Herceptest 3+ or FISH positive) on survival in this otherwise very good prognostic group. The group were 89% ER positive, with 72% smaller than 20mm. 80% were aged over 50 and 10% received chemotherapy and 91% received endocrine therapy (tamoxifen).

Results: The overall hazard ratio (HR) for HER2 positivity was 6.78 (95% CI 2.9-15.7, p<0.001) with 5yr breast cancer specific survival rates of 96% (HER2 negative) and 68% (HER2 positive). This reduction in survival in HER2 positive cases persisted when patients were split into subgroups by ER status, tumour size and age (table 1).

Conclusion: These results provide support for the use of adjuvant trastuzumab in this group of patients who are typically classified as very good prognosis, not routinely offered standard chemotherapy, and as such do not fit current prescribing guidelines for trastuzumab. A clinical trial to assess the benefit of adjuvant trastuzumab alone within this subgroup of HER2 patients would resolve this. These results are in keeping with those from HERA trial that suggested that patients with the best prognosis tumours (node negative and size 1-2cm) had benefit similar to the overall cohort.

The persistence of a reduction in survival in our ER positive subgroup despite endocrine therapy confirms the recent trans-ATAC analysis based on HER2 status and suggests that we cannot not rely solely on adjuvant endocrine therapy in these largely ER positive patients.

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Structure-guided design of inhibitors of the eukaryotic initiation factor 4E (eIF4E) mRNA-cap interaction as anti-cancer agents

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Protein synthesis in eukaryotes is regulated by eIF4E together with the other components of the translation initiation complex eIF4F. eIF4E anchors the mRNA by recognition of the 5' cap structure m7GpppN (where N is the first transcribed nucleotide and p represents phosphates), which contains a N-7-methylated guanine base. The role of eIF4E in cell proliferation and tumour progression is well documented, thus making eIF4E an attractive cancer drug target.

eIF4E recognizes the 5' cap structure through a characteristic cation-pi interaction involving the delocalized charge of the at N-7-modified guanine in the cap and two tryptophan residues in the eIF4E binding site, along with H-bonding interactions of the guanine base and electrostatic interactions with the phosphates. Our work aims to find non-nucleotidic cap-binding antagonists, and we look mainly for moieties in such inhibitors that could maintain the critical cation-pi interaction and would avoid the need for phosphate groups that render compounds membrane-impermeable and metabolically labile.

We follow a structure-guided drug design approach that consists of defining binding site constraints and performing in silico docking of small ligands into the eIF4E cap-binding site. We find that most of the scoring functions used to rank docked ligands fail to reward for the cation-pi interaction and we have implemented a quantum mechanical (QM) scoring strategy for the scoring of docked ligands. Hits from these virtual docking and scoring approaches were screened for binding eIF4E by Electrospray Ionization mass spectrometry (ESI-MS).

Several small molecules have been identified that bind to eIF4E and the results from these binding studies, as well as the effects of hit compounds on protein translation will be discussed.

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Gene expression profiling in formalin-fixed paraffin-embedded primary melanomas

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Melanoma is an aggressive highly metastatic disease arising from epidermal melanocytes. Diagnosis and prognosis of this disease is currently limited to histological factors such as measurement of tumour invasion by Breslow Thickness and few successful treatments are available due to our poor understanding of the metastatic phenotype.Studies investigating the molecular basis of melanoma initiation and progression have been limited in the past due to the heterogeneous nature of melanoma and limited availability of fresh primary tumour. New techniques such as whole genome gene expression profiling are improving knowledge of many human cancers, however this is often a DNA intensive method resulting in most studies of this kind on melanomas involving ether small numbers of fresh tissues or melanoma cell lines. We have used a novel method for gene expression profiling of 500 cancer genes in formalin-fixed paraffin-embedded primary melanomas. For this study, we selected 27 (FFPE) primary tumours from 27 patients; 15 of who had relapsed from their primary tumour within 5 years and 12 who had not relapsed after 5 years. The deepest, most invasive part of the tumours was sampled with a core biopsy needle and total RNA was isolated for analysis with the cDNA-mediated annealing selection, extension and ligation (DASL) assay (Illumina®). This assay is designed for use on partially degraded RNA for measurement of relative gene expression levels of up to 1536 sequence targets using as little as 25ng of total RNA. We used a Cancer Panel to target 502 genes commonly altered in cancer with three probes per gene. The results were visualised with Beadstudio analysis software (Illumina®) and normalisation was carried out using cubic spline methods, prior to export of results into STATA for further analysis.All tumours produced satisfactory results for the 502 genes in the Cancer Panel after quality control tests for average fragment length and amplification ability by qPCR. Genes found to have significantly different levels of expression between the group who had relapsed and the group that had not relapsed were SKI, PAI-1, BRCA2, WT1, MLLT4, NFKBIA and FGF8 (t-test and wilcoxon p

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Diagnosis of thyroid cancer by gene expression profiling on thyroid nodule biopsy

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The diagnosis of thyroid cancer relies on cytological examination of material collected from nodules by fine-needle aspiration biopsies (FNAB). Due to the absence of markers, it is difficult, even for experienced cytologists, to discriminate benign from malignant thyroid tumors. Thus, only 35% of patients undergoing thyroidectomy for cancer or suspicion of cancer, actually have a cancer. The diagnostic procedure must be improved to reduce the number of thyroid ablation subsequently proved to be unjustified (about 10,000 per year in France). With the aim of identifying marker genes capable to discriminate benign from malignant thyroid tumors, we designed an oligonucleotide-based nylon macroarray formed from 200-potentially informative genes. Gene expression profiles of normal and tumoral (adenomas, carcinomas) thyroid tissue were generated with the macroarray and validated by real-time PCR. In this study, we built tumor classifiers from macroarray data and we tested their performances on a series of samples corresponding to FNAB. Gene expression data deriving from samples of the Lyon Thyroid Tumor Bank, representing the "training set", were subjected to a weighted voting algorithm to generate prediction models or classifiers capable of assigning a sample to one of the two classes: benign or malignant. Three prediction models were built by considering either all thyroid carcinomas (the commun classifier) or only follicular carcinomas (the F classifier) or only papillary thyroid carcinomas (the P classifier). The classifiers were composed of 9 to 12 genes and brought into play a total of 19 "marker" genes which were used to compose a fourth predictor, the global classifier. The capacity of the 4 classifiers to discriminate benign from malignant tumors was tested on a series of FNAB (carried out on nodules after surgical resection) used as "validation set". In 23 out of 26 FNAB, the 4 classifiers gave a diagnosis similar to that of the pathologist used as "gold standard"; in the 3 other cases, the correct diagnosis was given by 3 of 4 classifiers. Thus, the combination of classifiers identified benign and malignant tumors with very high sensitivity and specificity. In conclusion, we developed a procedure of molecular diagnosis of benign versus malignant tumors applicable to the material collected by FNAB. This molecular test which complied with a pre-clinical validation stage is now subjected to a prospective, large-scale (800 patients) evaluation study.

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Identification of drug-sensitive prediction genes by an epigenetic reactivation screen of cisplatin-resistant NSCLC cell lines

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Non-small cell lung cancer (NSCLC) shows resistance in tumors that are initially chemo-sensitive, which is a serious problem in cisplatin-based adjuvant chemotherapy. CDDP is the paradigm of cytotoxic drugs in NSCLC treatment, however, it also induces, de novo DNA hypermethylation in vivo. Histone deacetylation and aberrant promoter hypermethylation are common epigenetic mechanisms for the silencing of

tumor suppressor genes (TSG) in many types of cancers, including lung cancer. However, their expression can be restored by demethylating and histone deacetylating inhibiting drugs such 5Aza-dC-2deoxycytidine (5Aza-dC) and trichostatin A (TSA). Platinum-induced DNA hypermethylation may be involved in the development of drug-resistant phenotypes by inactivating genes required for drug cytotoxicity. We aim to identify the global profile of TSG silenced by epigenetic mechanisms in NSCLC cell lines after CDDP treatment, and therefore potentially involved in the development of chemotherapy resistance. The present study is based on an expression microarray analysis of genes reactivated in a set of CDDP-resistant and sensitive NSCLC cell lines after 5Aza-dC and TSA treatment. CDDP-resistant cells were established by treating two NSCLC cell lines, H-460 and H-23, with increasing concentrations of CDDP. Then, cells were exposed to 5Aza-dC (5μM) and TSA (500nM) before RNA extraction. Total RNA from the different cell lines was extracted, reverse-transcribed and hybridized into an array platform containing the whole human genome. We selected for validation those genes upregulated after 5Aza-dC and TSA treatment, which expression was previously downregulated in CDDP-resistant versus cisplatin-sensitive cell lines. Next, we confirmed the presence of CpG island in the promoter region, the expression in normal lung cells and excluded those genes located in imprinted areas. Gene expression changes were confirmed by semi-quantitative RT-PCR. Promoter methylation was validated by bisulfite sequencing. Finally, methylation of validated genes was analyzed by methylation specific PCR (MSP) in NSCLC specimens with known CDDP response. Epigenetic regulation of selected genes was further studied in the abstract presented by M Cortes (back to back Poster). We have identified, a panel of genes with altered expression as a result of CDDP and epigenetic reactivation treatments. After validation of five, we confirmed one gene as a potential clinical marker, able to detect with 80% specificity, sensitive versus CDDP-resistant tumors in a panel of 30 paraffin embedded NSCLC samples. This study provides information regarding de novo promoter hypermethylation of potential TSG involved in the development of resistance and their potential use as targets enabling the diagnosis and chemotherapy treatment of NSCLC to be approached at the molecular level.Supported by Health Investigation funding (FIS/ISCIII). Supprted by FIS project number: PI061234 and by an unrestricted educational grant by Fundación Mutua Madrileña

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Differential expression of DNAmethyltransferases in sensitive versus cisplatin resistant NSCLC cell lines

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Aberrant promoter hypermethylation is a common epigenetic mechanism for the silencing of tumor suppressor genes (TSG) in many types of cancers, including lung cancer. In eukaryotes there are three families of DNA methyltransferase enzymes (DNMT) that catalyzed the DNA methylation process. DNMT3 family is involved primarily in methylation of new sites and the DNMT3B member is more highly expressed in human cancer cell lines and primary tumors than in normal tissue. In addition it has been recently reported a new subfamily of DNMT3B (delta-DNMT3B) that are the predominant forms in non-small cell lung cancer (NSCLC), suggesting an important role in DNA methylation control in lung tumorogenesis. One of the main problems in this tumor type is the frequent development of acquired-chemotherapy resistance. Genetic and epigenetic alterations are known to underlie the initiation and progression of neoplasia, therefore, one of the possible reasons for the development of chemotherapy resistance in NSCLC might be the epigenetic inactivation of certain TSG as a consequence of chemotherapy treatment. In addition, cisplatin (CDDP), the paradigm of cytotoxic drugs for NSCLC treatment, has been reported to induce, de novo DNA hypermethylation in vivo.

We analyzed the potential role of the DNMT family in the development of chemotherapy resistance to CDDP in NSCLC. The study is based on an expression microarray analysis of genes reactivated in a set of CDDP-resistant and sensitive NSCLC cell lines after 5Aza-dC and TSA treatment. Resistant cells were established by treating two NSCLC cell lines, H-460 and H-23, with increasing concentrations of CDDP. Then, cells were exposed to 5Aza-dC (5μM) and TSA (500nM) before RNA extraction in order to reactivate those genes epigenetically silenced in the resistant cell lines. Total RNA from the different cell lines was extracted, reverse-transcribed and hybridized into an array platform containing the whole human genome. The first part of this study is accessible in the abstract presented by I Ibanez de Caceres, in which we show very promising results regarding de novo promoter hypermethylation of specific genes and their relevance in the development of chemo-resistance to CDDP in NSCLC. In order to confirm a possible role of DNMT members silencing the selected genes in chemoresistance, we analyzed the differential expression of the DNMT family on sensitive versus CDDP-resistant cell lines. We found a marked increased expression of DNMT3B in the resistant cell lines compared with the parental ones. We confirmed this result by RT-PCR in both NSCLC cell lines, and in the ovarian human cancer cell lines 41M and 41MR, sensitive and resistant to CDDP respectively. Those results indicate a possible role of DNMT3B on the epigenetic regulation of specific genes responsible of the CDDP-acquire-resistance process; defining possible innovative treatment strategies for platinum resistant tumors.

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EGFR mutation in renal cell carcinoma confers sensitivity to gefitinib treatment

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Intragenic microdeletions and selected missense mutations located within tyrosine domain of epidermal growth factor receptor (EGFR) are known to be associated with the pronounced response to low molecular weight EGFR tyrosine kinase inhibitors (TKI), gefitinib or erlotinib. Unfortunately, these TKI-sensitizing mutations have been detected almost exclusively in lung adenocarcinomas, while their occurrence in tumors of other histological types or other organs is exceptionally rare. We applied EGFR mutation test as the last hope option to a heavily pretreated patient G., 60 years old, who suffered from the progression of renal cell carcinoma (RCC) and was administered to the hospital due to life-threatening condition. Unexpectedly, PCR and sequencing analysis revealed “lung-type” 15 base pair deletion, so the therapy by gefitinib was applied. This treatment led to a dramatic symptomatic response within the first week of therapy; the reduction of dyspnea was so evident that allowed the patient to return to the work. Clinical examination demonstrated complete disappearance of extensive pleuritis and pericarditis. Computed tomography measurement of metastatic lesions revealed minor response which could be classified as disease stabilization (RECIST criteria). The duration of clinical benefit was 4 months. The above observation led to a question, whether EGFR mutations occur at a noticeable frequency in RCC and whether their testing has to be considered in the routine clinical setting. Available literature indicates that only 38 RCC samples have been tested for the presence of EGFR mutations up to now, and one of those contained “lung-type” EGFR deletion. Therefore, we collected 118 RCC cases and subjected the tumor material to molecular analysis. However, none of these tumors contained TKI-sensitizing EGFR mutation. Taken together with published data, the following conclusions can be drawn from this study: 1) By now, 3 cases of gefitinib treatment of EGFR mutation-containing non-lung tumors (1 thymoma, 1 ovarian carcinoma, and 1 RCC from this study) have been reported, and all of them demonstrated evident clinical benefit from the therapy. Therefore, intragenic deletions of EGFR confer sensitivity to TKI treatment independently of the tumor type. 2) EGFR mutations in RCC are rare, thus the utility of the appropriate test for clinical management of kidney cancer remains questionable.

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MLH1 promotor hypermethylation, BRAF and K-ras mutation analysis on tumours suspected from Lynch Syndrome to prioritize mismatch repair gene testing

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Background: Microsatellite Instability (MSI) testing and immuno-histochemistry (IHC) are powerful tools that help identify individuals at risk for having LS and current diagnostic strategies can detect almost all highly penetrant Mismatch Repair (MMR) gene mutations. Our goals were to compare the performance of two panels of microsatellite markers in relation to IHC, as well as BRAF V600E mutation- and MLH1 promotor hypermethylation assays, and the determination of KRAS mutations in the Microsatellite Stable tumours (MSS).

Methods: Patients with a family history suggestive of Lynch syndrome (n=524) in the time period of 2005 till 2007 were tested for MSI and IHC staining of the MMR proteins MLH1, MSH2, MSH6 and PMS2. MSI-high tumours without expression of MLH1 in IHC, were tested for BRAF V600E