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

K. Jacobsen[1], A.-V. Laenkholm[2], Q. Tan[2], H. Ditzel[1,2]. *[1]Medical Biotechnology Center, Institute of Medical Biology, University of Southern Denmark, Denmark; [2]Odense University Hospital, Denmark*

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

C. Jakobsen[1], N. Rasmussen[1], A.-V. Laenkholm[2], H. Ditzel[1]. *[1]University of Southern Denmark, Denmark; [2]Odense University Hospital, Denmark*

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

J. Jessup, J. Jacobson, T. Lively, I. Lubensky, D. Segal, S. Taube. *National Cancer Institute, Bethesda, MD, USA*

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?

B. Jordan, B. Gallez. *Université Catholique de Louvain, Brussels, Belgium*

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