11 INVITED

Is mTOR a good target for chemoprevention and what patients could benefit from mTOR inhibitors?

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Molecular targeted therapy refers to treatment strategies directed against molecular targets considered to be involved in neoplastic transformation. Such molecularly-targeted agents (MTA) are currently under study in all treatment settings including that of chemoprevention, defined as the use of natural or synthetic agents to interrupt the carcinogenic process, thereby nipping tumours in the bud.

Numerous studies have demonstrated that the Akt pathway is critical for cell survival via phosphorylation of a number of downstream proteins. It plays a very important role in promoting growth and blocking apoptosis in cancer cells such as SCLC. There is increasing evidence that PI3-K/Akt plays an important role in breast cancer tumourigenesis. Akt activity was found to be constitutive in breast cancer cell lines with either HER2 overexpression or a PTEN mutation. Active Akt can be detected in head and neck squamous cell carcinoma (HNSCC) whose pattern of expression and localization correlate with disease progression. Many other transforming events which do not directly modify PI3-K, Akt, or PTEN genetically still cause activation of the PI3-K/Akt/PTEN pathway. Three examples of such transforming events are the BCR/ABL translocation, which is the causative event in chronic myelogenous leukemia, amplification of HER2, and amplification of the EGFR.

Several preclinical studies have indicated that rapamycin or its derivatives specifically inhibit the transforming effect of the PI3-K/Akt pathway. For example, rapamycin inhibits the transforming activity of the oncogenic variants of PI3-K and Akt. There are several mTOR inhibitors under clinical development for cancer therapy. CCI-779 is a rapamycin derivative developed by Wyeth-Ayerst which has completed phase I studies as a single agent administered intravenously or orally. It is currently being investigated in combination studies with other anticancer agents and in a broad spectrum of phase II single agent studies. RAD001 (Novartis) and AP23573 (Ariad Pharmaceuticals) are other mTOR inhibitors undergoing clinical development which are being tested in phase II trials. There is no ongoing chemoprevention trial with these agents. However the PI3K/Akt pathway appears to be a very appealing target for chemoprevention. Indeed, the target is present and abnormal as compared to normal epithelium. The target should influences tumor biology, and the preclinical data obtained with the drug aiming at the target appears favourable (efficacy, toxicity). The key results of the drug in human studies are currently being gathered in terms of efficacy and toxicity from phase I, and phase II trials. While efficacy appears very promising, toxic issues (mucositis, hypertriglyceridimia, hyperglycemia, anorexia) warrant further analysis before embarking on chemoprevention trials with the current agents.

Scientific Symposium
Molecular profiling for early detection and prognosis

12 INVITED

Micrometastases: detection, characterization and clinical relevance

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Bone marrow (BM), which can be easily collected from the iliac crest, is the most important site for detecting micrometastatic epithelial tumor cells, which are present in BM samples of 20–40% of patients with carcinomas at various primary sites; even in the absence of lymph node metastases (stage N0) or clinical signs of overt distant metastases (stage M0). BM samples can also be monitored for the presence of micrometastatic tumor cells after primary surgical treatment, to detect tumor recurrence. Gene expression profiling studies of breast cancer cells indicate that specific molecular pathways are associated with haematogeneous dissemination of tumour cells to BM, whereas these pathways were not involved with lymphatic dissemination. Disseminated tumour cells found in the BM of patients with various types of solid tumours (e.g. breast, colon and lung) can be detected by sensitive immunocytochemical and molecular assays. For epithelial tumors, cytokeratins have become the best marker for the immunocytochemical detection of micrometastatic tumor cells. The presence of disseminated tumour cells in bone marrow predicts the development of overt metastases – both in the bone and other organs.

Phenotyping of disseminated tumor cells in BM of early stage patients has also yielded additional prognostic information. In patients with gastric cancer, the presence of micrometastatic cells that express the urokinase-type plasminogen activator receptor (uPAR) correlates with an unfavourable prognosis. Similar observations have been made in patients with breast cancer; Her2/neu overexpression by tumor cells that have disseminated to the BM predicts poor clinical outcome. So uPAR and Her2/neu might be important for the survival and growth of disseminated tumor cells. Recently, Jonathan Uhr's group presented evidence that the genotype of persistent circulating tumor cells may change towards a Her2-amplified genotype, which might be in line with our previous findings on the selection of a Her2-positive phenotype in BM micrometastases.

The genetic characterization of single disseminated tumour cells isolated from the BM, along with gene expression profiling studies of primary tumour cells, indicate that haematogeneous dissemination is often a very early event in tumour progression. The cells appear to first disseminate from the early primary lesions and then acquire additional genetic defects.

Peripheral blood would be an ideal source for the detection of disseminated tumor cells because of an easy sampling procedure. However, the prognostic significance of circulating tumor cells (CTC) is much less clear than for DTC in BM. An important progress seems to be the development new enrichment systems for CTC. Using new detection systems, it was shown that the presence of CTC in breast cancer patients detected by immunocytochemical or molecular methods correlated with stage and course of the disease. We and others have recently shown a correlation between the detection of CTC in the blood and DTC in BM of patients with primary breast cancer. These findings indicate that CTC detection could have clinical relevance.

Functional analysis of micrometastatic cells remains a challenge, because even after short-term culture their numbers are still small (on average between 1,000 and 10,000 cells). Permanent cell lines have therefore been established, and show gene expression and genomic characteristics that are typical of epithelial tumor cells *in situ*. These could therefore serve as models for functional studies on dormancy of micrometastatic cells.

Single disseminated tumour cells in the blood and bone marrow are targets for adjuvant therapy. These cells show often different properties than cells of the primary tumour, so further molecular analysis will provide additional information and will help to develop antimetastatic therapies.

13 Abstract not received

14 INVITED

Expression profiling of peripheral blood cells (PBC) for early detection

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Existing methods to detect breast cancer in asymptomatic patients have limitations, and there is a need to develop more accurate and convenient methods. Especially, an accurate method for breast cancer detection based on peripheral blood as a clinical sample will be highly desirable because of the easy accessibility and less-invasive nature by which samples can be obtained.

Results demonstrating that peripheral blood can be used to develop a gene expression based test for early detection of breast cancer will be presented. The rationale for using blood cells as monitors for a malignant disease elsewhere in the body is based on the hypothesis that a malignant growth will cause characteristic changes in the biochemical environment of blood. These changes will affect the expression pattern of certain genes in blood cells.

We initially conducted a pilot study where the expression pattern of 1368 genes in peripheral blood cells of 24 females with breast cancer and 32 females with no signs of this disease were analyzed using macroarrays and the expression data analyzed by PAM. The results were validated using a standard leave-one-out cross-validation approach. We were able to identify a set of genes that correctly predicted the diagnostic class in at least 82% of the samples. The majority of the identified genes had a decreased expression in samples from breast cancer patients, and predominantly encoded proteins implicated in ribosome production and translation control. In contrast, the expression of some defense-related genes was increased in samples from breast cancer patients.

In order to revalidate these findings and to increase the repertoire of informative genes, we have now extended the study with a larger number of breast cancer and non-breast cancer samples and used Agilent WG oligo arrays for large-scale gene expression analysis. The preliminary analysis of the data supports our previous finding that a blood-based gene expression test can potentially be developed to detect breast cancer in asymptomatic patients.

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15 INVITED

Profiling clinical behaviour of tumours using DNA methylation markers

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Aberrant DNA methylation is the most common molecular lesion of the cancer cell. Neither gene mutations (nucleotide changes, deletions, recombinations) nor cytogenetic abnormalities are so omnipresent in human tumors as DNA methylation alterations. The most studied change of DNA methylation in neoplasms is the silencing of tumor suppressor genes by CpG island promoter hypermethylation, that target genes so relevant as $p16^{INK4a}$, BRCA1 and hMLH1. There is a profile of CpG island hypermethylation according to the tumor type and genes silent by methylation represent all cellular pathways. The introduction of bisulfite-PCR methodologies combined with new genomic approaches is providing us with a comprehensive spectrum of the genes undergoing this epigenetic change across all malignancies. However, we still know very little about how this aberrant DNA methylation "invades" the previously unmethylated CpG island and is maintained trough cell divisions. Furthermore, we should remember that this occur in the context of a global genomic loss of 5-methylcytosine. Initial clues to understand this paradox should be revealed from the current studies of DNA methyltransferases and Methyl CpG binding proteins. From the translational standpoint, we should make an effort to validate the use of some hypermethylated genes as a biomarkers of the disease such as it may occurs with MGMT and GSTP1 in brain and prostate tumors, respectively. Finally, we must expect the development of new and more specific DNA demethylating agents that awake these methyl-dormant tumor suppressor genes and prove their therapeutic values. The expections are high.

16 INVITED

Expression of replication origins firing genes predicts clinical outcome of primary cutaneous melanoma patients

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Gene-expression profiling in human cutaneous melanomas is impaired by the difficulty in getting access to a retrospective collection of frozen tumors. Thus, compared to other solid tumors, gene expression profiling data on human cutaneous melanomas are scarce, and data with prognostic implication are entirely lacking. In order to better understand the progression of this tumor and to identify key genes involved in melanoma prognosis, we correlated gene-expression profiles with clinical outcome in a cohort of 83 patients with primary melanoma of the skin, and applied a multiple random validation strategy to identify genes with a high probability to predict 4-yrs. distant metastasis free-survival. Profiles were also compared in primary melanomas and paired metastases. We identified a signature based on the top 60 genes discriminating between primary melanomas associated with good and poor prognosis. Some of these genes are key-genes in the regulation of replication origins firing, such as *mini-chromosome maintenance (MCM)* genes and *geminin*. Results have been validated at the mRNA and protein levels in independent populations of 17 and 176 primary melanomas with long-term follow-up respectively, showing that the prognostic value of overexpression of replication origins firing genes is independent from thickness, ulceration, age, sex, and anatomic site.

Scientific Symposium
FECS/ASCO – Malignant lymphomas

17 INVITED

Molecular pathogenesis of lymphomas, where do we stand?

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Much progress has been made into our understanding of the pathogenesis of the non-Hodgkin's lymphomas (NHLs), largely due to advances in molecular biology resulting from completion of the first iteration of the human genome project coupled with technical innovation. Genome-wide gene expression profiling (GEP) has provided a landslide of data regarding the molecular pathogenesis of many NHL subtypes. This presentation will focus on 3 specific lymphoma subtypes, including diffuse large B cell (DLBCL), mantle cell (MCL) and follicular lymphoma (FL).
DLBCL is the most common NHL subtype worldwide, accounting for 30–40% of all lymphomas. Clinical and morphological heterogeneity have long been appreciated, but recent GEP studies have helped to elucidate important aspects of pathogenesis. DLBCL is made up of at least 3 major subtypes, including germinal center B cell type (GCB), activated B cell type (ABC) and primary mediastinal (PMBCL). Specific oncogenic mechanisms are associated with these molecular subtypes. For example, the t(14;18) that characterizes about 15% of *de novo* DLBCL is only found in the GCB-type. Both the ABC subtype and PMBCL are characterized by constitutive activation of the NF-κB signaling pathway, providing a potential target for therapy. Moreover, distinguishing these subtypes has clinical relevance, as the molecular distinctions translate into survival differences and a different disease course. PMBCL shares features of GEP in common with classical Hodgkin's lymphoma, a finding that helps to explain some unique clinical features of this lymphoma subtype.
MCL accounts for ~6% of NHLs and has an aggressive clinical course. It is characterized by the presence of the t(11;14) that leads to deregulation of cyclin D1 expression. GEP studies highlight the importance of the proliferation signature, with widely disparate survival characteristics between groups. The relationship between proliferation and a number of oncogenic alterations will be explored. GEP in FL has shown the importance of the microenvironment to the biology and outcome prediction for this tumor. Distinct gene expression signatures that have a profound impact on overall survival in FL appear to be derived from non-neoplastic cells, in particular, reactive T cells and macrophages. Hypotheses can be generated based on this new knowledge that suggests a role for the immune response in FL. These data will be reviewed.
Finally, new techniques that allow high-resolution analysis of the human genome are being applied to NHL clinical samples. When cases with prior GEP are studied, there is an opportunity to begin to explore the relationship between altered gene expression and chromosomal imbalances. These combined approaches provide some insight into the mechanisms of disease progression and clonal evolution in NHLs. More importantly, they help to identify potential new targets for therapy.

18 INVITED

On the way to a new biological molecular prognostic index: The Lunenburg Lymphoma Biomarker Consortium (LLBC)

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Biological prognostic markers in non-Hodgkin's lymphoma (NHL) exist, but have not gained acceptance in clinical practice. Clinical prognostic indices, such as the IPI in the aggressive lymphomas and the FLIPI in the follicular lymphomas, are widely used, in part following their validation in large cohorts of patients from international trials. However, clinical factors represent surrogates for the underlying biology of NHL and as such do not identify potential targets for novel therapies. Important biomarkers have been described in both diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL), but require validation and systematic study before they can be accepted for clinical usage.
The LLBC is an international effort with a mission to move risk stratification beyond the IPI. The ultimate goal of this consortium is to validate a list of important biomarkers initially in patients with DLBCL and FL. We aim to standardize the methodology for routine measurement and determine their independent contribution to prognosis using tissue microarray (TMA) techniques and immunohistochemistry. We will take advantage of large numbers of patients from both recent and remote randomised clinical trials from both North America and Europe. Standardization of the reagents and methodology are performed using test TMAs, further establishing the thresholds for determining positivity. Upon achieving this goal, a candidate list of biomarkers will be analysed using recent clinical trials of DLBCL comparing CHOP chemotherapy vs CHOP + Rituximab. Older studies employing CHOP as the standard arm will also be analysed, shedding light on the impact of therapy in determining prognostic marker relevance. Similar studies in FL will also be performed. Our goal is to determine a list of relevant and independent biomarkers that could be used in conjunction with clinical factors in the design of new prognostic models for the NHLs.