of time to progression and survival. This prediction algorithm was then validated in a blinded manner in two independent cohorts of NSCLC patients treated with EGFR TKIs. This classification algorithm did not predict outcome in three independent cohorts of patients who did not receive treatment with EGFR TKIs.
Thus, if upheld in prospective clinical trials, this analysis of pre-treatment peripheral blood might be useful in selecting therapy for advanced non-small cell lung cancer patients. We are currently in the process of testing this signature in sample sets from past randomized clinical trials, and a prospective trial is underway. New technologies, such as shotgun proteomics, we are now able to achieve a depth of information comparable to expression microarray analysis, with improving reproducibility. This is allowing for the more practical analysis of single samples, and definition of activated pathways in tumor cells in real-time. Direct quantitation of specific peptides of interest in the serum as candidate biomarkers can also be achieved. It is likely that as the technology improves, proteomic signatures of cancer will be a significant source of information enabling the development of clinically useful individualized of risk assessments and therapeutic decision-making.

SP158
The use of autoantibodies in the early detection of cancer

C. Chapman. *The University of Nottingham, UK*

Measurement of cancer associated antigens (eg CA15-3, CA125, CEA) in serum are often performed but, as they are essentially markers of disease bulk, they are of limited use in the early identification of a cancer. Early detection, at a stage when the tumour is still localised and treatable, is the goal of any screening tool and new approaches are required which do not rely on already circulating tumour cells, or disease bulk.
Cancer cells often present a number of novel, aberrantly expressed or mutated proteins, or even abnormally large amounts of normal proteins. The immune system is uniquely adapted to detect such changes and even small quantities of such proteins can lead to the production of a specific immune response in the form of specific autoantibodies. A very small tumour bulk, that could not be measured using conventional tumour marker assays, could therefore be identified following measurement of such antibodies.
Due to the heterogeneous nature of most solid tumours the measurement of autoantibodies to only one cancer associated antigen is unlikely to be sufficiently sensitive to make this approach useful as a screening test. Whereas measurement of autoantibodies to a panel of such antigens, if correctly managed, could provide a simple tool that is both sensitive and specific.
Autoantibodies to cancer antigens have been shown to be detectable in a number of different solid tumours. In some cases these autoantibodies have also been identified 4−5 years before the cancer could be diagnosed using more routine methodologies (eg mammography for breast cancer and CT for lung cancer).
Recent work has reported that approximately 40% of lung cancers can be detected by measuring autoantibodies to a range of tumour associated antigens, when compared to an age, gender and smoking matched group of 'normal' individuals (with a 90% specificity). This panel identified both small cell (SCLC) and non-small cell lung cancers (NSCLCs) and also picked up both early and late stage disease. Work is ongoing to try to identify a different panel of antigens which will be useful in the earlier detection of other solid tumours like breast, colorectal and hepatocellular carcinomas.
It will also be interesting to determine whether measurement of such antibodies following surgical resection and treatment, may also provide prognostic information for the clinician.

SP153
Stem cells and breast cancer: treatment resistance, markers and novel therapeutic targets

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There is emerging evidence that breast cancer stem cells (CSCs) are resistant to radio, chemo and endocrine therapies suggesting that CSC-specific treatments are needed. We investigated breast CSCs and established that breast cancer cell lines and primary tumours contain a CSC population that can be enriched for using cell surface markers such as ESA+CD44+CD24low.
Due to their relative insensitivity to treatment, we and others have demonstrated that CSCs are also enriched by radio, chemo and endocrine therapy. Increases in the proportion of CSCs after therapy is measured using the above markers and mammosphere colony assays of stem cell activity. DNA repair, survival and stem cell signalling pathways are strong emerging candidates for the underlying mechanisms of resistance.
With regards to endocrine treatment, we have established that CSCs in oestrogen receptor-β-positive (ER+) breast cancer are ER− and therefore inherently resistant to the direct effects of endocrine therapies. However, CSCs still respond to therapy-induced changes in microenvironmental signals. One candidate pathway known to regulate normal stem cells is Notch receptor signalling.
The Notch pathway comprises five secreted ligands, Jagged1/2 and Delta-like 1/3/4 and four receptors, Notch1−4. In breast cancer, we have shown that this pathway is activated by oestrogen and inhibited by tamoxifen and faslodex. We therefore investigated Notch receptor signalling within the CSC population and tested the effects of Notch inhibition on stem cell activity in breast cancer.
We have evidence that activated Notch4 is higher than activated Notch1 in CSCs, compared to the differentiated populations. Notch inhibition using gamma secretase inhibitors (GSI) had no significant effect on the cleavage of the Notch4 receptor but potently inhibited signalling through Notch1 receptor. GSIs caused decreased CSC activity in vitro, and reduced the growth of MCF7 and MDA-MB231 tumours by up to 50%. However, blocking all four Notch receptors using Numb cDNA or specific knockdown of Notch4 using shRNA completely prevented breast tumour formation.
Our findings indicate that Notch4 plays a key role in tumour initiation by CSCs while Notch1 is more active in differentiated proliferation. Thus, therapies targeting Notch4 receptor are likely to be more effective in preventing treatment resistance than those targeting Notch1.

SP165
Optimizing information obtained from fine needle aspiration (FNA) biopsies

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Fine Needle Aspiration Biopsy (FNAB) is a minimally-invasive and cost-effective method for sampling human tumors that is widely used around the world. Historically, FNAB samples have provided adequate material for microscopic examination; however, the successful development and application of molecularly targeted agents (MTAs) against cancer will also demand the robust and reliable detection of novel molecular biomarkers in FNAB samples. Molecular characterization of FNAB samples has been relatively limited and typically confined to a single molecular marker analyzed in a fixed sample. Expansion of such studies to more comprehensive analyses, such as gene expression profiling or multiplexed protein arrays, would significantly enhance cancer research and clinical diagnostics. However, such studies will require preservation of biospecimen "information content" through specialized specimen handling as well as sensitive, multiplexed analytical platforms.
FNA samples offer several advantages over surgically-excised or core biopsy samples: 1) Obtains viable cells; 2) Allows immediate assessment of specimen for adequacy; 3) Minimal preanalytical variability; 4) Can be performed repeatedly over time, permitting temporal studies within a single animal or human; 5) Less invasive and more cost-effective than surgical excisional biopsies. Challenges to the molecular analysis of FNAs include the small number of cells and the heterogeneity of the cellular composition. Potential technological solutions to these challenges will be presented.
One additional opportunity presented by the FNA sample is functional profiling of live cells through ex vivo biomarkers. The term "ex vivo biomarker" has been used to define a novel class of biomarkers − those which are evoked by live tumor cells after they have been removed from the patient. This involves removing viable cells from a patient through an FNA then stimulating the cells in vitro with growth factors that are relevant to the signal transduction networks targeted by MTAs. The biomarkers are typically newly modified phosphoproteins or newly expressed mRNAs in the signaling network. Such assays offer exciting possible applications: 1) patient stratification based on functional information to inform clinical trial design or clinical management; 2) novel pharmacodynamic assays for use in the development of targeted therapies.

SP175
Application of high resolution mass spectrometry for cancer biomarker discovery and validation

T. Conrads. *University of Pittsburgh, USA*

Developments in high resolution mass spectrometry (MS) and nanoflow chromatography have made possible high-throughput proteomic investigations of myriad clinically relevant samples in the expectation of identifying peptide or protein biomarkers for disease. Conventional protein biomarker discovery investigations are predominantly performed with samples such as serum or plasma. While serum or plasma samples may be more desirable from a clinical standpoint, tissue likely possesses a greater abundance of readily identifiable proteins directly reflective of disease. This lecture

will highlight the application of laser capture micro-dissection and high resolution MS for conducting proteomic investigations of formalin-fixed paraffin-embedded archival tissue for cancer biomarker discovery and validation.

SP156
New targets and cancer therapy: successes and failures

J. Dancey. *OICR, Canada*

In 1998, trastuzumab's approval for the treatment of HER2 postive metastatic breast cancer patients, successfully launched the era of targeted therapy and beginnings of the concept of personalized medicine. Within the decade additional successes have occurred: imatinib, initially approved in 2001 for the treatment of chronic myelogenous leukemia and since approved for the treatment of gastro-intestinal stromal tumours, systemic mastocytosis, idiopathic hypereosinophilic syndrome, dermatofibrosarcoma protuberans; the fall and rise of epidermal growth factor receptor inhibitors in lung, head and neck, and colorectal carcinoma, and vascular endothelial growth factor ligand and receptor inhibitors renal cell carcinoma. More recently, striking activity has been seen in early phase trials for inhibitors to PARP in BRCA deficient tumours and and triple negative breast cancer, to EML4-ALK translocations in NSCLC, to BRAF mutations in melanoma and to Hedgehog in multifocal, metastatic basal cell carcinoma, and to JAK2 in myelofibrosis. Swift drug development can occur when there is a successful linkage between a pharmacologically sound drug that effectively interacts with its target, target activation is a significant contributor to the malignancies of trial patients and that there is an accurate means for identifying such patients. Results with imatinib and trastuzumab, which has recently been shown to improve survival in HER2+ gastric carcinoma patients, suggest that activation due to mutations or amplification correlate with activity across histologies. Results from targeted agents also suggest that mutations within and between pathways are often mutually exclusive, activation of a specific target may correlate with activity for the target specific agent and resistance to other agents to targets that are upstream or in parallel pathways. Unfortunately, the simple linkage of good drug, good target and good test has remained elusive for many agents. Our challenge is narrow the gap between cancer biology, drug, and diagnostic test discovery and evaluation. Clinical trials and studies need to be conducted efficiently in rare tumours − to look for genetic links and activity across histologically and molecularly defined subsets. Further integration of activities in cancer target identification and validation, drug and diagnostic test development, is required for the efficient and ultimately successful cancer therapeutics.

SP147
Pharmacogenomics in pancreatic cancer

R. Danesi. *University of Pisa, Italy*

Cancer of the pancreas is a relatively common malignancy and a leading cause of cancer related deaths. Progress in diagnosis and treatment has been disappointing but improvement in understanding of pathogenesis and of molecular changes may offer some ground for rational and etiological approach [1]. The first evidence about the benefit of targeting dysregulated pathways was provided by the study on the addition of the EGFR inhibitor erlotinib to gemcitabine. Since then, despite other numerous negative studies, various agents have been investigated in the preclinical and clinical setting and are currently through drug development pipeline. Advances in the understanding of pancreas cancer biology have been made over the past decade, including the discovery of critical mutations in oncogenes (i.e., K-Ras) as well as the loss of tumor suppressor genes, such as TP53 and p16(INK4). Other studies showed the dysregulation of the expression of proteins involved in the control of cell cycle, proliferation, apoptosis, and invasiveness, such as Bcl-2, Akt, mdm2, and epidermal growth factor receptor. These characteristics might contribute to the aggressive behavior of pancreatic cancer and influence response to treatment [2]. Indeed, the inactivation of p53 may explain the relative resistance to 5-fluorouracil, whereas Bcl-2 overexpression is associated with reduced sensitivity to gemcitabine. However, the future challenge of pancreas cancer chemotherapy relies on the identification of molecular markers that help in the selection of drugs best suited to the individual patient. Recent pharmacogenetic studies focused on genes encoding proteins directly involved in drug activity, showing the role of human equilibrative nucleoside transporter-1 as prognostic factor in gemcitabine-treated patients [3]. Finally, inhibitors of signal transduction and angiogenesis are under extensive investigation, and several prospective trials have been devoted to this area. Pharmacogenetics is likely to play a central role in the personalization of treatment, to stratify patients based on their likelihood of response to both standard and targeted treatments. Thus, molecular analysis should be implemented in the optimal management of the patient affected by pancreatic adenocarcinoma.

References

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SP157
Informed consent for future research: how much can/should we ask upfront & afterwards

E. Eisenhauer. *NCIC CTG, Queen's University, Canada*

In cancer research, studies of biological samples hold great promise for identifying new targets, understanding mechanisms of action/resistance to therapy and, when linked with clinical data, prognostic and predictive factors for treatment selection. Issues related to informed consent for research on biological samples include: whether consent is required at all, the nature of research to be conducted, mechanisms for assuring confidentiality, how/if patients will receive results of the study(ies), withdrawal from the study, and oversight mechanisms for scientific and ethical review of research. There are variations in ethical guidelines and legal requirements from country to country that can affect the consent templates. This presentation will focus on tissue collections undertaken as part of a prospective research project (e.g. clinical trial or biobank) where a consent process will have taken place to address many of the key issues noted above. One problem that arises is how to handle the situation where research other than that initially agreed to by the patient is now proposed? In the biobanking situation this should be a rare phenomenon if the collection is obtained from patients appropriately consenting to a wide array of future research. However, many collections from clinical trials, particularly those from 5−10 years ago, obtained consent for an explicit research question and did not reference future use of tissue. In these circumstances, guidance must be sought not only from the legal regulations in the country(ies) from which the samples were obtained, but also from the ethical committee for the project. In some cases, the "new" project is simply an extension of the initial one; e.g. a patient has consented to EGFR expression studies in tissue and now FISH and mutational studies are proposed. In this example, re-consent is seldom required since the new project is in keeping with the original intent, and it carries no new risks to patients. If, however, the proposal is for genetic testing for cancer susceptibility genes, re-consent would be required in most jurisdictions. Interestingly, empiric studies of patients who have consented to tissue studies show most would agree to secondary research use provided confidentiality is maintained. Many now believe that, to avoid such problems, consent forms should state clearly the possible future uses for the collected samples and allow patients to authorize (or refuse) future research.

SP174
Gene signatures: Are we ready to change clinical management in cancer patient treatment?

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Introduction: Gene expression can be used to understand the biology and anticipate the clinical behavior of cancer. While effective for discovery, expression microarrays present significant challenges for clinical application.
Purpose: Review the approach to developing and validating gene signatures. Underscore specific successes and highlight their potential to improve cancer care. Discuss current trials and available tests that use gene expression signatures to guide therapy. Identify the important elements required for successful clinical application of gene signatures.
Main message: Successful application of gene signatures requires rigorous standards, meticulous execution, and the adoption of standard operating procedures so as to ensure robust and reproducible application to clinical samples.
Conclusions and Recommendations: While representing a significant challenge, gene signatures can be used to guide therapy. Development and application of gene signatures to guide clinical care requires a team approach and investigators with complementary expertise including oncology, molecular biology, pathology, laboratory medicine, and biostatistics.

SP168
Clinical relevance of circulating tumor cells (CTC) in primary and metastatic breast cancer

T. Fehm. *Dept. Gyn/OB, Germany*

A subclinical tumor cell spread can be assessed in breast patients with the detection of disseminated tumor cells (DTC) in bone marrow aspirates or