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.

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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.

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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