level of fibrinogen, D-dimer or fibrin, antithrombin III and prothrombin time (hypercoagulation group). Second group (n = 28) had normal values of coagulation (control group). All pts had received at least 2 cycles low-dose immunotherapy (IL-2, 1 MIU, i.v, 3 tiw + IFN, 5 MU, s.c, 3 tiw – 3 weeks on, 3 weeks off). Tumor response was assessed radiographically every 2 cycles using RECIST criteria. Median overall survival (OS) was estimated according to Kaplan-Meier method.
Results: Hypercoagulation was present at study entry in 38.8% of MRCC pts. 71.4 and 75% of pts were male, median age at on-study was 62 and 60.1 years in hypercoagulation and control group, respectively. 46.4% of pts had poor prognosis by MSKCC score in both groups (13 = 13 pts), and 53.6% of pts had good or intermediate prognosis. 25 (89.3%) pts of control group and 26 (92.9%) pts of hypercoagulation group had clear-cell histology. Pts with normal coagulation and treated with IL-2+IFN had a statistically longer survival and higher response rate than those who had abnormal coagulation (Table). Pts with hypercoagulation had predisposition to disease progression after 2 cycles of immunotherapy.

	Hypercoagulation group	Control group
CR	–	1 (3.6%)
PR	1 (3.6%(	5 (17.9%)
OR	3.6%	21.4%
SD	11 (39.3%)	14 (50.0%)
PD	16 (57.1%)	8 (28.6%)
Median OS*, months	7.1	14.5
95% CI	6.0−8.2	10.4−18.6

*Cancer-related survival; logrank p < 0.001.

Conclusions: These early results demonstrate that abnormal coagulation can be an independent prognostic factor for survival and efficacy to therapy in pts with MRCC.

543 POSTER

Soluble E-selectin levels and CEA expressing blood-borne cells in colorectal cancer patients. A causal relationship?

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Purpose: The recognition of E-selectin by colorectal cancer (CRC) cells is an essential step for adhesion to activated endothelium and metastatization. Increased expression of E-selectin has been found in small vessels surrounding lesions in CRC and elevated levels of soluble (s) E-selectin have been found in metastatic compared with non-metastatic CRC patients. One of the newer areas being explored in the management of CRC is the use of reverse transcription-PCR (RT-PCR) to analyze the blood of cancer patients for the detection of mRNA expressed in tumor cells. Thus, this study was aimed to verify whether CEA mRNA levels in blood-borne cells correlate with cytokines and adhesion molecules involved in the haematogenous spread of CRC cells.
Methods: CEA mRNA (by RT-PCR), proinflammatory cytokines (IL-6, IL-1beta, TNF-alpha) and sE-selectin levels (all by R&D immunoassays) were analyzed in blood samples obtained from 64 CRC patients, treated at "Tor Vergata" Clinical Center, 40 patients with benign CR diseases and 59 control subjects. Patients were histologically diagnosed with primary [Dukes' Stage A (n = 4), Stage B (n = 27), Stage C (n = 17) and Stage D (n = 2, with a single resectable liver metastasis)] or relapsing (metastasis to the liver: n = 8, peritoneum: n = 2, lung: n = 2 and multiple metastasis: n = 2) CRC. The study was performed under the appropriate institutional ethics approvals, and informed consent was obtained from each patient.
Results: Median sE-selectin levels were higher in patients with CRC (44 ng/ml) compared to controls (34 ng/ml) or patients with benign CR diseases (31 ng/ml, H = 18.5, p = 0.0001). Increased levels of sE-selectin were significantly associated with CEA mRNA positivity by RT-PCR (p
Conclusions: The findings obtained suggest that circulating cancer cells, or their released products, might be responsible, through cytokine release, for the elevation of circulating adhesion molecules in CRC patients.

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Searching for susceptibility alleles: emphasis on bilateral breast cancer

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Background: Polygenic inheritance plays an essential role in cancer susceptibility, however identification of at-risk alleles is compromised by poor reproducibility of case-control studies. We and others suggested earlier that the use of subjects with "extreme" characteristics of cancer risk, e.g. patients with multiple tumors, may provide highly demonstrative results of molecular epidemiological analysis.
Materials and Methods: Literature has been searched for case-control gene-association studies which analyzed both bilateral and unilateral breast cancer (BC) cases. 8 relevant reports have been identified, including 6 papers involving our contribution and 2 articles published by independent groups. The results of these investigations were compared against reference studies (i.e. meta- or pooled analyses) for each at-risk allele.
Results: Good concordance has been observed between the data obtained on limited number of bilateral BC and the larger data sets involving unilateral BC cases: all at-risk alleles (e.g., BRCA1 5382insC, CHEK2 1100delC, NBS1 657del5, ATM Ser49Cys) demonstrated some degree of overrepresentation both in women with a single tumor and in those with multiple cancers, while the "negative" studies (e.g., those for p53Arg72Pro, CYP17 −34 T/C polymorphisms) failed to reveal an effect in either of the patient groups. Most importantly, in all instances where a gene-disease interaction has been firmly established, the odds ratio observed for bilateral BC patients evidently exceeded the one calculated for unilateral series. Furthermore, the results of the analysis of bilateral BC corresponded well with the published reference studies.
Conclusions: For truly at-risk alleles, comparison of bilateral BC against controls always provides higher odds ratio estimates than the traditional analysis of non-selected BC cases; therefore, use of bilateral BC relaxes the requirements for the study size. Emphasis on bilateral form of breast cancer may significantly facilitate the search for genetic determinants of BC predisposition.

545 POSTER

Possible participation of fragile sites in her2/neu gene amplification on 17q12−21 chromosome in breast cancer

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Background: Overexpression of HER2/neu protein which plays a significant role in breast cancer development and progression was strongly associated with amplification of its coding gene her2/neu. A number of genes located around her2/neu were shown to be co-amplified with it in breast cancer. However initial events and mechanism of amplification in this locus is not clear until now. The aim of this study was to investigate 17q12-q21 chromosome region for potential fragile sites that could play a major role in amplification of her2/neu in a group of patients with breast cancer.
Materials and Methods: We examined genomic DNA from fresh frozen breast cancer tumor samples of 130 patients for amplification of HER2/neu by Real Time PCR with TaqMan technology. The sequence of the region around her2/neu was investigated by TwistFlex software to reveal loci with high level of flexibility.
Results: 35 out of 130 cases (27%) had increased her2/neu gene dosage. Among these 35 cases we analyzed amplification level of genes located in 17q12−21 chromosome region around HER2/neu: LASP1, MLN64, PPARBP, CASC3, TOP2A. Gene dosage of genes located in HER2/neu-TOP2A region was higher as compared with normal tissue in 14 out of 35 cases. PARBP-HER2-GSDML region had high level of amplification in 21 out of 35 cases.
Recent publications described involvement of fragile sites in various chromosomal rearrangements. One of the basic features of fragile sites is sequence flexibility. We analyzed the region around her2/neu gene for the presence of flexible sequences. Two loci with high flexibility were detected. First locus located within intron sequence of ZNFN1A3 gene, which situated 36 kb telomeric to her2/neu, the second locus was found within FBXO40 gene, which located on 720 kb centromeric to her2/neu gene. Interestingly, both genes were described as tumor suppressor genes in different tumor types.
Conclusion: We proposed that these two sites with high level of flexibility might play a critical role in amplification of this region consistent with the

BFB model for amplification in breast cancer. Fine mapping of borders of amplified regions, which including her2/neu, will open new goals for target therapy of advanced beast cancer.

546 POSTER

Prognostic value of CCN3 in osteosarcoma and Ewing's sarcoma

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Patients with osteosarcoma or Ewing's sarcoma, the two most common bone tumor, still suffer from the paucity of prognostic markers that could distinguish patients before therapy and drive treatment choices. Herein, we assessed the prognostic value of CCN1−3 genes, a group of genes involved in fundamental biological processes as well as in mesenchymal differentiation. Expression of CCN1−3 was detected by means of quantitative PCR in a series of newly diagnosed osteosarcoma or Ewing's sarcoma. In osteosarcoma, CCN1 and CCN2 expression was found statistically associated with genes involved in the commitment of mesenchymal stem cells toward osteoblasts and in the early phases of osteoblastic differentiation (RUNX family genes; cadherin 4, 11, and 13; jun and fos; collagen I and SPARC). CCN3 is highly expressed in osteosarcoma and its level of expression did not correlate with any specific osteoblastic differentiating genes. While neither differentiation genes nor CCN1 and 2 expression were statistically associated with survival, high expression of CCN3 significantly correlated with worse prognosis in osteosarcoma. CCN3 overexpression also showed a prognostic adverse relevance also in Ewing's sarcoma, either at gene and protein levels. Therefore, assessment of CCN3 expression levels at diagnosis may represent a useful molecular tool to early identify subgroups of patients with different prognosis either in osteosarcoma and in Ewing's sarcoma.

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Prognostic significance of p53 and Ki67 in Ewing's sarcoma tumours

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Background: Ewing's sarcoma (ES) is a heterogeneous neoplasm in which several genetic alterations involving cell cycle regulators have been described. Around 10% of ES suffer p53 alterations, which may be demonstrated by immunohistochemistry and/or mutational analysis. Many studies have demonstrated the prognostic significance of p53 in ES, but no large series of cases have been analyzed to validate its clinical use. Ki67 is a molecule that is detected in growing cells. Several papers have revealed the prognostic implication of Ki67 in sarcomas, but which needs to be confirmed in the case of ES. The aim of the present study is to evaluate the prognostic significance of p53 and Ki67 in a large series of ES.
Material and Methods: Paraffin-embedded material from 226 ES subtyped as follows: 66% classic ES, 10% large cell ES, 10% PNET, 3% clear cell ES, 3% atypical ES, 6% spindle cell ES and 1% hemangioendothelial ES. Seven tissue arrays (TA) were performed and immunohistochemical expression of p53 (clon DO7, DAKO) and Ki67 (MIB1, DAKO) was determined using a 1:50 dilution of each antibody.
Results: Follow-up was available from 132 patients with a median of 49 months (range: 1–306 months). 22% of cases expressed Ki67 in more than 5% of tumor cells. Expression of Ki67 was correlated with the progression of ES, being higher in recurrence (30%) and in metastasis (42%) than in primary tumors (20%) ($p = 0.042$). In the case of p53, 31% of ES expressed the protein but no relationship with progression was observed. Log rank test for progression-free (PFS) and overall survival (OS) showed the following results: Antibody: Ki67 ($\leqslant$5%, >5%), %PFS: 68 vs. 31, p: 0.001, %OS: 68 vs. 37, p: 0.009 Antibody: p53 (negative, positive), %PFS: 62 vs. 59, p: 0.366, %OS: 60 vs. 78, p: 0.040. In contrast to the expected, immunostaining of p53 was correlated with a better OS, suggesting that a mutational analysis of these cases should be performed in order to detect those with real mutant behaviour.
Conclusion: Ki67 immunostaining defines a subgroup of ES with a poor outcome and should be taken into consideration in the pathological staging of ES patients.
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548 POSTER

Micronuclei in exfoliated bladder cells of gynecological cancer patients receiving pelvic radiotherapy

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Background: The micronucleus (MN) assay in human exfoliated cells has been widely used to detect the genotoxic effects of environmental mutagens, infectious agents and hereditary diseases. This study clarifies the usefulness of the MN assay in exfoliated bladder cells to show normal (not related to the cancer) tissue damage of pelvic radiotherapy.
Materials and Methods: We measured the MN yield in exfoliated bladder cells of 20 gynecological cancer patients received pelvic radiotherapy. These patients were non-smokers, had no urinary tract disease and previous chemotherapy or radiotherapy. They received pelvic irradiation with 2 Gy daily fractions for 5 weeks. Urine samples were taken from the patients before commencement of radiotherapy (0 Gy), 24 hours after completion of the first fraction (2 Gy) and at the end of the therapy (50 Gy). In addition, to determine whether exfoliated bladder cells could be used as a sign of a genomic instability in cancer patients, baseline MN yields of the patient group before the therapy were compared with the healthy control group.
Results: We have detected significant difference between results of three different time periods ($P < 0.01$). The yield of MN after radiation doses of 50 Gy (2.93 ± 2.29) has increased when compared with the 0 Gy (1.37 ± 1.13) ($P < 0.01$) and 2 Gy (2.1 ± 1.92) ($P < 0.05$). There was no significant difference between the MN frequencies of 0 Gy (1.37 ± 1.13) and the control group (1.29 ± 0.74).
Conclusion: Exfoliated bladder cells which can be taken non-invasively could be used to show normal tissue damage after cumulative doses of pelvic radiotherapy but it is not an indicator of a genomic instability in gynecological cancers.

549 POSTER

Malignant mesothelioma (MM): prognostic risk factors and immunohistochemical markers in correlation with pathological changes and prognosis

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Background: Malignant mesothelioma (MM) is known to be a fatal malignancy that is related to asbestos exposure. A number of clinical prognostic factors have been described in the last 20 years, including histological cell type, performance status and clinical stage. However, much of the data have been conflicting probably because many of the studies have been of small size from a single centre. We conducted a population based study in Nova Scotia, Canada to examine the potential prognostic factors, as well as the protein expression of EGFR, VEGFR, and SV40 in MM and their impact on patient's survival.
Methods: All cases of MM diagnosed in the province of NS between 1990–2005 were identified through the Nova Scotia Cancer Registry. Clinical and laboratory data, including known prognostic factors such as WBC, LDH, platelet count and hemoglobin level, were abstracted through a retrospective chart review. Tissue microarray (TMA) with immunohistochemical (IHC) staining for EGFR, VEGFR and SV40 was performed. Survival, with Kaplan Meier analysis, and a multi-factorial model will be performed to detect prognostic factors.