of the acquired resistance while MET amplification is responsible for about 20%. Irreversible inhibitors including HKI-272 and PF-299804 can cause growth inhibition in NSCLC cell lines with both the resistance and sensitizing mutations, while gefitinib and erlotinib do not. HKI-272 and PF-299804 entered directed phase I and phase II trials in patients previously treated with gefitinib and erlotinib and mutation testing has been prospectively incorporated into the trials.

Conclusions: Biomarkers of response to EGFR-TKIs have been identified in retrospective studies of patients with non-small cell lung cancer and are now being prospectively incorporated into clinical trials of gefitinib and erlotinib. None of the biomarkers has yet been successful in these prospective trials to identify the subsets of patients who derive clinical benefit from the treatments but we await the results from additional ongoing clinical trials

S24
ERCC1 and response to chemotherapy

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Introduction: Today, cisplatin (and its analogs, carboplatin and oxaliplatin) remain the scaffolding of chemotherapy in many solid tumors including lung, head and neck, bladder, ovarian, and colon carcinomas. After several decades of clinical trials, a therapeutic plateau appears to have being reached with standard chemotherapy in most solid tumors. A re-evaluation of strategies to improve clinical outcomes is needed. At present, research in cancer survival is partly focused on translational pharmacogenetics, with the goal of providing individualized chemotherapy based on different genetic traits, such as polymorphisms, gene mutations, and overexpression of drug target gene transcripts. Also, in some instances, downregulation of crucial gene transcripts has been linked to enhanced chemotherapy response. At this time, one of the most relevant issues for cancer patients is the need for a reliable method to determine which chemotherapy combinations will have better chances of improving survival based on genetic markers. On that regard, defining the predictive and biological determinants of cisplatin response represent an important endeavor. The application of pharmacogenomics to cytotoxic chemotherapy could lead to the development of "individualized" drugs for patients with cancer. Numerous studies have reported the role of ERCC1 expression in the repair mechanism of cisplatin-induced DNA adducts in cancer.

Main Message: Numerous studies have reported the role of ERCC1 expression in the repair mechanism of cisplatin-induced DNA adducts in human ovarian cancer cells, in primary gastric tumors, in colorectal and esophageal cancer. ERCC1 expression has been negatively associated with response to cisplatin or oxaliplatin chemotherapy in gastric and colon cancer. High tumor tissue levels of ERCC1 mRNA in ovarian and gastric cancer patients have been associated with cisplatin resistance.

Taken altogether, these data suggest that ERCC1 is a potentially useful marker for predicting clinical resistance to cisplatin, carboplatin and oxaliplatin. Studies linking ERCC1 to resistance to platinum compounds have been conducted mainly by analysis of RNA or DNA. Nevertheless, recently ERCC1 protein expression was studied in resected NSCLC tumors from 761 patients from the International Adjuvant Lung Trial (IALT). Patients with ERCC1 negative tumors who were randomized to chemotherapy had significantly prolonged survival compared to those who were randomized to observation (test for interaction, $P < 0.009$; $HR = 0.65$; 95% CI [0.50−0.86]). In contrast, there was no survival difference between treated and none-treated patients among ERCC1 positive patients ($HR = 1.14$; 95% CI [0.84−1.55]). It was concluded that NSCLC patients with completely resected ERCC1 negative tumors seem to be stronger candidates for adjuvant cisplatin-based chemotherapy than those with resected ERCC1 positive tumors.

Conclusions: Based on these results, it is very probable that in the near future platinum-based chemotherapy could be chosen according to pharmacogenomic criteria such as ERCC1 expression on tumor tissue. Nevertheless, additional studies are warranted to standardize and optimize methodologies for ERCC1 analysis in tumor samples in order to define a biomarker profile predictive of patient outcome

S25
Gene signatures and response to chemotherapy in breast cancer: statistical artefact or reality?

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Introduction: Systemic chemotherapy for breast cancer improves overall survival, whether given pre-operatively or as post-operative adjuvant therapy. Newer chemotherapy regimens containing taxanes further improve survival compared to standard regimens but taxanes are expensive, toxic and may benefit only a small group of patients. Therefore, identification of regimen-specific predictive factors is a research priority.

Main Message: Several single arm neo-adjuvant chemotherapy trials have reported gene expression signatures obtained from tumour biopsies taken at diagnosis using conventional biostatistic methods (Chang et al. 2003, Ayers et al. 2004, Hannemann et al. 2005, Gianni et al. 2005, Hess et al. 2006, Cleator et al. 2006). Most of these studies reported signatures that predict clinical or pathological response. We will review briefly these studies and discuss their potential weaknesses.

Another approach is to use predictive signatures developed from cell lines (Potti et al. 2006). We used this approach to confirm the ability of these signatures to predict the response to chemotherapy of the ER negative breast tumours within a large series of patients enrolled in a recently completed phase III neoadjuvant trial (Bonnefoi et al. 2007). This sub-study was restricted to ER negative tumours because studies containing both ER positive and ER negative tumours are easily confounded by cell type bias linked to ER status. This trial compares a non-taxane regimen (fluorouracil + epirubicin + cyclophosphamide $\times$ 6; FEC arm) with a taxane regimen (docetaxel $\times$ 3 followed by epirubicin + docetaxel $\times$ 3; T $\rightarrow$ ET arm).

Pathological complete response was used as a surrogate measure of chemosensitivity.
One hundred and twenty-five ER negative tumours (55 pCR) were tested: 66 in the FEC arm (28 pCR) and 59 in the T → ET arm (27 pCR). RNA was prepared from sections of frozen biopsies taken at diagnosis and hybridized to Affymetrix X3P microarrays. In vitro single agent drug sensitivity signatures were combined to obtain FEC and T → ET regimen-specific signatures.
The regimen-specific signatures significantly predicted pCR in patients treated in the appropriate arm ($p < 0.0001$). The FEC predictor had a PPV of 68% (27/40, 52−80%) and NPV of 96% (25/26, 81−99%). The T → ET predictor had a PPV of 71% (25/35, 55−84%) and NPV of 92% (22/24, 74−98%). Analysis of tumour size, grade, nodal status, age and the regimen-specific signatures showed that the genomic signatures were the only independent variables predicting pCR at $p < 0.01$.
Conclusions: We have validated the use of regimen-specific drug sensitivity signatures in the context of a multicentre randomised trial. Selection of patients with these signatures would increase the pCR rate from 44% to around 70% in the patients tested here. The high NPV of both signatures (the NPV for each regimen-specific genomic signature is over 90%) indicates the potential to select patients who should be considered for trials with new agents. Organising clinical trials on this basis would have important implications for the subsequent use of the new agents tested.

S26

High throughput expression studies in primary cutaneous melanoma

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Main Message: Genes identified in a validated and reproducible signature prognosticating metastases or death are mainly associated with replication or DNA repair. For replication, two pathways are over-represented: the replication origins firing genes (ROF) and the separation of sister-chromatids by securin. Poor prognostic melanomas are characterized by a global overexpression of ROF-related genes. MCM-4 and MCM-6 immunoexpression is strongly correlated with metastasis free survival and OS. This prognostic value is maintained when age, sex, location of the primary tumor, thickness and ulceration are introduced in the multivariate model. The whole ROF system is locked by geminin that complexes CDT1 and CDC6. When CDT1 and CDC6 are released, they can recruit MCMs at the replication origins. When this interaction is altered, for instance when BRCA1-IRIS relieves geminin-CDC6 interaction, the helicases cascade becomes overactive leading to replication increase. hPTTG gene, coding for securin, is among the top genes of the prognostic signature. Securin has three known activities: it blocks the sister-chromatids separation in stabilizing separase, it stimulates angiogenesis and it decreases p53 transcription. Securin acts as an oncogene and provides a positive growth advantage as it downregulates sister-chromatids separation and therefore avoids the cells to enter into aneuploidy. P53 transcription inhibition leads to a decrease in p53-mediated apoptosis. Securin immunohistochemical expression is observed in vertical growth phase whereas melanomas in radial growth phase do not express securin.
Overexpression of DNA-repair genes is associated with metastases or death. Increase in DNA repair capacity could explain spontaneous resistance of most melanomas toward radiotherapy and alkylating agents. In this DNA repair genes list, NER and BER family genes are not represented. On the contrary, most of the DNA repair genes present in this group are involved in post-replicative repair of DNA lesions. This is in accordance with the hypothesis that aggressive melanomas need a fast and effective replication, and need to repair mistakes induced during replication. One of the genes of which overexpression is most evidently associated with poor prognosis is topoisomerase 2A (Top2A). Top2A codes for an enzyme that is essential for replication and chromosomal segregation. Actually Top2A expression seems to be a consequence rather than a cause of cell proliferation. However, cells that overexpress Top2A, and PCNA with which it interacts, are much more resistant toward alkylating agents.
In parallel with expression genomics studies, CGH array studies have provided important informations to refine the melanoma classification. The expression signature associated with B-Raf mutations reveals a strong association between B-Raf mutation and CD63 overexpression ($p = 10^{-14}$!). These results and their consequences will be discussed during the presentation as well as the intergration of expression and aCGH data.