CD34+ cells. In 16 p a positive selection of CD34+ cells was performed. CCbE consisted of cyclophosphamide 2,5 gr/m^2/day (days -5, -4), carboplatin 500 mg/m^2/day and etoposide 200 mg/m^2/12 hours (days -5, -4, -3).

Results : They had a median (m) age of 42 years (23-60). Estrogen receptor was positive in 35 p. The operable stage II-III BC p presented a m number of 10 positive nodes (6-44). In locally advanced BC, the m number of nodes was 10 (5-13). In the metastatic disease setting the sites of metastasis were as following : 10 in bone, 5 in soft tissues, 3 visceral metastases, 2 bony and visceral and 1 in soft tissue and visceral. Eleven p were enrolled with complete response and the other 10 p showed a partial response. The mean of obtained CD34+ cells was 6,1±0,58 x 10^6/kg. P mobilized with cyclophosphamide obtained a mean number of CD34+ cells comparable to those mobilized with G-CSF (7,8±1,74 vs 5,8±0,62 x 10^6/kg. P>0,2). The p purged received the lowest amount of progenitors (2,17±0,36 vs 5,14±0,53 x 10^6/kg. P<0,001). The grade III-IV toxicity consisted on vomiting in 54 p (60%), hepatic in 41 (45%), diarrhea in 14 (16%), mucositis in 10 (11%) and cutaneous in 1 (1%). M days to 0,5 and 1,0 x 10^9/L neutrophil was 11 and 12 and to 20 and 50 x 10^9/L platelet was 10 and 14 respectively. Two RBC and 2 platelet units were transfused per p. The m hospital stay was 22 days (17-37). Those p who received G-CSF obtained a significantly faster rate of granulocyte engraftment (10,9±0,26 vs 14,4±0,5 days. P<0,05). Transplant-related mortality was encountered in 2 p (sudden death and septic shock).

Conclusion : This combination high-dose CT with PBSC support is a feasible option for the treatment of BC patients and its toxicity results moderate.

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INFLAMMATORY BREAST CARCINOMA (IBC). CLINICAL OR PATHOLOGICAL DIAGNOSIS?

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Introduction: Inflammatory breast carcinoma diagnosis is based in typical clinical symptoms presence. Clinical signs are not always associated with pathologic characteristics (subdermal lymphatics involvement). If exclusively pathologic findings without clinical symptoms are sufficient for IBC diagnosis (occult inflammatory carcinoma)(OICB), is still controversial.

Methods: 163 clinically diagnosed IBC patients (CIBC) and 99 OICB diagnosed between May- 77 and May 93 were analyzed. The following clinical and pathological characeristics were anlyzed: age, menopausal status, clinical axillar node involvement, symptoms duration before diagnosis, grade, estrogen receptors, presence of metastasis at diagnosis, local recurrence, metastasic dissemination,disease-free (DFS) and overall survival (OS).All these characeristics were compared between both groups.

Results: Median age was significatively lower in CIBC (52.3 y vs 63.8 y)(p<0.001). Symptoms duration before diagnosis was also significatively shorter in CIBC (3.4 m vs 6.8 m) (p>0.0001). Visceral(36.2% vs 17.2%)(p=0.001) and CNS (7.4% vs 1%)(p=0.02) was significatively more frequent in CIBC. Negative estrogen receptors were more frequent in CIBC (34.9% vs 65.1%)(p<0.004). The 5-year DFS (25.6 vs 51.6%) (p<0.0001) and OS (27% vs 60,2%) (p<0.001) were shorter in CIBC.

Conclusion: CIBC (either when subdermal lymphatics involvement is present or not) must be clearly differenciated from OIBC. Prognosis of CIBC patients is poorer, so this two entities should be cleraly differenciated when therapeutic results are reported.

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INFLAMMATORY BREAST CANCER PRONOSTIC FACTORS FOR OUTCOME OF RESPONSE AND RELAPSE.

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Introduction: Inflammatory breast cancer (IBC) represent a group of patients with a poor prognosis.

Patients and Methods: From 1977 to 1983, 145 patients with clinical criteria of IBC treated with induction chemotherapy and surgery (119 patients) + adjuvant chemotherapy ± radiotherapy were analyzed. Several prognostic factors in realtionship with response and relapse were evaluated: age, hormonal status, inflammatory signs extension, estrogen receptors (ER), dermal lymphatic invasion (DL), pathological and clinical lymphatic node invasion, and induction chemotherapy scheme.

Results: Clinical objetive responses were observed in 118 patients (81,4%) with 2 complete response. Predictive factors of clinical response were: ER +, and absence dermal lymphatic invasion. Pathologycal response of 119 pts who had resection after chemotherapy was studied. 22,6% pathologycal breast response and 20% axillary lymph node response was obtained. The overall pathologycal response in both breast and axillary lymph node was only 10,9%. None analyzed variable showed stadistical significance in relationship with pathological response. Age and antracyclin treated group trends to stadistical significance.

126 out of 145 pts relapsed. The main localization was locoregional 22,6%, visceral 41,6% and bone 26,3%. Relapse was more frequent in patients with ER negative, not treated with antracyclin chemotherapy and with no pathological response after chemotherapy. Local relapse was higher in DL +, patients not treated with antracyclin , non pathological response and more than four lymph node axillary invasion after chemoterapy and patients without radiotherapy.

Conclusion: knowledge of clinical and pathologycal prognostic factors can provide information about relapse probability and treatment response in this high risk group of patients.

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ADJUVANT CMF IN NODE POSITIVE BREAST CANCER. ANALYSIS OF 173 PATIENTS.

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Introduction : The CMF chemotherapeutic regimen is widely used in the adjuvant treatment of the nodal positive breast cancer. Bonnadona et al. showed overall survival (OS) and disease free survival (DFS) improvement compared with no additional treatment after radical breast surgery. Five and ten years OS were 78 and 55% in treated patients. Five and ten years DFS were 59 and 43 % (NEJM 1995 ; 332 : 901-6).

Purpouse : To evaluate retrospectively OS and DFS and toxicity in patients treated in our institution since 1980.

Material and methods : We analized 173 patients with surgically resected breast cancer with axilar nodal involvement treated with adjuvant CMF beetween 1980 and 1996. Other treatments (surgery, radiotherapy or hormonotherapy) were applied using well-established protocols. Characteristics of the 168 evaluable patients were : median age : 49 years (27-77) ; pre-menopausal status : 58 % ; conservative surgery : 27 % ; median tumor size : 25 mm. (3-120) ; median number of involded nodes : 3 , N+ 1-3 : 65 % , N+ 4-9 : 22 % , n+ > 10 : 10 %. CMF-regimen used : oral : 57 % , intravenous / 21: 37 %.

Results : The median follow up is 52 months. Five and ten years overall survival are 85 and 56 % respectively. Disease free survival are 56 and 46 % at 5 and 10 years. Grade III or IV toxicity were observed in 13 % of the patients. We have observed 7 second neoplasms (all were carcinomas), in 7 patients (4%). Two of them were in the contralateral breast.

Conclusions: The 5 and 10 years OS and DFS in our study are similar to that obtained by Bonnadonna in its clinical trial. Treatment tolerance is good. The main toxicities were haematological and emesis (both < 10% grade III-IV). We haven't observed second neoplasms induced by treatment.

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SENTINEL NODE DETECTION IN BREAST CANCER PATIENTS

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Introduction Axillary lymph node metastasis is the most important prognostic factor in breast cancer patients. Nowadays, axillary lymphadenectomy is the treatment of choice in these patients. The sentinel lymph node (SN) concept presumes that primary tumour drains to an specific lymph node within a regional lymphatic basin. It has the highest likelihood to present lymph node metastasis from primary tumour. By performing a biopsy of SN we could predict the stage of all other regional lymph nodes, avoiding unnecessary lymphadenectomies.

Method We estudied prospectively 24 patients with breast cancer (10 in rigth breast, 13 in left breast and 1 bilateral). Seven tumours were categorized as T_1, 8 as T_2, 9 as T_3 and 1 as T_4. The day before surgery a lymphoscintigraphy with 74 MBq of ^{99m}Tc-nanocolloid was performed. Twenty minute dynamic flow images were obtained immediately after radiotracer injection followed by static images at 2 and 16 hrs. The first lymph node identified was considered as SN and was marked on the skin. During surgical procedure a hand-held gamma probe and blue day were used in order to locate more accurately the SN.

Results SNs were successfully identified in 21/24 patients (87.5%). The three cases in which SN was not detected, pathological study demonstrated a lymph node close to primary tumour. In the other patients 28 SNs were detected, being 6 of them metastatic (21%) (6 patients). A total amount of 254 axillary lymph nodes were harvested in 24 patients, being 17 of them positive for breast cancer, corresponding to the 6 SN positive patients. It was found only a false negative SN in a patient with T_4 tumour.

Conclusions These preliminary results support that the SN detection technique could be useful for lymphadenectomy patient selection in early stage breast cancer.

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OCCULT EPITHELIAL TUMOUR CELLS DETECTED BY IMMUNOCYTOCHEMICAL ASSAY IN BONE MARROW OF BREAST CANCER PATIENTS OBTAINED AT DIAGNOSIS

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INTRODUCTION. Immunocytochemical diagnostic techniques seem to be more sensitive than conventional histology and cytology techniques in showing the presence of carcinoma cells in bone marrow (BM) samples from breast cancer patients.

MATERIAL AND METHODS. A bone marrow aspirate from 46 high-risk breast cancer patients received in our Institution was studied from May-95 to Sept-98. Tumour cells of epithelial origin were analysed using the monoclonal antibody CK19 against cytokeratin (CK) with the alkaline phophatase anti-alkaline phosphatase method (APAAP). The presence of positive cells was compared in patients grouped by age, stage and positive/negative estrogens or progestagens receptors.

RESULTS. All samples were negative by conventional methods. Preliminary results showed CK positive tumour cells in 39 % (18/46) of the analysed samples. This positivity was not related to any of the previous studied factors. Nevertheless, when patients are segregated by stage, a higher proportion of CK positive specimens was described among metastatic patients (75% vs 30% in stage II/III).

CONCLUSION. The APAAP technique is more sensitive than histologycal or conventional cytological techniques. A higher detection of positive CK cells in BM samples from patients with advanced stages (metastatic or stage IV) was not significant but there is a statistical trend in this relationship.

Unfortunately, the immunocytochemical method is laborious and follow-up studies are required to assess its prognostic relevance.

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MORBIDITY ASSOCIATED TO EXTENSIVE LOCORREGIONAL RADIOTHERAPY (RT) AFTER HIGH-DOSE CHEMOTHERAPY (HDCh) WITH PERIFERAL-BLOOD STEMCELL (PBSC) RESCUE IN HIGH-RISK BREAST CANCER (BC).

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BACKGROUND: Following the observation by Peters that patients (pt) with high risk stage II BC (>10 + nodes) had a 33% locorregional relapse rate following surgery and HDCh, and that in a subsequent group of pt given Rt post HDCh the rate dropped to <10%, locorregional Rt is routinely scheduled for all high-risk BC pt inmediately after HDCh. Little is known about the toxicity of extensive Rt given early after hematological engraftment post HDCh.

METHODS AND RESULTS: We included 31 BC pt from March 1996 to September 1998, 7 pt (22,5%) with stage II (4-10 + nodes); 7 pt (22,5%) with stage II (>10 + nodes); and 17 pt (54,8%) with stage III, treated with HDCh (CTCb as described by Artman) after complete tumor resection either by mastectomy (29 pt) or lumpectomy (2 pt), with axillary node resection in all cases. All pt were scheduled to receive 45-55 Gy in 5-6 weeks to the chest wall (or breast in lumpectomy) plus axillary, internal mammary and supraclavicular lymph nodes.

Median time from PBSC infusion to initiation of Rt was 49 days (range 19-84), and to completion was 92 days (range 55-138). Acute toxicities were esophagitis in 4 pt, epithelitis in 20 pt, and acute radiation pneumonitis in 2 pt. No hematological grade 3-4 toxicity was observed. Rt had to be temporarily interrupted in 5 pt (2 due to acute pneumonitis, and 3 due to mild fever of unknown origin) for a median of 14 days; however, all pt received full doses of Rt. Delayed toxicities included radiation pneumonitis in 8 pt (7 with radiological evidence and 6 with symptoms).

DISCUSSION: Rt for high-risk BC patients can be given early after HDCh without compromising life or hematological engraftment.

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LOSS OF HETEROZYGOSITY AND MICROSATELLITE INSTABILITY IN BREAST CANCER.

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Background: Microsatellites are short repetitive nucleotide sequences that, through mutation, can undergo either expansion or contraction (MI). Allelic imbalance or loss of heterozygosity (LOH) studies have been used extensively to identify regions on chromosomes that may contain putative tumor supressor genes. MI and LOH may play a role in carcinogenesis. We investigate the incidence of MI and LOH in a series of breast carcinomas on chromosomes 11, 13 and 17, using 7 polymorfic microsatellite markers.

Methods: Fluorescent polymerase chain reaction (PCR) coupled with DNA fragment analysis in an automated DNA sequencer. We analized 40 paired breast cancer-peripheral blood DNA samples at seven different microsatellite loci (D11S904, D13S267, D13S153, D17S579, INT2, NM23 y ATM).

Results: Twenty of our 40 tumors (50%) exhibited LOH, while 11 specimens (27,5%) exhibited MI in at least one microsatellite marker. These MI and LOH data were analysed using a range of clinicopathological parameters. Tumors displaying MI with no evidence of LOH and tumors exhibiting MI and LOH belonging to stage II and III were found, however none were at stage I. These data suggest that MI may be an early event in mammary tumorigenesis whereas LOH occurs at a late stage

Conclusions: These findings indicate that MI and LOH are present in breast cancer and although the mechanism of action has yet to be elucidated, may play a role in breast carcinogenesis.

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HIGH-DOSE CHEMOTHERAPY (CT) AND PERIPHERAL BLOOD STEM-CELL TRANSPLANTATION (PBSCT) IN HIGH-RISK BREAST CANCER (BC). HOSPITAL UNIVERSITARIO LA FE EXPERIENCE.

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INTRODUCTION: Although no randomized trial has compared it with conventional therapy, high-dose CT plus PBSCT may benefit some subsets of patients with high-risk, localized BC.

PATIENTS AND METHOD: Between 1994 and 1998, 37 BC patients underwent PBSCT. Inclusion criteria were: 1) stage II and III disease with ≥10 involved axillary nodes (n=22); 2) locally advanced or inflammatory BC with >3 involved nodes after neoadjuvant CT (LAIBC, n=10); and 3) solitary relapses after curative therapy or stage IV disease in complete response after conventional CT (n=5). Neo- and adjuvant CT was FEC-75 (6 courses); for patients with stage IV or relapses, FEC-75 or CDDP-Paclitaxel were used. Cytaphoresis were performed after the 3rd course with G-CSF mobilization. The conditioning regimen was STAMP-5 (CTX-Tiothepa-CBDCA). After PBSCT, local irradiation and hormonal therapy were considered. The present analysis included the first 25 transplants (>18-month follow-up).

RESULTS: The median patient age was 45 years (26-61). Stage classification: II-B (4 patients), III-A (10), III-B (7) and IV (4; bone metastases in 3 cases, lymph node metastases in 1). The interval from diagnosis to PBSCT was 9 months (6-13). A median of 3.4 (0.24-16.37)x10^6 / CD34+ cells/kg were infused. Hematological recovery data: N>500/ul day 11 (7-18), N>1000/ul day 13 (10-23), P>20.000/ul day 10 (2-33) and P>50.000 day 13 (3-45). One patient died from lung bleeding. Median hospitalization time was 20 days (14-30). After a median follow-up of 30 months (18-59), 3-year disease-free and overall survival are, respectively (standard error: 18%) of 52 and 71% for N+≥10 BC, 32 and 42% for LAIBC, and 0 and 33% for stage IV BC (overall, 36 and 55%).

CONCLUSIONS: PBSCT-related toxicity is mild in BC. In comparison with historic controls, these results seem acceptable for high-risk operable BC, modest for LAIBC and disappointing for relapsed or stage IV disease.

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ADJUVANT CHEMOTHERAPY FOR LOCALY ADVANCED BREAST CANCER INVOLVING 4-9 AXILARY LYMPH NODES WITH DOSE INTENSITY FAC REGIMEN.

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Aim: to evaluate the therapeutic activity, feasibility and tolerance of high-dose intensity FAC regimen in locoregional breast cancer patients (pts) with 4-9 node positive.

Methods: 40 pts were treated with cyclophosphamide 600 mg/m^2 iv adriamycin 60 mg/m^2 iv and methotrexate 600 mg/m^2 iv on day 1 every 3 weeks for 6 cycles. All pts. Received radiation therapy and adyuvant Tamoxifeno if hormonal recepto (HR) was positive. Mean age was 49 years. Twenty pts. (50%) were premenopausal HR positive: 49%.

Results: the median follow-up was 36 month 6 pts. have died and 9 pts. have experienced recurrences. Relapse-free survival at a follow up of 24 and 36 month was 91% and 75% respectively. A total of 230 cycles were infused. The relative dose intensity (RDI) was >95% in 21 pts. In this group 2 pts. have experienced recurrence. RDI 90-95%:12 pts. with 4 recurrences. RDI <90%: 7 pts with 3 recurrences. Two pts. developed neutropenic fever. There was one case of congestive heart failure. Main toxicities (WHO graded) observed by cycles were as follow:

	GRADE I	GRADE II	GRADE III	GRADE IV
CARDIAC	2	-	-	1
NAU./VOMITING	13	16	11	0
STOMATITIS	1	4	3	-
ANEMIA	10	5	-	-
LEUKOPENIA	23	1	2	0
NEUTROPENIA	15	15	5	2

Conclusion: this dose-intensity FAC regimen was generally well tolerated, may be administered over multiple cycles in ambulatory patients and is highly effective, in terms of relapse-free survival in these patients.

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TREATMENT WITH TOREMIFENE IN WOMEN > 65 YEARS WITH T > 3 CM AND + HORMONAL RECEPTOR BREAST CARCINOMA. PHASE II STUDY (PRELIMINARY RESULTS).

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Objectives:
Primary: To evaluate the response rate (RR) of toremifene on primary breast tumors with positive hormonal receptors in patients > 65 years.
Secondary: Mean time to progression, mean duration of response and rate of conservative surgery following neoadjuvant therapy.

Materials and methods:
Inclusion criteria: Histologically or cytologically proven breast cancer. Positive estrogen or progesterone receptors. Age > 65 years. Karnofsky performance status > 50. Presence of any of the following criteria: T > 3 cm, T4a, T4b, T4c, or N2. The exclusion criteria were patients with M1 and male patients.
Treatment: Continuous toremifene, 60 mg/day PO. Six to 24 months after the initiation of therapy, an appropriate surgical treatment is recommended according to tumoral size and patient's status. If a response is demonstrated, treatment will be continued up to 5 years.

Results:
Seventeen patients have been enrolled from October '97 to October '98. Mean age, 77 (68-84). The mean maximum radiologic tumoral ϕ was 38 mm (25-70). Patients: 7 T2; 3 T3; 6 T4b and 1 N2. The mean duration of treatment was 5.4 months (0-12). At 3 months of treatment, 15 patients were clinically evaluable with 3 partial responses, 11 stable diseases and 1 progression. At 6 months, 6 patients have been radiologically assessed, with a RR of 83% (5/6).
Only one case of grade 2 vaginal dryness has been reported as adverse effect. Results for longer enrollment and treatment periods will be reported in April '99.

Conclusions:
Toremifene treatment provides a high percentage of radiologic responses with minimal toxicity. A more prolonged treatment period will allow us to evaluate whether longer treatments facilitate conservative surgery and a good systemic control of the disease.

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HEMATOLOGICAL TOXICITY OF RADIOTHERAPY AFTER HIGH-DOSE CHEMOTHERAPY (HDC) WITH STEM CELL TRASPLANT (SCT)IN BREAST CANCER PATIENTS.

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Introduction: Hematologic toxicity of radiotherapy in patients treated with HDC and SCT is highly suspected. Exact knowledge of the problem is important in order to stablish better schedule for radiotherapy.

Patients and methods: Hematologic evolution of 43 breats cancer patients treated with radiotherapy after HDC and SCT has been stablished. (Chemotherapy scheme was STAMP V). Radiotherapy treatment was iniciated after HDC and when sufficient hematologic recovery was reached. Total dose Radiotherapy was 50 Gy with cobalt and electrons all along a month of treatment. Hemogram was performed at the beginning , 2^{nd} and 3^{rd} week, and at the end of treatment.

Results: Median age was 47 years. All patients were treated as adjuvant high-risk (29 N+>10; 14 : locally advanced or inflammatory). Median values are shown in the table:

	Initial	2^{nd} week	3^{rd} week	End
Leucocites ($x10^9$/l)	3.8	2.8	2.6	2.9
Hemoglobin (g/l)	11.7	13.5	11	11.3
Platelets ($x10^9$/l)	163	137	117	116

Radiotherapy after HDC and SCT has an impact on leucocites and platelets numbers. A 75% value reduction is observed. Hemoglobin is scarcelly affected. This results may be important to stablish the best moment to iniciate radiotherapy treatment after HDC and SCT.

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CONSERVATIVE TREATMENT IN INITIAL STAGES OF BREAST CANCER

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The surgery with breast conservation (CS) and radiotherapy (RT) has demonstrated to be as effective as mastectomy in the control local treatment of initial stages of breast cancer (BC).

Purpose: To evaluate the inicidence of local relapses in the patients with BC with initial stages treated with CS and RT.

Material and methods: Retrospective study of the patients treated with CS/RT in our hospital between and june 1998.

Results: Were evaluated 139 patients.the median of age was 55 (range: 35- 85). A 32% of patients were premenopausal and 68% were postmenopausal. A total of 61 were stage I, 56 stage IIA and 12 stage IIB.The mean of the tumour size was 2.25 cm. The most frequent surgery was the tumorectomy and axillary dissection. During the follow-up period, relapsed 3 patients (2.27).

Conclusions. The conservative surgery with radiotherapy is an effective treatment in the initial stages of breast cancer, with low rate of local relapses.

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CONCURRENT CHEMO-RADIOTHERAPY TREATMENT FOR POST-CONSERVATIVE SURGERY PATIENTS OF BREAST CANCER; FIVE-YEARS OBSERVATION

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PURPOSE: Patiens of breast cancer treated by conservative surgery run an important risk of metastasis relapse, locally as well as systemically, as and optimun sequence between chemical and radiotherapy is unclear. From 1985 to 1992, we simultaneusly initiated a treatment protocol of both therapies.

METHODS: 73 pts. with a median age of 49yrs. (28-77) with the stage I(13%),II(78,3%),and III(8,7%) presenting risk of metastasis relapse received chemotherapy (CMF/CAF) concurrently radiotherapy (cobalto-60).Toxicidity was acceptable 2 pts hematological G-IV(OMS).

RESULTS: At this time (December 1997) 73,6%(54) pts are free of illness and 26,4%(19) have shown a relapse of metastasis. Locations: 7pts with breast and/or regional lymph nodes, 6 pts with distant metastasis and 6 pts with local and distant metastasis. Median time for freedom of illness was 63 moths(6-109) and median global was 73 months(11-126).

CONCLUSIONS: Give that we have observed that simutaneous-treatment of chemo-radiotherapy offers a good response rate and on acceptable toxic level, we belive it to be advantageous for post-conservative surgery patients of breast cancer, to whom local and systemic metastasis relapse present risk

3. Lung Cancer—Head and Neck Cancer

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CISPLATIN PLUS 5-FLUOROURACIL vs CISPLATIN PLUS VINORELBINE AS NEOADJUVANT TREATMENT IN LOCALLY ADVANCED HEAD AND NECK CANCER. PRELIMINARY RESULTS OF A RANDOMIZED TRIAL.

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INTRODUCTION: Preliminary results of a randomized trial are presented in order to compare two chemotherapy schedules, cisplatin plus 5-fluorouracil (PF) vs cisplatin plus vinorelbine (PV) as neoadjuvant treatment for patients with locally advanced head and neck cancer.

METHODS: Between 10/96 and 10/98, 29 patients were included in the study, and randomized to receive PF (cisplatin, 100 mg/m2 e.v. d1 plus 5-fluorouracil, 1000 mg/m2 continuous e.v. infusion d 1-5) or PV (cisplatin, same dose plus vinorelbine, 30 mg/m2 e.v. d 1,8). After 3 chemotherapy cycles, patients received local treatment (surgery and/or radiotherapy).

Patient features: 25 males and 4 females; median age of 50 years (range, 43-73). The histopathology was squamous cell carcinoma in 26 cases, undifferentiated carcinoma in 2, and cystic adenoid carcinoma in 1. Twenty-five patients were classified stage IV and four stage III disease.

RESULTS: Fifteen patients received PF and 14 PV. In PF arm, there were 1 complete (CR) and 9 partial responses (PR), 1 stable disease (SD), and 2 progressions (PD), while 2 patients were not evaluable (NE). The objective response rate was 67%. The median of courses delivered was 3 (range, 2-4). WHO grade 3-4 toxicity: mucositis in 3 patients, emesis in 4, leukopenia 4, and trombopenia in 2. Four patients received radiotherapy and 5 radiotherapy plus surgery. Local treatment improved 3 PR into RC. Eight patients are alive (4 of them disease-free) and 7 have died (5 due to PD and 2 due to myelotoxicity.

In PV-treated group, there were 3 CR, 7 PR, 3 PD, and 1 NE. The objetive response rate was 71%. Median number of courses: 3 (range, 2-4). WHO grade 3-4 toxicity: mucositis in 3 patients, leukopenia in 6, trombopenia in 1, and anemia in 1. Eight patients recieved irradiation and 2 surgery plus irradiation. Local treatment turned 1 PR into CR. Nine patients are still alive, four of then disease-free, while 5 have died (four due to PD, and 1 due to myelotoxicity).

CONCLUSIONS: a) PV is at least as effective as PF in the treatment of locally advanced head and neck cancer; b) digestive toxicity is greater for PF and myelotoxicity is greater for PV; c) serious adverse infections are similarly frequent in both arms; d) PV administration is easier than that of PF.

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CORRELATION OF p53 ONCOPROTEIN EXPRESSION WITH CHEMOTHERAPY RESPONSE IN SMALL CELL LUNG CARCINOMAS

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Introduction: p53 oncoprotein is essential in cell cycle control, cell cycle arrest and induction of programmed cell death, also called apoptosis in DNA damaged cells. Mutations in p53 gene are very frequent in small cell lung carcinoma (SCLC). Many studies correlate p53 mutations with chemoresistance in many neoplasms.

Methods and Material : 1.-Analysis of p53 expression by immunohistochemistry in paraffin-embedded tissue of 50 patients with small cell lung carcinomas (SCLC) 2.- Correlation of the results of the analysis with the clinical and evolutive features

Results: 1.- Hiperexpression of p53 (> 15% nuclei cell stained) was observed in 23/50 patients. 2.- Correlation with clinical characteristics (age, sex, performance status, weight loss, LDH serum levels, stage, number or localization of metastases) was not observed. 3.- Strong correlation of p53 status and chemotherapy response (p=0.00212) was observed: if p53 was abnormally accumulated in the cell nucleus the possibility of having complete responses to chemotherapy was lower than if p53 was normally expressed. 4.- p53 and status of illness were independent prognostic factors of chemotherapy response in multivariate analysis.

Conclusions: The study confirms the role of p53 in the chemoresistance phenomena in SCLC. The sistematic detection of p53 by immunohistochemistry could be useful in the clinical practice. We could then select those patients where an abnormal p53 expression could predict a worse response to standart treatment and we could choose other treatment strategies.

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PHASE I-II STUDY CONCOMITANT TAXOL AND CARBOPLATIN WITH RADIOTHERAPY IN ADVANCED HEAD AND NECK CANCER. PRELIMINARY RESULTS. A. Jaén, M. Martos, E. González, B. Medina, F. Sánchez, A. Cabrero, I. Porras, N. Moliedano, M. Capllonch, M. Fernández, A. Lozano, MA. Casanova. S. Oncology. Hospital General "Ciudad de Jaén". Jaén.

Introduction: Surgery and RT for advanced head and neck cancer yields poor results. Phase I-II study of the carboplatin and taxol combination has demonstrated the efficacy of both drugs as radio-sensitizers. The aim of our study was to determine the efficacy both drugs used concurrently with RT in terms of response rate and toxicity profile in advanced head and neck cance (HNC).

Materials and methods: From Dec 96 to Jan 98 25 patients were enrolled. Median age was 59,3 (41- 74) years, PS: 0-1. Sites of primary tumor nasopharynx 2, larynx 10, oropharynx 11, and unknown 2. The treatmen administered was: Taxol 175 mg/m2 day 1 and Carboplatin AUC=6 day 1 every 3 weeks through 2 induction cycles; followed by Taxol, according to th dose escalation (see Table) toghether with Carboplatin AUC=2, weekly concurrently with RT Co 60 (50 Gy). Subsequently the Carboplatin dose wa reduced to AUC=1, according to toxicity grade 4.

Results: Seventeen patients were assessable for response and toxicity An OR of 71% was obtained with CR 23% and 47% PR. Grade 3-4 mucositi was the main toxicity 58%, 1 patient presented grade 3 neurotoxicity, grade 4 neutropenia 41%. Another toxicities (radiodermitis, nausea and vomiting were low. Deaths due to toxicity were not observed. At a median follow-u duration of 14 months, 10 patients progressed, there were 6 local and 4 distal relapses with a SG 53%.

Levels (n= n° patients)	T(mg/m2)	CBDA	RT (Gy)	G3-4 Tox Hem	G3- 4 Tox.Non Hem
1 (n=6)	40	AUC=2	50	1	4
2(n=3)	45	AUC=1	50	2	0
3(n=4)	50	AUC=1	50	2	3

Conclusions: Concomitant taxol, carboplatin and RT achieve ar acceptable overall response rate in spite of high toxicity. This study contin for necesary accrual.

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PHASE I/II STUDY OF CDDP+VINORELBINE+ GEMCITABINE IN NON-SMALL CELL LUNG CARCINOMA (NSCLC).

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Gemcitabine and vinorelbine, two active agents in NSCLC, were combined with CDDP in a phase I/II study to determine the MTD and the activity of this regimen. Eligibility criteria: non-surgical NSCLC, adequate renal, cardiac, neurologic and hematologic functions, PS ^{3} 60, measurable or evaluable disease, and informed consent. Treatment: Vinorelbine followed by gemcitabine (both by iv infusion) was given on d 1 & 8, and CDDP at a fixed dose of 50 mg/m^2 on d 2 & 9, q 3 wks as outpatients. From Jan 98 to Sep 98, 36 pts entered the study: M/F 33/3, median age 59 yrs (33-73), PS 80 (60-100); stage IIIA 8, IIIB 10, IV 18; squamous 17, adenoc. 17, anaplastic 2; median no. of cycles 3 (1-7). Dose levels (mg/m^2):

Level	Pts	Cycles	Vinorelbine	Gemcitabine	G4 tox. (pts)
I	8	27	25	1000	1
II	18	60	25	1250	4
III	6	19	30	1000	5
IV	2	2	25	1500	0

Toxicity (WHO grading, no. of pts) in 33 pts: hemoglobine G2 9, G3 7, G4 1 (level III); granulocytes G2 1, G3 10, G4 10 (1 in level I, 4 in II, 5 in III), platelets G2 5, G3 3, G4 5 (1 in level I, 3 in II, 1 in III), neutropenic fever 3 (with 1 toxic death at level III); nausea/vomiting G2 10, G3 2; alopecia G2 1, G3 1; hepatic (always reversible) G1 2, G2 1, G3 1; neurologic G1 4, G2 2; cutaneous G1 2, G3 1; asthenia / moderate flu-like syndrome 17. Objective remissions: 17/30 (56%, 95% C.I. 36%-76%), including 2 pathologic CR. Hematologic DLT has been found in 5/6 pts at dose level III, and the study continues at dose level IV.

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PHASE II TRIAL OF GEMCITABINE, IFOSFAMIDE AND CISPLATIN IN THE TREATMENT OF ADVANCED NON-SMALL CELL LUNG CANCER. FINAL REPORT.

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We present the final results obtained with the combination of gemcitabine, ifosfamide and cisplatin (GIP) in the treatment of patients with locally or metastasic non-small cell lung cancer (NSCLC).

Patients and Methods: Between march 1996 and december 1997 sixty patients were included in this study, 59 were evaluable for response. They were 56 males and 4 females, of 38 to 75 years old. The predominant histology was scamous cell carcinoma (27), followed by adenocarcinoma (21) and poorly differentiated carcinoma (12). Five patients were staged as III A, 32 as III B and 23 as stage IV. The Karnofsky Performance Status (PS) was superior to 80% in 22 patients, equal to 80% in 22 and 70% in 16 patients. Therapeutic schedule: Gemcitabine 1000 mg/m^2 on days 1and 8, cisplatin 50 mg/m^2 on day 1 and ifosfamide 3 gr/m^2 on day 1, all of them administered intravenously, every 21 days.

Results: 255 cycles were administered, with a median of 4 cycles per patient. In 112 cycles a dose reduction was necessary. As a mean, it was administered 84% of scheduled dose of gemcitabine, 99% of ifosfamide and 100% of cisplatin doses.

Two complete remission (3%) and 24 partial remission (40%) were obtained. Thirteen patients presented a stable disease, and 20 patients progressed during the treatment. In 13 patients we observed an improvement and in 27 an stabilization of PS during the treatment. The toxicities registered (Grades III and IV of WHO) were alopecia 23/0, emesis 6/0, neurotoxicity 1/0, hemoglobin 9/0, leukocytes 28/0, granulocytes 25/9, platelets 2/2. Seven episodes of neutropenic fever were registered, all with a favorable evolution with antibiotic treatment.

Conclusions: GIP chemotherapy is useful in the treatment of NSCLC, with an objective response index of 43%, with an acceptable toxicity.

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SPANISH LUNG CANCER GROUP RANDOMIZED TRIAL OF PREOPERATIVE CHEMOTHERAPY (CISPLATIN EITHER 100 mg/m^2 OR 50 mg/m^2) IN STAGE IIIA NON-SMALL CELL LUNG CANCER(NSCLC).

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Cisplatin is one of the most active agents in NSCLC. However the importance of cisplatin dose in combination is unclear. This trial addresses whether higher cisplatin doses result in improved survival and increased pathologic complete remission in patients (pts) with clinically and mediastinoscopically staged IIIA(N2) NSCLC. From March 1993 to February 1997, 83 pts were randomized to receive either high-dose cisplatin (HDCP) (100 mg/m^2 iv day 1) or moderate-dose cisplatin (MDCP) (50 mg/m^2 iv day 1) in combination with ifosfamide (3 g/m^2 iv day 1) and mitomycin (6 mg/m^2 iv day 1). Three chemotherapy cycles were given at 21-day interval, then surgery, and postoperative irradiation. Forty-six pts received HDCP, and 37 MDCP. Clinical characteristics were well matched. Grade 3-4 anemia was more common in HDCP (p=.01). Clinical response rate was 59% for HDCP, and 30% for MDCP (p=.01). Thoracotomy was performed in 71 pts (86%), 58 of whom had resectable disease. Postoperative mortality was 11%. Pathologic complete remission was observed in one patient who received MDCP. Overall median survival was 13 months. Median survival in the HDCP and MDCP was 13, and 11 months, respectively (p=.3).

Preoperative mitomycin, ifosfamide, and cisplatin achieved moderate response rate, and acceptable resectability. In pts treated with HDCP, anemia was significantly higher and there was no significant improvement in either pathologic complete remission or overall survival.

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EXPERIENCE IN OUR CENTRE TREATING ADVANCED NON SMALL CELL LUNG CANCER (NSCLC) WITH GEMCITABINE-CISPLATIN

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Introduction: Since gemcitabine has shown antitumoral activity in NSCLC its use has been widely spread. The combination of cisplatin-gemcitabine has shown promising results.Purpose: to analyze toxicity profile and response rate achieved with this combination in unselected patients (p) with unressectable NSCLC. Regimen: Cisplatin 100mg/m^2 day 1°; Gemcitabine 1250mg/m^2 days 1° y 8°, every 21 days. Treatment was given as outpatients. Pacients. 53 p had stage IIIB (27p) and IV (26p). Histologic subtypes were: Squamous carcinoma 18p, adenocarcinoma 21p and mixed histologies 14p (large cell carcinoma, undifferenciated, others). 47 males y 6 females. Mean age, 60 (45-63). ECOG: 0 (6p), 1 (32p), 2(13), 3(2). Mean number of courses per p was 3'5(1-7). Total number of courses was 195.
Toxicity: all patients were elegible for toxicity, which has been estimated per course. Anemia grade III-IV: 7(3,5%); neutropenia grade III-IV: 26(13,3%); thrombocytopenia grade III-IV 24(12,3%). Mucositis grade II-IV 1p. Nausea and vomiting grade II-IV 11p(5,6%), diarrhoea grade III-IV 3p (1,5%). Three p had ototoxicity. There were 7 febrile neutropenias that required admission. There were no toxic deaths.
Results: 46p were elegible for response. Partial response (PR) 30,2% (9pIIIB and 7pIV); No Change (NC) 22'6% (9p IIIB and 3p IV). With a median follow up of 190 days, median overall survival was 352 days. Median survival for stage IIIB was 390 days and for stage IV, 269 days. Taking into account the histologic subtype, median survival for squamous was 440 days, 260 days for adenocarcinomas and 118 days for mixed tumors.
Conclusion:In our experience, Cisplatin-Gemcitabine combination has shown significant antitumoral activity in locally advanced and metastatic NSCLC
Further studies should be encouraged using it as an induction regimen in less advanced stages. Toxicity in this regimen is moderate and manageable.

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CHEMOTHERAPY IN SMALL-CELL LUNG CANCER (SCLC): A RANDOMIZED TRIAL OF HIGH-DOSE EPIRUBICIN-CISPLATIN (HDEP) versus ETOPOSIDE-CISPLATIN (EP).

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INTRODUCTION: In the previous experience of the Spanish Lung Cancer Group HDEP is a feasible and active combination for SCLC.
OBJECTIVES: To compare the antitumor activity (survival and response), toxicity and health costs of the HDEP combination versus the standard EP in the treatment of SCLC.
MATERIAL AND METHODS: Prospective, randomized, phase III-IV trial. Patients (pat.) were stratified according to center and disease stage and randomized, with informed consent, to receive HDEP [Cisplatin (100 mg/m2) + Epirubicin (100 mg/m2) day 1] or EP [Cisplatin (100 mg/m2), day 1 + Etoposide (100 mg/m2) days 1-2-3]. Those pat. with disease limited to thorax (LD) and who responded subsequently received thorax and prophylactic *holocranial* irradiation.
RESULTS: From June-94 to March-98, 404 pat. have been included, 199 in the HDEP arm and 205 in the EP arm. By stages, 205 pat. had LD and 194 Extended Disease (ED). The patient's characteristics (age, gender, general condition, weight loss, stage and metastasic sites) were well balanced between the arms of the study. At this moment 383 pat. are evaluable for toxicity, 333 for response and 379 for survival. The overall objective response rate obtained in the HDEP arm was 87% and 78% in the EP arm (p=0.03). By stages, in LD, the responses were CR 44% and PR 47% for HDEP and CR 35% and PR 47% for EP. In ED there were CR 20% and PR 62% for HDEP and CR 17% and PR 55% for EP. The medians for time to progression (11 months in LD and 9 months in ED) are similar between the compared treatments. The predominant toxicity was hematological, with neutropaenic fever in 5% of cycles and in 8% of treatment related deaths, without differences between treatments.
CONCLUSIONS: The overall response rate with HDEP has been superior to the one obtained with EP. Whoever both schedules are comparable in survival.

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GEMCITABINE, IFOSFAMIDE AND CISPLATIN (GIP) IN THE TREATMENT OF NON SURGICAL STAGE III NON-SMALL LUNG CANCER (NSCLC).

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Introduction: There are evidences suggesting that a combined treatment has a positive impact on the survival of locally advanced NSCLC patients (pts), even though there is some controversy about treatment sequence and the induction regimen.
Aim: To analyze the GIP efficacy and toxicity in non surgical stage III NSCLC.
Patients and Methods: Pts with non surgical IIIA/IIIB NSCLC, measurable disease, <71 years, performance status (PS,ECOG) <3 and adequate cardiac, renal and liver function were elegible. The treatment was Gemcitabine 1 g/m2 IV days (d) 1&8, Ifosfamide (/Mesna) 4 g/m2 IV d.1 and Cisplatin 80 mg/m2 IV d.1, q3wk, with radiologic response assesment after 3-4 courses. We have used profilactic G-CSF in all patients. Finnaly, radiotherapy was administered (60 Gy) at conventional fractionation. We have considered for surgical assesment prior to radiotherapy: IIIA stage with partial (PR) or complete response (CR), IIIB stage with CR or maximal PR and not T4 because of pleural effusion. Since 3/97 to 9/98 43 pts have been treated. Stage IIIA: 17, IIIB: 26. Median age: 60 (range: 33-71). Histology: squamous 25, adenocarcinoma 11, undifferenciated 9 pts. PS 0: 6, PS 1: 32 and PS 2: 5.
Results: 140 courses [median 3 (range 1-6)] have been administered. 41 pts were evaluable for response. Overall response rate was 70.7% (CI 95% 56-84): 7/41 CR (17%, CI 95% 6-28) and 22/41 PR (53.6%, CI 95% 38-70). All the pts were evaluable for toxicity: WHO grade 3-4 neutropenia was present in 46/140 courses (32.8%, CI 95% 25-40), grade 3-4 trombopenia in 53/140 courses (37.8%, CI 95% 30-46), febrile neutropenia in 6/140 courses (4.2%, CI 95% 1-7) and reversible grade 3 asthenia in 32/140 courses (22.8%, CI 95% 16-30). No toxic deaths were observed. With a median follow-up of 37 weeks we haven't reached the median duration of response.
Conclusions: 1) It is confirmed that GIP is a highly active regimen in NSCLC, with a response rate of 70% and a moderate hematological toxicity though manageable. 2) Its role in the multimodal treatment of the stage III NSCLC would have to be confirmed in the future comparative studies.

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Sequencial Chemotherapy (CT) and Radiotherapy (RT) vs Chemotherapy followed by concomitant Radiotherapy and daily cisplatin in locally advanced non small cell lung cancer (NSCLC) (no sergical III-A and III-B).

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Aim: To evaluated the efficacy (local control and survival) of the treatment with concomitant cisplatin and RT vs secuencial RT in NSCLC.
Characteristics: 38 patients (pts) were included in two arms Arm A.: 21 pts were evaluated for response and toxicity (III-A 7 pts, III-B 14 pts), treated with 3 cycles of CT follow by sequencial RT (60 Gy). Median age: 63 y (30-75). Epidermoid 15 pts, adencarcimoma 1 pts, giant cells 2 pts, undifferenciated 3 pts. Arm B: 17 pts, 15 were evaluated for response (1 rejected the treatment and 1 exitus) (III-A 2 pts, III-B 15 pts), treated with 3 cycles of CT followed by concomitant RT with daily cisplatin (4mg/m2 iv) during 30 days. Median age: 62 y (38-75). Epidermoid 12 pts, adenocarcinoma 3 pts, giant cells 1 pts, undifferenciated 1 pts. The CT protocol was: Ifosfamide 4 g/m2 iv 1st day, Cisplatin 100 mg/m2 iv 2nd day, Etoposide 80 mg/m2 for 3 days, every 28 days for 3 cycles.
Results:

	Arm A	Arm B
OR	43% (9/21)	60% (9/15)
PR	33% (7/21)	60% (9/15)
CR	10% (2/21)	0% (0/15)
NR	57% (12/21)	40% (6/15)

After RT 2 pts passed from PR to CR, in arm A (total CR 20%) and 1 pts passed from NR to PR in the arm B (total OP 66%). The medium survival was12 m in arm A (actuarial survival 45%) and 13 m in arm B (acturial survival 50%), with no significance (ns). The medium disease free survival was 9 m in both groups, ns. Toxicity was mild except 1 exitus due to a febrile neutropenia; neutropenia grade IV (7%) and anemia grade II (16%). There were two patients with neumonitis and 1 pts with esophagitis in arm B.
Conclusions: In our study, RT with concomitant daily cisplatin does not improve local control and survival in patients with locally advanced NSCLC. It was showed higher toxicity in arm B.

Si hay algún problema les rogamos nos manden notificación. Un saludo.
Raquel Salazar

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COMBINED CHEMOTHERAPY AND RADIOTHERAPY IN THE TREATMENT OF LOCALLY ADVANCED UNDIFFERENTIATED NASOPHARYNGEAL CARCINOMA (LAUNC)

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Introduction:
LAUNC compose a distinct subgroup of head and neck neoplasia. Although the best treatment is not known, combined chemotherapy (ChT) and radiotherapy (RT) have been better than RT alone in randomized trials. Our experience with combination ChT and RT in the treatment of LAUNC is presented.

Materials and methods:
From April 94 to November 97 nineteen patients with LAUNC (WHO 3) were treated with neoadjuvant ChT followed by radical RT. Fifteen males, four females. Mean age 49 years (y) (range 15-70). PS (ECOG) 0-1. Stage III: 1, IV (M0): 18; T4: 10 (53%) N2-N3: 14 (74%). Treatment: Cisplatin 100 mg/m2 d1, Epirrubicin 70 mg/m2 d1, Bleomycin 15 mg d1 and Bleomycin 12 mg/m2 24 hours continous infusion (port-a-cath) d 1 to 5, every 21 d (BEC). Sixteen patients were treated with RT 60-70 Gy to nasopharynx and 50-70 Gy to cervical nodes (1 pt 40 Gy due to severe mucositis); 2 pts received brachitherapy to nasopharynx (1050 and 1125 cGy). Three pts were not irradiated, 2 deaths and 1 lung metastases.

Results:
ChT: 60 cycles, mean 3.15 (range 2-4). Overall response: 11 (58%), complete response: 3 (16%), partial response 8 (42%), stable disease: 6 (31%) progressive disease: 1, early death: 1. After RT: Overall response: 15 (94%), complete response: 9 (56%) and partial response: 6 (38%). Two partial responses were converted surgically to complete response. Toxicity ChT (60 cycles): Emesis grade (g) II: 11, gIII: 4, gIV: 1. Neutropenia gII: 3, gIII: 9, gIV: 0. Mucositis gII: 4; Interstitial neumopathy: 1, neumonia (without neutropenia). Two early deaths, one from bilateral neumonia, other from colonic perforation. RT: mucositis gII: 10, gIII: 3 and gIV: 1. Median survival 62 weeks. Actuarial survival (3 y) 41%. Nine pts of 11 in complete response are alive without disease.

Conclusions:
LAUNC are tumours with high sensitivity to ChT and RT. In spite of this, local recurrence and distant metastases are frequent. Toxicity of combined treatment is significant. Although neoadjuvant ChT is active, standard treatment of LAUNC is not defined yet. Unselected series may have worse results than controled studies (perhaps due to Will Rogers phenomenon). Standard staging is also not defined for N2-N3 cases. Our results indicate that neoadjuvant ChT with this scheme may not be preferred to other schemes concomitant to RT, as evidenced recently in other studies (INT0099, VUMCA1). New active agents, also, may improve long term results.

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TREATMENT WITH CISPLATIN AND VINORELBINE IN PATIENTS WITH SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK.

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Cisplatine and vinorelbine are actives in monochemiotherapy and in combination with others drugs antineoplastic. The overall response rate are acceptable. Although this rate response is high we don't achieve increase the survival, maybe the reasons are the low complete response rate and short time to progression.

Aim: To assess the efficacy and safety of vinorelvine plus cisplatine in patients with metastatic or locoregional squamous cell carcinoma of the head and neck.

Patients and Methods: From May 1997 45 patients were included. The main characteristics were average age: 57 (Q25:50-Q75:66). The 37.4% (17) was local recurrence and 62,8% (28) primary tumours (.82% (23) was stage IV).

Untreated by chemotherapy the 75,5%(34). **Treatment:** Cisplatine: 100mg/m2 day 1° plus Vinorelbine 25 mg/m2 day 1° and 8°, after to value at third cycle the response we completed with locoregional treatment (Radiotherapy and/or Surgery).

Results: At the moment we administered 130 cycles(The 68% (31) have received at least, 3 cycles). In the untreated patients by chemotherapy the overall response obtained are a 80% (21% CR and PR 59%) and only 10% in the previously treated patients. The median duration of response was 30 weeks (8-52+). In general the tolerance has been good (Anaemia GIII: 11,8%, Leucopenia GIII: 8,9%, Neutropenia GIII: 17,7% y GIV: 13,3%. No case of severe neurological and gastrointestinal toxicity. We don't get yet the median of survival, in the patients with response (CR or PR) the average response is 49 weeks CI95%(44-541). 5 patients received surgical treatment and 18 received radiotherapy. In the 80% of patients improved the symptoms.

Conclusion: Thus combination is active in the first line of treatment of squamous cell carcinoma of the head and neck, with a acceptable toxicity.

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A NOVEL ANTI-APOPTOSIS GENE: SURVIVIN AS A PROGNOSIS MARKER IN NON-SMALL CELL LUNG CANCER

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The survivin gene is a novel apoptosis inhibitor, related to the baculovirus gene, which is believed to play a pivotal role in fetal development and in cancer in much the same fashion that the Sonic hedgehog gene is involved in the morphogenesis of the embryonic lung.

We investigated survivin expression in non-small cell lung cancer (NSCLC) and its influence on clinical outcome in patients up to stage IIIA NSCLC who had undergone radical surgery.

We designed a reverse transcriptase polymerase chain reaction (RT-PCR) assay to study the expression of the survivin gene in 83 NSCLCs (42 squamous cell carcinomas, 37 adenocarcinomas and 4 large-cell carcinomas) and neighboring normal lung tissue. The RT-PCR identified survivin gene transcript in 71 (85.5%) of the tumors samples and in only 10 (12%) of the paired histopathologically normal lung samples.

There was no relationship between histologic subtype (squamous vs non-squamous) and survivin gene expression. A Kaplan-Meier analysis of the 83 patients showed that the 12 patients without survivin expression had significantly better overall survival than the 71 patients with survivin expression (p=0.01 by univariate analysis; relative risk, 2.1).

We conclude that analysis of survivin transcript could prove to be both a useful diagnostic marker and an important source of prognostic information in NSCLC ; moreover, as an apoptosis inhibitor, it is a potential new target in anti-cancer therapy.

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ALTERED PACLITAXEL-INDUCED CYTOTOXICITY IN NON-SMALL CELL LUNG CANCER LINKED TO ß-TUBULIN MUTATIONS.

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The mechanisms causing chemoresistance in non-small cell lung cancer (NSCLC) patients have yet to be elucidated. Paclitaxel's unique anti-tumour mechanisms enhance the assembly of microtubules. We investigated the connection between ß-tubulin mutations and primary paclitaxel resistance. Constitutional genomic DNA and paired tumour DNA were isolated from 49 biopsies from 43 Spanish and 6 North American stage IIIB and IV NSCLC patients who had been treated with a 3-hour, 210mg/m^2 paclitaxel infusion and 24-hour, 200mg/m^2 infusion respectively. Specific ß-tubulin primers were designed for PCR amplification and sequencing of paclitaxel and GTP-binding ß-tubulin domains. Of 49 patients with NSCLC, 16 (33%; 95% CI, 20,7%-45,3%) had ß-tubulin mutations in exons one and four. None of the patients with ß-tubulin mutations had an objective response, whereas 13 of 33 (39,4%; 95% CI, 22,8%-56%; P=0,01) patients without ß-tubulin mutations had complete or partial response. Median survival was 3 months for the 16 patients with ß-tubulin mutations and 10 months for the 33 patients without ß-tubulin mutations (P=0,0001). In conclusion we have identified ß-tubulin mutations as a novel mechanism of resistance to antimicrotubule drug paclitaxel.

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ANALYSIS OF RESPONSE, KARNOFSKY PERFORMANCE STATUS MAINTENANCE AND SURVIVAL IN ADVANCED NON SMALL CELL LUNG CANCER (NSCLC) PATIENTS TREATED WITH GEMCITABINE (GEM).

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We analyze 44 chemonaive patients (pts) aged 65 yrs (45-77); 25 staged IIIb and 19 IV; 15(34%) with an initial KI>70% (groupA), 18(40%) KI=70% (group B) and 11(25%) KI=60% (group C).

Pts were treated with GEM 1200 mg/m^2 on days 1, 8 and 15 every 4 weeks until progression or inacceptable toxicity. Subsequent radiotherapy (RT) was applied to IIIb pts with PR or SD if feasible. Serial Karnofsky index (KI) was used to estimate the quality of life (QL). The end points for actuarial curves were loss of KI70% and death. Prognostic factors analysed were age, weight loss, stage and initial KI. **RESULTS:** Median (M) follow-up was 5 (1-24) months. 100 cycles were administered, M 2 (1-6) per patient. Toxicity was: grade 3-4 hematological in 2/100, grade 3 astenia 2/100, grade 3-4 fever 1/100, rash 8 pts (18%), thrombocytosis 11 (25%)pts. 7(16%) PR, 17 (39%) SD and 20 (45%) progression were observed. Only initial KI correlated with response: group A: 36% vs group B:11% vs group C: 0%. 6 pts with SD and 2 with PR received 45-50 Gy, 3 converted to PR. 1 with SD received RT and converted to CR. M time to KI 70% loss was 3 (2-24) months and OS 5 (1-24) months. 10 pts increased 1 level of KI for a M of 2 (1-5) months. Only initial KI was a significant prognostic factor for OS and KI70% maintainance. Group A: 11 (6-24) months OS and 8 (2-24) months KI70% mantainance. Group B: 3 (2-6) OS and 3 (2-4) KI70% mantainance. Group C: 2 (1-3) OS and 2 (1-2) KI70% maintainance.
CONCLUSIONS: GEM is relatively active and non toxic in initial KI>70% pts with advanced NSCLC. Initial KI was the only significant prognostic factor for response, survival and maintainance of KI≥70.

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FUNCTIONAL ANALYSIS OF MUTANT P53 ALLELES FROM NON-SMALL CELL LUNG CANCER (NSCLC) SURGICAL SPECIMENS.

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The p53 tumor supressor gene coordinates responses to DNA damage that include G1 phase cell cycle arrest, DNA repair and apoptosis. Mutant p53 is often associated with resistance to chemotherapy or radiotherapy and with a poor prognosis in various types of malignancies. p53 mutants are found in 30%-50% of NSCLC but they differ in their functional abilities with some showing loss-of-function and others gain of funtion phenotypes. As reported previously, we used the SSCP analysis but due to the presence of SSCP-false positive and its low sensitivity, we analysed the region of p53 encompassing codons 67-347 using the functional assay of separated alleles in yeast (FASAY) system. In this methodology, the wild-type function p53 induces the ADE2 gene, yielding white colonies and loss-of-function yields red colonies. RNA was extracted from 15 selected fresh-frozen NSCLC surgical specimens (stages I-IIIA) and they were amplified by RT-PCR and scored by FASAY. The p53 plasmids were recovered and DNA sequencing is in progress. Twelve specimens yielded between 64% and 98% red colonies suggesting that the majority of tumor cells contained mutant p53. The other three specimens yielded between 28% and 44% red colonies suggesting heterogeneity of the tumor with respect to p53 status. Median disease-free survival time was eight months for the patients with p53 mutations and has not been yet reached for the patients without mutations. These results demonstrate that FASAY analysis is an improved methodology to examine the status of p53 in the tumors of individual NSCLC patient because it 1) has a high sensitivity for detecting mutant p53 alleles in surgical specimens and 2) provides analysis of their biological function which will contribute to an improved understanding of the role of mutant p53 in the prognosis and chemoresistance of individual NSCLC patients.

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QUALITY OF LIFE (QL) IN NON-SMALL-CELL-LUNG-CANCER (NSCLC) PATIENTS (p) TREATED WITH HIGH DOSES OF CISPLATIN

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Introduction: Chemotherapy regimen based on Cisplatin is considered best adyuvant treatment in p. with NSCLC, althought is a potentially toxic treatment. The aim of this study was to assess high dosis of Cisplatin in MIC regimen impact on QL during treatment in p. with advanced NSCLC.

Methods: 67 p., 58 males. 60 yrs. (30-70). Epidermoides=30, Adenoma=20, Big Cells=7. IIIA=6, IIIB=43, IV=18. Treatment procedure: 1st day Mitomycin C 6mg/m2, Ifosfamide 3g/m2 y Cisplatin 100 mg/m2 every 21 days. Antiemetic profilaxis. Uroprotection with Mesna. QL was assessed using two different scales: Functional Living Index Cancer (FLIC -Schiepper et al., 1984) y EORTC QLC-30 (Aaronson et al., 1993). QL measures were taken on 1st, 2nd and 3rd cycles.

Results: No signifficant diferences were found in QL areas assessed in both scales, except for a worse Social Functioning and a reduction of Pain Symptoms. Response to treatment was as follow: IIIA, Partial Response 17%, Stable Disease 17%; IIIB, Complete Response 8%, Partial Response 36%, Stable Disease 39%; IV, Partial Response 17%, Stable Disease 49%. Toxicity Levels III-IV; Alopecia in 6 p., Diarrhea in 1 p., Fever in 2 p., Vomiting in 5 p. and Neutrophenia in 9 p. Median survival time was 12 months (IIIA: 14, IIIB:10 and IV: 8).

Conclusions:
1) MIC regimen of chemotherapy with high dose of Cisplatin does not significantly dimisnish QL of NSCLC p. during treatment.
2) Specific scales of QL should be added to the clinical assessment in the treatment of NSCLC.
3) Althought QL does not diminish it is necessary the development of new less toxic therapeutic schemas for NSCLC p.

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CISPLATIN, FLUOROURACIL AND VINORELBIN IN SQUAMOUS CELL HEAD AND NECK CARCINOMA

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Objective: To assess response and toxicity of cisplatin, fluorouracil and vinorelbin in squamous cell carcinoma of the head and
Patients and Methods: Between June 96 and June 98 all patients with stage III and IV histologically proven squamous cell head and neck carcinoma had been included. Therapeutic squeme was cisplatin 100 mg/m^2 day 1, 5-fluorouracil 650 mg/m^2 continuous infusion days 1 to 5 and vinorelbin 20 mg/m^2 days 1 and 8. After third course response was assessed. In neoadyuvant patients local therapy was added and in the remaining if any response three aditional chemotherapy courses were added.
Results: 47 patients have been included with a mean age of 55 years (34-73), only two were female. 23 patients were neoadyuvant and the remainig paliative. Neoadyuvant: 5 CR, 11 PR, 1 SD, 6 non evaluable due to short follow up. Paliative: 6 PR, 4 SD, 10 PE, 4 non evaluable because early death. Median survival for neoadyuvant patients can not be evaluated., in palitive patients 8.13 monthss with a 95% confidence interval between 5.68 and 10.5
Toxicity:Neutropenia in 11 patients beeing grade III to IV in 6, with a sepsis toxic death. Grade I to II anemia in 8 and grade III in one. Mucositis grade III in one. Four had renal toxicity and one motor
Conclusions: This combination offers a respo nse rate similar to that achieved with estándar cisplatin and fluorouracil. Toxicity is manageable. Survival is aparently not modified.

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GEMCITABINE (GEM) AND CARBOPLATIN (CBDCA) COMBINATION AS FIRST LINE TREATMENT IN NON-SMALL CELL LUNG CANCER. V. Alberola, A. Carrato, B. Massuti, O. Juan, J.J. Sánchez, J. García Gómez, N. Díaz Fernández, A. Meana, A. Rodríguez-Iescure, M.L. González. Grupo Español de Cáncer de pulmón.

Introduction. Gem and CBDCA are two of the most active single drugs in NSCLC treatment. He feasibility of the combination was examined in 13 NSCLC patients (pts) in a phase I trial (J. Carmichael, Proc ASCO 1995:351). MTD for carboplatin was determined as AUC 5.2. To assess the activity the activity and toxicity of the combination a prospective multicenter phase II trial was started in september 1996.
Méthods: Non-surgical advanced/metastatis NSCLC pts from 4 different Hospitals were treated with Gem 1000 mg/m2 d1, 8 and 15, abd CBDCA 5 AUC iv d1, after Gem, every 28 days (q4wk) (scheme A). Treatment was continued until progression or unacceptable toxicity. After including 32 pts in the study, a high percentage of grade 3 and 4 thrombocytopenia was observed. In order to ameliorate toxicity, the schema was modified for subsequent 43 pts as following: Gem 1000 mg/m2 iv d1 and 8, and CBDCA 5 AUC iv d1, every 21 days (q3 wk) (scheme B).
Results: From the total of 75 pts, median age was of 68 years (range 34-76), median ECOG was of 1 (range 0-2). 11 pts were stage IIIA (5 in scheme A and 6 in B), 30 pts stage IIIB (13 in A and 17 in B), 34 stage IV (14 in A and 20 in B). No statistically differences were observed in neutropenia grade 3 (52% vs 39%) o grade 4 (38% vs 23%) between both schemes. Thrombocytopenia was statistically significative for grade 3 (45% vs 24%, p=0.04) and grade 4 (61% vs 17%, p<0.0000002). No differences were detected in response rate or survival.
Conclusions. The Gem-CBDCA combination is very active for NSCLC treatment. When given every 28 days, thrombocytopenia is the dose limiting toxicity. When given in the 3 weeks schedule, hematological toxicity is acceptable and response rate achieved (37% with 2 pts CR) is in the range of most active regimens.

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A PHASE II STUDY OF 3 SEQUENCE-DEPENDEN DOCETAXEL (D)/VINORELBINE(V) DNA DAMAGE-INDUCE APOPTOSIS IN PATIENTS WITH NON-SMALL CELL LUN CANCER (NSCLC)

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Introduction: D and V are both active agents for NSCLC. However, i combination, their mechanisms may be either synergistic or antagonisti depending on the schedule used. The first objective of this study is t evaluate toxicity and activity of 3 different schedules of administration. second objective is to analyze DNA damage-induced apoptosis b analyzing the sFas in patient sera.

Methods: Three sequential phase II studies are being evaluated: Schedul A: D 75mg/m2 day 1 and V 20mg/m2 day 1 and 6; Schedule B: 1 75mg/m2 day 6 and V 20mg/m2 day 1 and 6; and Schedule C: 1 75mg/m2 day 1 and V day 1 and 15. sFas is analyzed with the ELIS method before starting chemotherapy and at every cycle.

Results: Schedule A: 14 patients (p) have been included , all evaluabl for toxicity and response. 75 cycles (c). Greatest toxicity: low nad neutrofil counts (20% grade 3-4 neutropenia, 42,9% neutropenic feve 35,7% needing G-GSF. Activity: CR 7,1%, PR 35,7%, SD 28,6%, PI 28,6%. Schedule B: 13 p, 9 evaluable for response, 42 c administrated, 3 evaluable for toxicity. Schedule C: 10 p, 1 evaluable for response, 17 administrated, 7 evaluable for toxicity.

Conclusions: Schedule A has nadir neutropenia as the principal toxicity Initial and final values of sFas seem useful in predicting clinical respons A low initial sFas value may indicate a better tumor evolution. It i planned to enroll more patients in order to draw more definite conclusion

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CLINICAL TRIAL IN NON-SMALL-CELL LUNG CANCER STAGES IIIB AND IV. THE RECRUITMENT: THE ONCOLOGIST TERRITORY

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Introduction: Only 2-3% of the cancer patients are enrolled in clinical trials (1-2% of the patients with the most common tumours). Data, especially from the United States, show that near 19% of the patients potentially candidates for a clinical trial are finally included. (10-30% in co-operative group studies).
One of the most necessary data for everyone who wants to carry on a clinical trial is the real recruitment possibility. Finally, the decision depends on the clinician within his hospital.

Material and methods: Between July 1994 to June 1995 we participated in a phase III clinical trial of Non-small-cell Lung Cancer, stages IIIb and IV (Two chemotherapy arms). The eligibility criteria were the usual in this type of study: Histological/cytological diagnosis, adequate renal, hepatic and bone marrow function, no previous chemotherapy treatment, informed consent, measurable disease, no other tumours, no CNS metastases. We prospectively recorded every consecutive patient we evaluate for the trial, and the reason for exclusion.

Results: We screened 56 patients in 12 months. 25 were included in the trial (44,6%). Complete data was only recorded during the first 8 months. The analysis in the 8 months showed: 48 patients screened, 17 patients included (35,4%). Recruitment rate by month varied from 0-75%. The main reasons for the exclusion were advanced age (> 75 years) (16,12%), low Karnofsky index 70% (12,9%), no measurable disease (9,6%), CNS metastases (9,6%) and severe concomitant diseases (9,6%). Only one patient refused to participate in the study.

Conclusion: The real recruitment rate in non-small lung cancer clinical trial in our setting can be more than 1/3 of the patients seen by the Oncologist. The recruitment can be quick if every patient is considered for the study. The main reasons for the exclusion are: low Karnofsky index, advanced age, CNS metastases, and lack of measurable disease.

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PHASE II STUDY OF GEMCITABINE-VINORELBINE (GV) IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC) OVER 70 YEAR-OLD OR WITH CONTRAINDICATION TO RECEIVE CISPLATIN.

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Objective: to assess the efficacy and toxicity of the combination GV in patients with advanced NSCLC who are either over 70 year-old or have some contraindication to receive cisplatin.

Patients and method: from November 96' to March 98', 46 patients with advanced NSCLC were included. Thirty-five were older than 70 (mean 74, range 70-81), 7 had an elevated creatinine level over 1.5 mg/dL and 4 congestive heart failure that could escape control with fluid overload. Stage: IIIA in 4 patients, IIIB in 14 and IV in 28. ECOG: 0 in 5 patients, 1 in 12 and 2 in 29. There were five female and 41 male.
Chemotherapy consisted of gemcitabine 1000 mg/m2 plus vinorelbine 25 mg/m2, both on days 1, 8 and 15 every 28 days. Response was evaluated after 3 courses of therapy.

Results: 43 patients are assessable for toxicity and 40 also for response. The overall response rate was 27.5% (95% CI: 14.6-44%), with 5% complete responses, 22.5% partial responses, 32.5% stabilization and 40% progression. Twenty-four patients did not progress (complete plus partial responses plus stable disease), 60% (95% CI 43.5-75%). A total of 158 courses were administered, with an average of 3.6 per patient. WHO grade 3-4 toxicities were as follows: neutropenia in 14% of patients, 3 of whom (7%) died due to neutropenia and sepsis, thrombocytopenia in 5% of patients, and renal toxicity in 2%. The median survival was 8.5 months..

Conclusions: the combination CV for the therapy of advanced NSCLC in patients older than 70 or with contraindication to receive cisplatin is active at the present doses but produces important toxicity.

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PHASE I/II TRIAL WITH CISPLATIN AND INCREASING DOSES OF GEMCITABINE IN STAGE IV NON-SMALL CELL LUNG CANCER (NSCLC) DISEMINADO.

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Aim.- To modify the combination of cisplatin (C) and gemcitabine (G), one of the most active in NSCLC to reduce C dose and increase dose of gemcitabine.

Objective.- To assess toxicity and efficacy of cisplatin 50 mg/m2 plus increasing gemcitabine trying to reach the maximum tolerable dose of G in this combination.

Patients and methods.- Eligible patients had confirmed NSCLC, measurable disease, stage IIIb (positive pleural effusion) or IV, with adequate performance status (PS 0-2), and normal haematopoietic, renal and liver functions and no contraindications for receiving chemotherapy.

Therapeutic scheme.- Cisplatin 50 mg/m2 day 1 and gemcitabine (doses from 1.400 to 2.000 mg/m2) days 1 & 8 every 21 days. The treatment was maintained until toxicity or progression. 15 patients (p) were included in the first level and 6p in each of the following.

Results.- Between August/97 and October/98 31p were included. All of the patients with stage IV: median age 59 years (38 – 76), male 25p (80,6 %). Characteristics: histology: squamous 13p (42%), adenoca 9p (29%), undifferentiated 9p (29%). ECOG 0 3p (9,6%), 1 19p (61,3%), 2 9p (29%). Weight loss >10% 13p (42%). High LDH 4p (13%). Metastatic sites: median 2 (1-3) (lung 10p, pleura 6p, nodal 8p, adrenal 7p, subcutaneous 2p, liver 6p, choroides 1p, bone 4p, CNS 5p, kidney 1p).

Toxicity: 82 courses were administered (median 3 (1-6)). Actually given dose was 99% (day 1) and 93% (day 8). Haematological Toxicity: Hb G3 2p, neut G3 1p, plat G4 1p; non-haematological: vomiting G3 1p, asthenia G3 3p, mucositis G2 1p. There was an episode of neutropenic fever an 2p showed thrombocytosis >1.200 $x10^{12}$/L.

Objective Responses.- Evaluable 27p: Partial 4p (14,8%), stable disease 17p (63,0%), progresive disease 6p (22,2%).

Conclusions.- The present combination showed modest activity in metastatic NSCLC. Overall response rate was lower than the obtained with higher CDDP doses but many enrolled patients had intrinsically very bad prognostic factors. Toxicity was mild. It is planned to complete inclusion in the level of gemcitabine 2.400 mg/m2.

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A PHASE II TRIAL OF HIGH-DOSE GEMCITABINE (GEM)+ CISPLATIN (CDDP) IN ADVANCED NON SMALL CELL LUNG CANCER(NSCLC). PRELIMINARY RESULTS.

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Standard dose intensity of GEM in early phase II trials in NSCLC was <1gr/m^2/week. Recent trials (Fosella F, J Clin Oncol 15:310-315;1997) have tested high dose GEM (>1gr/m^2/week) as single drug in patients(p.) with untreated advanced NSCLC. In September 1997 we initiated a phase II trial to evaluate tolerance and activity of a combination of high-dose GEM and CDDP. Treatment scheme was: GEM 1750 mg/m^2 iv on day 1 and 8 and CDDP 100 mg/m^2 iv on day 1, courses were repeated every 3 weeks until progression, unacceptable toxicity, or to a maximum of 6 courses.

RESULTS:

Nineteen patients were included. Median age 62 years (range 46-72). Sex:Male 89%. Perfomance Status (Zubrod):1:63%,2:37%. Histology: squamous cell carcinoma 68%, adenocarcinoma 21%, large cell carcinoma 11%. Stage: IIIB(pleural effusion) 11%, IV 89%. 11 patients had weight loss less than 10%. Total number of cycles administered was 63. Toxicity(WHO Grades 3-4):Anemia 5%,Neutropenia 11%,Thrombocytopenia 11%,Nausea/Vomiting 0%,Renal 0%,Mucositis 0%,Diarrhea 0%,Peripheral neuropaty 0%. There were no neutropenic fever episodes. Number of patients requiring red blood cell transfusion: 11%. Peculiar GEM-related toxicities included:Fatigue 21%,Flu-like syndrome 11%,Skin rash 21%,Peripheral edema 5%. For 13 evaluable p. Overall Response was 46% (CI 95%: 19-73%),Complete Response 0%,Partial Response 46%,No Change 30%,Progression 23%.Response duration (median): 8 months (range 1-10). Palliation of symptoms was seen in 63% of patients. Time to Progression(median):7 months (range 1-12). Median actuarial survival has not reached (68% at 11 months). Median follow-up: 4 months or to death.

CONCLUSIONS:

Combination chemotherapy was well tolerated. Peculiar GEM-related toxicities were seen, including fatigue and skin-rash. Response rate and palliation of symptoms are noteworthy.

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GIP II, PHASE II STUDY IN ADVANCED NON SMALL CELL LUNG CANCER. (GEMCITABINE IFOSFAMIDE and CISPLATIN).

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The chemotherapy treatment constitutes the first strategy in the majority of the cases of non small cell lung cancer (NSCLC) that are not subsidiary of surgery. Of possible drugs that can employ seems fundamental the employment of cisplatin. As gemcitabine as ifosfamide have demonstrated to be drugs assets in the treatment of the advanced NSCLC, both present synergy in the combination with cisplatin.

We have treated 83 patients (75 men / 5 women) with the following scheme :

Gemcitabine :	1.200 <mg> / <m2>	Days 1 and 8
Ifosfamide :	3.000 <mg> / <m2>	Day 8
Cisplatin :	75 <mg> / <m2>	Day 8

administered every 21 days.

The characteristis of tumors are: squamous 61 %, adenocarcinoma 22 %, and large cells 22 %; and by stage: IIIB 44 % and IV 37 %.

Until the moment are to value for response 50 patients, we have obtained 62 % of objective responses :1 C.R., ,25 P.R., (C.I., 95 % : 37, 4- 66, 3 %).

Ten patients have presented digestive toxicity G III (nausea or vomiting). The hematologic toxicity (by patient) has been :Anemia GIII :7, GIV :1 ;Granulopenia GIII :23, GIV :19 ;Trombopenia GIII :7, G IV :1,

At the moment of this analysis the 54, 2 % of the patients are lives, with an interval of survival of 6 to 60 weeks.

The preliminary data of toxicity and activity do to suppose that schedule is ussefull in the treatment of the advanced NSCLC.

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PROGNOSTIC IMPACT OF BULKY MEDIASTINAL LYMPH NODES (N2 >2.5CM) IN LOCALLY ADVANCED NON SMALL CELL LUNG CANCER (LA-NSCLC) TREATED WITH PLATINUM-BASED INDUCTION CHEMO-THERAPY.

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INTRODUCTION: LA-NSCLC has a dismal prognosis with local treatment alone (surgery or radiotherapy) with less than 5% 5 years survival. Recently several phase II trials using induction cisplatin-based chemotherapy or chemo-radiotherapy before surgery have improved survival (17-37% 5 y OS). These conflicting survival data could be derived from different treatment strategies or also due to different prognostic factors in patient selection.

PATIENT AND METHODS: 70 patients (p) with LA-NSCLC treated in different phase III trials in two hospitals have been evaluated in order to asses prognostic factors in survival. Patients have ECOG PS 0,1 without loss weight >5%. 37p. with stage IIIA(N2) (T3N0,1 excluded) were treated with induction chemo (MIC 73%) followed by surgery plus radiotherapy (RT) and 33 p. Stage IIIb (malignant pleural effusion and supraclavicular node excluded) were treated with induction chemo (PE and CbE 100%) followed by RT. Mediastinoscopy was performed in 29/37 (78%) of patients with stage IIIA-N2. We analysed the following variables: age, gender, histology, clinical stage, primary tumour size, type of consolidation treatment (surgery vs RT), induction chemo schedule and size of mediastinal lymph nodes (<1.5 vs 1.6-2.5 vs >2.5 cm).

Results.- 29/37p (78%) stage III-A N2 underwent radical surgical resection and 17/33p (51%) were treated with RT 50 – 65Gy. Overall response rate to induction chemo was 40% (28/70p) and 2p (3%) achieved CR. Median survival (MS) was 13 months. 12p remained disease free with median follow-up of 2.5 years (10-61 months). At the univariate analysis only response rate to induction chemo (CR/PR with MS 22months vs SD 11months vs PD 5 months; p<:0001) and bulky mediastinal lymph node N2> 2.5cm (MS 7 months vs 14 months; p=.01) had statistically significant value.

CONCLUSIONS.- In prospective randomised trials stage IIIA-N2 & IIIB should be treated in the same way. Bulky mediastinal lymph nodes (N2 > 2.5cm) had a dismal prognosis and this should be considered in further prospective randomised trials.

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PALLIATIVE CHEMOTHERAPY WITH CISPLATIN (C) AND IFOSFAMIDE (I) FOR RECURRENT OR METASTATIC SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK (SCCHN).

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Purpose. We undertook a prospective pilot study for patients(pt) with recurrent or metastatic SCCHN to test the activity and toxicity of a combination with C and I, trying to identify an alternative regimen to methotrexate or cisplatin/5-FU. **Patients and methods.** From 9/95 to 5/97, 21 pt (20 men) were treated with C 25 mg/m²/d iv bolus and I 1000 mg/m²/d iv c.i., on days 1-3 and repeated every 3 weeks for 2-6 courses. Median age was 59 (44-74), median performance status 80% (70-100%). Nine (43%) pt had distant metastases. Sixteen (76%) had prior surgery and 18 (86%) prior radiotherapy. 13 (62%) had measurable lesions in preirradiated area. **Results:** Six (28%) pt received only 1 cycle of chemotherapy: 3 because of disease progresion, 1 toxic death, 1 persistent stomatitis and 1 refused further treatment. These last 3 pt were not evaluable for response. Response rate: PR: 8/21 (38%); NC: 3/21 (14%) and PD: 7/21 (33%). Median survival: 6 (0-17) months. A total number of 63 cycles were given, median of 2. Doses were reduced in 6 cycles (10%) and 12 (19%) of them were delayed 1 week. Main toxicities (WHO criteria): neutropenia grade III-IV: 6 cycles, with 3 (5%)febrile episodes. Thrombocytopenia grade III: 1; anemia III-IV: 4; nausea/vomiting grade III: 2; stomatitis grade IV: 1. Five pt presented grade III alopecia. **Conclusions:** The combination C + I in pt with recurrent or metastatic disease has a moderate response rate with similar toxicity to other chemotherapy combinations.

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NON-SMALL CELL LUNG CANCER SECOND LINE CHEMOTHERAPY WITH GEMCITABIN PLUS VINORELVINE

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Objectives: To assess therapeutic efficacy and toxicity of gemcitabin plus vinorelvine as second line therapy of advanced non small cell lung carcinoma.

Patients and Methods: Between September 97 and March 98, stage IIIb and IV non small cell lung patients previously treated with chemotherapy were included. ECOG had to be less than 3 and lesions measurable bidimensionally. Therapeutic scheme was gemcitabin 1200 mg/sqm days 1, 8 and 15 and vinorelbin 25 mg/sqm days 1 and 8, both intravenously. Courses were repeated every 28 days.

Results: 16 patients have been included, males 13, with a median age of 62 (42-72). Histologic type was adenocarcinoma 6, squamous 8 and undifferenciated 2. Histological Grade G3 in 3, G2 in 4, Gx in 9. Stage IIIb in 6 and IV in 10. Previous chemotherapy was MIC (mytomicin, cisplatin and ifosfamide) in 12, cisplatin plus vinorelvine in 3 and taxol plus etoposide in 1. Median number of previos chemothery courses was 4.14 months and median time to second line therapy 8.5 months (1-27).

16 patients are evaluable for response, with 10 progressions, 4 stabilizations and 1 complete response demostrated histologicallyand 1 PR(OR= 12.5%). At present median survival has not been reached due to short follow up.58 courses have been administered with a mean of 3.25 (1-5). It has been necessary to delay 35% of the courses and reduce dose by 75% in 36.5%. Main toxicity has been hematologic specially on platelets, with trombocytopenia G2 in 14% and G3 in 11%, neutropenia G2 in 6% and G3 in 14%, anemia G3-4 in 14%. No toxic death have ocurred.

Conclusions: This combination has moderate activity as second line therapy in advanced non small cell lung cancer patients. Due to its high hematologic toxicity its administration at initially planned doses is not posible.

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GEMCITABINE (G) PLUS CISPLATIN (C) IN ADVANCED NON - SMALL CELL LUNG CANCER (NSCLC): FINAL PHASE II RESULTS.

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Fifty consecutive chemonaive patients (pts), with locally advanced or metastatic NSCLC, were recruited between January/96 and December/97, in a phase II study of G at higher dose (1200 mg/m2 as a 30 minute iv infusion on days 1,8 and 15) and C (100 mg/m2 over 60 minutes on day 15, before G) of a 28 day cycle for six , if no progresion .

Pts characteristis were: median age 61,2 years (39-74); 45 pts were males and 5 pts were females; performance status 0-1 in 36 pts and 2 in pts; 2 pts had stage IIIA, 24 pts had stage IIIB and 26 pts stage IV; histology: adenocarcinoma in 19 pts, squamous in 18 pts and large cell in 13 pts. 47 of 50 pts are evaluable for response and toxicity.

Twenty partial responses were reported , for and overall RR 42.5% (95%CI 28-57), 11 in stage IIIB (RR 50%;95%CI 30-70) and 9 in stage IV (RR 36%; 95%CI 18-54). 16 pts (34%) had stable disease and 11 pts (23.5%) progresive disease . With a median follow-up of 11 months (m), the median duration of response was 9.1 m and the median time to disease progresion was 7.1 m . The median survival was 8.9 m (for stage III 12.5 m and for stage IV 8.2 m) with actuarial 1-year survival of 39% (stage III 54% and stage IV 24%).

The main toxicities, over 219 cycles were: grades (G) 1-2 neutropenia 41.9%; G 1-2 anemia 50.1%; G 1-2 thrombocytopenia 27.3%; G 3 nausea and vomiting 13.6% . 5 pts developed febrile neutropenia and 1 toxic death ocurred The mean dose per infusion was G 1056 mg/m2 (89%) and C 82 mg/m2 (82%).

Our results indicate that higher doses of G and C was confirmed to be a very active and well tolerated regimen, in the treatment of locally advanced or metastatic NSCLC.

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GENETIC SUSCEPTIBILITY TO LUNG CANCER ASSOCIATED WITH RARE *HRAS1* VNTR ALLELES IN SPANISH LUNG CANCER PATIENTS.

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Background : The highly polymorphic *HRAS1* VNTR (variable number of tandem repeats) mapped 1 Kb downstream from the human H-*ras 1* has been described as an inherited predisposing factor in many human cancers.

Methods : 478 *HRAS1* VNTR alleles from patients with lung cancer, and 892 from unaffected controls were typed using PCR-long agarose gel electrophoresis assay of peripheral blood lymphocyte DNA. Rare alleles were differentiated from common alleles (a1, a2, a3 and a4) by shifts in electrophoretic mobility.

Findings : A higher percentage of rare *HRAS1* VNTR alleles in lung cancer patients than in unaffected controls (31.8% vs 21.9%) was confirmed. The presence of rare alleles was associated with an increased risk of lung cancer (*odds ratio* 1.65 [$p \leq 0.0001$]), indicating a genetic predisposition to lung cancer. No differences based on other clinicopathological variables (stage at diagnosis, histological subtype, gender) were observed. Furthermore, a meta-analysis showed a higher distribution of rare alleles in our study of Caucasian Spaniards than in other studies of American or Northern European Caucasian populations.

Conclusions : The presence of rare *HRAS1* VNTR alleles may serve as an inherited genetic predisposition marker for lung cancer. This presence can be easily determined from peripheral blood samples by PCR-based methods. Furthermore, interracial variations in allele frequencies and variations between Caucasian subpopulations suggest that genetic variations may be involved in susceptibility to lung oncogenesis, especially in certain ethnic populations.

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TAXOL-CARBOPLATIN IN ADVANCED NON SMALL CELL LUNG CANCER (NSCLC).
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Purpose: To evaluate efficacy and toxicity of this scheme with Taxol and Carboplatin in advanced non small cell lung cancer (NSCLC)

Methods: From November 97 to September 98, 23 patients (pts) were included, 21 males and 2 females. Median age was 53.3 (34-69), ECOG PS 0/1/2 was 4/15/4. Stage IIIB/IV was 6/17. Histology: squamous cell carcinoma/adenocarcinoma/large cell carcinoma was 12/9/2. This scheme was repetead every 21 days: Taxol 200mg/m2 day 1, Carboplatin AUC 7, day 1.

Results: 18 patients were evaluable for response and 23 for toxicity. 11 PR (61 1%). 2 SD (11.1%) and 5 PD (27.7%) were achieved. A total of 85 courses (85 sessions) were administered with a median of 4 cycles per pts (1-6). The dose intensity delivered was a 100% of the dose-intensity projected in 18 pts. Hematological toxicity was observed in 9 pts: (34.7%): Neutropenia G4 in 1 pt (4.3%), thrombopenia G2 in 2 pts (8.2%) anemia G1-2 in 5 pts (21.7%), leucopenia G1 in 1 pt (4.3%). One toxic death was reported, patient died due to neutropenia febrile G4. Non hematological toxicities: nausea-vomiting G2-3 in 5 pts (26%), alopecia G2-3 in 16 pts (73.9%). Neurotoxicity G2 in 5 pts (4.3%). GOT/GPT G2 in 1 pts (4.3%). Fever G2 in 1 pts (4.3%).

Conclusions: To present data on survival or response time is not possible due to short follow up. This study confirms that Taxol-Carboplatin is a well tolerated schema and offers favorable results in the management of the advanced non small cell lung cancer.

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COMBINED TREATMENT OF CHEMOTHERAPY AND RADIOTEHERAPY IN LOCALLY ADVANCED HEAD AND NECK CARCINOMA.
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Squamous cell carcinoma of the head and neck generally presents in stage III-IV , in most of cases being unresectable. Last years combined treatment of chemo-radiotherapy accounts with the aim of improving organ preservation and global survival.
Between 10/93 and 3/98, 29 patients with locally advanced carcinoma , inoperable, were treated in our service with chemotherapy and radiotherapy. Mean age was 52(range 34-72), 27 males(93%) and 2 females(7%). Stage III :6 pt (20.7%), and IV: 23 pt (79.3%). Primary tumors were located in: oral cavity 3 (10.3%),oropharynx 13 (44.8%), larynx 2(6.9%), hipopharynx 9(31%), nasopharynx 2(6.9%)
Pts received :cisplatin 20mg/m2/d, 5-fluorouracil 800mg/m2/d and Iv 20 mgr/m2/d IV in continuous infusion of 120 hours every 21 days, for four cycles. Sequential RT: 65-70 Gy.
Results: 29pt were evaluable for toxicity and 27 for response (93.1%).
Toxicity: No pt died due to toxicity. Main toxicities were(grade III-IV): neutropenia 7 pt, diarrhea 4 , mucositis 12pt, trombopenia 3 pt.
Response: RG 88.9%: 16 RC(59.3%), 8RP(29.6%) and 3 NC (11.1%). Global survival at 16m: 55%, and 11 pt remain free of disease. Time to progression: 13.7 m.
Conclusion: with induction chemotherapy and sequential radiotherapy a high response rates is achived, with tolerable toxicity in locally advanced squamous carcioma of head and neck.

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CISPLATIN (P), IFOSFAMIDE (I) AND ETOPOSIDE (E) (PEI) COMBINATION IN THE TREATMENT OF SMALL CELL LUNG CANCER (SCLC).
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Servicio Oncología. Hospital Meixoeiro. Vigo. Spain.

PURPOSE: In our past study with Cisplatin and Etoposide (PE) combination in the treatment of SCLC (Oncologia 1995;18, 3:50), 80% OR and 35% pCR was achieved. We present, the ifosfamide impact, in previous PE protocol for SCLC.
METHODS: Between May 1995 and Setember 1998, twenty-seven patients (pts) with previously untreated and histologically proven SCLC were treated with a combination PEI: P 100 mg/m2 day 2, E 100 mg/m2 days 1-3 and I 3000 mg/m2 day 1 every 21 days for six, if no progresion. Pts were evaluated after 3º and 6º cycle. Pts with pCR received prophylactic cranial irradiation and if limited disease (LD) received also thoracic radiotherapy after completion chemotherapy. Median age were 58.8 years (range 39-74). 25 pts are males and 2 pts females. 20 pts had ECOG 0-1 and 7 pts ECOG 2. 12 pts with LD and 15 pts with extensive disease (ED). 25 pts are evaluable for response and 27 pts for toxicity.
RESULTS: The overall response rate was 92% (23/25 pts) with 13 pRC (52%), 10 PR (40%) and 2 PD (8%). LD: 11 pCR (92%) and ED: 2 pCR (15%) were observed. With a median follow-up of 9,4 months (mo), the median duration of response is 8.5 mo and the median time to disease progresion is 6,7 mo. Median duration of suvirval is 9,2 mo (in the LD has not been reached and in the ED is 8,5 mo, with a actuarial 1-year survival of 45% (79% in LD and 21% in ED). The main toxicities, over 134 cycles (4,9 for pts) are: grade (g) 3-4 neutropenia 12,7%, g 3-4 anemia 6,7%, g 3-4 thrombocytopenia 4,5% and g 3-4 peripheral neuropathy 1.5%. 12 pts developed febrile neutropenia and 2 toxic death ocurred.
CONCLUSIONS: We concluded that this PEI combination is very active, with a considerable toxicity but manageable, in the treatment of SCLC.

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PRELIMINARY ASSESSMENT OF A PHASE II STUDY OF PACLITAXEL AND GEMCITABINE IN COMBINATION IN PATIENTS WITH ADVANCED NSCLC.
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H. Clinico de Zaragoza[1], H. Germans Trias i Pujol[2], H. Clinico Universitario de San Carlos[3], H. Vall de Hebron[4], H. General de Valencia[5], H. General de Elche[6], H. Servet de Zaragoza[7], H. Arnau de Vilanova[8], H. Clínico de Valencia[9], H. General Alicante[10], (Grupo Español de Cáncer de Pulmón).
Paclitaxel (P) and Gemtabicine (G) are two of the drugs, non-platinum derived, more active in NSCLC treatment. A phase II study was developed to evaluate efficacy and toxicity of this combination. Treatment schedule: P 150mg/m^2 (3h-infusion) followed by G 2000 mg/m^2 (1/2 h-infusion), days 1 and 15 every 28 days. The maximum number of cycles was 8. Chemo-naive patients (pts) with advanced NSCLC diagnosis, stage IV and IIIb (pleural infusion), performance status (Karnofsky $\geq$ 70), with measurable disease, normal hepatic and renal functions and normal blood count were included.
Results: 90 pts were recruited from Dec 1997 to Jun 1998. To date 85 pts have been evaluated. Sex: 81 pts male (95%), performance status: 100%, 10 pts (12%); 90%, 26 pts (31%); 80%, 37 pts (43%); and 70%, 12 pts (14%); Smokers: 75 pts (88%), significant decreased weight: 39 pts (46%). Histological diagnosis: Squamous, 39 pts (46%); adenocarcinoma 32 pts (38%), large cells 8 pts (9%), mixed 1 pt (1%), others 5 pts (6%). Differentiation grade: non-differentiated 7 pts (8%), poorly differentiated 28 pts (33%), moderate differentiated 5 pts (6%), well-differentiated 4 pts (5%), unknown 41 pts (48%), Disease stage: IIIb, 30 pts (35%); IV 55 pts (65%).
Conclusions: Toxicity assessment of the first 179 cycles shows a very tolerated haematological toxicity grade 3-4, except anemia grade 3 in 1 cycle and anemia grade 4 in another cycle. Toxicity non-haematological: alopecia grade 3 in 60 cycles and nausea-vomiting G3 in 4 cycles. This is a combination with and excellent and tolerated profile. Final analysis of data is ongoing.

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REGULAR TREATMENT WITH VINORELBINE (VNR) , IFOSFAMIDE (IFO) AND CISPLATIN (CDDP) IN PATIENTS (pts) WITH INOPERABLE NON SMALL CELL LUNG CANCER (NSCLC).

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GECP Grupo Español de Cáncer de Pulmón

INTRODUCTION: VNR, IFO and CDDP are considered among the most active drugs for the treatment of non small cell lung cancer (NSCLC). From January to September of 1998 has been conducted a study with pts diagnostically proven with inoperable NSCLC in stage IIIB and IV, age less than 75 years old and performance status ≤ 2. The pts were treated with the schedule: VNR 25 mg/m^2, D1 and D8; IFO 3 gr/m^2, D1; and CDDP 80 mg/m^2 D1 of a 21 days cycle.
The primary objectives were defined to asses the efficacy of this combination by the response rate and the overall survival, and to define its toxicity spectrum.
PATIENTS AND METHODES: The number of pts included has been 74 (70 males and 4 females), mean and median age are 60 (27-75) years; PS: 0-12 (16%9, 1-46 (62%), 2-16 (22%); stage: IIIB-37, IV-37 (50%), Histology: Adenocarcinoma 23 (31%), squamous 39 (53%), large cell undifferentiated 11 (15%) and anaplastic 1 (1%); 12 (16%) pts had relapsed from complete surgical resection that had been considered as curative.
RESULTS: This study is still ongoing and the results obtained from an interim data shows that the total number of cycles administered are 266, with a mean of 3.64 cycles per pt; and with an intensity of dose calculated only from the pts that finished the treatment and expressed in mg/m^2/week: VNR 13,25; 12,02; 5,8. IFO 923,24; 842,91; 322,7. CDDP 24,98; 23,33; 8,6 mean, median and standard deviation respectively.
The response rate has been calculated from 47 pts that have finished the treatment: complete response 1 (1,8%); Partial response 32 (58,2%); stable disease 3 (5,5%).
The most frequent toxicity was nausea and vomiting (G1-33%, G2-23%, G3-24%),but the limiting toxicity that had influence in the dose was the neutropenia (G1-4%, G2-11%, G3-20%, G4-8%), and of them acute infections 5,5%; anaemia (G1 -11%, G2 9%, G3 -10%); other events without clinical significance G1 and G2 were: asthenia, constipation and neurotoxicity: and alopecia G3 occurred in all the pts after the 3rd cycle.
CONCLUSION: We can assure after this first evaluation that this therapeutic scheme is efficient, and the toxicity can be beared by this group of pts. The overall survival still has to be defined.

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QUALITY OF LIFE IN NON SMALL CELL LUNG CANCER PATIENTS TREATED WITH CHEMOTHERAPY.

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JUSTIFICATION AND OBJECTIVES: The aim of our study is to assess the QL in Non Small Cell Lung Cancer Patients treated with Chemotherapy (ChT) and examine the patient appraisal of the extent to which each aspect of QL affects overall QL.

MATERIAL AND METHOD: 16 Patients with non small cell lung cancer treated with ChT were interviewed using the Functional Assessment of Cancer Therapy Scale. This scale produces subscale scores for physical, functional, social and emotional well-being, as well as satisfaction with the treatment relationship.
This instrument was administered at baseline, during the ChT treatment, and when the treatment was finished.
The degree and significance of differences between various measurements was calculated using W of Kendall method. Statistical significance was set at the 5% level.

RESULTS AND CONCLUSIONS: On the one hand, social-family and functional well-being decreased during the treatment and improved post-ChT, being better than baseline. As far as patient appraisal is concerned, he diminished the importance of the area functional and put the emphasis on social-family area, across the treatment and when it was finished.
On the other, equally, physical well-being decreased during the treatment and improved post-ChT, but it was worse than baseline. Patient attached great importance to this area during the treatment, however it neither was important at baseline nor post-ChT.
Finally, emotional well-being, as well as satisfaction with the doctor relationship were on the decrease across the treatment and even when it had finished. And furthermore, as far as patient appraisal is concerned, a great increase arose in importance.

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SEPTIC PULMONARY EMBOLISM COMPLICATING A PERIPHERAL VENOUS CATHETER SIMULATING METASTASES OF LUNG CARCINOMA

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INTRODUCTION:

Secondary bacteremia to peripheral venous catheters is a relatively frequent process in the hospital means. Gram positive germs are usually implied. Commonly, it is a self-limited process but in immunocompromise patients or with serious illness underlying they can to produce complications like endocarditis with right valves prevalence, septicaemia or like in our patient, septic pulmonary embolism.

CLINICAL CASE:

We report a 55 year-old male with small cells lung carcinoma in stadium IV (bony and hepatic metastases) that he is admitted because he present fever 4 days ago, without apparent focally. Fever didn't give with amoxicillin-clavulanic acid orally. In radiological study, a right parahilar mass and a condensation area with air bronchiogram in right base it is observed. The patient is entered with diagnosis of basal right pneumonia and treatment begins with curoxime intravenously and erythromycin orally, disappearing the fever in second day of treatment. One week later he begins with cough, greenish expectoration and fever (38.5°C). A chest radiograph of control showed multiple small lung nodules mainly in left lung, some of them cavities. CT scan confirmed this discovery. A cutaneous-mucous paleness, decreased breath sounds in left lung and hepatomegaly (3 cm) highlighted in physical exploration. A reddenly and pain were observed in periphery venous catheter area. Blood cells count sowed 6.600-leukocytes/ mm3 with 94.3 % of granulokytes. Echocardiogram was normal. Several blood cultures were negative. Antibiotic therapy of wide spectrum (imipenem) began remaining the patient without fever from the fifth day of treatment beginning. Disappearance of the nodular lesions was observed in later radiographic study, as well as resolution of the initial pneumonic process.

DISCUSSION:

Infectious processes (septic embolism, cavity pneumonia, and abscess) included mushrooms and mycobacterium granulomas are the most frequent cause of cavity images in lung. As non-infectious causes, we can remark non-septic emboli, vasculitic granulomas, primary or metastatic neoplasms, rheumatoid nodules and congenital bullaes. A detailed clinical history and the radiological discoveries can be helpful in differential diagnosis.

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PHASE III STUDY OF TREATMENT WITH ASSOCIATION OF CHEMOTHERAPY PLUS RADIOTHERAPY WITH OR WITHOUT SENSIBILISATION WITH TAXOL IN NON SMALL CELL LUNG CANCER IN STAGE III

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Introduction: Evaluation in patients with non small cell lung cancer of the antitumoral activity of a schedule of treatment with a combination of chemotherapy plus radiotherapy with or without secuential Taxol as radiosensitizer.

Material and Method: 16 patients have been included with histology of non small lung cancer in stage III, of wich 11 have been evaluated. All patients must have adequate hematologic, renal and hepatic functions, measurable or evaluable lesion and ECOG of 0-2. Patients who had received previous systemic chemotherapy or radiation therapy were excluded from the study. In arm A, treatment consisted in Cisplatin-based combinations x 3 courses each 21 days, 4 weeks after finished the chemotherapy, radiotherapy was administered with total doses of 60 Gy during 6 weeks. In arm B, also Cisplatin-based combinations x 3 courses each 21 days were administered. 4 weeks after having finished the chemotherapy, radiotherapy was administered with total doses of 60 Gy with Taxol 60 mg/m2 weekly during 6 weeks. Median age was of 64 years (range 50 to 75 years). All patients were men. Nine patients was squamous cell carcinoma, one adenocarcinoma and one large cell carcinoma. The median of ECOG was 1. Of 11 patients 5 were T3 N2 M0 (45,45%), 3 T4 N2 M0 (27,27%), 1 T4 N0 M0, 1 T4 N3 M0 and 1 T3 N0 M0.

Results: Up to date 11 patients are evaluable for toxicity and 10 for response. The number of cycles administered with induction chemotherapy was 29. The number of administrations in arm with Taxol and radiotherapy was 32. Toxicity during induction chemotherapy was low and during concurrent treatment with Taxol and radiotherapy was : 7 cycles with grade III mucositis (21,8%), 5 with grade II-II myalgia (15,6%), 4 with grade II-III paraesthesia (12,5%). Response rate obtained to induction chemotherapy was 50%, increased to 66,6% in arm A and after radiotherapy plus Taxol to 71,4% in arm B. Time to progresion and overall survival is the following: In arm of treatment with radiotherapy only, 2 of the 3 patients are exitus and 1 is alive in progression. Of the 8 patients who received Taxol and radiotherapy, 4 continue alive and without progression (7+, 10 +, 7+ y 3+).

Conclusion: Though the sample size of this study is small, the response rate obtained and the duration of response until this moment show that association of Taxol to radiotherapy is active.

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A PHASE II NON-RANDOMIZED TRIAL OF THREE COURSES OF PREOPERATIVE DOCETAXEL/CISPLATIN/GEMCITABINE IN STAGE III NSCLC PATIENTS

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Introduction: The rationale of neoadjuvant chemotherapy lies in the possibility of eradicating micrometastatic disease, which is almost invariable manifest when ipsilateral mediastinal or subcarinal lymph nodes are involved. We detected aberrant methylation of p16, DAP-Kinase, GSTP1, and MGMT of at least one gene in 68% of NSCLC resected tumors. 73% of these positive primary tumors also had abnormal methylation in matched serum samples.

Patients and Methods: The primary endpoint of the study was to measure response, toxicity, and operability when three courses of pre-operative docetaxel/cisplatin/gemcitabine were administered in patients with T1-3N2M0 or T4N0-1, as follows: docetaxel 20mg/m^2 weekly on days 1, 8, and 15; cisplatin 75mg/m^2 on day 1; gemcitabine 1000mg/m^2 on days 1 and 8. Two additional adjuvant courses were administered if positive mediastinal lymph nodes were found at surgery or if serum tumor DNA was detected. Patients with positive resection margins or involvement of the first mediastinal lymph node received 60 Gy (lineal accelerator 6 MV) plus docetaxel 20 mg/m^2 weekly.

Results: Since July 1998, nine male patients have been included: mean age = 60 years (range 39-76), performance status 0/1, 5 squamous cell carcinoma and 4 non-squamous. Six patients have completed treatment; 5 had PR and 1 had progressive disease. All five responders had complete resection and one had pathologic complete response. Myelotoxicity was mild, and non-hematologic grade 3 and 4 was not observed.

Conclusions: This interim analysis indicates that a high response rate is attained with mild toxicity. A multi-center study is being planned by the Spanish Lung Cancer Group to confirm these results.

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EXPERIENCE WITH NEOADJUVANT CHEMOTHERAPY IN STAGE IIIa NON-SMALL CELL LUNG CARCINOMA (NSCLC).

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Introduction : Stage IIIa NSCLC, with a mean survival (SV) of 12 months, is considered potentially resectable and curable. According to the new TNM staging, T3N1 cases have a better prognosis than T1-3N2 ones, thus Mediastinoscopy plays an essential role in their accurate diagnosis.

Objectives : We administered neoadjuvant chemotherapy (CHT) to increase tumoral resectability and treat the micrometastases in order to improve global SV.

Material and methods :We recruited 16 patients (15 males and 1 female, mean age : 60 years and PS : 0-1) with clinical stage IIIa between March 95 and September 97, of whom 56% had squamous cell ca., 25% adenocarcinoma and 19% other histology; 14 were N2 and the rest T1-3. The protocol was 3 cycles of CT with 100 mg/m2 Cisplatin day 1 and 120mg/m2 Etoposide Day 1-3 every 3 weeks, with subsequent radiological or histologic reevaluation of the disease.

Results : The response to CHT was 6% Complete Remissions, 31% Partial Remissions and 4 cases of Tumoral Progression. After CHT, 2 patients underwent Radical Surgery (3 others were inoperable for medical reasons, thus resectability rate: 31%) obtaining 12.5% Pathological Complete Responses. The remaining patients, except 3 who died earlier, were treated with RT at doses of 40-60 Gy on primary tumor and mediastinal and supraclavicular nodal chains. In October 98, with a median follow-up of 12.6 months, mean SV of group is 12.6 months and median SV 12 months. Mean time to Progression has been 4.4 months and Disease-Free Interval (DFI) 12 months. At the end of the study, patient outcomes are: 12 dead from the disease, 3 alive with tumor (all due to local recurrence) and 1 disease-free.

Conclusions : There have been randomized trials pointing out the benefits of pre-surgical neoadjuvant CHT in terms of Disease-free-survival and global SV. However, patients must be standardized according to stage to be able to generalize treatment according to prognostic subgroups. Neither the role of RT associated to pre-surgical CHT nor the comparability of neoadjuvant CHT plus radical RT with CHT plus surgery have yet been determined.

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CUSHING SYNDROME ASSOCIATED TO THYMIC CARCINOID.

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Thymic carcinoid is an infrequent malignant neuroendocrine tumor of the mediastinum. Less than 150 cases have been reported in the medical literature. It follows a very aggressive course, with frequent local relapses and distant metastases. Thirty cases of paraneoplastic Cushing syndrome associated to thymic carcinoid has been previously reported, but most involved patients with widespread distant metastases. We report a case of thymic carcinoid with locoregional invasion and no distant metastases associated to Cushing syndrome.

A 50-year old male presented with Cushing syndrome including typical phenotype, hiperglycemia and high blood pressure. Intensive work-up showed that the syndrome was due to ectopic ACTH secretion. The search for a tumor responsible for the ACTH secretion disclosed an anterior mediastinal mass (17x18x8 cm) in the CT scan. No distant metastases were found. A macroscopically complete resection was performed. The pathological diagnosis was thymic carcinoid reaching surgical margins. Pericardial fluid drained intraoperatively was positive for malignant cells. The patient had a stroke (talamic infarction) in the postoperative period with. Signs and symptoms of Cushing syndrome subsided after surgery. The patient was treated with chemotherapy (6 courses of cisplatin/VP16) followed by mediastinal radiotherapy and is currently disease-free and with minimal neurological sequelae 24 months after surgery.

Even though thymic carcinoid is an infrequent cause of Cushing syndrome, extensive work-up for an occult malignancy may be justified in patients with Cushing syndrome due to ectopic ACTH secretion since active treatment of the tumor may induce remission of Cushing syndrome.

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LAPAROSCOPIC COLECTOMY *Vs* CONVENTIONAL SURGERY IN THE TREATMENT OF COLON CARCINOMA. PORT-SITE METASTASES AND RECURRENCE RATE.

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Introduction: The most important treatment of colon carcinomas is the surgical treatment. Some concern exists about the accuracy of the laparoscopic surgery in performing appropriate oncologic treatment and staging of the colonic malignancies. However, the role of laparoscopic techniques in the treatment of colon carcinomas is questionable. Some concern exists about the accuracy of the laparoscopic surgery in performing appropriate oncologic treatment in colonic malignancies and the influence in survival. **Aim.** To study the impact of the laparoscopic approach to the patterns of port site metastases (PSM) and recurrence rate (RR) of resected colon carcinomas. **Patients and Methods:** A prospective, randomized study was conducted comparing laparoscopic assisted colectomy (LAC) versus open colectomy (OC) for colon cancer. We present the preliminary results in relation to: 1) the short-term outcome and the feasibility of the laparoscopic procedure to perform accurate oncologic resection and staging; 2) tumor implantation during laparoscopic surgery; 3) recurrence rate after colon resection and, 4) the cost of the laparoscopic procedures. Inclusion criteria are: patients diagnosed of colon carcinoma above 15 cm from anal verge. Exclusion criteria are intestinal obstruction, carcinomas localized at transverse colon, infiltration of adjacent organs and/or metastasic disease (Dukes D1 and D2) at the time of the surgery were excluded. Follow-up in the outpatient clinic was done every three months for a minimum of 12 months. **Results:** Out of 149 colorectal resections performed from November 1993 to October 1997, 73 LAC and 76 OC. Patients data were similar in both groups in relation to age, gender, Dukes stage, type of surgical resection (71±9 in LAC and 68±12 in OC). The mean follow-up was 32.6 months with a range of 12 to 60 months. No abdominal wall recurrence after laparoscopic resection of colonic cancers was seen (no trocar site recurrence or incision to remove the specimen). Recurrence rate was similar in both groups (LAC 5 patients; 6.5% and OC 15; 19.7%). **Conclusions:** The laparoscopic approach improves the short-term outcome of segmental colectomies for colon cancer. The laparoscopic colectomy is associated with a similar recurrence rate than open procedures for colon cancer. However, the further follow-up of these patients will allow us to answer in the near future whether or not the laparoscopic approach may influence the long-term outcome (five years).

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INCIDENCE OF HEREDITARY NON-POLYPOSIS COLORECTAL CANCER (HNPCC). MOLECULAR SCREENING. A PROSPECTIVE STUDY

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Introduction: HNPCC is the most frequent predisposing colorectal cancer (CRC) syndrome. In contrast to the adenomatous polyposis syndrome (where more than 100 adenomatous polyps are located along the large bowel), the HNPCC is difficult to identify phenotipically since it lacks distinctive clinical characteristics. Germ-line mutations in the mismatch repair genes (MMRG) is a common feature of the HNPCC syndrome. Moreover, tumors from this patients show a higher percentage of instability in microsatellite sequences (MSI) than the sporadic cases (80% vs 10%). Actual diagnosis is based basically on family history. The Amsterdam Criteria (AC) proposed to identify he HNPCC families have been criticized to be e too restrictive. Some less strigent criteria have been proposed although in that case the rate of mutations in the MMRG is quite low. In the view of this controversy, there is a need of a molecular marker for HNPCC. The aim of this study is to determine the frequency of the HNPCC syndrome among patients with CRC and to test a screening strategy for the disease in those patients.

Methods: We used a similar strategy to the devised by Aaltonen et al. (N Engl J Med 1998;338:1481-7). We screened tumor specimens from 101 consecutive CRC patients for MSI. Eight loci containing dinucleotide repeat sequences, one tri and one tetranucleotide markers representing different chromosomes were studied in each case. Tumors showing instability at two or more loci were scored as MSI+. Normal tissue from the patients with MSI+ was screened by direct sequencing for germ-line mutations of the hMSH2 annd hMLH1 genes.

Results: Nine out of 101 patients (9%) were MSI+. Two out of these nine patients showed germline mutations in the MMRG. From these MSI+ patients 3 were AC positive. 4 does not completely fulfill the AC although there was a familial history of CRC and two of them were AC negative. From the two patients with mutated MMRG (2% of 101), one completely fulfill AC , whereas the other had had colorectal and endometrial cancer and also a familial history of gastric cancer.

Conclusions: In our serie of patients, at least 2% had HNPCC. We recomend testing form MSI in tumors from patients with CRC and a family history of HNPCC. Analysis for germline mutations in MMRG sould be done in those patients with MSI+ tumors

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A PHASE II TRIAL ON THE ADDITION OF CISPLATIN TO HIGH DOSE WEEKLY 48 HOUR-CONTINUOUS INFUSION 5-FU IN ADVANCED GASTRIC CANCER.

Cervantes A, Navarro M, Carrato A, Sastre J, Antón A, Vicent JM, Tabernero JM, Escudero P, Aparicio J, Torregrosa MD, Rifá J, Maeztu I, Pellegrí A , Campos JM, Grávalos C, Diaz-Rubio E, on behalf of the TTD group.

To assess the activity of a combination of cisplatin (70 mg/^{2}) given every 21 days together with the classical weekly schedule of 48 hour continuous infusion of 5-FU (3g/m^2), the TTD group developed a phase II trial with the following inclusion criteria: histologically confirmed gastric cancer, locally advanced or metastatic and bidimensionally measurable, PS<3, good liver, kidney, heart and bone marrow function, absence of brain metastasis and second tumors. From October-96 to June-98, 153 patients were include in 22 different hospitals. 141 cases are fully evaluable for analysis. Mean age is 60 (32-75), 76% were males and 24% females. 65% were initially diagnosed as metastatic disease. The median number of metastatic locations is 2 (0-4). 58% had liver metastasis, 40% lymph node metastasis and 29% had a non resected primary tumor. Seven complete responses and 52 partial responses have been observed in 114 evaluable patients, with an objective response rate of 52% (IC95%:49-69). 20% showed progression and 28% got stable disease. Grade 3-4 toxicity were: nausea and vomiting 10%, diarrhea 7%, mucositis 11%, alopecia 4%, neutropenia 11% and thrombocytopenia 15%. Median time to progression was 7,3 months and median survival reached 9,4 months. The present schedule is an active and well tolerated combination in patients with advanced gastric cancer.

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COLO-RECTAL CANCER: TEN YEARS' EXPERIENCE IN MULTIDISCIPLINARY TREATMENT

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Introduction: descriptive study over 423 patients diagnosed with CRC attended in our center from February 1986 to October 1996.

Material and methods: all p were diagnosed with colon or rectal cancer after histologycal confirmation. Global survival(GS), relapse index (RI) and free-relapse time (FRT) were studied regarding tumor localization, stage and adyuvant treament when any. Median **age** was 68 years (22-95). 209 (49,4%) were males and 214 (50,6%) females. **Localization**: colon in 278 p (66.7%), rectum in 118 (27,9%) and both in 25 p 85.9%). **Histology**: 87.7% were adenosquamous-cell type. Dukes' **Stage** (known in 415 p): A:18, B: 175, C:110, D:112. All p went thru surgery, followed by some kind of adyuvant treatment in 157 (radiation therapy in 3 p (0,7%), chemotherapy in 140(66,6%) and combine treatment in 14 p (3,3%)).

Results: GS analysis was performed on 414 p. The median survival time was 31 months (m) (CI 95%:24-38). **Relapse** was observed in 109 p, consisting in a local recurrence in 22 cases (20,2%), metastatic in 54 (49,5%) and local and metastatic in 20 (18,3%). The **TRL analysis** was performed on 398 p, with a median time of 76 m. 75% of p were relapse-free after 24 months' follow-up. Rergarding **localization**, the **GS analysis** shows that the median survival was 39 m (CI 95%:25-53) in the group with colon cancer, versus 27 m(CI 95%:20-33) in the rectal group, which was statistically significant (p:0,05, Log-Rank test). A **relapse** took place in 24,3% of colon localization and in 21,2% of rectal or colo-rectal, with no statistical significance (p: 0,83). GS according to **stage** showed a higher mortality range for stage D compared to any less advanced stage (p<0,001). Statistical significance was also found comparing stage A+B1 to C (p:0,009), but not comparing stages B2+B3 to C (p:0,08). We found a statistical significant (p:0,02) benefit in survival but not (p:0,09) in RI or TRL among those p in stages B2, B3 and C receiving some kind of **adyuvant treatment**.

Conclusions: these results obtained in our sanitary area (400.000 inhabitants), with 40-45 cases of CRC annual incidence are consistent with already described aspect about epidemiology, histologycal characteristics, natural history and prognostic factors of this tumor.

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PHASE II STUDY OF IRINOTECAN (CPT 11) IN PATIENTS WHIT NOT PRETREATRED ADVANCED COLORECTAL CANCER.

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Purpose: To assess the efficacy and toxicity profile of CPT-11 in patients with advanced colorectal cancer, not pretreated with a previous first line chemotherapy. Methods: Since October 96, until October 97, 65 patients were recruited, 61of whom are currently evaluable for toxicity and 60 for efficacy. The planned CPT-11 dose was 350 mg/sqm q3w.Treatment for delayed diarrhea was based on a high dose loperamide schedule. Previous adjuvant treatment with 5-FU was allowed. Results: Median age: 59.01 years (27-70); M/F:36/25. Tumor sites 44.3% colon, 55.7% rectum, 86.9% of the patients had one metastatic site (77.4 % liver, 13.2% lung, 7.5% locoregional, 1 9% other), and 13.1% had >= 2 sites (37.5 liver + locoregional, 25% liver + lung, 37.5% other); 31.1% of the patients had received a previous 5-FU adjuvant treatment and 68.9% were chemotherapy naive.345 cycles had been assessed (median 6, range 1-12), 2 (0.6%) of them had delayed and 11cycles (3.2) required dose reduction due to toxicity. Diarrhea (NCI grade 3 /4) appeared in only 6.69% cycles. Neutropenia (NCI grade 3/4) was observed in 3.48 cycles. 3/61 patients had febril neutropenia (1.15%cycles). A Mild collinergic syndrome, easily controlled with atropine, was observed in 77% of patients (44.6% cycles). 8 toxics episodes required hospitalization (5 severe diarrhea, 1 neutropenia with diarrhea, 1 acute pancreatitis and 1 died after the first cycle of treatment due to severe neutropenia with renal failure. 60 patients are evaluable for efficacy after 3 cycles per patient. 4/60 patients achieved complete response (6.7%), 11/60 patients achieved partial response (18.3), 24/60 maintain stable disease (40%) and 21/60 (35%) have progressed. 4 patients are still under treatment. Conclusion: Results show CPT-11 to be a useful drug in not pretreated advanced colorectal cancer. Preliminary data shows an overall response rate of 25 % and a tumor growth control of 65%. Survival rates are not yet available. Updated results will be reported.
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EXPERIENCE IN THE TREATMENT OF RECTAL CANCER USING RADICAL SURGERY AND TOTAL MESORECTAL EXCISION. RESULTS OF 69 CONSECUTIVE PATIENTS.

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Introduction: Surgery for rectal cancer must be considered as a main prognostic factor when considering recurrence rates and survival. According to different reported series, 5-year local relapse rates from up to 20-45%, in spite of adjuvant treatments like chemotherapy (CT) and radiationtherapy (RT), can only be explained by microscopic disease persistance at the rectal compartment. Surgical techniques involving a total mesorectal excision and a radical lymphadenectomy to the inferior mesenteric artery (IMA) root have shown, by themselves, a significant decrease of local and distant recurrence rates, as well as an improvement on the overall survival (OS). In addition, questions arise about the need for adjuvant treatments in those patients (pts), specially radiationtherapy.

Targets: To determine local (LR) and distance (DR) relapse rates and survival rate for resectable rectal cancer by means of techniques involving total mesorectal excision (TME). To assess surgical morbidity rate when compared to own previous series where only conventional radical surgery was performed.

Material and methods: From Jan 92 to Jan 98, a total of 69 consecutive radical resections including TME have been performed. Of these, 59 pts (85,5%) underwent a low anterior resection (LAR) and the remaining 10 pts (14,5%) underwent an abdominoperineal resection (APR). Distribution by site was as follows: 46 (75%) upper 1/3 of rectum, 13 (18%) middle 1/3, and 10 (7%) lower 1/3 of rectum. The APR procedures were all done for lower 1/3 tumors. Distribution by Stage was: Stage I 13 pts (19%), II 24 pts (35%), III 23 pts (33%) and IV 9 pts (13%). The TME was performed through avascular dissection under direct vision of the posterior and lateral side-walls of the pelvis. Peritoneal reflection was included with the anterior plane. All patients underwent radical lymph node dissection up to the IMA take off. Patients elegible for adjuvant treatment received chemotherapy and/or radiationtherapy based on the standard regimens being used.

Results: TME techniques did not show a higher morbidity and mortality rate when compared to previous series of the same surgical team. Nerve-sparing procedures were feasible for most patients. Neither operative blood transfussional needs and surgical time were increased nor the mean postoperative hospital stay. Only one patient undergoing a LAR developed an anastomotic leak (1,6%). Adjuvant treatments (QT and/or RT) were delivered according to standard time schedules, without reported added toxicities. After a mean follow-up of 43 months, range (9-71 mo) and only considering patients undergoing curative surgery (Stages I,II, III; 60 pts) the LR rate was 7,5% and the DR rate was 15%. Overall 3,5 year survival for the whole series reached 77%.

Conclussions: The knowledge of anatomic features of this area makes feasible the excision of the rectum-mesorectum complex with the likelyhood not to leave residual disease behind at the locoregional stage of rectal cancer. The TME procedure does not increase surgical morbidity or mortality. Application of adjuvant treatments are not disturbed by this technique either, and allows a substancial improvement for disease free survival (DFS) and overall survival (OS).

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OXALIPLATIN, ISOVORIN (IV) AND 5-FLUOROURACIL (5FU) AS SECOND-THIRD LINE TREATMENT IN ADVANCED COLORECTAL CARCINOMA (ACC).

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Oxaliplatin has shown activity in the treatment of resistant fluoropyrimidine colorectal cancer, either used alone or in combination with 5FU. The aim of this study is to evaluate the oxaliplatin clinical efficacy in second-third line treatment.

MATERIALS AND METHODS : in 21 patients with advanced colorectal carcinoma in progression, pretreated at least with a fluoropyrimidine regimen, was administered this treatment scheme: Oxaliplatin 100 mg/m² I.V.in 2 hours day 1 and Isovorin (IV) 250 mg/m² I.V. in 2 hours with 5-FU 1500 mg/m² I.V. in 20 hours days 1 and 2, every 15 days. Reevaluation was performed every 3 cycles and treatment was continued if response or stabilization was achieved until progression or unacceptable toxicity. Since the sixth cycle oxaliplatin was administered monthly in alternatives cycles. 13 patients were male and 8 females, median age 61 years (29 to 75), 15 had a sigmoid-rectal carcinoma and 6 a colon carcinoma. The most frequent metastatic localizations were liver, (11 pts), peritoneum (6) and lung (5),and 9 patients (43%) had more than one localizations. It was second line treatment in 11 patients (53%) and third line in the other 10 (47%) after CPT-11 administration.

RESULTS : 138 cycles were administered (median 6 cycles, 1 to 18).16 patients had stable disease (76%), 2 achieved partial response (10%) and 3 had a progressive disease (14%). 12 patients (57%) achieved clinical improvement (better performance status, reduction of symptoms or CEA or Ca 19.9). The main toxicity was nausea-vomiting G1-2 in 53 cycles (38%) and G3-4 in 14 cycles (10%) and neurological problems G1-2 in 29 cycles (21%) and G3 in 3 cycles (2%). The free-progression time was 4 months, median survival was 8 months and 7 patients (33%) were alive one year after starting the treatment.

CONCLUSIONS : This preliminary results show the combination with oxaliplatin, isovorin and 5FU is active in the treatment of progressive advanced colorectal patients, pretreated with fluoropyrimidine regimens. The toxicity profil is very moderate and the stabilization is achieved in 60% of patients.

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VALUE OF CHEMOTHERAPY (CTX) AFTER FAILURE TO 5 FLUOROURACIL (5FU) IN PATIENTS WITH METASTATIC COLORECTAL CARCINOMA (MCC). **M. Zorrilla, V. Alonso, T. Puertolas, J. Martinez-Trufero, A. Artal, J. Maurel, A. Herrero, A. Antón. Servicio de Oncología Médica. Hospital Miguel Servet. Zaragoza.**

Introduction.- The value of systemic second line CTX for patients with MCC after failure to 5FU has been recently shown (Proc. ASCO 17: 984, 1998). Few information about other CTX salvage regimens and patients suitable for this treatment have been published.

Material y Methods.- 35 assesable patients after failure to 5FU CTX have received second line CTX with Irinotecan (CPT11) 350 mg/m^2 every 21 days and 15 patients who subsequently received salvage CTX based on oxaliplatin (LOHP) with the FOLFOX3 schedule: LOHP 85mg/m^2 day 1°, folinic acid 500mg/m^2 days 1°-2° and 5FU 3 gr/m^2 in 48 hours continous infusion, every 2weeks.

Results.- Median age 63 years (31-74). Median ECOG 1. Adjuvant CTX 11%. Metastatic sites: 1 40%, 2 or more 60%. All patients received CTX for advanced disease with 5FU and 23% received 2 lines of 5FU. Response rate in first line CXT based on 5FU was 17% and 65% SD. Response rate after CPT11 CTX was 11,5% PR, 40% SD, with a median time to tumor progresion (TTP) of 3,5 meses and a median survival since 2nd line (CPT11) of 7,5 months and 24 months since first line CTX. 15 of 35 patients received third line CTX with LOHP, with 20% PR (3/15) and 33% SD (5/15) with a median survival since third line CTX of 7 months. 12 patients of the whole cohort survived more than 24 months from diagnosis of MCC, with a median survival of 31 months (24-60).

Conclusions.- A high number of patients with MCC are eligible for second and third line CTX, and are those with a long time survival. Although response rate is low, tumor stabilization may be achieved up to 40-50% of the patients. In a future randomized trials will show the best way to combine these drugs and the secuence of treatment of MCC.

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BOLUS 5-FLUOROURACIL + LEUCOVORIN (FU-LV) IN FIRST-LINE TREATMENT OF ADVANCED COLORECTAL CANCER.

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The Oncology's Department started to work at our Hospital in 1.993. At that moment, biochemical modulation of 5-Fluorouracil with low dose Leucovorin was considered standard treatment of Advanced Colorectal Cancer. We report the experience for five years with this treatment.

From march-1.993 to february-1.998, we used FU-LV in the first-line treatment of patients with Advanced Colorectal Cancer, out of any clinical assay. The treatment scheme was Leucovorin 20 mg/m2 (2 h infusion) + 5-Fluoruacil 425 mg/m2 (bolus), for 5 days, every 4 weeks, until progression of disease or serious toxicity. A total of 53 previously untreated patients (male 21, female 32 median age 63 years (range 34-75), were enrolled in this 5 years. There was 30 pts. with stage IV at diagnosis, and 23 pts. with recurrent disease (from 8 to 36 months after the initial diagnosis). Only 8 pts. had received any adjuvant treatmen: pelvic radietherapy in 3 pts, FU-LV in 4 pts, and FU-Levamisol 1 patient. The ECOG at the beguining of the FU-LV was 0: 36 pts, 1: 12 pts, 2: 4 pts, a 3: 1 patient. Metastatic organs was liver (33 pts), lung (14 pts), primary tumor (13 pts), abdominal lymph node (12 pts), peritoneum (10 pts), local and presacra recurrance (5 pts. both), and 2 patients had metastasis ant bone, pleural and spleen. There was 22 pts with only one organ involved, 22 pts with 2 and 11 pts with more than 2 organs.

For response evaluation, If measure of disease was difficult by radiology, we accepted a raising level of tumor markers (CEA, CA 19.9 or both) as Disease Progression. There was 2 early deaths and 1 toxic death who also were included as Disease' Progression.

The median number of cycles by patient was 6 (range 1 to 12), and a total of 371 cycles was administred for all the group. The median follow-up was 30 months (range 8 to 41 moths). The response rate observed was 26.6% (95% IC 19%-34%): 3 Complete Response, and 11 Parcial Response (one of these obtained complete remission after resection of lung metastasis on response). There was Estable disease in 19 pts (36%) and Progression in 20 pts (38%) This included 2 early and 1 toxic deaths). In the group with response, the estimated median duration of response was 15 moths (95%IC 12-19). For all the patients, the estimated median time to progression was 8 monts (95%IC 3-13) and global survival was 12 14 months (95%IC 8.5-17).

Toxicity of 5FU-LV was mild. Grade 3 or 4 leucopenia ocurred in 2 pts (4%), and grade 3-4 neutropenia in 8 (14%), with 3 episodes of febrile neutropenia (5% of patients). Grade 3 or 4 anemia and thrombocytopenia ocurred in 2 (4%) and 1 (2%). The grade 3-4 non-hematologic toxicities were mucositis: 3 pts (5%), emesis: 2 pts. (3%), diarrhea: 8 pts (14%). There was 1 toxic death by grade 4 mucositis and febrile neutropenia. There was also another 12 pts (25%) who need dose reduction or treatment delay about persistent or recurrent grade 2 toxicity (mucositis or diarrhea), specially in prolongued tratments.

Conclusions: Our Response Rate (26.6%) is similar to other reports for phase II and III 5FU-LV assay. The toxicity was generally mild, but 30% patients need dose reduction about toxicity. More active and less toxic schemes are needed in the treatment of Advanced Colorectal Carcinoma. Quality of life must be too evaluated in the paliative treatment of colorectal carcinoma.

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HIGH RATE OF COMPLETE REMISIONS (CR) WITH CONCOMITANT CHEMORADIOTHERAPY(QRT) IN ESOPHAGEAL CANCER(EC). PRELIMINARY RESULTS IN A PHASE II TRIAL.

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Introduction. Concomitant treatment with 5FU-CDDP+RT is considered as one of the theraples with more antitumoral activity in EC. Twenty five- fifty percent of pathological complete remisions have been published . However, toxicity is severe and it is not yet determined the best concomitant treatment. Our purpose was to investigate a new combination therapy in order to get high rate of response and treatment compliance. **Patients and methods.** Study design: a sequential phase II trial with a minimal 10% and optimal 30% activity of CR.Treatment: CDDP (100mg/m^2 day 1) and 5FU(1000mg/m^{2}x5d); day 28 5FU(1000mg/m^{2}x5d)concomitant with RT; last 5 days with RT CDDP (100mg/m^2 day 1) and 5FU(1000mg/m^{2}x5d). RT dose was 45 or 59, 4Gy depending on resecability. Inclusion criteria: squamous cell or adenocarcinoma T2-3N0-1M0. Fourteen pt have been enrolled, 10 have already finished the treatment and 8 are evaluable for response. **Results.** Toxiciy 1 cycle: G-3: nausea and vomiting 9%, mucositis 9%; neither haematological nor G4 toxicity. Combined QRTtoxicity:G3:haematological 30%, esophageal 30%; G4:haematological 30%, esophageal 20%; 5 neutropenic fever and 1 pneumonia. No death for acute toxicity; no interruptions to RT. We performe 25% dose reduction in 66% of pt. No acute complications in any of the 4 pt who underwent surgery. Response: subjective improval in 90%pt; biopsy:cCR 5/7.After surgery:pCR 3/4, pPR 1/4. 7 c+pCR and 1PR out of 8pt evaluable for response.**Conclusions:** Our therapy provides a high rate of CR (75%pathological). Toxicity is severe, but manageable. Treatment compliance is 100% and concomitant QRT does not increase postsurgical morbility.

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Dose finding study with irinotecan (CPT-11) in previously treated colo-rectal cancer

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From May 1997 to July 1998 20 previously treated patients with colorectal cancer have been treated with increasing doses of CPT-11 until grade II toxicity (except alopecia). The goal was to identify the adequate dose in the clinical practice. we began with a dose of 250 mg/m2 and was escalated until 350 mg/m2 or grade II toxicity. In the case of detect a grade III toxicity with the initial dose we considered as tolerable dose the dose of the inferior steep. Characteristics of the patients: median age 59 years (range 42-60), Karnofsky 70%; 5 patients progressed to a complementary treatment and 15 was diagnosed as stage IV at the begging; 4 patients received pelvic radiotherapy; grade III toxicity with previous treatment in 4 patients; 3 partial responses, 13 no-change and 4 progressive disease as responses to previous treatment. The metastatic disease was located in the liver in 11 patients; pelvic recurrent disease, 3; lymph nodes, 2; lung, 2; multiple organ metastasis in 2 patients.
Results: 5 patients progressed with the first cycle and they only received one cycle of chemotherapy; 6 patients debuted with grade II-III toxicity in the first cycle; 2 of the 4 patients who received previous radiotherapy tolerated 350 mg/m2, one of 300 mg/m2 and another one of 250 mg/m2; we founded grade II-III toxicity in 19 pätients (7 cases of vomits grade III, 4 leukopenia grade II-III, 3 diarrhoea, 2 anaemia and one case of mucositis); The median tolerable dose was 300 mg/m2, only 2 patients reached 350 mg/ m2; We documented 2 partial responses for an overall activity of 10% (4-24%).
conclusions: In the clinical practice and with unselected patients it is not recommendable to begin with a dose of 350 mg/m2

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SQUAMOUS CELL CARCINOMA OF THE ANAL REGION: FIFTEEN-YEAR EXPERIENCE AT A SINGLE CENTER.

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INTRODUCTION: Squamous cell carcinoma of the anal region is a rare neoplasm. Surgery (SUR) according to Miles' technique (abdominoperineal resection) was the most frequently employed treatment until recent years. The introduction of radiotherapy (RT), alone or in combination with chemotherapy (CT), has offered similar results than SUR with the advantage of sphincter preservation. Today, multimodal treatment is the therapy of choice. Transrectal ultrasonography development has led to a better disease staging and a higher percentage of local resections.

MATERIAL Y METHODS: A retrospective study was performed to review the clinical features, prognostic factors, and treatment options employed in patients with anal region carcinomas diagnosed at our institution between 1983 and 1998. The following parameters were analysed: age at diagnosis, sex, initial symptoms, disease stage, first-line therapy, local and/or systemic relapse incidence, and patient status. The actuarial survival was analysed through the Kaplan-Meier method.

RESULTS: From 1983 to 1998, 16 pacientes with squamous cell carcinoma of the anal region were diagnosed. There were 11 females and 5 males. Median patient age was 65 years (range, 52-85).

The most common initial symptoms were rectal bleeding (8 patients), a perianal mass or swelling (3), and ulceration (3 cases). Stage classification was: stage I (5 patients), II (6), III-A (2), and III-B (3).

The first-line treatment consisted of SUR in 6 patients (3 Miles' resections and 3 local resections), RT in 4, SUR+RT in 3, and CT+RT in 3 cases. At the time of this analysis, 8 patients have relapsed (7 local, 1 systemic recurrences).

Eleven patients are still alive (8 of them disease-free), and 5 have died (4 due to tumor progression, 1 due to intercurrent causes). Of note, one patient relapsed locoregionally more than 10 years from the initial diagnosis.

The actuarial 5-year survival is 56%, and median survival has not yet been reached.

CONCLUSIONS: Our results are in concordance with epidemiologic data from the literature. We confirm a low incidence of systemic metastases, both at diagnosis and at relapse. The most commonly used therapy was SUR alone or in combination with RT. However, in the last years, RT alone or in combination with CT has been employed with increasing frequency. In contrast with other reports, most of our patients were diagnosed at early (I and II) disease stages. Survival is similar to other published series.

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PROGNOSTIC VALUE OF THE EXPRESSION OF BCL-2, BAX AND BCL-X IN PATIENTS WITH ESOPHAGEAL CANCER

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Introduction: Expression of apoptotic related proteins is involved in prognosis and chemotherapy response in several tumors. To date, the prognostic value of the Bcl-2 family proteins in esophageal cancer has not been established. The purpose of the present study was to determine the expression of Bcl-2 and Bcl-X (anti-apoptotic proteins) and Bax (pro-apoptotic protein) and their impact on the clinical outcome of 43 patients with esophageal cancer. The study was carried out with patients treated at the Darmouth Hitchcock Medical Center (Lebanon, NH) between 1994 and 1997.

Patients and Methods: We analyzed 43 newly diagnosed patients (p): 31 males and 12 females. 65% of the tumors were adenocarcinomas. Patients were classified as Stage I(2p); II(19p); III(19p); IV(3p). 22p were treated with chemoradiotherapy. Esofaguectomy was performed in 23p. Follow-up was between 3 and 44 months. The expression of Bcl-2, Bax and Bcl-x was assessed by immunohistochemical analysis with a specific antibody for each protein. A staining of more than 25% of the tumor cells was recorded as a positive case.

Results: Expression of Bcl-2, Bax and Bcl-X was detected in 30% (13 of 43), 65% (26 of 40) and 88% (37 of 42) of patients, respectively. A correlation with clinical characteristics was not demostrated. Bcl-2 expression was not predictive for survival. Median survival time (MST) in patients with Bax expression was 8 months compared with 14 months in patients without Bax expression (p=0.2). MST in patients expressing Bcl-2-/Bax+ (19p) was worse than any of the other groups (P=0.1). MST in patients with high level expression of Bcl-X (more than 50% of tumor cells positive) was 8 months compared with 15 months in Bcl-X negative patients. The one-year survival rates were 36% and 72% respectively (P=0.05).

Conclusions: Bcl-2, Bax and Bcl-X proteins are frequently expressed in esophageal cancer. Our results suggest that both the Bcl-2-/Bax+ combination and a high level expression of Bcl-X are associated with unfavorable prognosis in esophageal cancer patients. The analysis of more patients is indicated to confirm these results.

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Phase II study of 5-Fluorouracil (5-Fu), Cisplatin (CDDP) and vinorelbine (VNR) in patients (pts) with advanced adenocarcinoma of oesophagus.

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INTRODUCTION: A preliminary study was conducted in 12 patients with locally advanced metastatic adenocarcinoma of the oesophagus. They were treated with 5-Fu 800 mg/m2 D2 to D5, CDDP 100 mg/m2 D1 and VNR 20 mg/m2 D1 and D5 every 21 days.

PATIENTS AND METHODS: Median age 61 years (55-68). 11 patients have prior lost of weight, 4 of them >10%.

50 % of pts had a poor or moderate histological grade of tumour.

4 pts had metastatic disease at first presentation. Non prior radiotherapy.

RESULTS: *Toxicity:* All pts were evaluables for toxicity: 2 toxic deaths (1 due of progression disease - oesophagus tracheal fistula with secondary bronchopneumonia, 1 due of septic shock: febrile neutropenia and pneumonia); G3-4 neutropenia, 4 pts; G3 anaemia , 1; G3-4 thrombopenia, 2. Without significant non haematological toxicity.

Of the 12 evaluable pts: 2 had a complete response; 2 partial response; 2 stable disease; 4 pts progressed.

The median time of follow-up was 121,8 days (≅ 4 months) range 9 to 248 days. At the moment 2 pts are alive without disease and 7 pts were alive with disease.

CONCLUSION: This chemotherapy regimen shows a 33 % response rate and high toxicity. The poor outcome could be ascribes to both patients characteristics and/or high dose of VNR. However this preliminary data requires further confirmation.

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PHASE II STUDY OF IRINOTECAN (CPT-11) IN PATIENTS WITH ADVANCED COLORECTAL CANCER RESISTANT TO 5-FLUOROURACIL BASED CHEMOTHERAPY.

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Aim: To assess the eficacy and safety profiles of CPT-11 in patients with advanced colorectal cancer (ACRC) 5-Fluorouracil (5-Fu) resistant.

Patients and methods: 33 patients were recruited and all are currently evaluable for toxicity and 27 for efficacy. Median age: 55 years (40-75). M/F: 18/15. PS 0-1/2: 24/9. Primary tumor: colon 22, rectum 11. At diagnosis, 17 patients were stage D and in 5 of them the primary tumor was not removed. All patients were resistant to 5-Fu based chemotherapy: 31 received 5-Fu based treatment as first line for ACRC and 2 progressed during adjuvant 5-Fu based therapy. Non resectable metastatic sites: multiple (22 patients), single (11). Liver (73%), lymph nodes (30%), lung (27%), soft tissues (18%), peritoneum (12%).

CPT-11 was given at 350 mg/m^2 IV during 60 minutes q3w. Treatment for delayed diarrhea was based on a high dose loperamide schedule.

Results: 137 cycles had been assessed (media: 4, range: 1-8). The planned CPT-11 dose was reduced en 14 patients (42%) because of severe toxicity. Major toxicities: grade 3/4 neutropenia: 19 cycles (14%); febrile neutropenia: 6 cycles (4%); grade 2/3 delayed diarrhea: 36 cycles (26%); grade 2/3 emesis: 41 cycles (30%); moderate-severe asthenia: 38 cycles (28%); mild cholinergic syndrome: 16 cycles (12%); grade 3 alopecia: 17 patients (51%). 1 patient died of septic shock after 2 cycles. 1/27 (4%) patients achieved complete response; 2/27 (7%) achieved partial response, and 14/27(52%) stable disease. 10/27 (37%) patients progressed and six patients were not evaluable for response (2 early deaths and 3 early withdrawal). Median survival was 8 months. 21 patients are dead: 19 from progressive disease, 1 from toxicity and 1 from no related cause.

Conclusions: in our group of 5-Fu resistant metastatic colorectal cancer patients, CPT-11 showed a 63% tumor growth control with acceptable safety profile with 20-30% moderate-intense toxicity, similar to previous studies.

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PHASE II STUDY OF CONTINUOUS TEGAFUR (TG) AND LEUCOVORIN (LV) IN ADVANCED COLORECTAL CARCINOMA

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Tefagur is a prodrug that its metabolized to 5-FU, with practically a 100% oral biodisponibillity that is susceptibler of biochemical modulation with folinic acid. Previos studies have shown the efficacy of a 750 mgs/m2/day dose + folinic acid (LV) 45 mgs/day, in squedules with a resting week after 14 to 21 therapy days.

Objectives: To assess in terms of response (R) survival (S), and toxicity (T) the combination of TG 500 mgs/m2/day + LV 30 mgs/day bid, in a continuous way up to progresion or inaceptable toxicity in patients (p) with colorectal carcinoma histologically proven and with measurable lesions. No previous chemotherapy (QT) for advanced disease.

Patientes and methods: Between november 96 and september-98 we have treated 35 p. (24 M / 11 F), median age 62 years (48-79). Adyuvant QT (FU + LCV x 6) in 11 p., RT in 8 p. Median DFS 12 months (0-110). ECOG 0: 9 p., 1: 19 p., 2: 7p. Metastatic places: 1: 20 p., 2: 11 p., 3 ór more: 4 p. Median CEA: 68 ngs/ml (0-3.164).

Results: At the moment of the analysis 25 p have finished therapy and 14 have died. 26 p. are evaluable for R and 33 for T. Mean duration of therapy is 19 weeks (3-52), with a mean dose intensity of 75%. T: Diarrhoea G2-3: 51%, nausea G2: 33.3%, mucositis G2: 12%, epigastric pain G2: 9%, neurologic G2: 1 p. Three patients refused therapy due to gastrointestinal toxicity. We have seen 1 PR (3.8%) and 13 (50%) SD. In 8 patients second line QT was used. Actuarial Survival is 10 months (95%CI: 4.35-15,66) In responding patients P R ór SD is 17,5 m. and if progression 8,5 m.

Conclusions: Very low response rate with a 50% SD and a median survival similar to parenteral QT squedules. Toxicity mainly gastrointestinal let us think that discontinuous schedule with a resting time of 2 to 3 weeks is the better aproach.

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CLINICAL EXPERIENCE WITH RALTITREXED (R) IN PATIENTS WITH ADVANCED COLORECTAL CARCINOMA (ACC).

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(R) (Tomudex) is the first specific inhibitor of thymidylate synthase with a wide clinical use in the treatment of ACC. Phase II and III studies have showed its safety and activity with an easy administration schedule.

Between August 1997 and August 1998 34 patients (pts) with ACC were treated with R (3.0 mgrs/m2 iv/3 weeks in a 15 minutes short infusion).

Patients: Females 17, Males 19; Median age: 64 years; Colon/rectal cancer: 23/11: Perfomance Status: ECOG 0-1=32 pts, ECOG 2=2 pts; Sites of metastases: Liver 22 pts (64.7%), Lung 4 pts (11.8%), Peritoneal carcinomatosis 3 pts (8.8%), Others 5 pts (14.6%). Two or more sites affected in seven pts; No prior chemotherapy in 17 pts (50%).

Results: Median number of cycles administered: 4.5 (1-11), total cycles administered: 153. Objective response rate was seen in 5 pts (14.7 %) and stable disease in 12 pts (35). The median survival was 10.6 months with a progression free survival of 3.8 months. The median follow-up was 7.2 months.

Toxicity: No haematological G III-IV toxicity was seen. The digestive toxicity was moderated: vomiting G III in 2 pts (5.9%), diarrhoea G IV in 2 pts (5.9%), stomatitis G IV in 2 pts (5.9%). The most important toxicity observed was asthenia G III-IV in 7 pts (20.6%) and anorexia G III-IV in 3 pts (8.8%). G III renal toxicity was seen in 1 pts.

Conclusion: The results of this study did not show any differences with the previously reported in terms of global responses, median of survival and time to progression. The toxicity associated was mild and this treatment was well tolerated.

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INFLUENCE OF SURGICAL RADICALITY ON SURVIVAL FOR LOCAL AND LOCALLY ADVANCED GASTRIC CANCER

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Objectives: To assess the eficacy for a supra-radical surgical procedure (SRSP) involving gastrectomy and extended lymphadenectomy up to gastroepiploic, hepatic, left gastric chain nodes and greater omentectomy for resection of local and locally advanced gastric cancers. Retrospective comparative study versus conventional radical surgical procedures (CRSP) (gastrectomy and lymphadenectomy) regarding morbidity, disease free survival (DFS) and overall survival (OS). To validate an SRSP in gastric cancer as an optimal treatment approach with impact in survival.

Material and methods: A retrospective study was performed of all patients with local and locally advanced gastric cancer who underwent surgery at the Creu Roja Hospital in Hospitalet from Jan 94 to Dec 97. An SRSP or CRSP was performed in each patient depending on patient status or surgical team skills. All patients with a performance status (PS) >70 and <75 years old underwent adjuvant chemotherapy in different regimens according to standars being used (FAMTX, ELF, CDDP+5-FU). Statistical analyses have been done through the SPSS windows system.

Results: The series comprise 70 pts who underwent surgery in this time period. There were 42 males and 28 females. Mean age was 70 years, range (32-99). About 26 pts (37%) were considered to be stages IA, IB, II, and 44 pts (63%) were IIIA, IIIB stages. A SRSP was performed in 34 pts (49%) and 36 pts (51%) underwent a CRSP. Both groups were considered to be comparatively homogeneous regarding sex, PS, and staging. No differences in surgical morbidity were found. The mean follow-up has been 34 months. By using the Kaplan-Meier cummulative life-tables we achieved a 2 year DFS and OS of 65% and 61%, respectively. Median survival has not been reached yet for the whole group. The SRSP group achieved a 2 year OS of 70% and the figure for the CRSP would reach 56,5% (p 0.09). 2 year OS for stages I and II was about 76,5% and 50% for stages III. Higher OS were found in both groups when a SRSP was performed.

Conclussions: 1. A supra-radical surgical approach for gastric cancer seems to have an impact on survival and, probably, a longer follow-up will reach statistical significance. 2. Supra-radical surgical procedures do not lead to a higher morbidity and mortality than the conventional surgical approaches. 3. The "type of surgery" should be considered as a prognostic feature regarding survival analyses of gastric cancer series. 4. Randomized prospective comparative studies should be undertaken with standard "type of surgery" in order to determine the role for adjuvant chemotherapy in gastric cancer.

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RESULTS OF POSTOPERATIVE ADJUVANT TREATMENT OF RECTAL CANCER

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Introduction:

On the basis of studies which showed the benefists of combining chemotherapy with radiotherapy (RXT) in postoperative adjuvant treatment of rectum cancer, several recommendations were published in 1991 for the adjuvant treatment of these patients.

Patients and methods:

Between 1991 and 1997, 66 patients radically treated for rectum cancer stage II and III began adjuvant treatment following the aforementioned recommendations. The treatment comprised two cycles of 5-FU 500mg/m2/ day IV for five days, every four weeks. Subsequently, RXT was applied over the pelvis 45-50 Gy in 4-5 weeks, with administration of 5-FU in the first 3 and last 3 days of RXT application. After this, two more cycles of 5-FU 450mg/m2/ day IV for five days, every four weeks, was administered.

Results:

The mean age of the patients was 63 years, 28 (42%)of which were women and 38 (58%) men. Surgery consisted in the abdominoperineal amputation of the rectum (AAP) in 33 (50%) of the patients, and an anterior resection of the remaining patients. Twenty-eight (42%) patients were stage II, whereas thirty-eight (58%) were stage III. The most frequent grade III toxicities were diarrhoea, which coincided with RXT in 4 (6%) patients, and leukopenia in 2 (3%). Six patients did not complete treatment, but only one of these was due to toxicity. In a 28 month follow-up period , 21 (32%) patients relapsed: 17 (81%) with metastasis, 3 (14%) with local recurrence, and 1 (5%) as both local recurrence and distant metastasis. Disease free survival at 3 years was 68%, and overall survival was 85%. Of the variables analysed, only the type of surgery showed any kind prognostic value, which was worse in patients with AAP.

Conclusions:

This adjuvant treatment schedule has little toxicity and offers several results which are similar to those described in medical literature.

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TRANSPLANTATION AND ADJUVANT CHEMOTHERAPY FOR HEPATOCELLULAR CARCINOMA.

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Background: Transplantation is sometimes recommended for hepatocellular carcinoma (HCC). Patients with stages II to IV-A cirrhosis have a median survival of 12 months following resection or other local therapies. More than 86% of patients have a recurrence, most within 2 years. Increases in post-transplant survival has not been established, and even less so when doxorubicin chemotherapy is administered.

Materials and methods: Between 1992 and 1998, 9 patients underwent transplantation and subsequently received weekly doses of adjuvant doxorubicin for 20 weeks. All patients had hepatocarcinoma. Eight patients had cirrhosis (44.4% C virus, 0% B virus, 22.2% alcoholic, 22.2% cryptogenic, 0% metabolic hepatopathy). One patient had chronic active hepatitis. Classification was 33.3% stage II, 22.2% stage III, and 44.4% stage IV-A. Major vascular invasion was seen in 11.1%, microscopic invasion in 22.2%, and 44.4% had capsular invasion (11.1% pT2). Single tumorous nodules were found in 33.3%, multiple tumorous nodules in 1 lobe 22.3%, and 44.4% multiple tumorous nodules in both lobes. 44.4% had incidental operative HCC. The larger nodules had a median diameter of 4.5 cm. Median Child and Pugh classification was B-7. Median αfetoprotein level was 16. Median prothrombin time was 70 %. Median patient age was 57 years. Male/female ratio 3:1. Median ECOG was 1. Preoperative treatment was received by 2 patients (1 underwent PIE and tamoxifen, and 1 chemoembolization). Mean time from transplantation to chemotherapy was 25 days. Doxorubicin, 10mg/m^2, was administered weekly for 20 weeks. Ventricular heart function was not monitored because no patient received doxorubicin dosage in excess of 200 mg/m^2 and there was no previous cardiopathy in the enrolled patients.

Results: 66% patients received full dosage and completed the scheduled treatment. 22% had 25-50% dose reductions for myelotoxicity (grade III-IV). 1 patient (11.1%) had treatment withdrawn due to toxicity after 12 doses (P. Carinii and CMV pneumonia). There was 1 treatment related death (P. Carinii pneumonia at 4 months). Reduced immunosuppression doses were administered to 4 patients during chemotherapy due to high serum levels. 4 patients relapsed (44.4%), 1 in the first year, 3 in the third year. 2 patients died without evidence of tumor (1 of pneumonia and 1 of acute Epstein-Barr hepatitis), and 3 patients are still living without evidence of disease at 4, 13 and 14 months. C virus recurrence occurred in 1 patient. Median survival following transplantation was 14 months (range 4 to 37 months), and median disease-free survival was 25 months.

Summary: Transplantation combined with adjuvant chemotherapy seems to be effective and appears to encourage improved disease-free survival and satisfactory quality of life (as seen by other authors) in this group of patients.

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PREOPERATIVE CHEMORADIOTHERAPY (QT-RDT) IN LOCALLY ADVANCED RECTAL CANCER. PRELIMINARY RESULTS.

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Introduction: The prognosis of locally advanced rectal cancer despite radical surgery is poor. To improve it we began a trial that incorporate RDT in the initial strategy of treatment. We present the results of preoperative QT-RDT administration in these tumours.

Methods: Patients with stage $T_{3-4}N_{x-2}M_0$ rectal cancer have been treated with continuous iv infusion of 5-fluoruracil (5-FU) 300 mg/m^2/day for 5 days every week concurrently with external beam RDT 45 Gy (fraction of 1,8 Gy/session) for 5 days every week. Total duration of combined treatment was 5 weeks. At the end of treatment radical surgery was planned followed by postoperative chemotherapy if they had no evidence of pathological progression.

Results: Between April 1996 and July 1998, 33 pts (23 m, 10 f) were included. Median age was 61 (45-76) and 26/33 were N+ (resectable, 27 and unresectable, 6). One out of 31 evaluable pts couldn't finished chemotherapy because an ischemic anginous pain. No III-IV toxicity was detected. Surgery was radical in 30 patients (13 anterior resection and 18 abdominoperineal resection). In 8 pts anal sphincter was spared. Pathological total response rate was 68%: 3 RC (9,7%), 18% PR. In 12 pts only microscopic foci of residual disease was detected.

Conclusions: Preoperative treatment with continuous infusion of 5-FU and radiotherapy in locally advanced rectal cancer was well tolerated with a remarkable pathological response rate. Results are too preliminary to ascertain if they have an impact on survival.

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ADJUVANT THERAPY WITH 5-FLUOROURACIL (5FU) PLUS LEVAMISOLE or 5FU PLUS RADIOTHERAPY IN RESECTED COLORECTAL CANCER.

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INTRODUCTION: The combination of 5FU and levamisole was the first adjuvant therapy demonstrating its efficacy in colorectal cancer (CRC). We have used it at our center between 1992 and 1997. Preliminary results are presented here.

PATIENTS AND METHOD: 78 patients (39 males and 39 females) with a median age of 56 ± 10 years were included. There were 45 (58%) cases of colon cancer, which received 5FU (450 mg/m2/week e.v.) plus levamisole (50 mg/8h p.o. x 3d/15d) for 1 year. The remaining 33 (42%) cases had rectal cancer and received 5FU (same schedule) plus pelvic irradiation (50 Gy, in the first 2-3 months from surgery). The main indications for adjuvant therapy were: nodal involvement (stage III, 65%), T3-T4N0 rectal tumors (14%), T4N0 colon tumors (9%), and occurrence of obstruction/perforation (5%).

RESULTS: Mean time from surgery to chemotherapy was 7 ± 3 weeks. 22 patients (28%) did not conclude the planned therapy (10 due to progression, 5 patient negatives, 4 intercurrent causes and 3 due to toxicity). In the remainder, the relative dose intensity was 96% (64-102%), and the total dose of 5FU received ranged from 69 to 106% of that planned (median 100%). Grade 3-4 toxicity consisted of diarrhea (12% cases), mucositis (4%), emesis (4%), leucopenia (3%), skin toxicity (2%), hepatic toxicity (1%), allergy (1%) and CNS toxicity (1%). 36 patients (46%) showed no adverse events. There were no toxic deaths. After a median follow-up of 22 months (13-71), 24 patients (31%) have relapsed. The 3-year actuarial overall survival was 81%.

CONCLUSIONS: The association of 5FU and levamisole or radiotherapy is a well-tolerated adjuvant therapy for CRC. The main inconveniences are its long duration and the need for an implantable e.v. device. Of note, a significant proportion (13%) of relapses during treatment has been observed

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NEOADJUVANT THERAPY OF RECTAL CANCER WITH UFT-LEUCOVORIN (LV) PLUS RADIOTHERAPY (RT).

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Objective: to assess the efficacy and tolerance of UFT-LV plus RT as neoadjuvant therapy in locally advanced rectal cancer.

Methods: from April 95' to January 98', 28 patients with locally advanced rectal adenocarcinoma (medium or lower third) were treated. In 12 patients (43%) the tumor was located less than 5 cm from the anal margin, and in the others at 5-10 cm. Pre-surgical stage: T3-4N0M0=20, T3-4N1-2M0=6, T3-4N0-2M1=2. Three courses of UFT-LV plus RT (45 Gy) were given before surgery. After surgery, 6 additional courses were administered. The therapeutic scheme consisted of intravenous LV 500 mg/m2 on day 1, UFT 350 mg/m2 (in 2-3 daily doses) on days 1 to 14, and oral LV 15 mg/12 hours on days 2 to 14. Courses were repeated every 28 days. Twenty-four out of 28 patients have been operated so far and are the subject of this communication.

Results: after 3 courses plus RT, 17 responses were obtained (71%, 95%CI 53-89%), 4 complete responses and 13 partial responses. Downstaging was observed in 14 patients (58%). Surgery consisted of low-anterior resection in 11 patients (46%) and abdominal-perineal amputation in 13. Two patients had an incomplete resection. There were 2 histological complete responses (8%), whereas microscopic tumoral foci were found in 11 cases (46%). Nine patients (38%) experienced grade 3-4 toxicity consisting of diarrhea, vomiting or mucositis. Two patients had cystitis and proctitis. As to surgical complications, dehiscenses appeared in 2 patients and an abscess in 4. After a median follow-up of 18 months, no local or distant relapses have appeared in all the patients with a complete resection.

Conclusions: neoadjuvant therapy with UFT-LV plus RT downstages some patients with adenocarcinoma of the rectum before surgery and allows a conservative procedure. However, due to the toxicity, we have decided to lower the dosis of UFT at 300 mg/m2 before surgery. As the follow-up is still short, it cannot be deduced whether this strategy reduces the relapse rate and improves survival.

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UFT PLUS LEUCOVORIN (LV) IN ADVANCED COLORECTAL CANCER (CCR). A PHASE II TRIAL.

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Oral fluoropyrimidines have proved their activity in CCR in Japan and later in the U.S.A. (Pazdur et al. JCO 1994). Continuous oral administration can simulate iv continuous infusion of 5-FU, although there are no Phase III trials which have demonstrated beyond doubt that it has similar activity clinically. We aimed to evaluate the activity and toxicity of UFT (Tegafur and Uracil) in a 4:1 molar concentration plus LV, in patient diagnosed with metastatic CCR with measurable bidimensional disease. Patients were treated with UFT 300 mg/m2 plus LV 150 mg/day, every 8 hours, during 28 days, resting one week. If Grade 3-4 toxicity appeared treatment was interrupted until recovery.
From January 1997 till August 1998, 144 patients were included. For analysis in November 1998 , 87 patients were evaluable for response, toxicity and survival. Patient characteristics were: median age 65 years (31-72); Karnosfky Index 90 (70-100), 27 women and 60 men, 64,7% of the patients had advanced disease at the moment of diagnosis, 72% of the patients had liver metastasis. Toxicity was low, Grade 3-4: nausea and vomiting 7%, diarrhea 12%.
Objetive responses were 5 complete response (CR) (5.7%), 12 partial responses (PR) (13.8%) with overall responses of 19.5% (confidence limits 95% = 11-28%). 35 patients stabilized (NC). (40.2%). The number of patients without progresion (CR +'PR + NC) was 52 (59.7%) (confidence limits 95% = 48.7-70%). With a maximum 21 month follow-up the actuarial median time to progression is 4.4 months (confidence limits. 95% = 3.8 -5 months) and the actuarial median survival is 13 months (confidence limits. 95% = 9.89 - 15.84 months).
The number of patients who did not progress and the median survival found in this analysis allow us to conclude that the regimen is active and the low toxicity makes it recommendable.

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ASSOCIATION OF COLONIC CARCINOMA AND INFECTION FOR *GEMELLA HAEMOLISANS*

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Introduction

Gemella haemolysans is a gram-positive coccus, commensal of the upper respiratory, gastrointestinal and genitourinary tract in humans. Occasional cases of systemic infection due to *G. haemolysans* have been reported in humans; these cases include endocarditis, septicaemia and meningitis.

Clinical case

We report the case of a 37 years-old woman with colonic adenocarcinoma stadium IV that presented fever of several weeks of evolution without apparent focal symptoms. The physical examination and the complementary studies, except for to 16300 leukocytes/mm3 (granulokytes 89.4%) in blood count, they were anodyne. Cultures of three separate blood specimens drawn over 72 hours yielded gram-positive cocci. The isolated organism was identified as *G. haemolysans* by the ATB - System. The organism was sensitive to penicillin, vancomycin, and gentamicin (MICs < 1mcg/mL). A transthoracic echocardiogram showed two vegetations on the mitral valve and one vegetation on tricuspid valve. The mitral and tricuspid valve function was normal. The patient's condition improved clinically after antibiotic treatment with vancomycin 500 mg. iv. 6 hourly and imipenem 500 mg. iv. 6 hourly was started, and several cultures of blood drawn after completion of antibiotic therapy (40 days) were negative. The fever did not recur after completion of treatment.

Conclusions

We report the first case of multiple healthy native valve endocarditis by *G. haemolysans* , being the second in the one that it objective association among infection for *G. haemolisans* and colonic adenocarcinoma. It is no clear how colorectal cancer predisposes to an increased risk of enterogenous by some bacteria such as *S. bovis* or *G. haemolysans*. This case suggest that patients with colonic carcinoma who have a prolonged fever history without any apparent source of bacteremia, should undergo a work-up to exclude a
G. haemolysans endocarditis. The election treatment is penicillin more an aminoglycosid, and like alternative in allergic rifampin more erythromycin. The answer to the treatment is good and it is uncommon to have to carry out substitution valvular.

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CDC GROUP IV C-2 INFECTION IN PATIENT WITH COLONIC ADENOCARCINOMA

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Introduction

The CDC group IV - c2 bacterium is a gram-negative bacillus rarely isolated like human pathogens that have been implied in the literature like infection producers in 8 patients the our included. In some cases the water and other fluids are recognised as reservoir. Almost all described cases correspond to patient with variable degree of immunocompromise or with serious illness underlying.

Clinical case

We present the case of a 36 year-old female with colonic adenocarcinoma stadium IV. She received several lines of QT after to palliative colonic resection. The patient was carrier of percutaneous right nephrostomy catheter for obstructive bilateral ureterohydronephrosis degree III due to intrapelvic masses. She began with fever. It is verified in several urine cultures the presence of G- bacillus. The organism was a short glucose-non fermentative gram negative rod which grew on Mcconkey agar and on Blood Agar at 37ºC and 42ºC, but did not grow on Salmonella-Sygella agar at 37ºC. They are mobility by peritrichous flagella and the following microbiological characteristics: the isolate was positive in test for oxidase, catalase, citrate and urease; It was negative in test for nitrate reduction indole production, gelatin and esculin hydrolysis, and oxidation fermentation of glucose, sucrose, lactose, maltose and xylose. The identification was completed with Urine Combo Panel 61in the automated system Micro Scan Walk-Away and strip ID 32GN. Treatment was realised with ciprofloxacin 750 mg. each 12 h. orally for fifteen days disappearing the fever in the fifth day. Percutaneous nephrostomy catheter replacement was not necessary.

Conclusions

In oncology patients, the immunodepression due to their own illness and the chemotherapy treatment, as well as to the cutaneous barrier disruption for catheters (veined, nephrostomy), and the treatment with wide spectrum antibiotics, they lengthen the periods of neutropenia and they favor opportunists infections for germs like the CDC group IV c-2 bacillus. They have only been described infection by CDC group IV c-2 in a patient with solid tumour, but our case is the first one described in the literature of secondary bacteremia due to urinary infection having a percutaneous nephrostomy catheter like entrance door. It is necessary to keep in mind the potential pathogenically of these germs in oncology patients.

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5-FU BY PROTRACTED CONTINUOUS INFUSION AND RADIATION THERAPY IN RECTAL CANCER. J.M. Baena, A. Rueda, A. Mateos, M.J. Gómez, J.A. Contreras, M.D. de las Peñas* , A. Senra. Medical Oncology and Radiotherapy * Departments, U.H. Puerta del Mar. Cádiz.

Background : The best chemotherapy schedule to be combined with radiotherapy for rectal cancer patients (p) is not known. The purpose of the present study is to determine the efficacy and toxicity of a combination of radiation therapy and protracted infusion 5-FU in rectal cancer.

Methods : Twenty p with the diagnosis of stage II-III carcinomas of the rectum and 2 p with rectal carcinoma recurrences were treated in our institution between January 1995 and June 1998. Continuous infusion of 5-FU (300 mg/m^2/day x 12 weeks) was given through central venous catheter connected to an ambulatory infusion pump. Radiotherapy total dose consisted of 50 Gy with conventional dose fraction. The p were treated with megavoltage photons (Co-60).

Results : Fifteen men and 7 women had a median age of 56 years (36-70). Ten p had anterior resection and 10 had abdominoperineal resection. Two had inoperable pelvic recurrences without signs of distant metastatic disease. The pathological stage was : T_1 in 1 p, T_2 in 1 p, T_3 in 15 p, T_4 in 3 p, N_0 in 8 p, N_1 in 8 p and N_2 in 4 p. The median number of metastatic nodes was 1,5 (1-15). The histological grading (G) was : G_1 in 11 p, G_2 in 9 p and G_x in 2 p. The projected dose intensity of 5-FU was 2100 mg/m^2/week and the received dose intensity was 2030 mg/m^2/week (relative dose intensity : 0,96). The toxicity consisted on diarrhea G_1 in 14 p, G_2 in 3 p and G_3 in 3 p, mucositis G_1 in 5 p, and G_2 in 5 p, nausea/vomiting G_1 in 5 p and G_2 in 1 p, alopecia G_1 in 2 p, dermal toxicity G_1 in 6 p and G_3 in 1 p (lupus-like : cutaneous reaction of photosensibility with ANA, anti-SSA and anti-Ro +), peripheral neuropaty G_1 in 1 p and haematological toxicity G_1 in 1 p and G_2 in 1 p. There was 1 p with axillary venous thrombosis on implantable access device. The median follow-up was 20 months (3-41). Of the 20 p with stage II-III, 2 developed recurrent disease (hepatic and pulmonary in 1 p and pelvic and peritoneal in other p) and 1 died. Two p with local recurrences obtained partial responses and its duration was 7 and 5 months.

Conclusions : This study, which used a simultaneous protracted continuous infusion 5-FU and radiotherapy, shows that this combination is an active and tolerable regimen for the treatment of rectal cancer patients.

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ADJUNVANT CHEMOTHERAPY OF COLORECTAL CANCER WITH FLUORACIL AND LEUCOVORIN.

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Aim: the purpose of this study was to evaluate the therapeutic activity and toxicity of adyunvant chemotherapy with 5-fluoracil (FU) and leucovorin (L) in patients (pts) with colorectal cancer.

Methods: between september 92 and september 95, 93 pts have been treated. There were 39 women and 53 men. Median age was 63y. Stage II: 59 pts (63%); stage III: 34 pts (37%). Colon cancer: 54 pts (58%); rectal cancer: 39pts (42%). Treatment consisted of FU 370 mg/m2 /d IV bolus plus L 200 mg/m2 /d IV for 5 consecutive days every 28d for one year. Rectal cancer pts received local radiation theraphy after second course.

Results: 74 pts (80%) completed the treatment. Toxicity delayed treatment in 16 pts. Grade 3-4 toxicity was: neutropenia: 7 pts (8%), stomatitis: 7 pts (8%). After maximum follow-up of 7 years, 36 pts (39%) had disease relapse (stage II: 32%; stage III:50%; colon cancer: 30%; rectal cancer: 44%). Local-regional relapse was seen in 5 pts, liver: 9pts, lung: 9 pts and multiple in 8 pts. Five and seven year survival was 67% (stage II: 84%: stage III: 36%).

Conclusion: we conclude that the results in this study are comparable to other regimens containing 5-fluoracil and leucovorin in adyunvant therapy of colorectal cancer and the toxicity was acceptable.

5. Urological Cancer

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SIGNIFICANT ACTIVITY OF THE MULTI-TARGETED ANTIFOLATE MTA (LY231514) IN ADVANCED TRANSITIONAL CELL CARCINOMA (TCC) OF THE BLADDER: RESULTS OF A PHASE II TRIAL.

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Background: MTA is a novel multi-targeted antifolate that inhibits multiple folate-dependent enzymes, including thymidylate synthase (TS), dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyltransferase (GAR. Γ). MTA has activity in preclinical models and human tumors (breast, colon, head and neck, cervix and lung).

Methods: We undertook a phase II trial of MTA in 23 patients (pts) with advanced TCC of the bladder. MTA was administered as 10 minute infusions every 3 weeks, at a dose of 600 mg/m^2 (first 6 pts) or 500 mg/m^2 (subsequent pts). Criteria for eligibility was: Patients with inoperable TCC; bidimensionally measurable disease; without prior chemotherapy for locally advanced or metastasic disease; prior radiotherapy as long as the irradiated area is not the only source of measurable disease; adequate hematologic , hepatic and renal function. There are 23 evaluable, 22 pts are men and 1 pt is a woman. Median age is 66 years (range 48-76). PS (ECOG) is 0 (8 pts), 1 (12 pts) and 2 (3 pts). All pts had metastasic disease, median number of disease sites : 2 (range 1-4).

Results: The main hematologic toxicity found was: neutropenia G4 in 8 pts (35%), 5 pts developed neutropenic fever; thrombocytopenia G3-4 in 2 pts(9%) and anemia G3-4 in 4 pts (17%). Non-hematologic toxicity included: skin rash G2-3 in 12 pts (52%), nausea and vomiting G2-3 in 6 pts (26%) and alopecia G1 in 5 pts (21%). There were 2 toxic deaths due to septicemia and renal failure. The responses found were: partial response in 7 pts (30%), stable disease in 8 pts (35%), progression in 7 pts (30%) and early death in 1 patient (4%).

Conclusion: MTA has definitive antitumor activity in advanced TCC of the bladder, but its toxicity is significant.

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GEMCITABINE-CARBOPLATINUM LOCALLY ADVANCED OR METASTATIC BLADDER CANCER PATIENTS WITH RENAL FAILURE.

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Introduction:
Bladder cnacer is a frequent tumour in our population and ussually affects elderly patients. For demographic reasons, the general state of many patients is bad and often accompanied by renal failure, and therefore cannot benefit from treatment which includes cisplatinum. The development of new combinations for treating such patients is thus of vital importance.

Patients and methods:
Between 1997 and 1998, 15 patients with locally advanced non-surgical or metastic bladder tumours were treated at our centers. Treatment consisted in administering 1000 mg/m2 of Gemcitabine on days 1 and 8, and carboplatinum (AUC=5) on day 1, every 21 days.

Results:
The mean age of the patients was 69 years (range:54-78 years), 4 (26%) of which were women and 11 (74%) men. Average IK was 70% (range:50-100%). Mean cretanine clearance was 44.9 ml/min (range:21-55 ml/min). Three patients had previously received chemotherapy. Metastic localisations were: 10 lymph nodes, 3 pulmonary, 1 bone, 2 liver and 1 pelvic. The mean cycles/patient was 4 (1-6). With reference to responses, 1 was RC, 4 RP (RO:38%) (11-65%), 6 EE and 1 progresion. Two patients have not been evaluated as yet. Hematological toxicities were as follows: grade I anemia in 2 patients, grade III in 2; grade I leukocytes in 2 patients, grade III in 1 and grade IV in 2 patients; grade three platelets in 2 patients. Toxic death ocurred in the course of one grade IV neutropenic event. Non-hematological toxicities were as follows: grade I vomiting in 1 patient, grade II in 2 and grade III in 1. One patient showed a grade III hepatic toxicity.

Conclusions:
The aforementioned treatment has a little toxicity, is easy to administer and offers promising results in this group of patients.

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BOMP/EPI INTENSIVE ALTERNATING CHEMOTHERAPY FOR IGCCC POOR-PROGNOSIS GERM CELL TUMORS: THE SPANISH GERM CELL CANCER GROUP EXPERIENCE (GG)

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Background: Patients with poor-prognosis germ cell tumors according to the IGCCC have a poor long-term survival. This study evaluates the efficacy and toxicity of the intensive alternating chemotherapy regimen BOMP/EPI in these patients.
Patients and methods: Patients with IGCCC poor-prognosis germ cell tumors treated at 13 centres were studied. Treatment consisted of bleomycin 30 mg, vincristine 2 mg, methotrexate 300 mg/m2 and cisplatin 100 mg/m2 (BOMP), alternating at 14 day interval with etoposide 120 mg/m2 d1-4, ifosfamide 1.3 gr/m2 d1-4 and cisplatin 25 mg/m2 d1-4 (EPI). BOMP was administered at 21 day interval from EPI. Bleomycin was administered weekly per 12 weeks.
Results: Thirty-eight patients were treated. The median number of cycles administered was 7 (1-10 cycles). Eighteen patients achieved complete response with chemotherapy alone (12 had necrosis and 2 mature teratoma at postchemotherapy resection), four achieved complete response with chemotherapy and surgical resection of viable cancer. Therefore, overall favorable response was achieved in 22 patients (60%). Four additional patients had marker negative non-resected residual masses. Eleven patients were considered as treatment failures, including one early death and one toxic death due to granulocytopenic sepsis and renal failure. Hematologic toxicity was most common, with 26 patients (70%) having grade 4 granulocytopenia. After a median follow-up of 41 months, the actuarial 2-year overall survival and progression-free survival were 64% and 58%, respectively.
Conclusion: BOMP/EPI is active in poor-prognosis germ cell tumors according to the IGCCC criteria. The results obtained compare favorably with those expected with conventional chemotherapy, and justify further studies.

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CONSERVATIVE TREATMENT OF T2 T3a N0 M0 BLADDER CANCER WITH TRANSURETHRAL RESECTION (TUR) AND CHEMOTHERAPY (CHT)

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CHT is effective in the treatment of advanced invasive bladder cancer (IBC). A trial was designed to assess the possibility of bladder preservation in T2 T3a N0 M0 tumors treated with chemotherapy after TUR.
PATIENTS AND METHODS: 48 patients were treated from January 1989 to December 1996. The median age was 61 (45-76).
Inclusion criteria were: 1) transitional or undifferentiated IBC; 2) T2 T3a N0 M0 after therapheutic TUR; 3) lack of ureterhydronephrosis, diffuse carcinoma in situ (CIS) or CIS in prostatic urethra, and 4)creatinine levels <130x1.25mmol/l. Most of them (79%) were grade III transitional or undifferentiated IBC.
Three courses of CHT (Methotrexate 30mg/m2 and Vinblastine 4mg/m2 on days 1 and 8, and Carboplatin 350 mg/m2 on day 2, every 4 weeks) were administered after deep TUR with a complete tumor resection.
Response was evaluated with a cystoscopy and a deep scar biopsy, and TUR when there was residual tumor. If complete remission (CR) was demonstrated, the patients were followed with cystoscopy every 4 months during the first 3 years, and every 6 six months afterwards. When there was persistent superficial tumor, 3 courses of CHT were repeated followed by cystoscopy reevaluation. Further finding of superficial tumors were treated with TUR, intravesical CHT or BCG. Cystectomy was performed when invasive tumor was found just after CHT or at any time during the follow-up
RESULTS: 46 patients were evaluable for response. 35 patients (76%) presented CR, 2 partial remission (PR) and 9 (19%) no response (NR). Toxicity was moderated. Median follow-up for 38 patients was 48 months (36-109); 29 of them had reached CR, and in 10 superficial tumor or CIS was eventually observed; 17 patients (45%) developed invasive tumor after primary TUR, 10 after the response and 7 without reaching it. Overall survival and survival free from IBC –the same at 5 and 7 years- were 71% and 53% respectively.
DISCUSSION: Chemotherapy after TUR in T2 T3a N0 M0 transitional or undifferentiated IBC without ureterhydronephrosys or diffuse CIS allows for bladder preservation in more than 50% of the patients without compromising survival.

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HOME-THERAPY TRIAL OF SUBCUTANEOUS INTERLEUKIN-2 IN METASTATIC RENAL CELL CARCINOMA: HOSPITAL CENTRAL DE ASTURIAS EXPERIENCE.

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We assessed the feasibility and toxicity of an outpatient subcutaneous (s.c.) interleukin-2 (IL-2) administration in metastatic renal cell carcinoma (MRCC). Therapy consisted of s.c. IL-2 at 18 x 10^6 IU three times or 9 x 10^6 IU four to five times per week in combination with corticoids/antipyretics p.o. premedication. Maintenance of treatment was carried out until progressive disease or unacceptable toxicity.
From February 93 to June 98, twenty-three consecutive patients (pts) with histologically confirmed progressive MRCC entered in the study. Patient's characteristics: Median age 61 years (range 34-75); Karnofsky index 70 (50-100); F/M: 4/19; prior nephrectomy 18; metastatic sites: Lung 17, soft tissue 7, kidney 5, bone 3, liver 3, CNS 1 case.
Results: 23 pts have been considered evaluable for toxicity and twenty one for clinical response. Only 2 pts tolerated the dose of 18 x 10^6 IU three times per week. The most frequent toxicities registered were: fever in 12 ptes (Grades (G); G1 in 1, D2 in 8 and G3 in 3), severe constitutional symptoms in 11, and injection site pain/cellulitis in 6 pts. Other toxicities as anorexia, nausea, diarrhea and skin rash were registered in ≤ 2 pts. In 18 pts reversible leukocytosis with a median leukocyte peak > 10.000 /mm³ was observed. No pts responded to treatment, 15 had stable disease and 6 progressed. Fifteen pts left the study due to progression disease and 6 for toxicity (4 for severe constitutional symptoms and 2 for fever not tolerated). Median time to treatment failure and overall survival was 10 weeks (range 3 – 44) and 40 weeks (15 days – 208 w) respectively.
Conclusion: This regimen of IL-2 has not activity and is associated with severe non haematologic toxicity in pts with MRCC.

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HIGH FRECUENCY OF PELVIC RELAPSE AFTER CYSTECTOMY IN INVASIVE BLADDER CANCER (IBC)

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Treatment of T2-T4a N0 M0 IBC is cystectomy, with 5 years survival of about 50%. Most frequent relapse pattern is considered to be distant metastasis and locorregional relapse is only present in 10% of patients. Therefore radiotherapy has been abandoned as a locorregional treatment in favour of quemotherapy. One preliminar phase III study of neoadjuvant quemotherapy versus surgery only, couldn't prove increased survival in the quemotherapy arm (Eur J Cancer 1995;31A:241). We present definitive results of that study, with stress on relapse pattern analysis.
PATIENTS AND METHODS: one woman and 78 men, median age 62 years (31-75) were included from January 1989 and October 1995, with a median overall survival of 72 months (36-116).
Inclusion criteria were transitional or undifferenciated IBC T3a-3b T4a N0 M0 ando T2a-2b with ureterhyronephrosis or diffuse carcinoma in situ.
Treatment: cystoprostatectomy and pelvic lymphadenectomy, with urethrectomy when the tumor infiltrated the prostate or when carcinoma in situ was detected in the prostatic urethra, and ileal neobladder substitution. 39 patients received neoadjuvant quemotherapy with MTX, VBL and CBDCA.
Follow-up: clinical examination and renal function every 4 months in the first and second year and every 6 months until the fifth year. Abdominal ultrasonography was alternated with urography and ductography every 6 months, and standard analysis, chest Xrays and abdominal CTscan were performed annually.
RESULTS: 71 patients were considered evaluable. 32 of them (45%) presented with tumoral relapse (TR). Isolated pelvic locoregional TR was detected in 15 patients (47%), distant TR in 12 (37,5%) and both in 5 (16%). Only 1/6 patients with pT2b N0 M0 and 0/24 ŌpT2a N0 M0 relapsed, which is in contrast with 20/29 (69%) in patients with pT3b T4a-4b N0 M0 and 11/12 (92%) with pN1-2. 91% and 97% of relapses ocurred in the first 2 and 3 years. 5th and 8th year overall survival were 54% and 51% respectively.
CONCLUSIONS: Locoregional relapse in IBC patients after cystectomy was more frequent than published: isolated pelvic TR was seen in 47% of bad prognosis invasive tumor patients. No TR was observed in tumors ŌpT2a N0 M0. These results suggest that radiotherapy may be contemplated in the adjuvant setting of high risk IBC, with the intend of diminish locorregional relapse.

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Phase II study of Vinorelbine (VNR) - Estramustine (E) in the treatment of patients (pts) with hormonoresistent prostate carcinoma.

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INTRODUCTION: A preliminary study was conducted in 12 patients with metastatic prostate cancer. They were treated withVNR 25 mg/m2 D1 and D8 every 21 days and E phosphate 280 mg p.o. D1 to 14.

PATIENTS AND METHODS: Median age was 69,5 years (60-84). Initial PS was ECOG 1-2 in 75% of pts. All pts had bone metastatic disease; 8 of them more than one metastatic site. All pts had received hormonal treatment, LHRH agonist and 1st. line antiandrogen treatment; 8 pts weren't treated with radiotherapy.

RESULTS: *Toxicity*: All pts were evaluable for toxicity: 2 toxic deaths (1 due to pulmonar tromboembolism, probably related with E; 1 stopped treatment due to COPD aggravation and disease progression. Haematological toxicity G3-4; only anaemia G3 (3 cycles); anaemia G2, 22; neutropenia G2, 6; ginecomastia was observed (G2 in 2 cycles). E must be stopped in 4 cycles due to related hipertensive crisis.

11 pts were evaluable for response: 1 CR, 4 PR, 2 SD and 4 progressed. Median time of follow-up was 221.6 days (≅7,5 months) range 21 to 570 days. At the moment 1 pts still alive without disease and 7 pts live with disease. PSA decrease in all pts on 3rd. cycle. % ↓ PSA= 51,7% (29,5) (mean/SD). T-test for paired data cycle 1-3 p=0,07. We observed in 5 pts a PS ECOG decrease of 1 or 2 degrees at 3 cycles. T Wilcoxon p=0,034.

75% of patients with pain at the entry become asymtomatic on 3 cycles. T McNemar p=0,031.

CONCLUSION:

Although this is a preliminary study with a well tolerated regimen, we observed a significant decrease of symptoms and improvement of PS on the 3rd. cycle of treatment.

The study should be continued to confirm these data and confirm the PSA decrease in these pts.

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UFT AND 4-EPIRRUBICIN IN HORMONOREFRACTARY METASTATIC PROSTATIC CARCINOMA

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Objective: To assess efficacy and toxicity of UFT and 4-epirrubicin in hormonorefractary metastatic prostatic carcinoma.

Patients and Methods: Histollogically proven hormonorefractary metastatic prostatic carcinoma were included when progressing. Therapeutic squeme was UFT 300 mg/m^2 continually and 4-epirrubicina 25 mg/m^2 on days 1, 8 and 15. Courses were repeated every 28 days.

Results: 16 patients have been included with a mean age of 68.5 years (52-84). All patients had bone metastases, 8 nodal and 2 liver. When symptomatic improvement was assesseded CR 9%, PR 45%, PD 27% and SD 18%. When evaluation was done on the basis of tumor markers PR 36%, SD 27% and PD 36%. Finally when response was assessed radiologically we found a partial response (6%) in a patient with liver metastases, a 64% stable disease and progresive disease in 30%.

Extrahematologic toxicity has been minimal with 2 patients with grade II mucositis and an extravasation of 4-epirrubicin asociated with a severe cutaneous necrosis. Febrile neutropenia ocurred in 3 patients and 23% of courses needed to be delayed.

Conclusions: This squeme is asociated with a significant activity in hormonorefractary prostatic carcinoma. Toxicity isa moderated. Patient inclusion is going to be continued.

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RENAL CANCER: REVISION OF 59 CASES.

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INTRODUCTION: the renal tumours are infrequent, they are 1.4% of all cancers in Europe and 1.5% of all deaths for cancer. The surgery is the only curative treatment used in initial stages only. About 60% of all patients with renal cell carcinoma develop metastasis along their evolution.

OBJECTIVE: To determine the characteristics of the group of patients diagnosticated in our hospital during a period of time.

MATERIAL AND METHODS: Retrospective study of the patients disgnosticated and treated in the Department of Medical Oncology in our hospital between Janaury 1988 and december 1995.

RESULTS: Were evaluated 59 patients. The median of age was 59 years (range 28 - 83). The most frequent clinical presentation was the hematuria. Tha carcinoma of clear cells was the most frequent histologic type. 17 of the patients had localized disease at the diagnosis, 9 locally advanced disease and 33 had metastatic disease. The initial treatment was nefrectomy in 26 cases. Were treated 14 patients with chemotherapy, 2 with inmunotherapy and 5 of them with hormonotherapy. The median survival was 12 months.

CONCLUSION: There was many patients in our serie with advanced disease, that verify the median survival obtained.

6. Gynaecological Cancer

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METASTATIC CANCER PRESENTATION. VALIDATION OF A DIAGNOSTIC ALGORITHM IN 212 PATIENTS.
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Introduction: Histologically diagnosed tumors from their metastases without clinically identified primary tumors (PT) are known as metastases of unknown origin (MUO). On the other side, those cases where metastases are the first sign, without evidence of the primary tumors previous to clinical examination, are known as metastatic cancer presentation (MCP). This situation generates anxiety and usually leads to undertake a great work up, sometimes unpleasant, and often not wothwhile, to reach diagnoses. Even if the PT is found, only few patients will get benefit from treatment. The use of a diagnostic algorithm (DA) would allow us to spare time and diagnostic procedures. In addition, an accurate diagnosis of PT likely to be treated should be feasible.
Objectives: Patients accrual for MUO and MCP conditions, presentation pictures, and PT of origin, when found. Validation of the usefulness of a DA in the MCP setting to assure diagnoses of all PT likely to be treated.
Patients and methods: A prospective study was undertaken from Jan 92 to Dec 97 for patients admitted and diagnosed of MCP. The whole group underwent a basic study (BS) consisting of clinical interview, complete physical examination including: ORL, rectal , breast, thyroid exams, standard blood tests along with tumor markers, and chest X-ray. For those patients where the BS was able to suspect a PT, a more directed study was performed (DS). Patients with negative BS or DS were considered for MUO, and these all underwent a protocolized study (PS) consisting of Abdominal CT scan and mamography (females). Those patients whose PT was not found after the DA process underwent an exhaustive study (ES) in order to validate the usefulness of the DA. Neoplastic histologic confirmation was obtained in all patients.
Results: The series included 228 patients (168 males, 60 females). Mean age was 61years, range from 18 to 84. The main guiding symptom was osseous (29%), neurological (24%), chest (17%), abdominal (15%). A positive BS was reached for 183 patients (80%). Of these, chest x-ray (78 pts) and physical examination (75 pts) lead to most of the diagnoses. The histology of metastases allowed diagnoses of the PT in 31 pts. Only the PSA showed a high sensitivity and specificity. The DS was able to achieve diagnosis of PT in 143 pts (63%), and 85 pts were filed as MUO. The PS reached diagnosis for PT in 23 pts (27%); 19 by means of abdominal CT scan, and 4 through mamographies, of these, only 9 (11%) (4 ovarian, 4 breasts and 1 lymphoma) were considered likely to be treated. The remaining 62 pts underwent an ES, and only in 11 (18%) diagnosis was made. However, none of them was considered for treatment. Finally, diagnoses was not found in 51 (22%) pts. The usual PT were lung (39%), MUO (22%), prostate (7%), and breast (6%). The most frequent metastatic sites, excluding lymph nodes, were bones (26%), CNS (16%), and liver (15%); the histologic types were adenocarcinoma (54%) and undifferentiated carcinoma (22%).
Conclusions: Lung cancer and MUO comprises 61% of the MCP. The BS can lead to diagnoses in 2/3 of cases, being physical examination and chest x-ray of great diagnostic usefulness. The histology of metastases and PSA are of most dignostic value. A PS based on abdominal CT scan and mamograpy (females) is able to achieve diagnoses for the remaining tumors likely to be treated.

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A PHASE I CLINICAL AND PHARMACOKINETIC (PK) STUDY OF PACLITAXEL (P) AND DOCETAXEL (D) IN PATIENTS (PTS) WITH SOLID TUMORS. M.A. Izquierdo, J.L. Pontón, M. García, M. Navarro, R. Mesia, F. Cardenal, M. Gil, J.R. Germá. Catalan Institute Oncology, Barcelona, Spain.

Introduction. Although P and D are structurally similar they have significant differences with respect to biological and pharmacologic characteristics, clinical activity (including absence of complete cross-resistance) and toxicity (TX). We initiated a phase I study of the combination of P and D to assess its feasibility. **Material and methods.** P and D were administered in 3 and 1 hr, respectively, every 21 days. No G-CSF was given. All PTD had received chemotherapy for advanced disease. The drug sequence was alternated in consecutive PTS and in the first two cycles within each PT. PK analysis was done in the first two cycles studying both sequences of TRE.). **Results.** To date 9 PTS have been included and received 46 cycles. At dose level I (P100 mg/m^2, D50 mg/m^2) 1/6 had, DLT (gr. 4 neutropenia [NEU] + fever) after cycle 1. At dose level II (P135 mg/m^2, D50 mg/m^2), 1/4 PTS had DLT (gr. 4 neutropenia [NEU] + fever) after cycle 1. Accrual continues at level II. Non-hematological toxicity gr.3/4 was not observed. In 4 PTS dose reduction was needed because of gr. 4 NEU + fever. All cycles were administered at day 21. As best response we observed 1 CR, 1 PR (nearly CR), 2 minor responses, 3 SD, and 2 PD. The sequence of treatment had influence on the PK of D. The PK study will be presented. **Conclusion.** The combination of P and D is feasible. NEU+fever seems the DLT of this combination. Responses have been observed in this group oh pre-treated PTS. Accrual continues to define MTD and further clarify sequence-dependent PK interactions.

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A STUDY OF PROGNOSTIC FACTORS (PF) FOR SURVIVAL IN CANCER OF UNKNOWN PRIMARY SITE (CUPS).

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INTRODUCTION: We present a retrospective series of 167 patients (p) who were diagnosed of CUPS in which we analyze PF for survival(S).
PATIENTS AND METHODS: Cases that had a suspected primary site or could be included in a specific clinical entity were not considered. Mean age was 63y (23-93). 56% were males and 36% had an ECOG (PS) ≥ 2. Main symptoms at diagnosis were: malaise (5%), tumor mass (22%), neurologic symptoms (14%), bone pain (10%), respiratory symptoms (11%), abdominal (23%), or more than one (15%). 33% were well-differenciated adenocarcinomas, 13% were squamous carcinomas and 54% were undifferenciated. Sites of metastasis(LOCMET) were: 78% lymph nodes (19% mediastinal, 22% retroperitoneal and 38% peripheral), 6% cutaneous, 23% pleuropulmonary, 34% intrabdominal, 20% peritoneal carcinomatosis, 16% brain and 23% bone. 54% had only one LOCMET. 29p (17%) were treated radically or with optimal cytoreduction. 67p (40%) received chemotherapy (CT)(55p were given cisplatin and/or adriamycin containing regimens and 12p had other regimens). 22p (13%) had CR, 11p (7%) had PR and 56p (33%) had no changes or progressed(NC-PG). 78p (47%) received only symptomatic treatment.
RESULTS: with a median follow up of 35 m, 135p (81%) had died of the tumor. Median S was 6 m (CI 95%, 4-8 m) and S at 1,2 and 3 years was 33%, 13% and 7% respectively. A multivariate analyses was performed to detect PF for S including sex, age, PS, histologic subtype, treatment, response to treatment and LOCMET. Only PR (p<0,016) and CR (p<0,001) were statistically significant as PF for S. Comparing to p who had not been treatment, risk of death for those who had achieved CR was 0.13 (CI 95%: 0.06-0.3) and 0.34 (CI 95%: 0'14-0'82) for p with PR. Considering response to treatment, median S was 2m (no treatment), 6 m (NC-PG), 19 m (RP) and 35 m (CR (p<0.001)).
CONCLUSION: In our group, response to treatment was the only factor associated with a longer S. Other PF are needed in order to select p who could benefit from treatment.

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A PHASE I TRIAL OF A COMBINATION OF A FIXED DOSE OF CARBOPLATIN PLUS PACLITAXEL AND ADRIAMYCIN IN FIRST LINE THERAPY FOR ADVANCED OVARIAN CANCER AND SUBOPTIMAL SURGICAL CYTOREDUCTION.

Cervantes A, Poveda A, González, Massutí B, Balañá C, Insa A, on behalf of the Spanish group for research in ovarian cancer (GEICO).

To develop a tolerable treatment of carboplatin at a fixed dose (AUC=5) plus paclitaxel, given in 1 hour, and adriamycin every 21 days, the GEICO group carried out a phase I trial. Inclusion criteria were: histologically confirmed ovarian cancer, PS<3 and normal liver, kidney, heart and marrow function. Patients after initial surgical debulking were included in 5 consecutive levels of dose:

Dose :	Paclitaxel	Adriamycin	Carboplatin
Level 0	135 mg/^{2}	40 mg/^{2}	AUC=5
Level 1	135 mg/^{2}	50 mg/^{2}	AUC=5
Level 2	150 mg/^{2}	50 mg/^{2}	AUC=5
Level 3	175 mg/^{2}	50 mg/^{2}	AUC=5
Level 4	175 mg/^{2}	60 mg/^{2}	AUC=5

From November-97 to September-98, 23 patients were accrued in 5 different institutions. Mean age was 61 (41-73). Three patients were respectively included at dose levels 0, 1 and 2. At dose level 3 8 cases were registered and 7 were accrued at dose level 4. Dose limiting toxicity was assessed after the first course of therapy and was febrile neutropenia at dose level 4. No other non hematological toxicity was detected as limiting. One patient at dose level 2 and 3 at dose level 4 had neutropenic fever. Level 4 was considered Dose limiting toxicity and the dose level 3 was the recommended dose for further phase II trials.

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PROGNOSTIC FACTORS IN ADVANCED OVARIAN CANCER. A RETROSPECTIVE ANALYSIS OF 135 PATIENTS.

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To assess the validity of a prognostic index (GPI) of Van Houwelingen (JCO 1989) in our series of 135 patients with advanced ovarian cancer (FIGO stage IIb-IV) and to study prognostic factors able to predict time to first progression and overall survival, a multiple regression analysis was performed. Factors studied were age, stage, histology, grade, Performance status (PS), presence of ascitis, tumor size before surgical debulking and residual tumor size after cytoreductive surgery. GPI was calculated after van Houwelingen, taking into account Karnofsky PS, grade, FIGO stage, residual tumor and ascitis. Patients were diagnosed between March, 1982 to December, 1996. Minimum follow-up was 22 months. Patients were treated with combination chemotherapy, most of them including cisplatin or carboplatin. Median follow-up was 83 months. In our series, GPI was a good predictor of survival ($p < 0.00001$) and of progression free survival ($p < 0.0001$). Progression free survival was predicted in the univariate analysis by age ($p=0.018$), Karnofsky ($p=0.009$), stage ($p<0.00001$) and residual tumor size ($p = 0.0001$). However, ascitis, grade, histology, and preoperative size had no effect on time to first progression. Survival was influenced by age ($p = 0.0002$), Karnofsky PS ($p < 0.00001$), stage ($p<0.00001$) and residual size after cytoreduction ($p < 0.00001$). These factors should be taken into account to stratify patients when included in randomized trials.

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TAXOL AND CARBOPLATIN IN FIRST LINE OF TREATMENT IN OVARIAN CANCER WITH STAGE III AND IV.

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Introduction: The aims of study are: determination of response rate to combination of Taxol and Carboplatin in first line of treatment of ovarian cancer in stage III and IV , evaluation in second laparotomy of response and determination the toxicity of this combination.

Material and Mhetod: From January -1996 to July -1998, 77 patients were included, 60 were evaluables for response and all for toxicity. The treatment : Taxol: 175 mg/m2 i.v.in 3 h., day 1 and Carboplatin : AUC 7, day 1, was given every 21 days. After 6 courses of treatment clinical response was valored and a second laparotomy was done to patients who enhanced clinical response. Mean age was: 59,8 years. Stage III: 52 patients (67,5%), stage IV: 25 patients (32,4%), stages III C and IV:61 patients(79,2%).

Results: The clinical response was valored by radiologic method and normal Ca-125 serum levels. The response rate was: CR: 23/60 (38,3%); PR: 28/60 (46,6%); SD: 8/60 (13,3%) and Progression 1/60 (1,6%). The RR was 51/60 (84,9%). A second laparotomy was done in 47 patients with the following results: Pathologic complete response 18/47 (38,29%); Pathologic partial response and residual minimun disease : 18/47 (38,29%). The number of patients without disease after second laparotomy (Pathologic CR patológica + CR postsurgery) was 36/47 (76,59%). The toxicity was evaluated by courses in 398 courses administered with mean of 5,16 courses (1-8). Hematologic toxicity :Neutropenia (G-3):10 (2,5%); Anemia (G-3): 10 (2,5%); Thrombocytopenia (G-3):4 (1%) and one febrile neutropenia. Non hematologic toxicity was: Nausea/Vomiting (G-3): 5 (1,2%) and Neurotoxicity(G-3): 6 (1,5%). G-4 non hematologic toxicity wasn't observed.

Conclusión: This regimen shows in this group of patients high rate of pathologic complete response with 78,3% of second laparotomy (47/60) and mild hematological and non hematological toxicity wich allows outpatient administration.

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In^{111} LABELED B72.3 IMMUNOSCINTIGRAPHY IN EPITELIAL OVARIAN CANCER. PROSPECTIVE STUDY. PRELIMINAR RESULTS.

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Objective: To evaluate the feasibility of Radioimmunoscintigraphy (RIS) at. 1) initial diagnosis of ovarian carcinoma (OC); 2) detection of residual disease after chemotherapy (CT); relapse.

Materials and methods: 24 patients have been studied: 16 with high risk pelvic mass (group 1); 7 with suspicion of residual disease (group 2); and 1 with relapsed disease. One mg of B 72.3 labelled with 5mCi de In^{111} e.v. was inyected e.v. and thoracic-abdominal gammagraphic scanning was performed at 24, 48 and 72 hours. Between 4 and 7 days later a laparotomic examination was performed.

Results: The Laparotomic finding were: In group 1: 7 OC, 2 borderline tumours (BOR) and 7 benign tumours were found; in group 2: 5 OC, 1 BOR y 1RCp; and OC relapse. The feasibility of RIS to detected OC or BOR was evaluated and the results were compared with others diagnostic methods.

Results Group 1	RIS	ECO	TAC	DOPPLER	CA 125
% Sensitivity	**100** (9/9)	100(7/7)	100(8/8)	100(7/7)	89(8/9)
% Specificity	**43**(3/7)	0 (0/7)	0 (0/3)	33(2/6)	40(2/5)
% + Predict. Value	**69**(9/13)	50(7/14)	73(8/11)	64(7/11)	73(8/11)
% - Predict. Value	**100**(3/3)	0 (0/0)	0 (0/0)	100(2/2)	67(2/3)
% Accuracy	**75**(12/16)	50 (7/14)	73 (8/11)	70(9/13)	71(10/14)

Results Group 2:	RIS	TAC	CA125
% Sensitivity	**50** (3/6)	67 (2/3)	40 (2/5)
% Specificity	**100** (1/1)	50 (1/2)	50 (1/2)
% + Predictive Value	**100** (3/3)	67 (2/3)	67 (2/3)
% - Predictive value	**25** (1/4)	50 (1/2)	25 (1/4)
% Accuracy	**57** (4/7)	60 (3/5)	43 (3/7)

Group 3: The case in this group had a RIS both: false negative and false positive.

Not toxicity was found.

Conclusions: The accuracy of I^{111}-B72.3 RIS was similar to other conventional diagnostic methods. In high risk pelvic mass a negative RIS showed benign tumour in 100% of cases. In residual disease, a RIS + predicts bulky cancer. These results should be confirmed in a larger study.

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SECOND-LINE CHEMOTHERAPY OF EPITHELIAL OVARIAN CANCER WITH PACLITAXEL-DOXORUBICIN-CYCLOPHOSPHAMIDE.

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Background: Paclitaxel monotherapy produces objective responses in 20-40 % of patients with ovarian cancer previously treated with a platinum-based regimen. The aim of this study was to examine the efficacy of a combination regimen of paclitaxel with two active agents in ovarian cancer.

Patients and Methods: A prospective phase II study was designed. Inclusion criteria were histologically confirmed epithelial ovarian cancer diagnosis, measurable disease and previous treatment with platinum-based therapy. Patients received paclitaxel 175 mg/m^2 as a 3-hour infusion, followed by doxorubicin 50 mg/m^2 and cyclophosphamide 500mg/m^2, every 21 days. G-CSF was given as a daily subcutaneous injection of $30 \cdot 10^6$ UI/day , days 5 to 15.

Results: Twenty-nine assessable patients were included: thirteen were platinum-sensitive patients (PSP) (considered as patients who had a disease free survival (DFS) > 6 months), and sixteen were platinum-resistant patients (PRP). One hundred and twenty-nine cycles were administered. Twelve (41%) out of 29 patients responded (2 CR, 10 PR); nine (31%) demonstrated stable disease and 8 (28%) progressed. The overall response rate in PSP was 69% (9/13) versus 19% (3/16) in PRP ($p<0,006$). The median DFS was 3,5 months (5 months in PSP and 3 months in PRP, $p=0,004$). The median overall survival was 11 months (19,3 in PSP and 9,3 in PRP, $p=0,01$). Toxicity was moderate ; eight episodes (6% of cycles) of febrile neutropenia were observed.

Conclusions: The combination regimen paclitaxel, doxorubicin and cyclophosphamide achieved a high rate of responses in platinum-sensitive patients. These results in PSP are superior to the observed with taxol in monotherapy.

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PHASE II TRIAL OF LIPOSOMAL DOXORUBICIN (CAELIX) IN RELAPSES OF CISPLATIN REFRACTORY OVARIAN CARCINOMA.

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In order to determine the activity of liposomal doxorubicin in patients with recurrences of ovarian carcinoma after treatment with cisplatin we designed a phase II trial. Elegibility criteria included normal hepatic, renal, cardiac and marrow function, WHO performance status <3, histologically confirmed ovarian carcinoma, resistance or progression after cisplatin treatment and measurable disease. Caelix at initial dose of 50 mg/m2 was given every 21 days. The starting dose was reduced to 40 mg/m2 after the 2nd cycle. 18 patients with median age of 60 years (32-76) received at least 2 cycles of treatment. Characteristics of the patients: serose histology: 14, vs other histology: 4; number of lesions <3 : 5, vs >3 lesions: 13; size of the largest lesion <5 cm: 10, vs >5 cm: 8; median number of previous treatments: 2 (2-5); presence of ascitis: 6, vs no ascitis: 12. Median PS was 2 (0-2). Median time from the last treatment was 2 months. 15 patients relapsed during cisplatin treatment and 2 patients did before 6 months from the last treatment with cisplatin. Only one patient had characteristics of probable sensitivity to cisplatin. 74 cycles were administered with median number of 3 (2-10). There were only one partial response and 10 stable diseases. 7 patients progressed before 3rd cycle. The most commonly observed toxicities were skin toxicity 1/2 (2/1) and mucositis 1/2 (5/6). Two patients had grade 3 mucositis, and no other grade 3/4 toxicity was detected. Treatment with Caelix has good tolerance but low activity in this selected population of cisplatin refractory patients.

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COMPARATIVE STUDY BETWEEN PERITONEAL SURFACE SEROUS PAPILAR CARCINOMA AND OVARIAN SEROUS PAPILAR CARCINOMA STAGES III AND IV.

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Introduction: Recent studies regarding ovarian cancer Stages III and IV have led to differentiate a new entity known as peritoneal surface serous papilar carcinoma (PSSPC) which would comprise up to 15% of the well known ovarian serous papilar carcinomas (OSPC). Controversy arises about whether those tumors are actually ovarian tumors with peritoneal surface spread, or could be a neoplastic proliferation of epithelial cells from peritoneal surface. Initially, this new entity has been considered more aggressive and with poorer prognosis when compared to OSPC. However, comparative studies concerning staging and histologic grade managed by radical surgical procedures and postoperative systemic chemotherapy show controversial results.
Aims: Incidence of PSSPC among ovarian epithelial carcinoma stages III and IV undergoing surgery in our Institution. Comparative analyses of overall survival (OS) concerning the OSPC of patients (pts) managed by the same approach (supraradical surgery using peritonectomy procedures and postoperative chemotherapy). To validate radical cytoreductive surgery as an optimal procedure for PSSPC.
Material and methods: Retrospective study of histologic samples from patients who underwent surgery at the CHCR for ovarian neoplasms stages III and IV. Supra-radical procedures involving peritonectomy procedures were performed. Surgical morbidity and mortality assessment as well as chemotherapy related toxicities. To identify PSSPC cases in order to assess overall survival figures compared to the OSPC group.
Results: From March 90 to Jan 98 a total of 47 pts diagnosed of stage III and IV ovarian cancer underwent supra-radical surgery. Mean age was 66 years, range (41-89). About 26 pts were considered to have serous papilar carcinomas. Nine of these patients (35%) were PSSPC and the remaining 17 pts (65%) were OSPC. Surgery was considered to be optimal (residual disease nodules less than 1cm) for 24 pts (92%). Cisplatin based adjuvant chemotherapy was delivered in 19 pts. Toxicity was mainly haematological (4pts) and GI tract (2 pts). After a mean follow-up of 2.8 years (0,4-6,6) median survival time reached 12 months (PSSPC 14,2 mo (4-34) and OSPC 9,5 mo (5-25+)). According to stage disease: Stage III 18 mo (1-81), Stage IV 9,7 mo (1-37). The 2-year OS was 20% (PSSPC 12,5; OSPC 17,6%). Perioperative mortality reached a 19% rate (5 pts). Mortality rate was higher during the first six months. However, the advanced stage of disease (15 pts satge IV), age of some patients, and the "learning curve" of those complex surgical procedures must be taken into account.
Conclussions: The present study tries to show that supra-radical surgery for stages III and IV OSPC using oncologic criteria is able to achieve higher survival rates than the standard surgical debulking approaches. According to our own experience PSSPC behaves as a different entity when compared to OSPC, and managed with supraradical surgery along with postoperative cisplatin-based chemotherapy has a better outcome than OSPC. Finally, PSSPC must be yet considered an entity not well-defined so far. Studies must be done addressing basic questions like histopatholocic differential diagnosis, optimal treatment approach and prognosis.

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PACLITAXEL-CARBOPLATIN AS FIRST LINE CHEMOTHERAPY IN ADVANCED OVARIAN CANCER.

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PURPOSE: To evaluate response rate and toxicity of the schema Paclitaxel 175 mg/m^2 and Carboplatin AUC 7.5 every 3 weeks in patients (pts) with advanced ovarian cancer.
METHODS: From December 1996 to Octuber 1998, 22 (pts) were included (1 pts stage IIIB, 16 pts Stage IIIC, 5 pts Stage IV) 15 out of 22 pts were evaluable for response after 6 cycles of treatment and 22 for toxicity. The treatment scheduled was: Paclitaxel 175 mg/m2 iv 3h-infusion day 1 and Carboplatin AUC 7.5 day 1. Courses evey 21 days. Median age was 58 (38-73). ECOG PS 1 (15 pts) and 2 (7 pts). Histologic subtypes were serous adenocarcinoma (17 pts), mucinous (2 pts), endometrioid (1 pt), others (1 pt). Differentiation grade: G1 (1 pt), G2 (5 pts), G3 (7 pts), unknown (9 pts). It was carried out initial surgery: radical: 11 pts (<2cm: 4 pts, >2 cm: 7 pts). Exploratory 8pts. Measurable diseases in 5 pts and evaluable one in 10 pts. 7 pts had measurable and evaluable disease.

RESULTS: The total number of cycles administered were 121. It was registered non-hematological toxicities (% per cycle): G3: nausea-vomiting: 0.8%, alopecia: 100%; G2: nausea-vomiting: 7.4%, diarrhea: 1.6%, stomatitis: 1.6%, peripheral neuropathy: 6.6%, arthralgia-myalgia: 12.3%; G1: peripheral neuropathy: 37.1%. With regards to hematological toxicity: anemia G3: 5.2%, G2: 27.3%, G1: 30.5%. Leucopenia 1.05%, G3: 12.6%, G2: 28.4%, G1: 24.2%. Neutropenia G4: 12.6%, G3: 22.1%, G2: 27.3%, G1: 9.4%. Thrombocytopenia: G4: 2.1%, G3: 9.4%, G2: 9.4%, G1: 8.4%. 1 case of febrile neutropenia had occurred ans in 2 cases were necessary blood transfussion support. In 4 pts were necessary dose reduction and in 5 patients were essencial the use of G-CSF. After 6 cycles of treatment it was evaluated the response rate (RR) (by CT and tumour markers) in 15 pts, with 7 CR (73.2%), 4 PR (26.6%), 4 SD (26.6%). Overall RR: 11 (73.2%). 10 pts were undertaken to second look laparotomy with four pathological (18.8%).

CONCLUSION: These results suggest that Taxol + Carboplatin combination is effective in this group of pts, associated with moderate toxicity though manegeable.

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RETROSPECTIVE TRIAL IN OVARIAN CARCINOMA STAGES III-IV TREATED WITH PACLITAXEL AND PLATINUM ANALOGUES.

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Introduction.- Paclitaxel (P) has revealed as a very active drug in advanced ovarian carcinoma. Aim: to asses efficacy and toxicity profile of a schedule with P and a platinum analogue.

Material and Methods.- 20 assesable patients (p) with ovarian carcinoma stages III-IV FIGO, were treated between March 95 and September 97. Median age 59 years (25-75 years). Perfomance Status (ECOG) < 2 in all cases. 15 p were classified as stage III and 5 p as stage IV. Histology: adenocarcinoma papillary serous in 17 p, endometroid in 2 p y mixed in 1 p. Histologic grade: G1 3 p. G2 5p y G3 9p. Surgery was optime (residual disease < 1 cm) in 5 p and suboptime in 15p. Treatment with chemotherapy (QT): CDDP 75 mg/m^2 + Paclitaxel 175 mg/m^2 18 p y CBDCA 4 AUC Paclitaxel 175 mg/m^2 2 p.

Results.- All p with stage III achieved clinical RC; in 6 p "second-look" was performed, with pathologic RC in 3 of them and residual disease in the other 3p. From the 15 p clasified inicially as stage III, 73% stay alive with a median follow-up of 17 months. Median survival for stage III has not yet been reached. Patients with stage IV achieved RP in 3p and SD in 2p. Toxicity G3-4 (OMS): Alopecia G3 100%p. Neurotoxicity G3 2p. Nausea/Vomiting G3 2p. Anemia G3 4p. Neutropenia G4 1 p. Neutropenic fever 1p.

Conclusions.- Chemotherapy schedule with paclitaxel and cisplatin or analogues is well tolerated with a low incidence of serious adverse events. We are not able to asses survival because of the short follow-up and the low number of patients included.

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Second-line treatment with Topotecan in ovarian cancer

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Topotecan (tpt) has demonstrated its efficacy in relapsed ovarian cancer after chemotherapy treatment .We have analysed the results obtained from 11 patients with ovarian neoplasm, which presented a failure to the chemotherapy treatment with cisplatin and/or taxol and were treated in a second line chemotherapy with topotecan.

The mean age of the group was of 60 years (49-72). 7 patients had presented early relapsed (< 3 months), 2 presented relapsed of 3 to 6 months and another 2 presented late relapsed (> 6 months). 9 of those patients received a previous treatment with taxol and all of them treatment with cisplatin. More than one line of chemotherapy treatment was given to 6 patients. With regards to the first line treatment the responses obtained were 3 complete responses (CR), 3 partial responses (PR) and 5 progression diseases (PD).

The topotecan doses were of 1,5 mg/m^2 x 5 days with a total of 60 cycles administered (mean of 5,4 range of 1-9). From this we obtained 1 CR, 6 PR, 2 estabilizations and 1 PD. One of the patients was not evaluated due to toxicity in the first cycle. With respect to the toxicity, it was mainly hematology which caused the interruption of the treatment in one patient, basically due to anemia (grade 2:50% of the cycles and grade 3:21% of the cycles) and leucopenia (grade 2 3% and grade 3:8%). 3 patients were diagnosed an infection that needed admission in hospital, but there were no toxic deaths. The survival analysis is not valid for appraisal due to the short monitoring interval that we have; today 9 patients are alive (+17, +4, +4, +11, +4, +7, +7, +4, +6) and 2 deceased (14,9) one of them with brain metastasis.

Though our results are quite preliminary, we find a large level of activity with topotecan (54%) in a group of patients that received an extensive previous treatment with recurrent ovarian cancer.

7. Lymphomas, Sarcomas, CNS, Melanoma

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HIGH-DOSE IFOSFAMIDE AND DOXORRUBICIN (HDI-DX) IN ADVANCED PREVIOUSLY UNTREATED SOFT TISSUE SARCOMA (STS) PATIENTS. A PHASE II STUDY OF THE SPANISH GROUP FOR RESEARCH ON SARCOMAS (GEIS). *A López Pousa, JM Buesa, J Montalar, J Martín, J Maurel, J García del Muro, R de las Peñas, J Cruz, J Cassinello, I Sevilla, C Balañá, A Casado, I Bover, A Paredes, J Carles, A Poveda.* H. Sant Pau, Avda Padre Claret 167. 08025 Barcelona. Spain.

Introduction: Doxorrubicin (DX) and high-dose Ifosfamide (HDI) are two active drugs in the treatment of STS with a similar response rate. We are performing a phase II trial in first line with HDI-DX (scalating dose of HDI), in order to evaluate the activity and toxicity of this regime.

Material and Methods: From Jul 97 to Oct 98 we have included 54 previously untreated advanced STS pts in a trial with HDI-DX, with a dose of DX 50 mg/m^2 and a continuous infusion of HDI, starting at 12 g/m^2 and scalating dose to 13 and 14 g/m^2 (2 g/m^2 in 2-hours d1, followed by 2 g/m^2/day d1 to d5-6), in absence of any grade IV toxicity or neutropenic fever, per cy in each pt. Otherwise a dose reduction to 10 g/m^2 has been allowed. GM-CSF (Leucomax®, Schering-Plough) 5 µg/Kg/d x 7 (d6-d13) was administered after each cy. Thirty-five pts are already evaluable: 16 M, 19 F; median age 55 (27-65) y. Performance status: WHO grade 0-13; 1-14; 2-8 pts. Histology: leiomiosarc 9, malignant fibrous h. 5, synovial 3, liposarc 3, fibrosarc 3, neurogenic 3, mixed müllerian 2, others 7. Target tumor location: non-resectable primary or recurrence 18 (exclusively 10), lung 16, nodal 2, liver 2, soft tissue 1, peritoneal 1, bone 1. Histologic grade: 1-6; 2-9; 3-17 pts.

Results: We have already administered 87evaluable cycles (cy), median 2 (range 1-5). Dose intensity of HDI: 2.80 (1.7-3.5) g/m^2/week. Dose of HDI per cycle: 12 g/m^2 in 51 cy, 13 g/m^2 in 12 cy, 14 g/m^2 in 5 cy and 10 g/m^2 (reduction dose) in 19 cy. Haematologic toxicity (grade 3-4 CALBG - % cy): Hbne 17%; Leuc 13% G3, 37% G4; Gran 9% G3, 41% G4; Plat 14% G3, 12% G4. Non-Haematologic toxicity (grade 3-4 CALBG - % cy): Nausea 13%; Vom 14%; Stomatitis 9% G3; Hematuria 1% Neurocortical 5% G3, moderate somnolence 9%; Infection 18% G3, 5% G4; Asthenia 19% G3, 2% G4; Anorexia 15%; Cardiac 3,5% G3. Neutropenic fever: 13 pts in 20 cy (23%). There was one toxic death due to a septic shock after the first cy. Transfusions: RBC in 22% cy; PLT in 5% cy.

Objective activity: 8 PR (29%), 7 SD (25%), 5 PD, 8 NE in 28 evaluable pts.

Conclusions: HDI-DX is an active regime in adult STS. Haematological toxicity limits the maximum dose of HDI (12-10 g/m^2 in 80% of cy) and together with cardiac and neurological toxicity may restrict its use.

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High-grade astrocytoma (HGA) of the corpus callosum (CC): survival and prognostic factors.

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OBJECTIVE: To evaluate outcome of HGA involving CC and to identify prognostic factors.

BACKGROUND: It is unclear whether aggressive treatment of CC-HGA may benefit to some patients.

DESIGN/METHODS: Fifty-eight patients with biopsy-proven CC-HGA were treated in four different, French hospitals between 1987-1997. All patients received a course of cerebral radiotherapy (RT) (60 Gy/1.8 Gy fractions) and/or nitrosourea-based chemotherapy. Survival was calculated from the date of histological diagnosis using the method of Kaplan and Meier. Univariate and multivariate analysis were performed using the Cox proportional hazards model.

RESULTS: There were 27 men and 31 women. Median age at diagnosis was 59 years (33-72 years). Tumor histology was glioblastoma (GBM) in 23 patients, anaplastic astrocytoma (AA) in 17 and anaplastic oligodendroglioma (AO) or oligo-astrocytoma (AOA) in 18. For the entire group the median survival (MS) was 33 weeks. For GBM MS was 31 weeks, for AA MS was 34 weeks and for AO/AOA MS was 153 weeks ($p = 0.0018$). On multivariate analysis, the only independent prognostic factors were treatment with RT ($p = 0.00001$), and Karnofsky performance status (KPS) after surgery ≥ 70 ($p = 0.019$). Histology ($p = 0.051$) and age (0.059) were near the statistical significance. Treatment with nitrosoureа was not considered because only two patients did not receive chemotherapy. Other like KPS before surgery, sex, surgery (subtotal resection vs. biopsy), and localization (fronto-callosum vs. middle vs. splenium) were not significant variables. Median KPS before and at the completion of treatment was 60 / 30 in GBM , 70 / 60 in AA and 60 / 60 in AO/AOA.

CONCLUSIONS: As for other malignant gliomas this study confirms that treatment with rt, KPS after surgery histology and age are important prognostic factors in CC-HCG. Very rapid deterioration of the KPS immediatly after or even during RT/CT suggest that aggressive treatment of CC-GBM is not useful. Stabilisation of KPS and the possibility of long-term survival support the use of conventional treatment (RT/CT) in patients with anaplastic gliomas of the CC, particularly CC-AO/AOA. Since CC-GBM and CC-AO/AOA often have the same aspect on computed tomography scan or MRI (butterfly glioma) a biopsy is mandatory in order to adapt the treatment to the histology.

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NON-HODGKIN'S LYMPHOMA (NHL) IN THE ELDERLY: AGE IS NOT ALWAYS A RISK FACTOR.

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INTRODUCTION: Age is usually considered as a poor prognostic factor for survival in NHL, but it is not known which is the influence of age among patients with other well recognized prognostic factors (PF).

OBJECTIVES: 1) To study the clinical characteristics of NHL in patients with more than 65 years old; 2) to analyse PF for survival in NHL arising in those patients; 3) to investigate the impact of age on survival within patient subgroups stratified according to the most important PF.

METHODS: The influence of histologic grade (according to the Working Formulation, but considering Immunoblastic Lymphoma in the Intermediate Grade group), and 54 clinical and biological pre-treatment parameters on cause-specific survival (CSS) was analysed by univariate (Kaplan-Meier model) and multivariate (Cox model) statistical methods in a retrospective series of patients treated at a single institution from 1977 to 1991.

PATIENTS: In this period, 521 patients with NHL were treated, but only 427 were evaluable (patients without histological samples for pathological revision, usually diagnosed in other centers and referred to treatment, were excluded). Among them, 95 were > 65 years old.

RESULTS: Median age: 72 yrs (range 66 to 84); male/female ratio: 43/52; Low/Intermediate/High grade: 38/47/10; Stage (I-II/III-IV): 36/59; Performance status (PS) (0-1 vs. >1): 39/56; B symptoms (yes/no): 47/48; LDH (normal/elevated): 33/62; serum albumin (low/normal): 20/75; tumor burden -MD Anderson criteria- (High/Intermediate/Low): 41/28/26. Median CSS was 52 months (120 for low-grade, 54 for intermediate-grade, and 11 months for high-grade).

In multivariate analysis, PF for CSS in patients older than 65 were: Histologic grade, poor PS, B symptoms, high LDH, and high tumor burden.

Analysing the influence of age (≤65/>65) on CSS within each of the patient subgroups (stratified according to these major PF), the results were interesting: There were no significant differences between young and old patients with bad PS, Stage I and IV disease, low serum albumin, and low or high tumor burden. The adverse prognostic value of age was only evident in patients with good PS; stage II and III disease; normal serum albumin and intermediate tumor burden.

CONCLUSION: 1) The clinical characteristics and PF of elderly patients with NHL are similar to the younger population; 2) Age is not always an adverse PF: Patients with other very good or very bad PF have a similar survival, independently of age.

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HIGH DOSE CHEMOTHERAPY (HDC) WITH STEM CELL TRANSPLANTATION (PBSCT) FOR LYMPHOMA. A SINGLE INSTITUTION EXPERIENCE. R Cajal, MJVaro*, JI Mayordomo, L Palomera*, A Yubero, JA Moreno*, MD Isla, J Herraez, P Bueso, JL Marti, L Murillo, A Sáenz, P Escudero, MD García, M Gutierrez*, A.Tres. Divs of Medical Oncology & * Haematology. University Hospital. Zaragoza. Spain.

Background: HDC and PBSCT is a promising treatment for non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD). **Methods:** From Oct/95 to Feb/98, 27 consecutive patients (pts) with NHL (22 pts) and HD (5 pts) underwent HDC with PBSCTat our institution. There were 17 males and 10 females. Median age was 46 years (range 22-63). Disease status: 16 pts (13 high grade NHL and 3 HD) were in first line; 9 pts were in first relapse (7 NHL: 6 high grade and 1 low grade and HD: 2 pts) and 2 in second or further relapse (2 NHL). Induction CHT for NHL was CHOP (+/- escalated dose) for newly diagnosed pts, and MINE or E-SHAP for relapse. HD pts received ABVD (first line)or MOPP for relapse. HDC regimen was BEAC or BEAM in 12 pts (12 NHL) and dose-escalated CBV for 15 pts (10 NHL and 5 HD). Median number of CD34 cells infused was 3.7 x 10^6/kg (1.4 -8.8). **Results:** Engrafment was prompt and predictable with a median 10 days (9-25) to reach ANC>500/ µl and 11 days (8-34) to platelet transfusion independency. All pts had neutropenic fever, mucositis and diarrhea (WHO grade 2-3).No grade 3-4 hepatic and renal toxicities were noted. One pt had Pn. Carinii pneumonitis. Four patients died with progression at day +42, +62, +90, and +101 and one died of urinary sepsis at day +70. 20 pts achieved CR and remain disease-free with a median follow-up of 23 months (range 2-35). 2 pts are alive with relapse. Actuarial 3-year disease free survival and total survival (DFS/TS) is 62.8/68.5%. 3-year OFS/TS according to disease status for NHL is 92.3/92.3% for first line, and 31.7/55.5% for first or further relapse. For HD, actuarial 3-year DFS/TS it is 100/100%. **Conclusion:** HDC with PBSCT is highly active in this setting of unfavorable NHL and HD pts with acceptable toxicity. Although results of further randomized trials are needed, early HDC with PBSCT for poor prognosis lymphoma is safe and encouraging.

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LONG-TERM RESULTS AFTER MULTIMODAL THERAPY FOR LOCALIZED OSTEOSARCOMA.
THE HOSPITAL UNIVERSITARIO LA FE EXPERIENCE

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INTRODUCTION: Since the introduction of combined modality treatment, the outlook of patients with non-metastatic osteosarcoma has significantly improved. A retrospective study was performed to assess the long-term results with this approach.
PATIENTS AND METHOD: From 1985 through 1993, 35 patients with high-grade central osteosarcoma (stage II-B) were treated with neoadjuvant chemotherapy (4 courses of high-dose methotrexate), wide functional surgery, and adjuvant chemotherapy (cisplatin-doxorubicin / cisplatin-doxorubicin / bleomycin-cyclophosphamide-dactinomycin, x 5) (modified T-10 A protocol). There were 19 males and 16 females; median patient age was 17 years (12-42). The primary site was located at the extremities in 83% cases (femur 20, tibia 4, fibula 2, humerus 2, scapula 1) and was axial in 17% (3 pelvis, 1 spine, 1 rib, 1 maxilla).
RESULTS: In spite of a low (1.2%), favorable (grade 3-4), histological response rate to neoadjuvant methotrexate, 31 (88%) patients underwent limb-sparing surgery. After the planned therapy, 28 (80%) patients achieved a complete response. However, 11 of them (39%) relapsed thereafter (3 recurrences were salvaged with surgery). There were no late relapses (more than 3 years from diagnosis). Median follow-up is 8 years (5-13). Disease-free and overall survival are, respectively, 49 and 64% at 5 years, and 49 and 59% at 10 years. Primary tumor site (extremity vs axial, p=0.0125) and tumor size (<10 cm vs ≥10 cm, p=0.0139) were significant prognostic factors.
CONCLUSIONS: Nearly 60% of patients with osteosarcoma are alive, disease-free after multimodality treatment, most of them with limb-sparing procedures. Although the prognosis of axial tumors is worse, they can be treated with extremity-directed protocols. Methotrexate scheduling should be individualized in order to optimize the percentage of chemotherapy-induced necrosis.

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VALIDITY AND APLICABILITY OF LYMPHOSCINTIGRAPHY AND A GAMMA PROBE IN IDENTIFICATION OF SENTINEL LYMPH NODE IN MALIGNANT MELANOMA

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Introduction The sentinel lymph node (SN) is the first node in a regional lymphatic basin that receive drainage from primary tumour. If SN is not involved by tumoural cells total lymphadenectomy of regional lymphatic basin can be avoided. The goal of this stydy was to assess SN detection technique and then, if it was successful, to perform it in a routinary everyday practice.
Method Fifty patients with malignant melanoma (MM) were prospectively studied (32 with stage I/II and 18 with stage III). The day before surgery a lymphoscintigraphy with 74 MBq of ^{99m}Tc-nanocolloid was performed. Twenty minute dynamic flow images were obtained immediately after radiotracer injection followed by static images at 30 min and 2 hrs. The first lymph node identified was considered as SN and was marked on the skin. During surgical procedure a hand-held gamma probe and blue day were used in order to locate more accurately the SN.
Results SNs were successfully identified in 49/50 patients (98%). In 32 patients with stage I/II 47 SNs were detected. Seven of them (16%) were positive for MM and 40 (84%) negative. A total amount of 225 regional lymph nodes was removed, all of them being negative for MM (no "skip metastasis"). In one patient of this group SN was not detected, being all the 6 regional lymph nodes harvested negative for MM. In 18 patients with stage III, 24 SNs were located, being 16 positive (67%) and 8 negative (33%) for MM. One hundred and ten regional lymph nodes were excised in SN positive patients, being 55 positive and 55 negative for MM. In SN negative patients 41 lymph nodes were harvested , all of them negative for MM.
Conclusions Surgical localization of SN is facilitated with lymphoscintigraphy and the use of the gamma probe, allowing to perform a biopsy and selection of patients for lymphadenectomy. Afterwards, we have included this technique in everyday practice. To date, 55 new patients have been estudied with this procedure.

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PRELIMINARY DATA OF REGISTRY FROM SPANISH GROUP FOR RESEARCH ON SARCOMAS (GEIS)

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We have analysed the first 425 soft tissue sarcoma (STS) patients registered in our data base from 1994 to 1998. These patients stemming from 19/26 hospital centers of GEIS.
OBJECTIVE: We present a descriptive analysis of diagnostic procedure data and no consideration has been made of treatment and survival data.
RESULTS: Median age 54.5 y.o. (15-91); Sex M/F: 199/226. STS were somatic in 325 patients (75.3%) and were of visceral origin in 105 (24.7%). The distribution by histology was similar to others published registries. The predominant somatic STS were: Liposarcoma (LPS): 85 (20%); Malignant fibrous histiocytoma (MFH): 53 (12.5%) and Synovial sarcoma (SS): 29 (6.8%).
The site distribution of somatic STS were: Extremities 53.4%; Retroperitoneum 22.5% ; Trunk 15.3%; Head and neck 6.5% and others 2.3%. The most frequent histologic types according to site was: for extremities MFH 24.8%, LPS 24.2% and LMS 16.6 %; for retroperitoneum LPS 35%, LMS 19%, and Neural sarcomas (NS) 8.4 %; for head and neck Rhabdomyosarcoma (RMS) 20%, Endothelial sarcomas (ES) 20%, LPS 15% and Fibrosarcoma 15%. At diagnostic time a 43.8% patients had a tumor greater than 10 cms. The most frequent histology between these tumors was LPS (40%) , while 67% of RMS were greater than 10 cm.
There has been a close relation for some histologic types and grade: for RMS 100% of patients were grade 3; for LPS 50% were grade 1 and for NS 44% grade 3. For stages IV B: ES 35%, LMS 9%, and LPS 8%.
Among visceral sarcomas the most frequent site distribution was: Uterus 44/105 (42%); Gastrointestinal 34/105 (32%) and Lung 8/105 (7%). For uterine sarcomas the predominant histology was mixed müllerian tumors in 20/44 (45%) of patients.
Diagnoses was made by excisional biopsy in 254/425 (59%), by incisional biopsy in 119/425 (28%), by fine-needle aspiration cytology in 32/425 (7%) and by tru-cut biopsy in 20/425 (5%). We realised in regard of this information that in the majority of patients the initial diagnosis of STS was not suspected. For an adequate therapeutic approach the percentage of tru-cut biopsy should be greater than the rest of procedures.

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DIAGNOSIS AND TREATMENT OF RELAPSED SOFT TISSUE SARCOMAS (STS).

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The objective of this retrospective analysis was to evaluate the symptoms (sym) and the method of diagnosis (dg) of local (LF) or distal failure (DF) in pts with STS initially submitted to a curative attempt, and to determine the efficacy of salvage treatment (trt). Seventy-six out off 152 clinical records randomly revised showed a failure (25 LF, 41 DF, 10 both) and the tumor was somatic (11, 26, 3), gynecologic (6, 12, 3), retroperitoneal (7, 2, 0), visceral (0, 1, 2) or of other origin (1, 0, 2). Metastases were located in lungs (36), liver (6), abdomen (6), bone (7), subcutaneous (3), lymph-nodes (3), mediastinum (1) or other (4). Thirty-five pts had sym, and the relapse was detected in a programmed visit (48) or in a visit asked by the patient (28). LF sym (pts): neurologic (1) or visceral pain (6), dyspnea (1), a lump (6), hemoptysis (1); DF sym: anorexia (1), asthenia (1), bone (5) or visceral pain (3), dyspnea (4), a lump (1), cough (2), hemoptysis (1); LF dg: physical exam(16), US (5), CT (9), MR (1); DF dg: physical exam (1), X-ray (36), US 2, CT 8, MR 2, bone scan 2. LF trt: surgery (S) 11, Qtx 11, Rtx 1, S + Rtx ± QTX 8, symptomatic 7; DF trt: S 4, S + Rtx 1, S + Qtx 13, Qtx 31, symptomatic 10. Time to any failure by grade of malignancy (G): G1 43, G2 12, G3 7 mos. Median follow-up: 45 mos. (range 7-218), median survival from LF 44 mos. (20-73) and from DF 43 mos. (7-218). In the moment of this analysis 14 pts (5 LF, 9 DF) were alive with NED. Follow- up of STS patients allows the diagnosis of local or distal failure in an asymptomatic phase in up to a 54% of the cases, and salvage therapy may rescue a 18% of patients. Grade of malignancy influences time to the local and distal failure.

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RITUXIMAB IN THE TREATMENT OF PATIENTS WITH RELAPSED LOW-GRADE OR FOLLICULAR NON-HODGKIN'S LYMPHOMA.

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The monoclonal antibody, Rituximab (Mabthera®) is capable of binding to the CD20 antigen of B-cell surface, inducing apoptosis, antibody and complement dependent cell mediated toxicity and sensitisation to chemotherapy of resistant/refractory lymphoma cells.

PATIENTS AND METHODS: Seven patients with mean age of 41 years (between 35 and 58) diagnosed of low-grade lymphoma or follicular lymphoma refractory to previous treatment including radiation therapy, chemotherapy (CHOP, MINE, ESHAP, NOSTE, DHAP, Fludarabine) or interferon, or relapsed after stem cell transplantation, were included in this study. Treatment consists of four 375 mg/m^2 weekly doses.

RESULTS: Clinical responses were observed in 3 patients (43%) (2 CR; 1 PR); 2 patients had stable disease (SD) and 2 were judged to have progressive disease (PD). The adverse effects were mild to moderate and reversible.

CONCLUSIONS: According to the results in this short series, Rituximab has proven a reasonably safe drug, and the response rate is similar to other reports. Nevertheless, its use in first-line therapy, its inclusion in combination chemotherapy regimes, and its effectiveness in the management of high-grade and aggressive lymphomas are questions to be answered after more ample series have been evaluated.

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PRIMARY SARCOMAS OF THE HEART AND MUTATIONAL STATUS OF K-RAS AND TP53 GENES.

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Aim: Primary heart sarcomas, the most prevalent of all cardiac malignancies, are very rare lesions. No satisfactory treatment strategies are available until the present time, and the survival of patients is short. There are very few studies concerning genetic alterations in these tumors.

Materials and Methods: We investigated the mutational status of TP53 and K-ras genes in primary cardiac sarcomas and analyzed the clinicopathologic features of the lesions. Five patients, 3 with angiosarcomas and 2 with rhabdomyosarcomas were tested for mutations at exons 5, 6, 7, and 8 of the TP53 gene and at exon 1 of K-ras. The mutational study was performed by PCR-SSCP method and direct sequencing in tumor DNA extracted from paraffin embedded tissue.Two cases of cardiac rhabdomyoma were also examined.

Results: Direct sequence analysis failed to detect point mutations in the TP53 gene in any of the 5 cases; however, at exon 1 of K-ras gene, 3 patients (60%) presented the same mutation at the first base of codon 13 (G to A transition) consisting of an amino acid substitution (Gly-13 to Ser). No changes were observed in the two rhabdomyomas. There were no significant differences in the clinicopathologic features displayed in the cases presenting the mutation except age under 50 years and sex: all three were men.

Conclusions: This surprisingly high rate of K-ras mutations in primary heart sarcomas suggests that there may be a specific pathogenetic mechanism involving ras activation in these cardiac tumors.

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ADJUVANT THERAPY FOR HIGH-RISK MELANOMA. A SINGLE INSTITUTION EXPERIENCE.

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BACKGROUND: The increasing incidence of melanomas, its high aggressivity and the importance of early diagnosis are the reasons for the increasing dedication of oncologists to some of this tumor. Ongoing studies are testing the value of adjuvant therapy with alpha-interferon (IFN). The most important randomised trial (by Kirwood, ECOG) found a significant survival benefit in the IFN arm. The experience of our institution is presented. METHODS: From 01/97 to 08/98, 16 patients(pts) with high-risk melanoma were treated with IFN following surgical resection. Eight patients with T4N0M0 received high-intermediate dose (HID) (10 MU sc, 5 days per week for 4 weeks followed by 10 MU sc 3 times per week for 48 weeks), and 8 pts with resected nodal metastases (N1-2) received high dose (HD) (20MU/m2 sc 5 days per week followed by 10 MU sc, 3 times per week for 48 weeks). Patients were followed weekly (induction phase) and later monthly (maintenance phase) with physical, serum biochemistry and full blood counts. RESULTS: In 8 patients receiving HD, predominant toxicity during induction phase was G 2-3 liver toxicity, that required IFN interruption in 75% of cases for a median of 14 days. Other toxicities included asthenia, myalgia, G 1-2 leukopenia and G 2 thrombopenia. Treatment was ceased during maintenance phase in 50% of patients due to relapse. In 8 pts receiving HID of IFN , the predominant toxicity during the induction phase was asthenia, that required interruption of IFN in 25% of cases. With median following of 15 months, 6 pts have died with disease, 2 are alive with disease and 8 are disease-free. Median time to progression is 12 months and median survival is 14 months. CONCLUSIONS: HD and HID IFN are well tolerated, and toxicities are easily manageable, but in high-risk melanoma patients, tumour progression remains the most frequent reason for treatment cessation.

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RETROSPECTIVE ANALYSIS OF OUTCOME OF KAPOSI SARCOMA (KS) HIV-PATIENTS ACCORDING TO ANTI-HIV THERAPY GIVEN.

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Protease inhibitors (PI) have improved outcome and survival of HIV-patients. This analysis was made to assess whether any difference between HIV SK-patients existed according to antiviral (AV) therapy (with or without PI).

Patients and methods.- HIV- SK patients treated between 1988 – 1997 were analysed (AV from 1988 to 1994, AV+PI from 1995 to 1997). Patients characteristics, therapy given and outcome are analysed.

Results.- Characteristics: AV(12p): male 12p; median age 34 (26-67) years; HIV-stage: C1 6p, C2 1p, C3 5p; time since HIV diagnostic: median 17m (2-60); previous opportunistic infections: median 1,5 (0-3); initial CD4: median 140 (10-334); AV drugs: median 1 (0-3) (AZT 10p, DDI 5p, DDC2p). KS was the first diagnosis in 2p; sites: skin 11p, mucoses 8p, visceral 4p, nodal 2p (median 2). AV+PI (6p): male 6p; median age 35,5 (27-58) years; HIV-stage: C1 1p, C2 1p, C3 4p; time since HIV diagnosis: median 0 months (0-36); previous opportunistic infections: median 0,5 (0-1); initial CD4: median 168,5 (77-487); AV+PI drugs: median 2 (1-4) (saknavir 4p, indinavir 5p). KS was the first diagnosis in 6p; sites: skin 6p, mucoses 4p, visceral 2p, nodal 0p (median 2).

KS therapy: AV: no treatment 3p, α-interferon (IFN) 6p, chemo (VPpo) 3p. Responses: IFN EE 1p, PD 5p; Chem PR 1p, SD 1p, PD 1p (OR 8,3%). 4p received 2nd line Chemo (VPpo 2p, doxo 1p, VPiv 1p). AV+PI: no treatment 0p, α−interferon-1p, Chemo (VP po) 5p. Responses: IFN SD 1p; VPpo CR 1p, PR 3p, SD 1p (OR 66,6%). 2p received 2nd line (VPpo 1p, liposomal doxo 1p). Survival: AV: exitus 11p (1p lost-follow-up in PD): median 4 weeks (2-84). AV+PI: exitus 0p: median not reached after 82 weeeks (48-188).

Conclusions.- PI therapy of HIV patients improved outcome of KS patients. In our series patients receiving PI showed a better performance status, a shorter time since previous HIV diagnosis and less opportunistic infections. Response rate and survival are much higher in the patients receiving AV+PI.

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MERKEL CELL CARCINOMA (MCC): DESCRIPTION OF TWO CASES WITH AXILLARY INVOLVEMENT AND UNIFORM TREATMENT.

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MCC, formerly called cutaneous trabecular carcinoma, was first described by Toker in 1972. Average age, 75 years. Very aggressive clinical evolution, with local relapse (26%-44% of cases), regional ganglionary involvement (up to 75%), and distant metastases (33%). The 5-year survival is 30%-64%. Its high sensitivity to radiotherapy has led to its systematically use in the initial treatment of the disease, after surgery. As a neuroendocrine tumour, the response rate to chemotherapy based on Cisplatin (CDDP) and Etoposide (VP16) is also considerable, to the extent that several authors recommend its systematic use, in conjunction with radiotherapy, with or without surgery, in those cases with locoregional ganglionary involvement.

We present two patients with axillary metastases, treated uniformly with the sequence of chemotherapy, surgery, and radiotherapy.

Case 1: Female aged 40. MCC at right scapular level, extirpated on 02.02.96. Negative extension study: thoracic X-ray and CT, abdominal ultrasound, and bone scan. She received local radiotherapy at a dose of 50 Gy. Disease-free interval 7 months, with right axillary metastases appeared in september 1996. Another screening study, negative for distant metastases. The patient was treated sequentially with six cicles of CDDP and VP16 chemotherapy, from october 1996 to february 1997, axillary lymph node resection on 04.11.97, and axillary radiotherapy, 50 Gy in 200 cGy fractions from may 26th to june 27th 1997. At present there is no evidence of disease, after 24 months from the axillary resection.

Case 2: Male aged 75. MCC at middle dorsolumbar level, extirpated on 06.10.97. Extension study for metastases negative. He received local radiotherapy, 45 Gy. Disease-free interval 6 months, with bilateral axillary metastases appeared in december 1997. Another screening study, negative for distant metastases. The patient was treated sequentially with six cicles of CDDP and VP16 chemotherapy, from december 1997 to may 1998, bilateral axillary lymph node resection on 06.01.98, and bilateral axillary radiotherapy 50 Gy in 200 cGy fractions from june 12th to july 27th 1998. At present there is no evidence of disease, after 10 months from the axillary resection.

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PHASE II STUDY OF DOXORUBICIN LIPOSOMIAL (CAELIX®) FOR THE TREATMENT OF ADVANCED SOFT TISSUE SARCOMA (STS) OF ADULTS PRE-TREATED WITH ONE LINE OF CHEMOTHERAPY.

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Introduction. Doroxubicin is considered, together with ifosfamide and dacarbazin (DTIC), the most active drug for the treatment of SPB. In different studies of phase I and II, the liposomal option has been proven to have a better tolerance level than the conventional treatment even when the doses were, higher, in proportion. Its activity in diverse tumours has been demonstrated, in spite the lack of experience with this drug for the treatment of STS.

Material and methods. The main objective of this study is to determine the therapeutic activity in second line treatment in patients with advanced STS in adults, with a 35 mg/m2 dose each 3 weeks. A compulsory questionnaire has been included QLQ-C30 of the EORTC (quality of life assessment), as well as, a revision of the histopathologic diagnosis. Among the usual exclusion criteria, we have also considered: more than one previous chemotherapy line, more than a previous dose of 300mg/m2 doxorubicin, FEV <50%, cardiotherapy ≥ type II of the NYHA ranking.

Results. Starting June 1998 until the writing of this study, 13 middle-aged patients have been included. 47 year-old (28-74). Histology: leiomiosarcoma 6, neurogenic 2, sinovial 2, malignant fibrohistiocitoma 2, and not classifiable 1. Previous treatments: doxorubicin 10, ifosfamide 3. Localised metastases = lung: only 2, multiple 5, with more findings 2: liver: only 2, with more findings 2, nodes 2, local relapse 1+ metastasis, intrabdominal: 1.

They are assessable for toxicity in 27 cycles. Toxicity (n° cycles/grade) Non-hematologic toxicity: nausea 3G1; constipation 2G1, stomatitis 6G1, 1G2; asthenia 8G1; anorexia 1G1, headache 2G1, cutaneous 2G1, 2G2; alopecia 1G2, pruritus 1G2, fever 3G1; hand-foot syndrome: 7G1.

Conclusions. Toxicity has been acceptable until now, without the need of modify in any treatment. The definite toxicity data and assessment results remain to be presented.

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NON-HODGKIN'S LYMPHOMA (NHL) OF MUCOSA-ASSOCIATED TISSUE (MALT) IN THE PAROTID GLAND (PG): REPORT OF THREE CASES.

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PURPOSE: NHL in the salivary glands account for 5% of the extranodal NHL, involving mainly the PG (80%). In more than 60% of the patients they belong to the low-grade MALT type. We present the clinical course, pathology, treatment and outcome in three cases.

PATIENTS AND METHODS: We show 2 male and 1 female patients (P), of 43, 62 and 64 years of age. All of them presented with a non-tender, low-growing, parotid mass. Only 1 P suffered from Sjogren's syndrome (SS). The pathologic study revealed low-grade MALT NHL in the 3 P. In 2 P no other affected locations were found after physical exam, complete serum chemistry profile, chest X-rays, toraco-abdominal CT scan and bone marrow biopsy. In the remaining P, parotid, node, stomach, lung and bone marrow NHL involvement were confirmed by biopsy.

RESULTS: Parotid superficial lobectomy was initially performed in 2 P, followed by 6 cycles of CHOP chemotherapy (C) in 1 P, and 3 cycles of CVP-type C and concomitant radiation therapy (RT) in the other. Both achieved a complete response, lasting 17 and 6 months respectively. The P with extensive involvement received parotid RT and needed 3 different salvage C regimens after sucesive relapses, finally suffering a histologic transformation into a large cell lymphoma.

CONCLUSIONS: Malignant lymphomas of the parotid gland are uncommon and often not suspected clinically. As previously described, not all three of our cases presented with an associated SS (which happens in 20% of historical series). The outcome in early stages is frequently favorable when treated locally. The indolent course, even with malignant histologic transformatión, poses a challenge regarding the choice of the best treatment regimen.

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PRIMARY CEREBRAL NHL IN IMMUNOCOMPETENT PATIENT: COMBINED TREATMENT

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Introduction: Primitive Lymphoma of the CNS appears with highest frequency among AIDS and immunocompromised patients. Among immunocompetent patients the incidence is increasing and although the prognosis is better it is still poor, with a mean survival (SV) of 1.5 – 3.3 months for untreated cases and a 3-year SV below 8%. It represents 1-2% of extranodal non-Hodgkin's Lymphoma (NHL) and generally presents in males in highly malignant histologic forms. Its treatment has changed in recent decades in an attempt to increase the global SV of affected patients.

Material and Methods: We present the case of a 63-year-old male with one week history of cephalalgia, asthenia and bradypsychia. The CT and MRI showed a right frontal expansive process suggestive of meningioma. This tumor was resected and the pathologic diagnosis was diffuse large cell B lymphoma. A wide-ranging study (thoracic-abdominal CT, bone-marrow biopsy, LDH, B2 microglobulin, RCL cytology, HIV serology and immunologic study) ruled out primitive lymphoma involvement at another level, thus the final diagnosis was primary NHL of the CNS in an immunocompetent patient. The treatment protocol was: ProMACE chemotherapy (CHT) with 1.5g/m2 Methotrexate x 3 cycles, intrathecal CHT with MTX, Ara-c and Prednisone x 5 weekly doses and holocranial RT with 40 Gy and bed tumor boost with 50 Gy.

Results: The patient is alive and disease-free. Global survival is 16 months and we are continuing the follow-up.

Conclusions: This case is a typical example of the entity that we treat, in which we initially performed surgery because it was confused with a meningioma. Despite the patient being over 60 years old we believe his SV to be optimal, attributable to the combined treatment received. It has long been difficult to define the treatment of primitive NHL of the CNS, since studied series were small and heterogeneous. Treatment was previously based on RT and corticoids, and even on surgery as sole modality. However, recent reports have identified variables with positive impact on SV such as: age < 60 years, systemic CHT treatment including high doses of Methotrexate, intrathecal CHT and holocranial RT at doses over 40Gy (in this therapeutic sequence).

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SUSTAINED REMISSION OF T CELL ANGIOCENTRIC LYMPHOMA (MIDLINE LETHAL GRANULOMA) WITH HIGH-DOSE CHEMOTHERAPY AND STEM CELL RESCUE.

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T cell angiocentric lymphoma (lethal midline granuloma) is a high-grade lymphoma associated to dire prognosis and terrible local morbidity (facial disfiguration). The outcome of patients treated with radiotherapy alone or chemotherapy followed by radiotherapy is not satisfactory. We report the case of a patient treated with multimodal therapy including high-dose chemotherapy with stem cell rescue.

A 59-year old male presented with nose bleeding. A CT scan disclosed a large mediofacial mass with destruction of the middle right turbinate. Biopsy showed T cell angiocentric lymphoma. The patient was treated with chemotherapy (2 courses of dose-escalated CHOP alternating with 2 courses of high-dose Ara-C plus methotrexate) with partial response. Immediately, he received high-dose chemotherapy (dose escalated CBV, including cyclophosphamide 7,2 g/m2; BCNU 600 mg/m2 and VP16 2,4 g/m2) with autologous peripheral blood stem cell rescue. The patient entered complete response and was then treated with local radiotherapy. He remains disease-free 37 months after diagnosis and 33 months after high-dose chemotherapy.

High-dose chemotherapy with stem cell rescue is a promising treatment modality for patients with relapsed high-grade lymphoma. The role of this aggressive therapy as first-line therapy for high-risk patients is the subject of ongoing trials. However, in patients with very poor prognosis such as those with angiocentric lymphoma failing to enter complete remission with conventional chemotherapy, administration of high-dose chemotherapy before consolidation radiotherapy is worth considering.

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METASTASIC MERKEL'S CELL CARCINOMA

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Merkel's carcinoma is an aggresive skin tumor. Although it's not strictly a small cell carcinoma it behaves similarly, with frequent relapses and poor prognosis (worse for males).

We present the case of a 46-year old man who presented with severe weight loss (18%) in the previous year, a 4.5 cm. skin tumor in the axilla and a 1.5 cm. cervical node. The skin tumor and nodal metastasis proved to be Merkel's cell carcinoma. On CT Scan, widespread metastases were found in both suprarrenal glands, measuring 8x5 and 7x5 cm.; in the right kidney with ipsilateral hiliar nodes and several metastases in the left kidney; left paraaortic nodes; a 3-cm. metastasis in the body of the pancreas; and several micronodular lung metastases.

The patient was treated with cisplatin (100 mg/m^2 iv. on day 1) and VP-16 (100 mg./ m^2 on days 1 to 3) q. 21 days. The patient is currently in partial response.

The unfavorable natural history of Merkel's cell carcinoma and available treatment options will be discussed.

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ISOLATED SKIN METASTASIS OF MAMARY ANGIOSARCOMA

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BACKGROUND: Mamary angiosarcoma an infrequent tumour (less than 200 cases reported) is probably the most aggressive breast tumour, whit 5 year survival < 10% for grade 3° tumour, and sites of metastases which are different from both peculiar breast carcinoma and soft - tissue sarcomas. A case of angiosarcoma of the breast whit cutaneous relapse is presented.

CASE REPORT: A 34-year old woman was diagnosed whit grade 3° angiosarcoma of the right breast in december/96. She was treated whit: modified radical mastectomy, axillary lymphadenectomy and immediate breast reconstruction; followed by 5 courses of adjuvant chemotherapy whit Adriamycin, and radiation therapy to the breast. Disease - free for 1 year, and them come with trombophlebitis of the right axillary vein, that resolved in 2 weeks with antiinflammatory drugs. That's when a small subcutaneous tumour measuring 8mm in diameter was felt along the axillary vein. Excisional biopsy confirmed relapse of angiosarcoma. No other metastases were found. The patient was treated with Adriamycin and Ifosfamide followed by high-dose Carboplatin, Cyclophosphamide and Etoposide with stem-cell rescue, and radiotherapy to the axilla. The patient is currently progression-free 1 year after relapse.

The natural history of the mamary angiosarcoma, the atypical pattern of sites of relapse and the treatment options will be reviewed.

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CONCOMITTANT SPLENIC PRESENTATION OF PRIMARY NON-HODGKIN'S LYMPHOMA (NHL) AND UNKNOWN PRIMARY TUMOUR (UPT). J. Buxó, J. Pérez de Olaguer, J. Vila, A. Domingo, E. Boada, and J.M. Raventós. Centro Médico Teknon. Vilana 12, 08022 Barcelona, Spain.

Splenic metastases from non-hematological tumours may be the result of direct invasion (stomach, pancreas or left kidney and suprarenal), or from hematogenous spread, in the case of disseminated neoplastic disease. Its frequency varies in the different series (1.6 to 30%), and it has been suggested that they are as common as in the liver if the relative weight of both organs is considered. Splenic metastases are usually detected at autopsy. In most of the cases they are deposits of primary tumours of the lung, breast, or malignant melanoma. Their pathological pattern varies from single nodule, multiple nodules, diffuse infiltration, to only microscopic involvement. UPT in the spleen is truly exceptional.

Primary splenic NHL is a rare localisation of extranodal cases of the disease (1% of the total). It's usually seen in patients over 60 years of age and with a female/male ratio of 2:1. Its clinical appearance is splenomegaly or non-justified hypersplenism with abnormal hemogram, and B-symptoms are infrequent. It usually appears as a splenic tumour. Pathological diagnosis and treatment are based on splenectomy. For cases of a high-risk histology, adjuvant chemotherapy could be considered, but this disease is too unusual to recommend a standard post-splenectomy treatment.

Case report: Male aged 62. Vertebral MRI with D10 (pathological fracture and medular compression), D12, L2 and S1 involvement. Previous clinical history of dorso-lumbar pain, without neurological symptomatology nor toxic syndrome, of two months of duration. The physical examination and blood test were normal. Screening study (thorax X-ray, bone scan, thoracic and abdominal CT): solid splenic nodule at parahilar level, and bone metastases. Tru-Cut biopsy of L2: bone rarefaction and osteonecrosis, not conclusive. Radiotherapy on D10, 30 Gy. A splenectomy was performed: 1. Diffuse, large cell tumour at parahilar level, cytokeratin and vimentin negative, CD45 and CD45R positive, and CD20 negative; 2. Microscopic focus of poorly differentiated large cells, negative CD45, CD45R, CD20 and vimentine, and positive cytokeratin. A second screening study (bone biopsy and ^{67}Ga scan) was negative.

Clinical diagnosis: 1. Primary splenic T-cell NHL, and 2. Splenic metastases from poorly differentiated adenocarcinoma, UPT.

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LONG-TERM SURVIVAL IN METASTATIC PARAGANGANGLIOMA (EXTRA-ADRENAL PHEOCHROMOCITOMA)

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BACKGROUND: Unlike its adrenal counterpart, extra-adrenal pheochromocitoma (paraganglioma) frequently behaves in malignat fashion. The most frequent primari site is the organ or Zuckerkandl. Even though the natural history of malignant pheochromocytoma is indolant, most patients die withm three years of the diagnosis of metastases. (Scott WM el al. Surg Gynecol Obstet 1982; 154:801). We report a patient surviving more than 10 years after diagnosis of malignant extra-adrenal pheochromocytoma with bone metastases is presented.

CASE REPORT: A 24-year old woman presented with malignant pheocromocytoma of the organ of Zuckerkandl with multiple metastases of bone scintigraphy and X- ray. She was first treated with chemotherapy (4 courses of Adriamicin+Ifosfamide alternating with 4 courses of Cisplatin+VP-16 q. 21 days) with partial response, and subequently underwant surgical resection of the primary tumor followed with 4 courses of I131- MIBG (770 Mc total dose). The patient has remaired progression-free whit residual bone images on bone scintigraphy and I131- MIBG scintigraphy.

DISCUSSION: Even though dissemined malignant pheochromocyoma is not aumently amenable to curative treatment, long-term survival symtoms is an achievable goul.

8. Support and Palliative

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RETROSPECTIVE ANALYSIS OF THE INCIDENCE AND OUTCOME OF ANAEMIA IN OUT-PATIENT TREATED WITH CHEMOTHERAPY INCLUDING PLATINUM.

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Aim.- To assess incidence of anaemia and transfusions in treated out-patients receiving platinum chemotherapy (cis or carboplatin) (PT).

Patients and Methods.- Characteristics of patients treated with PT in our Day Hospital during 1997 have been assessed. Differences in baseline parameters have been analysed according to transfusional requirements during chemotherapy.

Results.- 119 PT-patients (p) were found. Characteristics: gender: male 96 p (80,7%), female 23p (19,3%). Median age: 60 years (23-76); ECOG 0 37p (31,1%), 1 68p (57,2%), 2 11p (9,2%), 3 3p (2,5%). Primary: lung 96p (80,7%), uro-gynecological 13p (10,9%), digestive 6p (5,0 %), other 7p (5,9%). Histology: squamous 42p (35,5%), adenoca. 52p (43,7%), small cell 13p (10,9%), other 12p (9,4%). Chemotherapy: cisplatin 116p (97,5%). Two-drug schemes 66p (55,5%), 3 or more 53p (44,5%). Number of courses given: median 4c (1-7). Previous RT 10 p (8,4%), concomitant RT 10 p (8,4%). Anaemia (mean): Hb 12,7mg/L (8,7-17,5), Hcte 38,9% (25,1-49,2), leukocytes 8.290/mm3 (1.800-30.800), platelets 310.000/mm3 (78.000-974.000); VCM 89,7fl (76,4-102,8). Response to chemotherapy: objective response 35p (29,4%), stable disease 31p (26,1%), progressive disease 53p (44,5%).

Anaemia: mean per course: Hb c1 11,7, c2 11,4, c3 11,4, c4 10,9, c5 10,6; VCM c1 89,6, c2 89,6, c3 92,3, c4 94,1, c5 94,6. Hb<10,5 baseline 11/121 (9,0p) c3 29/85 (23,9%), c5 17/41 (14,1%). Transfusions were given to 28 p (23,5%) (23p 1t, 5p >1t): median 2 Units per patient.

Differences between patients given and not given transfusions: mean dose of CDDP (p =.04), baseline Hb (p<.001) and previous chemo or radiotherapy (p= .04, p=.02) were statistically different. Sqm CDDP and dose-intensity were higher in patients requiring transfusions but without statistical differences. Age, baseline Hcte, ECOG, stage, histology or drugs given were similar between groups.

Conclusions.- Anaemia is a frequent complication in oncology out-patients. It worsens as the number of courses given increases and an important percentage of patients eventually require transfusions. Some baseline characteristics may help to identify initially patients most probable to precise transfusions, according to previous therapy, CDDP dose and baseline HB.

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SMALL-CELL LUNG CANCER IN THE ELDERLY: IS AGE OF PATIENT A RELEVANT FACTOR?

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INTRODUCTION For small-cell lung cancer (SCLC) a different management for diagnosis and treatment in the elderly have been reported. It seems that the diagnostic work-up and treatment may be less intensive in that subgroup of patients, with negative discrimination against inclusion in cancer protocols and lesser overall survival.

PATIENTS AND METHODS. The patients' age was categorized in two groups: under 70 years and 70 years or more and a comparison for treatment variables, toxicities, response and time to event measures has been performed. Information on clinical parameters such as weight loss, co-morbidity, performance status and investigative procedures for staging of disease and inclusion in clinical trials were recorded for patients in the province of Albacete (Spain).

RESULTS. There were 123 (69/54, <70/ 70 years) cases diagnosed. Ninety five patients were referred for treatment to our Unit. Of these, 86%% (36/54) patients were aged under 70 years and 67% (36/54) were in the older age category. Clinical variables and staging procedures did not differ between groups. Trial assignment showed a bias in favour of younger patients (11 vs 1, P=.02). No differences in the number of patients without treatment were found, but the older group presented less cases of optimal (4 cycles) therapy, less chemotherapy delivery (smaller mean total doses of cisplatin and etoposide) and smaller mean total dose of radiotherapy (57/45 Gy). The response to treatment (46%/50%), toxicity G3-4 registered and overall survival (33/19 weeks) did not differ between age categories.

CONCLUSION. Elderly patients receive less agressive treatment, but Response Rate is not to be different. The bias for inclusion of elderly patients on Clinical Trials should be modified. Age seems not to be a relevant prognostic factor in this disease. Carefully calculated dose reductions for chemotherapy in elderly patients based on initial performance status and/or toxicity during treatment may be a useful policy without detrimental implications on the outcome.

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SUBCUTANEOUS INFUSION IN THE FINAL PHASE OF CANCER DISEASE.

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INTRODUCTION The final phase of cancer disease is plurisymptomatic (median of 9.3 symptoms in the first visit), being the pain one of the more frequent symptom (about 80-90% of patients) during the evolution of the illness. Moreover, in the last week of life the patients suffer for dysphagia (60%) , disturbances of level of consciousness (22%) dyspnea (16%) and others symptoms that make impossible the use of the oral route . In domiciliary palliative care the subcutaneous infusion is elective since we can achieve continual plasmatic levels of the drugs with a technique that is generally free of complications and that we can use in ambulatory patients.

MATERIAL AND METHODS: In Sanitary Area 11 in Madrid, there is a co-ordinated palliative program of attention to terminal oncologic patients. In this work we analyse 1150 terminal cancer patients who were attended in this program until death. The median age was 66.8 years (range 13-94) ,being the distribution by sex men 61% and woman 39%. The more frequent tumoral sites were digestive (284) and lung (245). A total of 619 patients (54%) required subcutaneous medication and 79.6% required morphine in the evolution of their illness. We use this technique to administer analgesics antiemetics and anxiolytic sedatives. We use devices lightweight and portable ,and in one case disposable with a life of 24 hours or 5 days, with a fluid less than 2 ml/hour because is the maximum dose absorbed by the subcutaneous tissue . The pain symptom is specifically analysed in function of the intensity perceived by the patient (VAS)

RESULTS: The total of devices employed were 1261. The median of devices per patient was 1.1 (range 0-40). The median time of use of subcutaneous infusion were 9 days per patient (range between hours until 102 days).The reasons to use the subcutaneous route were dysphagia (60%) dyspnea (18%) , vomiting (8) pain (5%) intestinal obstruction (3%) haemorrhage (3%), agitated delirium of the final phase (3%). The median survival was 52 days. In this work we analyse the median doses of the drugs employed.

CONCLUSIONS: We conclude that subcutaneous infusion is the first choice when parenteral analgesia is required in palliative care patients. More that the 50% of our patients need the subcutaneous infusion during the evolution of their illness with a good symptomatic control (85% of the patients died in complete absence of pain) . The technique is generally free of complications and very useful in the treatment of ambulatory patients.

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DYING FOR BREAST CANCER

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Breast cancer is one of the most isert causes of dying for women in the developed countries. Its incidence in Spain range between 40 and 55/100.000 habitants and is the 20% of total dyby cancer, in female population.

Inside the integral attention it is nessry to cover and pay attention to the final stage of this disease in its biopsyoial aspect. In this study we present the most frequent symptoms and its col in a group of patients dead by this disease.

MATERIAL AND METHOD

In Sanitary Area 11 in Madrid, this a co-ordinated palliative program of attention to terminal oncologic pati Since November 1992, 1140 patients have been attended until death. 104 v diagnosed of breast cancer and they are the base of the study. All of them ireceived at least two visits in order to evaluate the response.

RESULTS

Medium age was 65,5 years ol 8-94). Women:103, men:1. All had demonstrated hystologic diagnosis, metastatic localizations were: osseous 49, hepatic 28, lung 26, NSC 17, gangry 16 and others 18. Median survival in palliative program is 93 days instee of total patients.

Media of symptoms in 1st visit is 7, 2nd 4 and in the last one is 3,7. The most frequents in physical sphere are mouth 79%, pain 77%, anorexia 71%, asthenia 67%, constipation 61%. he psychological sphere the sume of sadness, distress, anxiety and depion reach the 60% of frequency in appearing.

The pain symptom is specifically anay in function of the intensity perceived by the patient (VAS) and in fum of its origen (visceral, osseous, neuropathic). Initial medium VAS is ,nd at the final 3,6. Type of pains with higher initial VAS are visceral 6,4 arluropathic with 7,1. In order to control the pain, drugs recommended by OMS its analgesic scale are used with a sequential method in the analgesia. M of the visits/affected patient of breast cancer is 13 instead of 9 of the total. Visits to psychologists have been done. 77% (80) die at home.

CONCLUSIONS

- The final stage of patients with bt cancer is long and with a lot of symptoms.
- There is an affectation in physic asychological plane that needs to be evaluated and treated.
- It is necessary to do active and cmed palliative care to improve, as possible, theur general situation.

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DEATH AT HOME: HOME-BASED PALLIATIVE CARE OF PATIENTS DYING OF CANCER

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INTRODUCTION: The aim of palliative care is achievement of the best possible quality of life for patients and their families. We try to demostrate that the quality of terminal care in the home and the possibility of a home death depend to a great extent upon the care provided by the home-base palliative care units.

METHODS: This paper reports on our experience of caring for dying people at home for a period of time between december 1993 and december 1997.

RESULTS: We retrospectively reviewed 338 consecutive admissions in our unit. 55.2% men and 44.8% women whose ages ranged from 5 years to 93 years; median age was 67.2. Median length of stay was 13.9 days (range 1-300).

The location of the primary tumor was: lung, 92 patients, colo-rectum, 39 patients, breast, 37, liver, 25 patients, stomach, 24, lymphomas and leukemias, 21 patients, pancreas, 16, prostate, 14, bladder, 14 patients, ovary, 10, esophagus, 7 patients, and others, 39 patients.

In our study, it was found that 332 patients (98.2%) died in their own home. Hospital attendance was required in 6 cases, 2 of them due to medical complications (neumothorax and severe hematuria), one patient due to inadecuate familiar support and 3 patients had expressed a preference for not to dye at home.

CONCLUSION: 98.2% of the terminally ill patients assisted in our unit died at home. In our experience, the majority of terminal care occurs in people's own homes and many dying people and their careerswould prefer the death itself to occur in the home. With an adequate medical support is possible the achievement of a home death for all who desire it.

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Treatment of malignant superior vena cava syndrome(SVCS) by endovascular stent insertion. C. Falo, R. Mesia, J. Dominguez*, A. Fernández, E. Escalante*, A. Montes, C. Sancho*, A. Arance, X. Pérez, F. Cardenal. Medical Oncology Departement. *Vascular Radiology Department. Institut Català d'Oncologia.CSUB..Barcelona.

We report our experience in the management of malignant SVCS with percutaneous endovascular self-expanding stent insertion. **Patients and methods:** Between 8/93 and 12/97 thirty one patients (pts), 26 men and 5 women, median (m) age 58 (29-75), m Karnofsky score index 60 (40-80), with malignancy (9 small cell lung cancer (SCLC), 21 non small cell lung cancer (NSCLC), 1 oesophageal cancer), complicated with SVCS were treated with endovascular stent insertion, prior radiologic confirmation.Eighteen pts had undergone previous treatment for their neoplasm, whereas in 13 (11 NSCLC, 2 SCLC) the stent insertion was the first therapeutic procedure. **Results:** A median of 1(1-2) stent were placed (33 Wallstent, 3 Memotherm), m diameter 12 (9-14) mm, achieving repermeabilization of stenosis in all patients. The relief of dyspnea and edema was successful in 28 (90%): complete in 14 pts and partial in 14 pts, most of them in less than 72 hours. Improvement of SVCS by stent insertion allowed 9/11 pts with NSCLC to receive full doses of chemotherapy with appropiate overhydration. Safety: inmediate complications: one death from the procedure, one asymptomatic migration of the stent, non fatal cardiac arrithmia and sepsis in 2 pts. Late complicacions secondary to anticoagulation in 3 pts (1 fatal pulmonary haemorrhage). There were 7 (23%) reobstructions, 4 of them could be resolved. The m survival from stent insertion was 4 (0-20) months. **Conclusions:** Stent insertion in pts with malignant SVCS achieves inmediate relief of symptoms related to obstruction. In selectes pts, this approach allows early initiation of anticancer therapy that would not have been possible with active SVCS. Complications of the procedurte were acceptable, considering the fragile condition of these type of pts.

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PREDICTIVE FACTORS FOR ANTIBIOTIC ADDITION IN PATIENTS (PTS) WITH FEBRILE NEUTROPENIA (FN) AFTER CHEMOTHERAPY (CT) FOR SOLID TUMORS AND TREATED WITH PIPERACILIN+TAZOBACTAM (P+T) MONOTHERAPY

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Introduction: In 30% of pts treated with antibiotic monotherapy because of FN, a second antibiotic needs to be added because of persitent fever. Predictive factors for a second antibiotic addition are studied.

Patients and methods: From March-96 to April-98, 152 FN episodes have been treated with P+T monotherapy (4/0.5 gr every 6 hours IV). Treatment was instituted in patients with fever > 38.5 ºC or >38ºC longer than 6 hours and neutrophils < 1000/mm3. Amikacin was added if persistent fever after 48h evolution. Predictive factors studies were: age, sex, days from CT to fever, leucocites, neutrophils and platelets initial numbers, response to treatment, central device, mucositis presence and intrahospitalary infection.

Results: Median age: 54.3 years (range 17-75). Females 113 (74.3%). Median days from CT to fever: 11.3 (3-23). Median number the day of fever: leucocites 940 /mm3 (0-3100), neutrophils 253 /mm3 (0-1000), platelets 131000 (7000-470000). Central device: 22 pts (14.5%); intrahospitalary infection: 24 pts (15.8%); No response to CT 67pts (44.1%); Mucositis 21pts (13.8%). Amikacina was added after 48h in 45 pts (29.6%). Univariate analysis showed significative predictive factors: number of neutrophils (p=0.002), number of leucocites (p=0.006), days from CT to fever (0.09). Multivariate analysis (logistic regression) showed as independent predictive factor the number of neutrophils (p=0.0006). 18.6% of pts wiht more than 500/ mm3 neutrophils versus 46.7% of those with less than 500/mm3, needed a second antibiotic.

Conclussion: Number of neutrophils when a FN is instaurated is a independent predictive factor for amikacine to be added to monotherapy with P+T in pts with FN after CT for solid tumors.

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ANALITICAL PRONOSTIC FACTORS IN PATIENTS WITH PROGRESIVE MALIGNANT DISSEASE (PMD).

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Objectives: In the group if patients (pts) with PMD symtoms and quality of live have been considered the main prognostic factors. Analitical data had been studied very few. The aim of our study is to identifie analitical variables that give a prediction to survival in PMD pts.

Patients and methods: 316 PMD pts were studied to achieve a possible relation between 22 analitical data (hemogram, liver and renal parameters, albumine, total proteins, LDH, Na, K etc.) and survival. We used actuarial methods to do survival curves and Log-rank test and Breslow test to compare theirs. Afterward multiple regression analysis was made by Cox tecnical step by step.

Results: Median survival was 26 days: 295 died and 21 alive. Uniovariable analysis shown an association between less survival and: hipoalbuminemia ($p<0.001$), hipocolesterolemia ($p<0.05$), lymphopenia ($p<0.01$), anemia ($p<0.01$), increase of LDH ($p<0.01$), increase of Phosphatse Alkaline($p<0.01$) and increase of GGT ($p<0.05$). Multiple regression analysis only identified albumine, colesterol, PA and LDH as independent variables with predictive value over survival in PMD patients.

Conclusions: Determination of albumine, colesterol, PA and LDH will be helpfull to give a pronostic more distinct in PMD patients.

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HIGH DOSE CHEMOTHERAPY IN BREAST CANCER WITH PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) SUPPORT: TOXICITY OF TWO DIFFERENT CHEMOTHERAPY SCHEDULES.

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OBJETIVE: Analysis of toxicity in breast cancer patients treated with two different high dose chemotherpy schedules.

PATIENTS AND METHODS:

Schedule A : Carboplatin 200 mg/ m^2 / day + Thiotepa 125 mg / m^2 / day + Cyclophosphamide 1500 mg / m^2 / day in continuous infusion, days –7, -6 –5 and –4.

Schedule B : Cyclophosphamide 1800 mg/m^2 / day (-6, -5 y –4) in 1 hour infusion + Cisplatin 55 mg / m^2 / day (-6, -5 y –4) in continuous infusion+ BCNU 600 mg/ m^2 / day (-3) in 2 hours following cisplatin.

PBPC mobilization was performed with filgrastim at a dose of 5 mcg / kg / day at least for 4 days following hematologic recovery from the last cycle of induction chemotherapy. PBPC were reinfused at day 0.

	SCHEDULE A	SCHEDULE B
10 or more axiliary nodes	50	6
Stage III	19	2
Stage IV	11	12

RESULTS :

Patients with neutropenic fever		60(75%)	17 (85%)
		Median - range	Median - range
Days with neutropenic fever		3 (1-11)	2 (1- 17)
Days with neutrophil count<0.5x10^9/l		9 (6-14)	8 (6-15)
>0.5x10^9/l neutrophil count day		+10 (7-12)	+10 (8-16)
Days with platelet count < 20x10^9/l		8 (2-18)	12 (5-18)
> 20x10^9/l platelet count day		+10 (5–15)	+13 (7-18)
Mucositis	Grade 1-2	54 (67%)	18 (80%)
	Grade 3	9 (11%)	0 (0%)
Emesis	Grade 1-2	18 (22%)	5 (25%)
	Grade 3	16 (20%)	6 (30%)
	Grade 4	1 (1.2%)	1 (5%)
Diarrhea	Grade 1-2	23 (28%)	3 (15%)
	Grade 3	17 (21%)	1 (5%)
Haematuria	Grade 1-2	9 (11%)	3 (15%)
	Grade 3	1 (1.2%)	0 (0%)
Cardiotoxicity	Grade 1-2	1 (1.2%)	2 (10%)

a death occurred in schedule A .

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ANALYSIS OF THE INFECTIOUS EVENTS DURING HIGH-DOSE CHEMOTHERAPY (HDC) AND PERIPHERAL BLOOD STEM CELL (PBSC) AUTOLOGOUS TRANSPLANTATION.

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Aim: To analyse the infectious complications associated to administration of HDC and PBSC autologous transplantation .

Methods: Prospective study that includes 206 patients (p) of 219p with solid tumors and lymphomas (13p not included by ex-vivo manipulation) treated with HDC and subsequent infusion of 7,49 (2,3-44,4) x 10^6 CD34/Kg, with G-CSF 5mg/kg in 196p. All p received oral antimicrobial prophylaxis with acyclovir 200 mg/6h, 157p itraconazole 200 mg/12h, 178p ciprofloxacin 500mg/8-12h and 29p ciprofloxacin 500mg/8h + rifampicine 300mg/12h, and stayed in single rooms with HEPA.

Febrile neutropenic patients were initially treated with empirical i.v. imipenem; vancomycin, amikacin and amphotericin B lipid complex was added if unexplained fever persisted during 2, 5 and 7-8 days (d) respectively.

Results: The median (M) recovery days to 0.5 x 10^9 neutrophils/L was 9 (7-30)d and to 20 x 10^9 platelets/L was 11 (7-42)d. Severe neutropenia (<0.1 x 109/L) lasted for a median of 5 (4-27)d, from -d+2 to +6-, in which 65% (147/226) of febrile episodes (FE) and 49% (32/65) of cases of bacteriemia occurred. M red cell and platelet transfusions were 2 (0-8) and 3 (1-34). M hospitalization days from d-0 was 13 (9-64). M antibiotics used was 2 (0-6) during a M of 6 (0-33)d and amphotericin B was used in only 7p (3%). 189p (91%) developed fever with a M duration of 2 (0-16)d. 226 FE were detected, 138 (61%) were unexplained fever (UF), 5 (2%) were microbiologicaly documented (MDF), 23 (10%) clinically documented (CDF) and 60 (26%) clinically and microbiologicaly documented (CMF). 24 FE were observed in the preneutropenic period, 18 (75%) of which were catheter-related. In the neutropenic period 179 (79%) FE were detected: 123 (68%) UF and 38 (21%) CMF. In the recovery period, 23 FE ocurred, 9(39%) of which were CDF (5 pneumonia). Overall, 65 bacteriemia episodes were observed: 46 cocci gram (+) (40 S.epidermidis) and 13 non fermenting BGN. Among clinical foci 55 (67%) catheter, 14 pneumonia, 2 lung invasive aspergillosis and 1 neutropenic enterocolitis, were observed. 3 (1,5%) p died during the procedure.

Conclusions: Most of the patients develop fever during the period of severe neutropenia. The FE documentation depends on the period of HDC/PBSC: in preneutropenic p most of the FE are catheter-related, in neutropenia are UF and in the recovery period attention must be paid to CDF without microbiologic detection. The most frequent bacteremia are secondary to cocci gram+ of catheter. The rapid hematologic recovery favors the resolution of the FE and the low morbi - mortality of the procedure.

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Introduction: In 30% of pts treated with antibiotic monotherapy because of FN, a second antibiotic needs to be added because of persitent fever. Predictive factors for a second antibiotic addition are studied.

Patients and methods: From March-96 to April-98, 152 FN episodes have been treated with P+T monotherapy (4/0.5 gr every 6 hours IV). Treatment was instituted in patients with fever > 38.5 ºC or >38ºC longer than 6 hours and neutrophils < 1000/mm3. Amikacin was added if persistent fever after 48h evolution. Predictive factors studies were: age, sex, days from CT to fever, leucocites, neutrophils and platelets initial numbers, response to treatment, central device, mucositis presence and intrahospitalary infection.

Results: Median age: 54.3 years (range 17-75). Females 113 (74.3%). Median days from CT to fever: 11.3 (3-23). Median number the day of fever: leucocites 940 /mm3 (0-3100), neutrophils 253 /mm3 (0-1000), platelets 131000 (7000-470000). Central device: 22 pts (14.5%); intrahospitalary infection: 24 pts (15.8%); No response to CT 67pts (44.1%); Mucositis 21pts (13.8%). Amikacina was added after 48h in 45 pts (29.6%). Univariate analysis showed significative predictive factors: number of neutrophils (p=0.002), number of leucocites (p=0.006), days from CT to fever (0.09). Multivariate analysis (logistic regression) showed as independent predictive factor the number of neutrophils (p=0.0006). 18.6% of pts wiht more than 500/ mm3 neutrophils versus 46.7% of those with less than 500/mm3, needed a second antibiotic.

Conclussion: Number of neutrophils when a FN is instaurated is a independent predictive factor for amikacine to be added to monotherapy with P+T in pts with FN after CT for solid tumors.

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ANALITICAL PRONOSTIC FACTORS IN PATIENTS WITH PROGRESIVE MALIGNANT DISSEASE (PMD).

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Objectives: In the group if patients (pts) with PMD symtoms and quality of live have been considered the main prognostic factors. Analitical data had been studied very few. The aim of our study is to identifie analitical variables that give a prediction to survival in PMD pts.

Patients and methods: 316 PMD pts were studied to achieve a possible relation between 22 analitical data (hemogram, liver and renal parameters, albumine, total proteins, LDH, Na, K etc.) and survival. We used actuarial methods to do survival curves and Log-rank test and Breslow test to compare theirs. Afterward multiple regression analysis was made by Cox tecnical step by step.

Results: Median survival was 26 days: 295 died and 21 alive. Uniovariable analysis shown an association between less survival and: hipoalbuminemia ($p<0.001$), hipocolesterolemia ($p<0.05$), lymphopenia ($p<0.01$), anemia ($p<0.01$), increase of LDH ($p<0.01$), increase of Phosphatse Alkaline($p<0.01$) and increase of GGT ($p<0.05$). Multiple regression analysis only identified albumine, colesterol, PA and LDH as independent variables with predictive value over survival in PMD patients.

Conclusions: Determination of albumine, colesterol, PA and LDH will be helpfull to give a pronostic more distinct in PMD patients.

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HIGH DOSE CHEMOTHERAPY IN BREAST CANCER WITH PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) SUPPORT: TOXICITY OF TWO DIFFERENT CHEMOTHERAPY SCHEDULES.

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OBJETIVE: Analysis of toxicity in breast cancer patients treated with two different high dose chemotherpy schedules.

PATIENTS AND METHODS:

Schedule A : Carboplatin 200 mg/ m^2 / day + Thiotepa 125 mg / m^2 / day + Cyclophosphamide 1500 mg / m^2 / day in continuous infusion, days –7, -6 –5 and –4.

Schedule B : Cyclophosphamide 1800 mg/m^2 / day (-6, -5 y –4) in 1 hour infusion + Cisplatin 55 mg / m^2 / day (-6, -5 y –4) in continuous infusion+ BCNU 600 mg/ m^2 / day (-3) in 2 hours following cisplatin.

PBPC mobilization was performed with filgrastim at a dose of 5 mcg / kg / day at least for 4 days following hematologic recovery from the last cycle of induction chemotherapy. PBPC were reinfused at day 0.

	SCHEDULE A	SCHEDULE B
10 or more axiliary nodes	50	6
Stage III	19	2
Stage IV	11	12

RESULTS :

Patients with neutropenic fever		60(75%)	17 (85%)
		Median - range	Median - range
Days with neutropenic fever		3 (1-11)	2 (1- 17)
Days with neutrophil count<0.5x10^9/l		9 (6-14)	8 (6-15)
>0.5x10^9/l neutrophil count day		+10 (7-12)	+10 (8-16)
Days with platelet count < 20x10^9/l		8 (2-18)	12 (5-18)
> 20x10^9/l platelet count day		+10 (5–15)	+13 (7-18)
Mucositis	Grade 1-2	54 (67%)	18 (80%)
	Grade 3	9 (11%)	0 (0%)
Emesis	Grade 1-2	18 (22%)	5 (25%)
	Grade 3	16 (20%)	6 (30%)
	Grade 4	1 (1.2%)	1 (5%)
Diarrhea	Grade 1-2	23 (28%)	3 (15%)
	Grade 3	17 (21%)	1 (5%)
Haematuria	Grade 1-2	9 (11%)	3 (15%)
	Grade 3	1 (1.2%)	0 (0%)
Cardiotoxicity	Grade 1-2	1 (1.2%)	2 (10%)

a death occurred in schedule A .

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ANALYSIS OF THE INFECTIOUS EVENTS DURING HIGH-DOSE CHEMOTHERAPY (HDC) AND PERIPHERAL BLOOD STEM CELL (PBSC) AUTOLOGOUS TRANSPLANTATION.

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Aim: To analyse the infectious complications associated to administration of HDC and PBSC autologous transplantation .

Methods: Prospective study that includes 206 patients (p) of 219p with solid tumors and lymphomas (13p not included by ex-vivo manipulation) treated with HDC and subsequent infusion of 7,49 (2,3-44,4) x 10^6 CD34/Kg, with G-CSF 5mg/kg in 196p. All p received oral antimicrobial prophylaxis with acyclovir 200 mg/6h, 157p itraconazole 200 mg/12h, 178p ciprofloxacin 500mg/8-12h and 29p ciprofloxacin 500mg/8h + rifampicine 300mg/12h, and stayed in single rooms with HEPA.

Febrile neutropenic patients were initially treated with empirical i.v. imipenem; vancomycin, amikacin and amphotericin B lipid complex was added if unexplained fever persisted during 2, 5 and 7-8 days (d) respectively.

Results: The median (M) recovery days to 0.5 x 10^9 neutrophils/L was 9 (7-30)d and to 20 x 10^9 platelets/L was 11 (7-42)d. Severe neutropenia (<0.1 x 109/L) lasted for a median of 5 (4-27)d, from -d+2 to +6-, in which 65% (147/226) of febrile episodes (FE) and 49% (32/65) of cases of bacteriemia occurred. M red cell and platelet transfusions were 2 (0-8) and 3 (1-34). M hospitalization days from d-0 was 13 (9-64). M antibiotics used was 2 (0-6) during a M of 6 (0-33)d and amphotericin B was used in only 7p (3%). 189p (91%) developed fever with a M duration of 2 (0-16)d. 226 FE were detected, 138 (61%) were unexplained fever (UF), 5 (2%) were microbiologicaly documented (MDF), 23 (10%) clinically documented (CDF) and 60 (26%) clinically and microbiologicaly documented (CMF). 24 FE were observed in the preneutropenic period, 18 (75%) of which were catheter-related. In the neutropenic period 179 (79%) FE were detected: 123 (68%) UF and 38 (21%) CMF. In the recovery period, 23 FE ocurred, 9(39%) of which were CDF (5 pneumonia). Overall, 65 bacteriemia episodes were observed: 46 cocci gram (+) (40 S.epidermidis) and 13 non fermenting BGN. Among clinical foci 55 (67%) catheter, 14 pneumonia, 2 lung invasive aspergillosis and 1 neutropenic enterocolitis, were observed. 3 (1,5%) p died during the procedure.

Conclusions: Most of the patients develop fever during the period of severe neutropenia. The FE documentation depends on the period of HDC/PBSC: in preneutropenic p most of the FE are catheter-related, in neutropenia are UF and in the recovery period attention must be paid to CDF without microbiologic detection. The most frequent bacteremia are secondary to cocci gram+ of catheter. The rapid hematologic recovery favors the resolution of the FE and the low morbi - mortality of the procedure.

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COMPARISON OF TWO ANTIEMETIC COMBINATION TREATMENTS IN BREAST CANCER PATIENTS UNDERGOING HIGH-DOSE CHEMOTHERAPY (HDC) WITH PERIPHERAL BLOOD PROGENITOR CELLS TRASPLANT (PBPCT).

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Introduction: High dose chemotherapy is highly emetogenic. Combination of antiserotoninergics with antidopaminergic drugs have been scarcely investigated patients treated with HDC.

Patients and methods: Emetic episodes have been analyzed in 67 consecutive breast cancer patients (pts)undergoing HDC treatment followed by PBPCT and treated with two different antiemetic combinations. Chemotherapy treatment consisted in ciclophosphamide 6gr/m2, carboplatin 800 mg/m2 and thioteps 500 mg/m2 in 4 day continuous infusion. (CTCb). Two antiemetic combinations have been tested consecutively. From November-95 to December-96 30 pts were treated with granisetron 3 mg bid I.V, dexametasone 12 mg I.V., loracepam 1mg p.o. and omeprazol 20 mg p.o. once daily, and haloperidol 0,5 mg bid p.o.(group H). From January-97 to October-98 haloperidol was sustituted by clorpromazine 12,5 mg tid p.o. (group C).

Nausea and emetic episodes were evaluated every 6h from the first day of treatment up to the 8th day after (peripheral porgenitor cells infusion day). Antiemetic response was evaluated as complete response (CR) if no emetic episodes were present, major response (MR) if patients had 1 or 2 episodes, minor response (mR) if had 3 or 4 episodes, and no response (NR) if more than 4 episodes were present.

Results: 67 patients were evaluated, 30 in group H and 37 in group C. Three (10%)pts in group H and 4 (14,3%) in group C presented CR for the whole 4 days treatment period.(p=N.S.) Responses are showed in the table:

	CR (%)	MR (%)	mR (%)	Failure (%)
Group H	2 (6.7)	0 (0)	2 (6.7)	26 (86.7)
Group C	3 (8.1)	4 (10.8)	1 (2.7)	29 (78.4)

No statistical significative differences were found between both groups, neither everyday nor in whole treatment period response. Nevertheles the number of patients with less than two emetic episodes is slighty higher in group C (19% vs 6.7%).

Vomiting is an important problem in patients undergoing high-dose chemotherapy treatment, even with heavy antiemetic treatment. Both antiemetic combinations show similar results.

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ADVERSE EFFECTS DURING INFUSION OF CRYOPRESERVED PERIPHERAL BLOOD STEM CELLS FOLLOWING HIGH-DOSE CHEMOTHERAPY.

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Background: Infusion of cryopreserved peripheral blood stem cells (PBSC) is frequently associated to adverse effects, related to dimethylsulfoxide (DMSO). **Aims:** To evaluate the frequency and severity of adverse effects associated to PBSC infusion and possible risk factors. **Patients and Methods:** From November 1994 to June 1997, 93 courses of high-dose chemotherapy with PBSC infusion were given to patients (pts) with breast cancer (51), non-Hodgkin's lymphoma (15), multiple myeloma (6), acute leukemia/mielodysplasia (5), ovarian cancer (4), Hodgkin disease (3), germ cell tumors (3), small cell lung cancer (SCLC) (2), others (2). PBSCs were cryopreserved in polyolefin bags with 10% DMSO as cryoprotectant and frozen at -80ºC in a methanol bath. Pts were premedicated with furosemide, ondansetron, dexclorfeniramine and 6-methylprednisolone before PBSC infusion. Right after defreezing in a 37ºC water bath, PBSCs were infused through a central venous line with serial measurement of vital signs. **Results:** Median number of bags infused was 3 (range 1-13). Median volume infused was 374 ml (100-1602). PBSCs were infused in 1 day (90 pts) or in 2 (3). Forty-five pts (48%) had adverse effects, including unpleasant taste (18%), pharingeal itching (16%), nausea/vomiting (16%), abdominal pain (15%), mild hemoglobinuria (15%), dry cough (8%), chills (7%), precordial opression (7%), flushes (5%) and vagal reaction (2%). One pt with SCLC and chronic bronchitis had a respiratory arrest secondary to vagal reaction. He was resuscitated but died in ICU 10 days later. All other pts required no medication. No pt receiving <200 ml had any adverse reaction(p<.001,2-tail).**Conclusions:** PBSC infusion was associated to adverse reactions in 48% of pts. Most were mild and did not require therapy. The volume infused was directly correlated with frequency of adverse effects.

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COPING STYLE: A PREDICTIVE VARIABLE OF THE FEARS RATE IN THE FAMILY OF CANCER PATIENT.

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JUSTIFICATION: We hipotetize a relationship between fears prevalence and mechanisms of coping in cancer patient's family. The aim of this study is to assess the prognostic factors of fears rate.

MATERIAL AND METHOD: Sample: 29 families of cancer patients were interviewed using the Mini Mental Adjustment to Cancer Scale (MINIMAC) and Fears Scale.
Analysis: Multiple Regression models were developed and applied to test independent variables that affected outcome.
Statistical significance was set at the 5% level.

RESULTS AND CONCLUSIONS: Coping style was a prognostic variable to fears rate of cancer patients' family. Ways of coping that increase fears rate are: Depression (ß= 761261), Fatalism (ß= 624711) and Anxious Preoccupation (ß= 157144). Ways of coping that reduce fears rate are: Fighting Spirit (ß= -203572) and Cognitive Avoidance (ß= -169151).

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A RANDOMIZED TRIAL ON THE HEMATOLOGICAL AND EXTRAHEMATOLOGICAL EFFECTS OF ADDING GM-CSF TO POSTRANSPLANT G-CSF AFTER HIGH-DOSE CHEMOTHERAPY WITH STEM CELL RESCUE.

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Background: Neutropenia after high-dose chemotherapy (HD-Cht) with stem cell rescue is significantly shorter with peripheral blood stem cell (PBSC) infusion than with bone marrow. However, neutrophil (ANC) recovery takes around 8 days(d) and the use of early postransplant granulocyte colony-stimulating factor (G-CSF) does not shorten this period significantly. Preclinical data show synergy between granulocyte-macrophage CSF (GM-CSF) and G-CSF when given sequentially. GM-CSF might also ameliorate Cht-induced mucositis. **Aims:** To test if adding early postransplant GM-CSF to late G-CSF results in earlier recovery of ANC, monocyte, platelet(PLT) and reticulocyte counts than G-CSF alone and it ameliorates Cht-induced mucositis, bleeding, need for total parenteral nutrition (TPN) or other complications. **Patients (p) and Methods:** From 11/95 to 11/97, 72 p with solid tumors (breast ca 52 p,non-Hodgkin's lymphoma 11 p, others 9 p) treated with HD-Cht+PBSC were randomized to GM-CSF 5 mcg/Kg/d s.c. d +3 to +6 followed by G-CSF 5 mcg/Kg/d s.c. d +7 to ANC >1000 *vs* G-CSF 5 mcg/Kg/d s.c. d +7 to ANC >1000. Daily complete blood counts and toxicity assessments were performed. **Results:** Thirty-six p were treated with GM-CSF+G-CSF and 36 with G-CSF. P characteristics were well balanced between both arms. Median time from PBSC infusion to ANC>100 was 7 d for p on GM-CSF+G-CSF vs 8 d with G-CSF (2-tailed-p<.1, logrank). Number of p taking >10 d to reach ANC>500 was 4/36 (11%) with GM-CSF+G-CSF vs 10/36(27%) with G-CSF(p<.05). There were also marginally significant differences in monocyte recovery, number of PLT transfusions, grade 3-4 mucositis and time with TPN favoring GM-CSF+G-CSF. **Conclusions:** Sequential GM-CSF+G-CSF after HD-Cht+PBSC results in marginally significant improvements in ANC recovery and mucositis over G-CSF.

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Anemia in cancer patients in one year period.

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Resumen

Purpose : to make a descriptive study and to find out the relation between the anaemia in cancer patients and the different factors it depends on such as the age, sex, treatment, types of tumor and its widespread. .

Pacientes y métodos : 413 patients diagnosted in our Medical Oncology departament of Hospital La Paz during one year (Marzo 96- Marzo 97) were analyzed. Serious anemia was defined as Hb <=10.5 and slight anemia between that value and Hb=12 for women, and Hb=13 for men. The least Hb value and its relations with the evolution of the tumor (remision , stabilitation, or progression was studied

Resultados : 70 % of patients developed anemia, and 68% of those during the active treatment. Half of them were slight anemia and 8% of them needed a blood transfusion. The cause of the anemia was the tumor itself in 19% of the cases, the chemotherapy treatment in 36%, and mixed (Surg+ ChT+RT) in the 42% of the cases. 82% of patients with lungcancer, 78% with colorectal cancer and 58% of other gastrointestinal cancers had anemia at any time of the disease. It resulted statically significant ($p < 0.0004$) in relation to the rest of tumors.The probability to have anemia is higher for those treated with concomitant ChT+ RT ($IC_{95\%OR}$: 1,42-6,52), cis o carboplatin OR: 12,77 ($IC_{95\%OR}$: 3,77-51,94) and the male sex ($IC_{95\%OR}$: 1,34-3,37). 77,3% (58/75) of the patients with metastasic disease developed anemia, while 64,8% (81/125) of the patienta with no metastasic disease developed it. It is at the limits of stadistical signification (p=0.06) de los que no. Similar results were obtained when only slight anemia was consider. **Serious anemia** is more probably in those treated withcis o carboplatin ($IC_{95\%OR}$: 4,33-14,88), in the presence of metastasc disease ($IC_{95\%OR}$: 1,93-7,12) and in those who are younger than 65 years ($IC_{95\%OR}$: 1,12- 2,69). Neither concomitanat ChT-RT nor male sex were statically significant. In those patients who developed serious anemia, the tumor was in progress in 60%, in remision in 10% and in stabilitation in 30%. In spite of 32% of progrssion, 58% of remision and 10% of stabilitation in who did not develop it ($p < 0.0001$)

Conclusiones : the incidence of anemia in oncology patients is very high and in more than a half of the cases is slight anemia. The most important cause is ChT treatment (specially cisplatin). It is more frecuent in the lung, colorectal and otrer gastrointestinal cancers, in this order. The probability of having a serious anemia is bigger in those patients with metastases, aged under 65 and those treatea with cis or carboplatin. The tumor progression was significally higher in those patients with Hb < 10.5 at any time of the illness.

Key words: Anemia. Cancer. Chemotherapy.

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PROSPECTIVE EVALUATION OF THE NEUROTOXICITY OF THE COMBINATION OF DOCETAXEL PLUS VINORELBINE.

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Background: Given that both docetaxel (DTX) and vinorelbine (VRB) induce peripheral neurotoxicity (PN), all patients (pts) with antracyclin-resistant metastatic breast cancer enrolled in a Phase II trial with DTX (75 mg/m^2)+VRB (30 mg/m^2), both i.v. on day 1 q 21 days for up to 6 courses, were prospectively evaluated with serial neurologic examination after each course and nerve conduction study (NCS) before course 1 and after course 3 and 6. PN was graded with the National Cancer Institute common toxicity criteria (NCI-CTC). **Results:** None of 23 pts treated had DTX+VRB discontinued due to PN, but 14 received less than 6 courses due to tumor progression (13) or neutropenic sepsis (1). Ten pts who did not receive at least 3 courses in the absence of detectable PN have been excluded from analysis. After 3 courses.DTX+VRB induced mild grade 1 sensory PN (absent tendon reflexes) in 12/13 pts (92%). Only 4/13 pts had symptoms (distal paresthesias in the feet). After 6 courses, PN was grade 2 (objective sensory loss in the feet) in 5/9 pts (55%) and grade 1 in 4. No pt reported motor or autonomic symptoms. All pts became asymptomatic within 1 month after treatment completion. NCS after 3 courses showed only decreased amplitude of sensitive evoked potentials in 54% of pts. NCS after 6 courses showed sensory axonal changes in 7/9 pts (77%) and decreased peroneal motor amplitude in 4/9 (44%). **Conclusions:** The combination of DTX+VRB induces symetric sensitive axonal PN which presents with distal paresthesias after 3 courses of DTX+VRB, progresses slowly with more courses and resolves within 1 month from the last course. In this small series, no case with motor symptoms or disabling PN was seen. The PN of DTX+VRB is less severe than expected given the neurotoxicity of each drug as a single agent, and does not preclude clinical trials with DTX+VRB.

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PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) MOBILIZATION WITH FILGRASTIM IN BREAST CANCER PATIENTS.

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OBJETIVE : Analysis of mobilization capacity of filgrastim administered at a dose of 5 mcg/kg/dia s.c to 75 breast cancer patients treated with high dose chemotherapy.

PATIENTS AND METHODS Filgrastim was administered at a dose of 5 mcg/kg/day for at least 4 days after hematological recovery from the last cycle of induction chemotherapy. Median age: 47 years. Stage II :16 patients. Stage III 24 patients. Stage IV : 35 patients. Induction chemotherapy : CAF (cyclophosphamide 500 mg/m^2 + adriamicin 50 mg/m^2 + 5-FU 500 mg/m^2) or sCAF (cyclophosphamide 600 mg/m^2+adriamicin 60 mg/m^2+ 5-FU 600 mg/m^2) 48 patients ; FEC (5-FU 600 mg/m^2+ 4-epirrubicin 60 mg/m^2+ cyclophosphamide 600 mg/m^2) 27 patients.

PBPC were collected on CS Fenwall. A median of 10 l were processed per procedure, with a mean of 2.93± 0. 79 leucapheresis per patient (median 3, range 1-5)

RESULTS

	Mean	Median	Range
CMN x 10^8/kg	4.83	4.8	3.18 - 7.47
CFU-GM x 10^4/kg	38.7	35.38	1.39 - 92.9
CD 34 x 10^8/kg	2.8	1.92	1,89 – 7.4

A second mobilization procedure was not neccesary.

CONCLUSION

In this estudy the dose of filgrastim at 5 mcg/ kg / day allows mobilization of suficient CPSP amount for support in the setting of high dose therapy in breast cancer patients.

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CHEMICAL PLEURODESIS WITH IODINATED POVIDONE IN PATIENTS WITH MALIGNANT PLEURAL EFFUSION.

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INTRODUCTIÓN: To assess the efficacy of iodinated povidone in the pleurodesis treatment of malignant effusion and assess it inmediate toxicity.

PATIENTES & MÉTHODS: Patients with relapsing malignant effusion were included if they were not fit to other systemic therapy. ECOG needed to be below 4 and life expectancy over 2 months

After emptying pleural cavity with a tube, 50 ml of iodinated povidone were administered, Xilocaina 1% was added to avoid pleuritic pain. After 24 hours, the tube was retired.

Response is evaluated the first month and every two months thereafter. CR was the disapareance of effusion on chest x-ray, PR, symptomatic improvement and effusion relapse in a volume less than 50% of previous, and non-response (NR), the relapse of effusion or clinical deterioration.

RESULTS: 23 pleurodesis were done (13 right and 10 left) in 20 patients, 11 of whom were female. Median age was 63 (30-88). 80% presented a PS (ECOG) less than 3. Primary tumor was lung (30,4%) and breast (26,1%).

	CR	PR	NR	Overall response
4 weeks	3 (20%)	7 (46,7%)	5 (33,3%)	10 (66,6%)
8 weeks	2 (13,3%)	5 (33,3%)	3 (20%)	7 (46,7%)
16 weeks	3 (20%)	1 (6,62%)	1 (6,62%)	4 (26,6%)

80% had toxicity: pain (69,6%), náusea (30,4%), vagal reaction (21,8%), fever (17,4%), disnea (13%) and cough (4,3%).

CONCLUSIÓNS: Intrapleural therapy with iodinated povidone is similar to other cytostatic agents. There is a high frecuency of toxicity, that however is manageable. It is a cheaper therapy.

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USE OF LENOGRASTIM IN ONE DOSE FOR CASES OF NEUTROPENIC FEVER IN SOLID TUMORS

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INTRODUCTION: Lenograstim is a recombining glicoprotein equivalent to G-CSF and separated from a human celular line of CHU-2. In clinical practice, its use is recomended to reduce serious neutropenia and associated complications in patients with malignant non-myeloid neoplasms undergoing citotoxic chemotherapy wich is associated with a high incidence of neutropenic fever. Various studies have demostrated the clinical benefits of this medicine, but in view of its high cost it was recomended to be used rationally.

MATERIAL AND METHODS: Between april 98 and july 98 twenty cases of neutropenic fever were registered. 60% (12) were woman and 40% (8) were men with an average age of 60 (45-77). In 45% (9) of the cases the basic neoplasm was breast cancer 25% (5) , lung cancer 10 % (5), 10% gastric cancer (2), 10% advanced colorrectal cancer (2), uterine leiomyosarcoma (1) and temporal osteosarcoma (19). In 60% of the cases (16) it was the first episode of neutropenic fever. The initial empiric antibiotic treatment was ceftacidime/amikacin i.v. in 75% (15)of the cases, imipenem i.v. 10% (2), ceftacidime i.v. 10% (2) and ofloxacin i.v. 5% (1). In 50 % of the cases, the focus of infection was not determinated, 25% lung/ respiratory tract (5), skin/ soft tissue 15% (3) and 10% digestive tract.

Two patients have been included twice in the study.

The average corporal surface of the sample was 1´8m² (1´4 -2). A total of 7 patients (35%) had a C.S > 1´75 m² and therefore received a dosis below the theoretically recomended dosis (19´2 MU for m² of C.S.)

Lenograstim is administered subcutaneously in doses of 33´6 MU /day regardless of the corporal surface. The basic criteria for inclusion was a total amount of neutrophils <ó= 500/mm³, without profilactic use of lenograstim or other G-CSF, and mesured fever of >ó= 38ºC.

RESULTS : The average amount of days required to obtain a number of neutrophils >ó= 1500 mm³ and to obtain apirexia was 1´85 days (1-4) and 2´1 days (1-8) rrespectively. The average duration of administration of lenograstim per episode was 4´9 days.

The most frecuent side effect was skin reaction in the injection area (15%) and pain in bones (10%).

Only one patient could not be evaluated due to progress of disease and exitus.

The group of patients who received a dose theoretically below the recomended dose required an average of 1´8 (1-3) to obtain an amount of neotrophils >ó= 1500 /mm³ and 1´4 (1-5) to obtain apirexia. The group of patients who received the theoretically correct dose was 1´7 (1-4) and 2´4 (1-8) respectively.

CONCLUSIONS : Due to the small scale of the sample analyzed, no significant differences can be established between both groups. Until a larger number of cases is completed, administration of a standard dose of 33´6 MU/day does not seem to have adverse effects in patients with CS > 1´75 m². Thanks to its low local and sistemic toxicity the lenograstim is a safe drug in daily clinical practice.

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EVALUATION OF THE ONCOLOGIC PATIENT'S SATISFACTION WITH SOCIAL WORKER UNIT

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PURPOSE: the aim of our study was to evaluate the satisfaction of both patients and relatives with the social service given by the Social Worker's Unit (SWUU) of the Department of Medical Oncology of the Hospital General in Valencia.

PATIENTS AND METHODS: A transversal study was done. 510 patients, admitted to the Medical Oncology Department from January 1994 to May 1997, were included. A questionnaire was sent by mail including two aspects: the patient's satisfaction with the SWUU, and the type of services offered by the unit and accepted by the patients.

RESULTS: 32% of the oncologic patients made at least one consultation at SWU. Only 25,9% answered the questionnaire, and a relative filled in most of them (64,1%). The information about the existence of the Social Worker Unit was given by the social workers themselves in 31%, the oncologist in 20%, and the rest by the staff and publicity. 90% of the patients did not find any difficulty in contacting with the social worker. Most of them (75%) were satisfied with the Unit. 80% of the patients considered that the facilities offered were suitable. Social services offered by SWU were economical aids (42%), technical equipment (22%) and nursing houses (15%). However, the patients requested mainly, nursing houses in 42%, technical equipment in 32% and economical aids in 20%.

CONCLUSIONS: The Social Worker Unit was easily available and provided a high degree of satisfaction. Most of the patients got the information from the social workers or the oncologist. The nursing houses and technical equipment were more requested by the patients than the economical aids.

9. Non-Therapeutic Aspects

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DOMICILIARY CHEMOTHERAPY IN ONCOLOGY (DCT): RESULTS OF A PHASE III / IV CLINICAL TRIAL

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Objective: To determine the life quality (LQ), the accomplishment of the treatment, the patient's satisfaction for the care, and the chemotherapy (CT) duration in comparison to the chemotherapy duration at hospital. A secondary objective is to find no differences in medical complications.
Material and Method: Randomized clinical trial to demonstrate the efficacy and security of the DCT versus a hospital treatment. Patients with colorectal cancer eligible to treatment with 5-Fluorouracil CT based, either as adjuvant or in disseminated disease, were selected. The analyzed variables were: LQ (EORTC-30 questionnaire), satisfaction with medical and infirmary care, treatment accomplishment and toxicity, and use of the health system resources.
Results: Between Oct. '96 and Oct. '97, 87 patients were included: 67 adjuvant treatments (39 colon and 28 rectum) and 20 disseminated diseases. 42 patients were treated at the hospital and 45 followed DCT. There weren't significant differences in the randomized assignation to these groups neither in sex, age or tumor type. 8 patients left voluntarily the treatment (7 from the group at hospital and 1 from the DCT group), and 22 treatments were suspended because of toxicity or disease progression (13 from the group at hospital and 9 from the DCT group). There weren't differences in LQ between the two groups during all the treatment. However, differences were found at the end of the treatment; the patients of the DCT group reported more satisfaction and a significant better accomplishment degree of the treatment.
Conclusions: DCT treatment with 5-FU in bolus improves the accomplishment degree of treatment and the patient satisfaction with the care without increasing risks. A program with these characteristics may suppose more costs to the health system but the patient's costs are minor due to the improvement of their time management.

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ESTIMATION OF COST-EFFECTIVINESS IN FOLLOW- UP PROGRAM IN PATIENTS WITH BREAST CANCER, RANDOMIZED TRIAL.

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The constant increasing of the sanitary expense, and the limitation in resources, make planning to define the efficacy of the routinary clinical practice. This is very important in breast cancer due to its high incidence.

Objetive: To evaluate the rate cost-effectiveness of the diagnostic methods in early detection of recurrences in patients with breast cancer.

Material and method: Randomized clinical trial allocating breast cancer patients to two alternative follow-up protocols: intensive with diagnostic laboratory tests (blood count, biochemistry and biomarkers) and image exams (liver ultrasound, bone scan and chest roentgenography) vs clinical follow up. Patients with no metastatic breast cancer, under 70 years old, after finishing the initial treatment of their disease and no subsidiares of high-chemotherapy, are eligible for randomization into this trial. Mammography is performed every year in all women.

Results: Since January 1996, 89 patients were randomized, 45 were asigned to the intensive follow-up group (IF) and 44 to the clinical follow-up (CF). A total of 250 follow-up visits were performed (128 IF/122FC). In 15 cases were done no programmed visits(7CF/8IF). Five patients have relapsed in the study period (2CF/IF), only in one patient the clinic and the verification of the relapse by image methods were simultaneous; the rest of patients asked for asistance due to sintomatology.The global cost of the program of CF has been 1.811.710 pesetas, and the cost of the if has been 3.978.081 pesetas.

Conclusions: The preliminary results shows an excesive cost in the IF programs in patients with breast cancer, without improvement in the early diagnosis of the relapses.

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OCCUPATIONAL REINTEGRATION OF CANCER PATIENTS

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OBJECTIVE

To determine the consequences of cancer related to work.

PATIENTS AND METHODS

Between March and September in 1998, we asked to cancer patients in the Medical Oncology Department, about their work-related problems.

We take a poll of 60 people, 33 men and 27 women. The median age was 46 years old (22-65). Twenty-seven patients (45%) were employed in Service Sector. We make the statistical analysis with the Chi cuadrado or Fisher tests.

RESULTS

Fifty patients (83%) were in short sick-leave at the time of the treatment, 6 (10%) maintain their laboral activity, 3 (5%) had a contract cancellation at cancer diagnosis, and 1 (1%) sought the early retirement. Of the 6 patients who stay laborally active, 4 (66%) were self-employed, while 4 out of 50 in short sick-leave (8%) were self-employed (p=0'0025).

Short sick-leave median time was 9'5 months (1-48). Nevertheless, median time during the treatment period was 7 months (1-14).

At the end of cancer therapy, 38 patients (76%) returned to their job, 7 (14%) stayed in short sick-leave and 5 (10%) passed to permanent inability.

Ten patients (42%) who returned to job notified some changes in the environmental of the workplace, 6 of them (60%) had a positive experience with their co-workers, while the other (40%) reported some discrimination.

Twenty-nine patients (48%) became some disability by cancer. Nevertheless, 16 (55%) of these returned to their workplace, 6 (20%) stayed in short sick-leave, 5 (17%) in permanent inability, 1 (3%) were dismissed, and 1 (3%) in early retirement.

Among patients became some disability, the 100% of sarcoma and head and neck cancers, 40% of melanoma and breast cancer, and 18-20% of lymphomas and gastrointestinal tumors were described.

CONCLUSIONS

Preliminary results show that 83% of these polled got the short sick-leave while treatment period. When it finished, 76% returned to their workplace. As a consequence of neoplastic disease or therapeutic process, only 11% of patients passed to permanent inability.

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PROGNOSTIC FACTORS IN PATIENTS WITH SPINE METASTASES.

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BACKGROUND: The spine is the most common site of skeletal metastasis. Failure of its structural integrity as a result of metastatic vertebral involvement often brings about severe pain and/or paralysis.

We analyzed factors affecting survival in patients with spine metastases from breast, lung, and prostate carcinoma. Survival was determined from the date of dignostic to the date of last follow-up.

PATIENTS AND METHODS: We analyzed data from 258 patients with spinal metastases treated from 1987 to 1997 at our institution. The breast was the primary malignancy site in 168 patients, the prostate in 29 patients and the lung in 41 patients. The median age was 60 years.

We analyzed 15 factors. Survival curves were calculated according to the method of Kaplan-Meier. Univariate survival analysis was performed with the Mantel-Cox test, Breslow test and Tarone-Ware test. Survival was recorded from the date of diagnostic of spinal metastases. Potential prognostic factors were analyzed in a multivariate analysis using Cox's regression.

RESULTS: In the univariate analysis significant variables to predict survival were anatomic site of primary carcinoma, serum levels of alkaline phosphatase, performance status (Karnofsky), clinical benefit after the initiation of treatment, brain metastases, liver metastases, time from primary carcinoma to spinal metastases (months), the clinical findings at the time of diagnosis, uptake had diminished demonstrated by skeletal scintigraphy. Multivariate analysis showed that the primary site of the cáncer ($p<0,00001$; $\beta=1,78$; Exp (β)= 5,96; SE= 0,27), clinical benefit after the initiation of treatment ($p<0,00001$; $\beta=1,1$; Exp (β)= 3,02; SE= 0,25), serum levels of alkaline phosphatase ($p<0,00001$; $\beta=0,83$; Exp (β)= 2,31; SE= 0,19), and performance status (Karnofsky) at the time of diagnosis ($p<0,0003$; $\beta=0,76$; Exp (β)= 2,14; SE= 0,21) were the most important prognostic factors.

CONCLUSIONS: For patients with spinal metastases, the factors found to affect survival from the date of diagnostic of metastases include anatomic site of primary carcinoma, clinical benefit after the initiation of treatment, serum levels of alkaline phosphatase and performance status (Karnofsky).

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COMPUTERIZED PRESCRIPTION OF ANTINEOPLASIC CHEMOTHERAPY

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Introduction:
The manual prescription of chemotherapy is a complex process. Computerization reduces the possibility of error and the prescription time . Furthermore, it eliminates the need for intermediaries and shortens the waiting time of the whole cycle.

Patients and methods:
Computerized prescription of chemotherapy was initiated at our institution in 1991. All prescriptions have been dealt with in this manner since 1993. In summary, after entering the weight, size, outline of cytostatic treatment and antiemetic standard, the program calculates the intervals and dosis to be administered. Both pharmacy and nurses can know preparation orders immediately.

Results:
In its first five years, 2052 treatments have been registered in 1559 patients, 886 (56.8%) of which were men, and 673 (43.2%) women. Mean age was 62.8 years (20 to 93 years). A progressive increase in the number of treatments per year has been observed, from 288 in the first year to 448 at present. The most frequent tumour was digestive, with 398 (25.5%) registered cases, followed by breast cancer, with 354 (22.7%) cases, lung cancer in 304 (19.5%) cases and head and neck cancers in 160 (10.3%) cases. We recorded 108 different treatment schedules, with the most frequent being: 159 (7.7%) FAC or FEC; 146 (7.1%) CMF in some of its variants; 145 (7.1%) Al-Sarraf; 140 (6.8%) 5-FU; 140 (6.8%) 5-FU and folinic acid; and 122 (5.9%) MIC. Thirty-three different antineoplastic agents were employed: 5_FU was used in 918 (44.7%) treatments; cis-platin in 631 (30.8%); cyclofosfamide in 490 (23.9%); adriamicine in 313 (15.3) and metotrexate in 249 (12.1%). An important change occured in relation to the antiemetic standards, which implied using ondansetron in 20% of the patients in 1994 to using it in 80% in 1997. Active treatments numbered 132 up until June 1998.

Conclusions:
As well as eliminating errors and accelerating the process, computerized prescription of antineoplasic chemotherapy makes it possible to mantain and analyze the database that is created. On the other hand, it opens the possibility of linking this database with others within a general hospital, such as the Tumour Register, or specific service databases such as those used for neutropenic fevers and others designed ad hoc.

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MAY A MEDICAL ONCOLOGY UNIT IMPROVE THE OUTCOME OF THE ONCOLOGIC PATIENT UNDERGOING SURGERY?

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Targets: Medical Oncology has become one of the most changing medical specialities over the last decades. The complexity of the oncologic patient often demand multimodality approaches in their management and probably the need for specialiced units to deal with those patients. Surgical Oncology is not an exception to this matter. A Surgical Oncology Unit was created at the Creu Roja Hospital in Hospitalet eight years ago. This unit initially depended on the Department of Surgery and its aims were to improve the oncologic patient care. Six years ago this unit became a functional unit directly supervised by the medical director and working together with the medical oncology unit as a mixed medical-surgical oncologic area. Other care proffesionals like social workers and psicologists are also involved. The purpose of this presentation is to assess the assistance advantages in the management of the oncologic patients in this setting over the past six years.

Material and methods: Over one hundred oncologic files have been revised in order to achieve results belonging to two different time periods. First, an historical period previous to the oncologic mixed area, and the other refered to patients managed by the specialiced oncologic area. Some standard measures have been used as control features like: preoperative hospital stay, clinical-pathologic correlations, morbidity, unscheduled addmisions, time to the adjuvant treatment delivery etc. and some other subjective measures, although of great academic and scientific value like application of protocols, quality of discharge reports, clinical meetings, boards, and information given to patients.

Results and Conclussions: The results of this study may contribute to show the great advantages achieved by the specialiced group working together to provide the best combined approach to the oncologic patient.

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PROSPECTIVE ANALYSIS OF FAMILIAL BREAST CANCER SYNDROMES.

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Introduction : The incidence of breast cancer would probably defers between different countries as a consequence of environmental influence and specific hereditary patterns. The analytic approach of our study is to model the incidence of familial breast cancer and associated cancers in our breast cancer population.

Materials and methods: Between January 95 and December 96, we registered 292 new breast cancers in our unit. They were prospectively interviewed about family history of breast cancer as well as the ages at which those family members were affected, and bilaterality status. Those cases in which it was not possible to obtein information of three generations were excluded, so we were able to analyse 265 recorded cases.

Results: 84% cases had no history of a family link to either breast cancer or breast-ovarian cancers; 14% cases had a family history which was not significant; 2% cases showed significant criteria for familial breast cancer or breast-ovarian cancer syndromes.

Conclusion: The present study highlights a very low incidence (2%) of familial breast cancer syndromes in our population.

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LUNG ASPERGILLOMA CAUSING FALSE POSITIVE ^{131}IODINE WHOLE BODY SCAN IN THE EVALUATION OF PAPILLARY THYROID CARCINOMA

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Introduction:
^{131}Iodine whole body scan is commonly used to evaluate the presence of metastatic focuses in patient with papillary thyroid carcinoma as well as after thyroidectomy control . False positive studies are uncommon but they have been reported in relation with inflammatory processes of the lung.
Clinical case:
We present the case of a 34 year-old female that was diagnosed in 1981 as having papillary thyroid carcinoma and underwent a total thyroidectomy. ^{131}Iodine whole body scan after thyroidectomy was negative. In 1989 she was intervened of a lung aspergilloma in left upper lobe by embolectomy and resection of the area. From 1990, several ^{131}Iodine whole body scans were positives with focal uptake in LUL having already received two radioiodine therapeutic doses (100 mCi). Several ^{99}Tc isonitrilo whole body scans have been negatives. The patient has remained asymptomatic during this whole time without hyperthyroidism neither pulmonary symptoms and with thyroglobulin levels inside the normality. Thorax IRM showed a 6 cm homogeneous south-tissue mass occupying a spherical cavity in left upper lobe and an opacity in right vertex that its corresponded with two mycetomas. In this moment she had no evidence of recurrent thyroid cancer.
Conclusions:
In the case of aspergilloma, the use of iodine to manufacture fungus endotoxins or enzymes released in a closed cavity, it could be the cause of the ^{131}Iodine abnormal concentration in this cavity. However, this it is not totally clarified. This case makes necessary to be careful when interpreting abnormal ^{131}Iodine whole body scan, especially in the setting of asymptomatic young patients and with thyroglobulin levels inside the normality. In these cases, the ^{99}Tc isonitrilo whole body scan can be useful to discard metastatic focuses of papillary thyroid carcinoma.

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HIGH INCIDENCE OF OBESITY IN YOUNG ADULTS SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION
We wanted to explore the evolution of the body mass index (BMI) in patients who had been treated of acute lymphoblastic leukemia (ALL) during childhood. We considered as well the influence of the following factors: treatment modalities, age at diagnosis, sex, and puberty.
PATIENTS AND METHODS
We examined the frecuency and pattern of obesity in 37 survivors of ALL after a median follow up of ten years. Decimal ages at diagnosis were between 0,9 and 14,6 years. They had received several chemoradiotherapy schedules: Pethema 78, BFM 83, BFM 86, and BFM 90. BMI was recorded at diagnosis, and after the following years: 1,3,5, and 10 years. We used the statgraphics plus 7.1 and BMPD vs PC90 statistical programs.
RESULTS
After a median follow up of ten years 79 % of the patients had increased their BMI. This index (considered normal from 80 to 120 %) increased 20,1 %, and it was significant ($p<0,0001$). During the first year of treatment the steepest increase in BMI ocurred, and this change was also significant ($p<0,0009$). There were no significant differences in BMI evolution in boys and girls. Same results were obtained when comparing those patients diagnosed before and after 7 years old. Although no significant difference were found, girls and youngers gained more weight than boys and olders, respectively. BMI didn't show differences according to chemoradiotherapy schedules. Menarchy was attained at 12,2 years old.
CONCLUSIONS
We conclude that most of our patients increased dramatically their BMI, becoming near obese young adults. Therapeutic modalities didn't appear to relate with obesity. Puberty seemed to be normal. Probably girls and childs diagnosed at an earlier age are on a higher risk of obesity and should be advised regarding a more active lifestyle and a healthy diet.

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STUDY OF THE VALIDITY OF LIVE SCINTIGRAPHY WITH RED BLOOD CELLS-Tc99m TO DETECT AND DIFFERENTIAL DIAGNOSE CAVERNOUS HAEMANGIOMA AND METASTASES.

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Cavernous haemangioma is the most frequent benign tumour of the liver. So, its detection and differentiation from metastases is of crucial importance in the diagnostic workout. Isotopic techniques may detect "in vivo" benign liver lesions and help in the staging of cancer patients.
PATIENTS and METHODS.- A series of 78 patients (p) studied by means of liver scintigraphy with red blood cells-Tc99m because of a suspicion (in the ultrasound or CT scan) of hepatic haemangiomas is described.
RESULTS.- In 55p (70.5%) a collection of red cells was considered positive for haemangioma diagnosis. All of these cases have been confirmed with follow-up, ultrasound controls, and in 1 case laparotomy. Specificity was 100%.
In 23p (29.5%) no lesions were detected with the scintigraphy and the diagnosis of haemangioma was ruled out. 19p were true negative cases and 4p angiomas. False negatives occurred in small, deep-located lesions. Sensitivity was 93.2% and global validity was 70.5%.
CONCLUSIONS.- Hepatic scintigraphy with red blood cells-TC99m have high specificity and sensitivity. It should be considered appropriate in detection and differential diagnosis of hepatic lesions suspected to be cavernous haemangiomas in oncology patients.

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LONGITUDINAL GROWTH AND FINAL HEIGHT IN LONG-TERM SURVIVORS OF CHILDHOOD LEUKEMIA

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OBJECTIVES: We attempted to analyze the longitudinal growth and final height of a cohort of long term survivors of acute lymphoblastic leukemias, considering the influence of the following factors: radiotherapy, chemotherapy, sex, age at diagnosis, and parent's stature. **PATIENTS AND METHODS:** We studied retropectively 37 survivors of acute lymphoblastic leukemia (23 males, 14 females), with decimal ages at diagnosis between 0,9 and 14,6 years. They had been treated with several chemoradiotherapy schedules: Pethema 78, BFM 83, BFM 86, and BFM 90. Auxometric data was recorded at diagnosis, and after the following years: 1,3,5,8, and at final height. Measures were obtained with a Harpender calliper, and compared with Tanner standards for sex and age. We used the statgraphics plus 7.1 and BMPD vs PC90 statistical programs. **RESULTS :** Height at diagnosis was + 0,38 +/-1,16 SDS, and – 0,58 +/- 1,45 SDS when skeletal maturity was achieved. Final height was reduced in -0,96 SDS ($p<0,0001$). During the first year of treatment the deepest fall in height ocurred (-0,51 SD, $p< 0,00001$). After therapy was concluded patients showed a slight catch up, that was not significant. There was a trend of patients treated at an earlier age to have a reduction in final height, compared to the older patients ($p<0,0007$)). Female subjects lost more SDS (-1,19)at attained heigths than males (-0,82), but the difference was not significant. Mean final height was lower in patients treated with higher doses of cranial radiotherapy (>24 Gy), and intensive chemotherapy schedules, but it was not significant. Patient's stature decreased 0,56 +/- 0,74 SDS over their genetic target, from diagnosis to the completion of growth. **CONCLUSIONS:** Our patients experienced a significant decrease in final height; it developed mainly during the first year of therapy. Girls and children diagnosed at an earlier age were the subgroups more severely affected. Intensive chemotherapy schedules, and higher radiotherapy doses had a greater negative impact on final stature. These results underline the need to optimise the chemoradiotherapy schedules in childs with acute lymphoblastic leukemia.

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THE ONCOLOGIC AREA STRUCTURE AT THE CONSORCI HOSPITAL CREU ROJA HOSPITALET (CHCR)

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Introduction: The implantation of Medical Oncology Units at median level (II) General hospitals often reveal the need to determine their role among other assistance departments. In addition, few data is avilable for an accurate assessment of eficiency standars regarding assistance management.
Aims: To describe the Medical Oncology Unit (MOU) structure as an integrated area along with the Surgical Oncology Unit (SOU) and a collaborative level III Reference Hospital.
Description: The MOU and SOU Units got started as Functional Units in 1991. They both work together as a medical-surgical oncologic area. They share hospital inpatient wards, outpatient facilities, weekly multidisciplinary tumor boards, in order to better coordinate patients care. In 1997 a collaborative programme was signed up with the Catalan Institute of Oncology (ICO), a III level reference hospital. Since 1997 both Units are composed by the following medical staff each: 1 Unit Chief and 2 attending physicians. Developed activity: Weekly clinical joint meeting (MOU-SOU), MOU clinical sessions, Breast, Lung, Urologic, Gynecologic, Colorectal and Hematology-Oncology tumor boards. Oncologic pain and ORL joint boards. Teaching programme for Internal and Family medicine residents in training. Follow-up of the aims and features of the Health Plan related to the Oncologic Care for the Regional Area involved. Assessment of the application of protocols and trials, and time interval diagnosis-treatment for colon, lung, and breast cancer.
Methods: Parameters like hospitalization, outpatient care, in-day hospital, inpatient costs, chemotherapy costs, were all analysed.
Conclussions: 1. A mixed medical-surgical oncology area has definetely contributed to set up oncologic criteria among the rest of the hospital staff. 2. The case load and oncologic coordination is not reflected in the activity standars used for the rest of specialities. 3. Quality standars for medical oncology itself must be stablished. 4. Productivity and quality palliative care standars must also be stablished.

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USEFULNESS OF BREAST SCINTIGRAPHY WITH THALLIUM-201 IN DIFFERENTIAL DIAGNOSIS OF BREAST NEOPLASMS.

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Mammography is the best technique to early identify malignant lesions in the breast but its sensitivity is far from 100% and other image tests are needed to contribute to the differential diagnosis (MRI, PET, ultrasound scan, thermography and scintigraphy with different radioisotopes).
Aim.- To assess the diagnostic validity of Tl-201 breast scintigraphy and its aid to other image tests looking for rational indications for use.
Material and methods.- 45 cases (44 female) have been evaluated, aged 28 – 68 years old. Any lesion, either palpable or not, previously examined by routine techniques (mammography, ultrasound and thermography) were selected for study. Tl-201 Chloride was injected (111 MBq i.v.) and planar images were taken from both breasts 20-30 minutes after injection. Irradiation dose was 3,1 mGy to the breast.
Results.- In the isotopic study of 18 breast with benign lesions a false positive was found (chronic non-specific granulomatous mastitis). From 27 breast carcinomas (70.3% of them were ductal carcinoma), 22 were positive in the scintigraphy and 5 showed weak positive uptake. Minimum size of a lesion to be detected was 8 x 7 mm. Sensitivity was 82,4%, specificity 94,4%, positive predictive value 95,6% and negative predictive value 77,2%.
Conclusions.- Breast scintigraphy with Tl-201 is useful and may help conventional radiology in the diagnosis of breast: a) dense, fibrous juvenile or displastic breast; b) lesions showing indirect radiological signs of suspicting malignancy; c)lesions with direct signs and radiologically doubtful.

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Long-term Venous Central Catheters with Subcutaneous Port. Experience in a Unit of Oncology.

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Between Mars 1996 and September 1998, they were placed in the Unit of Medical Oncology , 70 Long-term Venous Central Catheters with Subcutaneous Port (LVCC), type Celsite 9.5 or Life-port. The catheters were used for blood-samples, chemotherapy and hemoderivate transfusions. Patients were diagnosed of: breast cancer, 29; colo-rectal cancer, 12; lymphoma,8; sarcoma,6; lung cancer,5; ovarian cancer,3; other tumors, 7. The complications experienced at insertion: transient arrhytmia, 16 (30%), arterial puncture, 5, not insertion, 2, local hematoma, 2 and pneumothorax, 1. 2 axilar and subclavian catheter-related venous thrombosis were restorated by anticoagulation. 4 catheters were removed due to infectious complications (5,7%), 2 out of 5 by multiple bacterias, 1 candida albicans, 1 bacilus spp.
Urokinase instillation (standar doses) was administered in 6 LVCC (8.5%) due to catheter oclussion, with resolution. The LVCC were removed in 3 patients after treatment and personal decision; 1 out of 3 had to be recovered by interventional radiology after catheter rupture and migration.
In conclusion, insertion of LVCC presents a very low percentage of severe complications during the insertion technique and treatment. The catheter removal was done due to severe infections in 4 patients. The coagulation-related diseases were resolved with standar treatment and normal function. Patients and nurses expressed their satisfaction due to easy handle of chemotherapy-administration after catheter insertion.