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

R. Clarke. *Paterson Institute, University of Manchester, UK*

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

D. Clark. *Johns Hopkins Medical Institutions, USA*

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