Material and Methods: We set a retrospective study including 13 patients with resectable NSCLC and N2 involvement. All patients underwent pre therapeutic mediastinal lymph node biopsy, neoadjuvant cisplatin-based chemotherapy, and complete resection of the primary tumor associated with mediastinal lymph node dissection. C-kit expression was measured by immunohistochemistry on lymph node biopsies and primary lung tumors. Immunopositive cells were counted and expressed as a percentage of tumor cells. The intensity of immunostaining was categorized as follows: 0, negative; +, low; ++, moderate; and +++, high.
Results: On pre therapeutic mediastinal lymph node biopsy, c-kit expression was found in one patient (1/13 = 7%), quantified as 30% of tumor cells, with low immunostaining intensity. On post chemotherapy lung tumor, c-kit expression was detected in 4 patients (4/13 = 30%), in 5 to 100% of cells, with low to high immunostaining intensity. Difference in c-kit expression between pre therapeutic mediastinal lymph node biopsy and post chemotherapy lung tumor was significant (McNemar chi-square test, P = .0455). Interestingly, the four patients with positive post chemotherapy lung tumor had negative pre therapeutic mediastinal lymph node biopsy.
Conclusion: Level of c-kit expression on pre therapeutic mediastinal lymph node biopsy does not predict its level of expression on post chemotherapy primary lung tumor. Additional studies should determine whether this discrepancy is linked to tissues heterogeneity or to neoadjuvant treatment.

642 POSTER

Organic anion transporting polypeptides contribute to prostate cancer progression

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The OATP family of transporters has been previously studied for their roles in drug elimination and pharmacodynamics. Recently, polymorphisms in family members have been implicated in prostate cancer disease progression and survival. The current hypothesis is that they effect clinical outcome by transporting growth hormones or chemotherapy agents. However, little is known about the prevalence and cause of OATP expression in cancer. Therefore, we completed a study on 321 primary tissue samples from 21 normal and cancerous patient samples examining the expression of three family members implicated in cancer; OATP1B3, OATP1B1 and OATP2B1. The results showed that OATP2B1 is more ubiquitously expressed in all tissues than OATP1B1 and OATP1B3. Based on expression frequency, OATP2B1 could be significant in or used as a biomarker for lymphatic and thyroid cancer. In contrast, OATP1B1 is expressed exclusively in some primary cancer tissue samples, but at a much lower frequency (<20% in 8 cancers) than both OATP1B3 and OATP2B1. OATP1B3 was also expressed in fewer normal tissue types, however it was expressed in 50% of cancerous prostate samples and there is a trend of increasing OATP1B3 expression with higher Gleason score. This supports previous data suggesting a role of OATP1B3 in advancing prostate cancer. Further experiments from quantitative PCR and western blot suggest that hypoxia elements in the OATP1B3 promoter are activated under tumor conditions and explain the increased expression in tumor cells. In addition, transport studies in *Xenopus* oocytes showed that optimal transport occurs at low androgen levels such as those seen in patients after androgen deprivation therapy. In summary, upon examining the expression of OATP1B3, OATP1B1, and OATP2B1 in primary tissue samples, there appear to be several cancers for which they may be progression or cancer biomarkers and warrant further study.

643 POSTER

Phosphorylated histone H3 and S6 proteins as biomarkers for targeted anti-cancer drug action measured using a combined IHC/Western method in skin biopsies

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In recent years there has been a rapid increase in targeted tumor therapy approaches. In contrast to classic chemotherapy, which non-specifically targets proliferating cells, these novel approaches attempt to specifically interfere with processes thought to be crucial for the tumor cell survival. Nevertheless, despite extensive validation, an immediate effect of target interference on tumor growth may not necessarily be expected. Thus, in order to demonstrate activity of these substances, a marker known to be modified upon inhibition of the target may serve as a surrogate to tumor inhibition. Taking in consideration the difficulties to perform sequential tumor biopsies, the use of surrogate tissues like blood or skin is being explored instead. Blood is relatively easily accessible, but unlike skin tissue, blood may not reflect the fact that drugs have to penetrate multiple layers of tissue in order to reach their target in the tumor. Also, other than cells in the dermis, which still proliferate, peripheral blood cells have largely exited the cell cycle. Effects of anti-mitotic drugs may therefore not easily be demonstrated. We have therefore developed a method which reproducibly allows the detection and quantification of potential target proteins via Western Blots and immuno-histochemistry (IHC) from human skin biopsy halves.
We looked at two phosphorylated marker proteins, phospho-histone H3, and phospho-S6. Histone H3 is phosphorylated on Ser 12, mainly by aurora B, which is a bona fide cancer drug target. It is thus a good marker for inhibition of aurora B kinase activity, but may also serve, albeit in a less direct manner, as a marker for cells arrested in M-phase, since this phosphorylation event is closely linked to chromosome condensation. S6 is the downstream target of p70S6K, which in itself may be a drug target, but lies downstream of AKT and mTOR kinases, both of which have been, and still are, exploited as drug targets.
In order to evaluate the above biomarkers, we employed this method using skin biopsies of mice treated with a variety of kinase inhibitors, i.e. Nexavar, Tozasertib, Sunitinib, and Everolimus. Data will be presented on the effect of H3 and S6 phosphorylation. Furthermore, data on the measurement of these proteins, and the stability of the signals in human biopsies from reduction surgery skin folds will be presented.
This is a versatile method to measure biomarkers to characterize the specificity of novel targeted drugs.

644 POSTER

Signaling pathways contributing to head and neck carcinoma radioresistance

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Radiation therapy plays an important role in the management of head and neck carcinoma. However, the problem of radioresistance and molecular mechanisms by which head and neck cancer cells overcome cytotoxic effects of radiation therapy remains to be elucidated. In order to investigate possible intracellular mechanisms underlying the head and neck cancer recurrences after radiotherapy, we have established three radiation-resistant squamous cell carcinoma cell lines, CAL27-IRR, SCC25-IRR and FaDu-IRR derived from the parental CAL27, SCC25 and FaDu head and neck cancer cells by repetitive exposure to ionizing radiation (summary dose was 100 Gy). CAL27-IRR, SCC25-IRR and FaDu-IRR (IRR cells) demonstrated pronounced radioresistance, enhanced oxygen consumption and activated epidermal growth factor (EGF) receptor related pathways, such as Ras-MAPK and PI3K-Akt and Jak-STAT. In order to elucidate additional mechanisms involved in the radioresistance development and increased oxygen consumption, we determined differences in the proteome profile of parental and IRR cells using two-dimensional differential gel electrophoresis (2-D DIGE) followed by computational image analysis and mass spectrometry. It was found that identified proteins were involved in the regulation of intracellular routs providing cell survival, release of angiogenesis-related factors, increased motility and invasiveness, enhanced mutagenesis, DNA repair and regulation of glycolysis. Our data suggest that some of the found proteins could be considered as potential biomarkers of head and neck cancer radioresistance and/or targets to improve radiotherapy outcome.

645 POSTER

External quality control for companion molecular diagnostics using cell lines

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Background: The use of companion diagnostics to ensure that cancer patients get optimal treatment is increasing, yet the development and implementation of such diagnostics often lags behind the development of new anti-cancer drugs. A prime example is the use of EGFR and KRAS mutation testing in lung and colorectal cancer respectively. Implementation to good laboratory practice standards is usually a requirement for laboratory accreditation and this needs quality assurance – both internal and external. The use of human tumour samples is difficult for many reasons, not least because of the variation inherent in such samples which renders them relatively poor controls. We have therefore developed a method using cell lines for a national external quality assurance scheme (NEQAS) which is also suitable to assist development of the tests themselves.
Materials and Methods: Cell lines containing specific EGFR and KRAS mutations were obtained from Horizon Discovery Ltd (Cambridge, UK) and passaged 2−3 times until sufficient cells were available to make fibrin (cytoclot) or agar embedded cell pellets. These were embedded in paraffin

wax using a Leica ASP or Pelorus system with >1 hour formalin fixation. Sections of these were then cut at 10 microns and four sections pooled for DNA extraction, performed using a Ambion Recoverall™ total nucleic acid isolation kit optimised for FFPE samples. Quantitiative PCR detection of mutations present was performed using the ARMS Scorpions kit (DxS/Qiagen, Manchester, UK) according to the manufacturer's instructions with an AB7500 PCR machine (Applied Biosystems Inc, Foster City, CA).
Results: Sufficient DNA recovery was obtained using both processing methods, despite the higher temperature and xylene free processing used by the Pelorus, though slightly higher recovery was observed using the ASP processor. Histology of the cell line pellets is feasible and routinely performed for each pellet, and the results can be rapidly compared with response to drugs or candidate molecules in vitro.
Conclusion: Cell lines containing specific integrated mutations provide an ideal alternative to tumour samples for target validation and drug discovery, to which one can also now add companion diagnostic design, initial validation, and quality assurance.

646 POSTER

Identification of biomarkers associated with tumor progression using laser microdissected tissues from colon adenoma and cancer

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Background: Colorectal (CRC) malignancies rank worldwide at third place for tumor diseases and account for an annual mortality rate of 492 000 cases. Although several molecular events are known to be involved in the transition from normal tissue to adenoma and finally to undifferentiated carcinoma it remains a challenge to discover new and more reliable biomarkers for diagnosis, prognosis and prediction of outcome. Towards this end a study was designed to identify potential biomarkers which are associated with the molecular events leading from epithelial adenoma to the early stages of carcinoma.
Material and Methods: A new biomarker discovery strategy was developed to combine the cell specificity and the selectivity of laser capture microdissection (LCM) with the resolution power and sensitivity of liquid-chromatography (LC)-matrix-assisted-laser-desorption/ionization mass spectrometry (LC-MALDI-MS). We carefully selected a group of closely matched patients (n = 10 for each group) afflicted with epithelial adenoma (high dysplasia) or early stages of carcinoma (stage I) and used the derived normal as well as the matched tumor tissue samples to reveal protein expression differences. According to this LC-MALDI-MS strategy microdissected cells were lysed and extracted proteins were digested with trypsin. Obtained peptides were separated by capillary reversed phase HPLC (Agilent). The resulting LC-fractions (300) were spotted on prespotted AnchorChip targets (PAC, Bruker) and tryptic fragments subsequently detected by reflector MALDI-MS (ultraflex III,Bruker) measurements. Differential peptide analysis was performed to discover robust and significant expression differences between patient groups. Therefore, only m/z ions displaying a minimum twofold difference and a p-value of 0.01 between groups were considered for further analysis. The selected peptides were subsequently fragmented by MS/MS experiments to reveal their primary sequence and protein identity.
Results: Up to 7000 ion signals ranging from m/z 800 to 4000 were generated and used for statistical analysis. Our targeted biomarker discovery approach resulted in the identification of more than 30 biomarker candidates.
Conclusion: The identified biomarker candidates are involved in diverse cellular functions and can now probably be linked to early or late events of tumor progression. Currently these biomarkers are being validated using antibody based assays to further analyze their potential as markers in a clinical setting.

647 POSTER

Detection of miR-302, an ES-specific microRNA, in cancer cell lines and tissues

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Background: The miR302–367 is a cluster of microRNAs that are exclusively expressed at high levels in embryonic stem (ES) cells. Indeed, miR-302–367 cluster may play an essential role in maintaining hESC pluripotency and self-renewal. In addition, promoter of miR302–367 is transcriptionally regulated by the ES cell master regulators, e.g. Oct3/4, Sox2, and Nanog (OSN). Previous studies indicated that expression of OSN could be detected in tumor samples. Therefore, expression of miR302s as a downstream component of OSN is also conceivable in the cancer cells and has the merit of being studied as a potent tumor marker.
Material and Methods: Generally, amplification and detection of microRNAs by PCR is not straightforward, due to their short lengths (20−24nt). In addition, high similarity of miR302-family members, make it difficult to specifically detect individual members. In the current study, a stretch of A-nucleotides were added to the 3′-end of the extracted RNAs by using poly-A polymerase. cDNA was then synthesized using an oligo-dT primer that was anchored to a tag sequence on its 5′-end. The tag could be used as a reverse primer in the subsequent stages. Additionally, the forward primer was selected such that it could specifically amplify miR302b.
Results: Specificity of the PCR was examined using a vector containing miR-302a, miR-302c, and miR-302d but not miR-302b. Based on our data, the miR302b-PCR system was specific, at least in the presence of 6×10^6 copies of the vector in 45 cycles. Consequently, the system was evaluated in different tumor samples and cell lines. Interestingly, the expression of miR302b was detected in some brain and bladder tumor cell lines in addition to tumor samples.
Conclusion: Recently, we have shown the functionality of miR302–367 promoter in a rare sub-population of brain tumor cell lines. The results of the current study also demonstrated a low expression level of miR-302 in cancer cells. These results may indicate that upstream regulators of miR-302, namely OSN, are expressed in a rare sub-population of cancer cells. In other words, an ES-like expression pattern and stemness properties may exist in this sub-population of putative cancer stem cells.

648 POSTER

Ex vivo assay to monitor response to chemotherapeutic agents in plucked human hairs

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Background: Plucked hair is a valuable surrogate biomarker tissue to monitor pharmacodynamic (PD) responses. Hair collection is also minimally invasive, simple and permits frequent sampling. We have previously developed immunohistochemistry (IHC) labeling protocols for plucked human scalp hair, enabling target response to treatment to be monitored directly. We now present an assay for the *ex vivo* maintenance, treatment and labeling of human hair. This provides effective proof of concept data for the detection in hair of a target protein response, before progressing to a clinical trial.
Methods: Donor hairs were plucked and immediately transferred to a maintenance medium containing vehicle or 200 nM Tarceva for 2 or 10 mins before being harvested and fixed (5 hairs per donor, per treatment) along with freshly plucked untreated hairs (5 per donor). Hairs were then embedded in wax, sectioned and IHC labeled for phospho-ERK1/2 (p-ERK1/2) using methods previously developed. The amount of p-ERK1/2 present was quantified using the Ariol® slide scanning system.
Results: In fresh hairs the p-ERK1/2 was restricted to distinct bands across the outer root sheath (ORS) of the hair. In control hairs maintained *ex vivo*, there was some diffusion of the banded labeling after 2 mins and complete diffusion after 10 mins, with labeling then presenting throughout the ORS. Labeling in hairs treated for 2 mins with Tarceva was similar to that of hairs treated with vehicle for 2 mins, whilst labeling was greatly reduced in hairs treated for 10 mins with Tarceva compared to 10 min vehicle treated hairs. Labeling was quantified on the Ariol® scanning system and after 10 min treatment with Tarceva a significant 73%, 59% and 61% decrease in donor 1, 2 and 3, respectively, was observed compared to 10 min vehicle treated hairs (Students *t*-test: donor 1 p = 0.0002, donor 2 p = 0.01, donor 3 p = 0.007).
Conclusion: This preliminary data indicates good reproducibility in the *ex vivo* hair assay and a significant reduction in p-ERK1/2 levels following 10 min Tarceva treatment. The *ex vivo* hair assay provides valuable proof of concept biomarker data, prior to collection of hairs within a clinical study, confirming whether the hairs are suitable surrogates for the target protein of interest.

649 POSTER

Epidermal growth factor receptor (EGFR) gene amplification is not the cause of protein overexpression in penile carcinoma

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Background: Squamous cell carcinoma of the penis affects mainly people with poor hygiene habits in undeveloped countries. Epidermal growth factor receptor (EGFR) is a well characterized tyrosine-kinase receptor that has