relative with breast cancer was detected 2.08 (95% confidence interval [CI], 2.0−2.2) in young women (<50 years) and it is dramaticaly decreased by age. The findings of the present study suggest that family history and age may have an impact on the incidence of breast cancer in Iranian women. Our analysis shows testing of 5382insC mutation in breast cancer can be utilized as one of prognosis factors of FBC development risk in combination with ER, PR and TP53.

P26

Tumor infiltrating lymphocytes in medullary breast cancer

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Background: Medullary breast cancer (MBC) has despite a high growth rate and anaplastic features a better prognosis compared to other types of breast cancer with a similar malignancy grade. Tumor infiltrating lymphocytes (TILs) is one of the characteristic features of MBC and it has been suggested that TILs contribute to the favorable prognosis. The immune response in MBC is thought to be TH1-based with high numbers of cytotoxic T lymphocytes (CTLs), but also includes significant numbers of plasma cells. Little is known about the signal pathways that is activated in MBC TILs.
Methods: TILs were isolated from tissue sections of 7 MBCs using laser capture microdissection. RNA was isolated from the TIL samples, amplified through two rounds including biotin labeling and genome-wide gene expression profiles were obtained by hybridization to HG-U133 Plus 2.0 GeneChips. Similarly, gene expression profiles were obtained from 5 samples of morphologically normal lymph nodes. Data analysis was carried out using the dChip software and the R programming package. Associations to certain functions or pathways were explored with the Ingenuity pathway analysis software.
Results: In all 600 genes were identified as significantly differently expressed (false discovery rate below 0.01). Of these 148 genes were upregulated and 452 genes were downregulated in TILs of MBC compared to normal lymph nodes. Among the upregulated genes functions associated with chemotaxis, homing and activation of lymphocytes, cytotoxicity of cells and cell death of T lymphocytes were identified as important functions. The genes that are downregulated in TILs were associated with early parts of the immune response such as development of lymphocytes.
Conclusions: Identification of genes upregulated in MBC TILs compared to normal lymph nodes showed that activated lymphocytes are present in the tumors and more specifically cytotoxic activity was seen. However, at the same time signs of termination of the immune response due to apoptosis of T lymphocytes was seen.
The apparent attraction of lymphocytes to the tumor and cell death of lymphocytes at the same time could indicate that the immune system is able to recognize the tumor but not mount an effective immune response due to suppression by the tumor.
Further studies comparing the gene expression profiles of MBC TILs with TILs isolated from other types of breast cancer will be performed.

P35

Phage display-derived human scFv antibodies isolated by binding to live primary breast cancer cells recognize GRP78

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Background: Clinical trials using monoclonal antibodies against cell surface markers have yielded encouraging therapeutic results in several cancer types. Generally, however, anti-cancer antibodies are only efficient against a subpopulation of cancers, and there is a strong need for identification of novel targets and human antibodies against them.
Methods: We have isolated single-chain human monoclonal antibodies from a large naive antibody phage display library by panning on a single-cell suspension of freshly-isolated live cancer cells from a human breast cancer specimen, and these antibodies were shown to specifically recognize cancer-associated cell surface proteins.
Results: One of the isolated human antibody fragments, Ab39, recognizes a cell surface antigen expressed on a subpopulation of cancer cell lines of different origins. Immunohistochemical analysis of a large panel of cancerous and normal tissues showed that Ab39 bound strongly to several cancers, including 45% breast carcinomas, 35% lung cancers, and 86% melanomas, but showed no or weak binding to normal tissues. A yeast two-hybrid screen of a large human testis cDNA library identified the glucose-regulated protein of 78 kDa (GRP78) as the antigen recognized by Ab39. The interaction was confirmed by co-localization studies and antibody-competition experiments that also mapped the epitope recognized by Ab39 to the COOH-terminus of GRP78.
Conclusions: The expression of GRP78 on the surface of cancer cells, but not normal cells, makes it an attractive target for cancer therapies, including monoclonal antibody-based immunotherapy. Our results suggest that the human antibody Ab39 may be a useful starting point for further genetic optimization that could render it a useful diagnostic and therapeutic reagent for a variety of cancers.

P55

Molecular diagnostics evaluation laboratories (MoDEL), a program to optimize assays for clinically useful cancer biomarkers

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Background: The Cancer Diagnosis Program (CDP) facilitates development of in vitro diagnostics (IVDs) to aid clinical decision-making forcancer patients. CDP pilot projects suggested that there are a number of barriers preventing effective development of diagnostic assays. Therefore, a Request for Information (RFI) was sent to the extramural community to define criteria for a program to help overcome these barriers.
Methods: The RFI was published in the NIH Guide with a range of questions about assay development, optimization and barriers to evaluation of clinical utility. In addition, e-mail solicitations were sent to translational researchers and small businesses.
Results: Responses were received from more than 50 investigators: 84% from academics, the others from small businesses or national laboratories. The respondents indicated that 90% of their assays were prognostic, 50% predicted response to therapy and 16% predicted adverse effects of therapy. Over 60% of assays measured proteins by either ELISA or immunohistochemistry while a third were RNA or DNA-based. Most of the assays/IVDs were in research laboratories. However, 16% have attained Level I−II evidence of clinical utility, and 11% were either performed in a CLIA-certified laboratory or used a commercial kit. Respondents identified resources needed to overcome barriers to effective assay development. These included the need for better access to tissue resources with more complete clinical annotation; assistance with reagent development and assay platform optimization; and statistical assistance and help with study or clinical trial design. The respondents indicated their plans for continued development of their assay/IVD included assessment in a definitive clinical trial or licensing for commercial development (64%) or offering the assay in a CLIA-certified laboratory (43%). 30% will seek FDA clearance.
Conclusions: These responses confirm the need for resources to aid assay development and maturation. MoDEL, will provide a suite of services and resources to meet these needs. MoDEL will be phased in over a 2−3 year period.

P11

Which MR parameters are relevant as predictive markers of tumor response to radio and chemotherapy?

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Background: DCE-MRI, intrinsic susceptibility weighted MRI, and EPR oximetry all reflect tumour microenvironment hemodynamic variables that influence tumor response. We tested whether these markers could have a predictive value in terms of tumor response to radio- and chemotherapy following treatments aimed at modulating tumor oxygen consumption and/or blood flow. Different classes of treatments were considered: vasodilators, anti-angiogenic agents in their normalization phase, and inhibitors of oxygen consumption.
Methods: Tumor oxygenation, perfusion, cell oxygen consumption, radiation sensitivity and chemosensitivity were studied in transplantable liver tumors after treatment with insulin, hydrocortisone, NSAIDs (NS-398), anti-angiogenic agents (thalidomide; SU5416; ZD6474), Botulinum toxin (BT) or vasodilators (Xanthinol nicotinate, XN; isosorbide dinitrate, IDN). Oxygenation and tumor cell oxygen consumption were measured using EPR oximetry. Perfusion parameters were assessed by DCE-MRI using P-792. A GRE-MRI sequence was used to evaluate the GRE signal intensity (SI) at 20ms, S0, and R2*. Regrowth delays were measured after irradiation or injection of of cyclophosphamide.
Results: All treatments induced an increase in tumor oxygenation. This effect was explained by an increase in tumor blood flow for some of the treatments (IDN, Thalidomide, XN, and BT), where the number of perfused voxels and/or Ktrans, Kep, and Vp parameters were increased. However, other treatments (insulin, hydrocortisone, NS-398, SU5416, and ZD6474) resulted in a lack of change or even in a decrease in perfusion parameters. In this case, the increase in oxygenation was explained by a decrease in oxygen consumption rate. If the SI in GRE sequences was increased for treatments such as IDN, it was decreased with insulin and NS-398 (concomitantly with a decrease in S0 and lack of change in R2*). All

treatments resulted in tumor radiosensitization while only those associated with an increase in blood flow resulted in chemosensitization. Treatments involving consumption effects did not improve sensitivity to chemotherapy.
Conclusions: It is necessary to evaluate a combination of MR parameters to be predictive in terms of tumor response to treatment. For example, the evaluation of BOLD parameters or DCE-MRI parameters alone could lead to a misinterpretation since a lack of change in perfusion or in BOLD SI are not always associated with a lack of change in oxygenation (and thereby in radiation sensitivity).

P14

Detection of novel biomarkers by plasma proteomic profiling of oesophageal adenocarcinoma mouse xenografts in response to epirubicin, cisplatin and 5-fluorouracil

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Background: Oesophageal cancer is the 9th most common malignancy worldwide with an increasing incidence in recent years. Use of neoadjuvant chemotherapy in locally advanced cancer prior to surgery has been shown to improve outcomes, but the response to therapy is variable and survival rates poor. Hence, the effective use of chemotherapy could be greatly improved by the availability of biomarkers that predict response to therapy. The purpose of this study was to identify candidate biomarkers in mouse xenograft models of oesophageal cancer.
Methods: OE19 (adenocarcinoma) xenografts were established in SCID immune-deficient mice and tumour growth rates recorded. A clinical dose of epirubicin, cisplatin or 5-fluorouracil was administered to xenografts (or controls), by once weekly peritoneal injection for up to 3 weeks. Plasma collected from treated and untreated xenografts and controls was analysed by SELDI-TOF MS using Ciphergen CM10 (weak cationic) and Q10 (strong anionic) protein chips. Protein peaks (m/z) were identified that differed significantly ($p < 0.05$) between the treatment groups for each drug. Samples containing statistically significant markers were fractionated on anion exchange spin columns and approximate pI determined. Searches were performed on the Swiss-Prot database for proteins with the target mass and pI.
Results: Tumour growth was suppressed in treated compared with untreated xenografts. A number protein peaks were identified that differed significantly ($p < 0.05$) between the treatment groups with each drug. Several of these protein peaks were also shown to be common to the three drugs. Determination of the approximate pI of the proteins by anion exchange fractionation has allowed a preliminary identification of two of these peaks.
Conclusions: These experiments have established a response to chemotherapy in oesophageal adenocarcinoma xenografts by proteomic profiling of plasma. A preliminary identification of two markers has been made. Candidate markers are being further identified and will be tested in clinical patients.

P9

Altered expression of plasma membrane proteins on breast cancer cells capable of forming metastasis. Identification by comparative proteomic analysis

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Background: Breast cancers often spread to regional lymph nodes and distal sites such as liver, lung and bone marrow and may appear many years after resection of the primary tumor. The formation of metastasis is a complex multi-step process. One of the steps includes the ability of disseminated cells to establish a secondary tumor at the distant site. The cancer cell proteins involved in this process, and cell surface proteins in particular, are poorly identified. To address this we identified proteins that exhibited altered expression level in a set of isogenic breast cancer cell lines; one cell line being capable of disseminate from the primary tumor by vascular channels and metastasize to distal sites, while the other was equally tumorigenic and able to disseminate single cells to distal organs, but remained dormant and did not metastasize.
Methods: Membrane purification and comparative LC-MS/MS proteomic analysis using 'stable isotope labelling of amino acids in cell culture' (SILAC) in a model system of two isogenic breast cancer cell lines (M-4A4/NM-2C5) derived from the MDA-MB-435 cell line by single cell cloning. Data was validated using protein chemistry methods, immuno-cyto- and -histo-chemistry analysis.
Results: Thirteen proteins were up-regulated while three proteins were down-regulated more than two-fold among more than three hundred validated membrane proteins. Among the cell surface proteins being up-regulated in the metastatic cell line compared to the non-metastatic one we found 5′nucleotidase, Ndrg1, integrin-β1 and MHC class II proteins. The up-regulation of these proteins on the metastatic cell line was validated using flowcytometry, immunocytochemistry and Western blotting. The expression of selected proteins was also examined with immunohistochemistry on breast cancer biopsies of the primary tumor from patients with a known medical history of recurrence status within a ten-year follow-up period.
Conclusions: The cell surface membrane proteins with altered expression level in the metastatic vs. the non-metastatic cell line may bring insight into the initial stages of metastasic development and potentially be clinical attractive for cancer diagnosis or therapy.

P34

Comparison of expression and distribution of eEF1A in normal and cancerous tissue

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Background: The eukaryotic elongation factor 1A is well known for its role in the elongation cycle of protein synthesis, where it catalyses the delivery of aminoacyl tRNA to the ribosome. It is however also believed to function in many other cellular processes including signal transduction, cytoskeletal organization and apoptosis. The protein is believed to play a role in tumorigenesis, as elevated levels of eEF1A has been shown to render rodent fibroblast cells highly susceptible to transformation induced by 3-methylcholanthrene and ultraviolet light, which is likely to make the cells more vulnerable to malignant transformation. Supporting this theory, elevated level of eEF1A mRNA has been found in pancreas, colon, breast, lung and gastric tumors compared to healthy tissue. We have examined whether if there is a correlation between protein expression and distribution of eEF1A, and the development of cancer.
Methods: Using the phage display technology, a recombinant Fab antibody reacting with eEF1A was isolated when searching for human autoantibodies from patients with Felty's syndrome. This Fab fragment called ANA15, is believed to bind a conformational epitope of eEF1A, present in the nucleus of cells. A commercial antibody (CBP-KK1), which was also used, binds to eEF1A in cell cytoplasma. Formalin-fixed paraffin-embedded tissue sections were incubated with diluted lysate of E-coli cells producing ANA15, and bound Fab detected with a goat anti-human Fab. Similarly, sections were incubated with CBP-KK1 and detected with anti-mouse IgG. Double staining with ANA15 and an antibody against Ki-67, which is a cell proliferation marker, was also performed.
Results: Many tumor types (e.g. endometrial and bladder carcinoma) exhibited stronger staining with ANA15 and CBP-KK1 than observed in the corresponding healthy tissue. The majority of colon, endometrial and thyroid carcinomas, however, showed a reduced staining with ANA15 when compared to normal tissue. Likewise colon and endometrial tumors showed decreased CBP-KK1 staining, whereas several thyroid carcinomas showed an increased staining with CBP-KK1. The double staining analysis showed that there was no correlation between the presence of nuclear eEF1A and Ki-67 expression.
Conclusions: The staining patterns varied between the tumor types, and therefore no general connection between the expression eEF1A and cancer was observed. However, differences in expression levels between some of the tumors (e.g. colon carcinoma) and the corresponding normal tissue were observed. No correlation between staining with ANA15 and anti-human Ki−67 was found, indicating that nuclear eEF1A is not only associated with cell cycle progression, but also reflect metabolic activity of the cell. Combining staining for eEF1A and Ki−67 may add valuable information when characterizing cancers.

P81

Targeted quantitation of lung cancer biomarker candidates by liquid chromatography–tandem mass spectrometry with multiple reaction monitoring

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Background: Currently there are no proven molecular strategies for the early detection of lung cancer of established utility in the clinic. In order to identify candidate tumor specific proteomic biomarkers we have employed liquid chromatography-tandem mass spectrometry (LC-MS-MS) with multiple reaction monitoring (MRM) in the tissue first and then in the serum of patients with and without lung cancer.
Methods: In this approach, MRM is used to detect MS-MS fragmentations of specific tryptic peptides derived from the proteins of interest. We used both Thermo LTQ linear ion trap and Thermo TSQ Quantum triple quadrupole instruments to monitor up to 10 (LTQ) and 240 (Quantum) MRM transitions to detect up to 10 (LTQ) and 60 (Quantum) proteins in a single