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Candidate biomarkers in neurooncology: determinants impacting on translation into diagnostic application

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Background: Brain cancer comprises a large spectrum of rare malignancies. In spite of considerable research efforts, candidate biomarkers hardly translate into diagnostic application, and typing of brain tumours remains to be based on conventional light microscopy. Linchpins to accelerate the translational pace would be desirable.
Methods: Based on principles defined by the National Cancer Institute Specialized Programs for Research Excellence (NCI-SPORE) for biomarker development and the cybernetic model of viable systems (VSM, Stafford Beer) we identified in a sequence of critical interdisciplinary discussions systemic determinants impacting on translation of neurooncological biomarkers into diagnostic application. Further, we analysed causes of the slow translational pace of the following candidate biomarkers: Ki-67 index in ependymoma; chromosome 1p status in oligodendroglioma; methylation status of the O6-methylguanine-methyltransferase (MGMT) promotor in glioblastoma; INI1 protein expression in malignant pediatric CNS tumours.
Results: We identified five systemic determinants influencing the translational pace: methodology, communication/cooperation, funding, research strategy, normative component. Further, we detected deficiences linked to these determinants as causes of protracted biomarker translation. For instance, methodology: focus on retrospective studies, insufficient statistical power, use of non-standardized laboratory methods, etc.; communication/cooperation: insufficient interdisciplinary interaction, fragmented research landscape, etc.; funding: insufficient funding of clinical neurooncological research; research strategy: neglect of patient-centered clinical research as distinct field of research; normative component: lack of platforms for implementation of broadly accepted analytical guidelines.
Conclusions: Multiple causes impede the translational pace of candidate biomarkers in neurooncology. These causes are linked to five systemic determinants. Promising initial linchpins to accelerate the translational pace seem to be: promotion of patient-centered research as distinct field of neurooncological research, and reinforcement of lively interdisciplinary scientific interaction between diagnostic and therapeutic neurooncological disciplines.

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Strategic directions for biomarker research: progress toward ensuring clinical relevance

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Background: An overview of the progress made toward implementing the recommendations from NBCCF's Strategic Consensus Conference, Shaping the Future of Biomarker Research in Breast Cancer to Ensure Clinical Relevance.
Methods: In 2005, the National Breast Cancer Coalition Fund (NBCCF) convened a multi-stakeholder, consumer-led conference with the goal of developing consensus on strategies to ensure that biomarker development in breast cancer leads to clinically meaningful applications. Participating consumers, clinicians, basic science researchers, industry representatives, and regulators developed five general principles that serve as the framework for six consensus priorities and 18 specific recommendations.
The following year, the consensus principles, priorities, and recommendations were published in a peer-reviewed journal (Nature Reviews Cancer, April 2007) along with a timeline for implementation.
Since then, NBCCF has monitored and reviewed various new private sector and government initiatives that pertain to biomarker assays. We have identified areas for which there are still no agreed-upon standards or guidelines, and compiled next steps for implementation of the consumer-led consensus panel recommendations.
Results: There has been much activity relevant to eleven of the eighteen consensus recommendations within the six priority areas.
The US federal government has published several new guidance documents, private sector entities have developed guidelines for the standardization of biomarker measurement, and several new collaborative initiatives have been formed among government, academia, and consumer advocates.
NBCCF's "Beyond the Guidelines" Project will address two recommendations by providing consumers with regularly updated information relevant to the level of scientific and clinical evidence and unresolved issues and controversies on specific biomarkers as part of a larger web-based breast cancer diagnosis and treatment information service.
Conclusions: Progress has been made in the past few years but much work is still to be done. NBCCF will collaborate with all stakeholders to ensure that progress continues toward implementing all of the strategic consensus panel recommendations on biomarker research.

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Affibody® molecules for molecular imaging of HER2-positive breast cancer lesions

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Background: Affibody® molecules are a novel class of small, non-immunoglobulin affinity ligands capable of binding to a wide range of protein targets. They are selected from combinatorial libraries based on a 58 amino acid protein A domain scaffold. Their small size holds promise for good penetration properties for in vivo diagnostic and they can be functionally produced both by peptide synthesis and by recombinant expression in E. coli. A HER2-specific Affibody molecule has been used to target HER2 in a mouse xenograft model showing outstanding tumor-to-blood ratio within one hour after injection. The Affibody® molecule was rapidly cleared from the blood via the kidneys leading to very low background and good biodistribution kinetics. Here, we present clinical data where a synthetic, monomeric, DOTA-conjugated Affibody® molecule, (ABY-002) was used to visualize HER2-expression in metastatic lesions in patients with recurrent breast cancer.
Methods: ABY-002 was labeled with 111-Indium or 68-Gallium at the clinic with a simple one step procedure and was injected into patients intravenously. High contrast SPECT and PET/CT images were obtained 2−3 hours post injection. Standard 18FDG-PET/CT images were also available for comparison.
Results: Molecular imaging with 111-In and 68-Ga conjugated ABY-002 showed specific tumor targeting with rapid blood clearance and allowed the detection of small metastatic lesions. No adverse effects were observed. One of the patients had received several cycles of Herceptin treatment; however, this did not preclude ABY-002-mediated imaging.
Conclusions: The ABY-002 Affibody® molecule shows promising imaging properties for both SPECT and PET; and further clinical investigations of this novel molecular imaging agent are warranted.

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Investigation of three founder mutations in BRCA1 and BRCA2 in Iranian breast cancer patients

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Background: Breast cancer (BC) is the most commonly diagnosed cancer in Iranian women, and is the leading cancer cause of death in this population. Mutations in the hereditary breast cancer suppressor gene BRCA1/2 account for almost half of the familial breast cancers (FBC) and the majority of the combined familial mammary and ovarian malignancies. The mutations with the highest number of registrations associated with breast cancer are 185delAG, 5382insC (in BRCA1) and 6174delT (in BRCA2). Mutation analysis of BRCA1/2 genes is helpful in the determination of developmental potential, early diagnosis and gene therapy for breast cancer. In our study, we used multiplex PCR to analyze breast cancer patients for three BRCA mutations in tissue samples using immunohistochemical features as criteria.
Methods: Patient samples were drawn from three medical centers in Iran. We retrieved formalin-fixed, paraffin-embedded tissue blocks from women with breast cancer diagnosed, the age of 25−80 years for the years 2004 and 2005. Eighty-four samples were used for multiplex PCR and immunohistochemical diagnosis. All cases were reviewed using a special questionnaire, which allowed taking into account the presence or absence family history of breast cancer and also other pathology information. CINAGEN Inc.'s DNA Extraction Kit was used to isolate blood and tissue DNA. A simple and rapid method was used to detect the simultaneous detection of 185delAG, 5382insC (in BRCA1) and 6174delT (in BRCA2). Morphological and Immunohistochemical diagnoses of breast cancer were retrieved from their hospital records.
Results: The proportions of cases for women with at least 1 first-degree relative with breast cancer were 32.1% in Iranian breast cancer pateints. One of three BRCA mutations (5382insC) was detected by multiplex PCR in 3 breast cancers samples. Comparison presences of 5382insC mutation in tumor samples with family history and without family history have shown that frequency of 5382insC mutation was higher in familial samples ($P < 0.001$) relatively non-familial breast cancer samples.
Conclusions: The incidence of FBC increases with age, doubling about every 10 years until the menopause, when the rate of increase slows dramatically. The relative risk of breast cancer conferred by a first-degree

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.

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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.

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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.

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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.

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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