Thursday, 17 April 2008 08:00–08:45

EUROPA DONNA TEACHING LECTURE

Young women and fertility in breast cancer

190 Invited

Young women and fertility in breast cancer

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Background: 15% of all breast cancers are diagnosed in women with fertility potential. Adjuvant chemotherapy in premenopausal women with breast cancer may induce premature ovarian failure which results in amenorrhea and affects fertility.

Results: Chemotherapy induced amenorrhea (CIA) is strongly related to the age of the patient, the duration and amount of chemotherapy given. The incidence of CIA resulting from taxane (T) based therapy is poorly characterized. A retrospective survey in premenopausal women determined the rates of CIA in women receiving AC followed by T compared with AC alone. Older age and the addition of a taxane to AC increased the risk of CIA.

There are several options available for fertility preservation for young women who wish to have a biologic child after breast cancer and are at increased risk for infertility. Options include cryopreservation of embryos, oocytes, ovarian tissue prior to treatment, and ovarian suppression through chemotherapy. However, most of these are considered experimental with the exception of cryopreservation of embryos. There has been concern that pregnancy after breast cancer may worsen prognosis in light of the endocrine manipulations used to treat breast cancer, particularly for women with hormone sensitive disease. Several studies addressing the potential risk of pregnancy after breast cancer have not revealed any negative effect on prognosis.

Ovarian stimulation as prerequisite for embryo or oocyte preservation leads to an increased estradiol level. Letrozole in combination with FSH resulted in significant lower peak estradiol levels than anastrazole combined with FSH and standard stimulation protocols, but in a similar fertilization rate.

The administration of GnRH agonists to women with Hodgkin's disease, breast cancer, and other malignancies, has demonstrated lower rates of premature ovarian failure in patients than in nonrandomized controls. Prospective, randomized studies are ongoing, the German ZORO trial in hormone receptor negative breast cancer has finished recruitment. A recent meta-analysis found that the administration of a GnRH agonist, in addition to chemotherapy, to patients with breast cancer was associated with less recurrence and superior survival.

Conclusion: There are several options but no standard for fertility preservation for women at risk for CIA. However, patients need to be informed about the risk and benefits of the ovarian function preserving modalities.

Thursday, 17 April 2008 09:00–10:30

KEYNOTE SYMPOSIUM

New tools for developing smarter drugs

191 Invited

Identification of biomarkers of therapy response using functional genetic approaches

R. Bernards[1]. [1]*The Netherlands Cancer Institute, Division of Molecular Carcinog. H-2, Amsterdam, The Netherlands*

Background: Unresponsiveness to therapy is remains a significant problem in the treatment of cancer, also with the new classes of targeted therapeutics. In my laboratory, we use functional genetic approached identify biomarkers that can be used to predict responsiveness to clinically-relevant cancer therapeutics. We focus on the well-established targeted cancer drugs such as Trastuzumab. This drug targets a specific molecule (HER2) that is over-expressed or in breast cancer. Nevertheless, it remains poorly explained why a significant number of tumors, which express the drug target, do not respond to the therapy. We aim to elucidate the molecular pathways that contribute to unresponsiveness to targeted cancer therapeutics using a functional genetic approach. This will yield biomarkers that can be used to predict how individual patients will respond to specific drugs. Furthermore, this work may allow the development of drugs that act in synergy with the established drug in the treatment of cancer.

Material and Methods: To identify biomarkers that control tumor cell responsiveness to cancer therapeutics, we use both genome-wide gain-of-function genetic screens (with cDNA expression libraries) and genome wide loss-of-function genetic screens (with RNA interference libraries) in cancer cells that are sensitive to the drug-of-interest. We search for genes whose over-expression or whose down-regulation in cultured cancer cells confers resistance to the drug-of-interest. Once we have identified such genes, we ask if their expression is correlated with clinical resistance to the drug-of-interest using tumor samples of cancer patients treated with the drug in question, whose response to therapy is documented.

Results: We have used BT474 human breast cancer cells (HER2 amplified), to find genes whose suppression confers resistance to Transtuzumab. We found that of 8,000 genes surveyed, only loss of PTEN caused resistance to Trastuzumab. In a cohort of 55 breast cancer patients, both loss of PTEN and mutation of the PIK3CA gene (which is controlled by PTEN) were predictive for poor response to Trastuzumab.

Conclusion: This study illustrates the power of genetic screens to identify biomarkers useful for predicting treatment response in the clinic. Our data demonstrate that activation of the PI3K pathway (caused either by loss of PTEN or by activating mutations in the PIK3CA gene) is predictive for poor responses to Trastuzumab-based therapy. Assessment of PI3K pathway activation in HER2+ breast cancer may help identify those patients that may benefit from drugs that inhibit the HER2 signaling pathway more downstream, e.g. by using PI3K inhibitor drugs or mTOR inhibitor drugs.

192 Invited

Gene expression profiling

C. Sotiriou[1], P. Dinh[1]. [1]*Institut Jules Bordet, Chemotherapy Unit, Brussels, Belgium*

With the advent of microarray technology, unprecedented opportunities have become available for performing comprehensive molecular and genetic profiling of breast cancer. This has resulted in a) a new molecular classification of breast cancer into clinically relevant subtypes b) various molecular "prognostic" signatures that have performed, as well as, if not better than traditional clinico-pathological prognostic factors in predicting clinical outcome and c) a number of molecular "predictive" signatures that bear the exciting promise of being able to identify subsets of patients who are particularly sensitive to a given drug or regimen of drugs. Perhaps reflecting similar oncogenic pathways, the different molecular signatures, in fact, carry very similar prognostic information despite having very few overlapping genes. Although the biological role of individual genes remains largely unclear, recent work suggests that those involved in proliferation and the cell-cycle may be the common driving force behind these signatures. Also recently, epithelial-stromal interactions are being increasingly recognized as important in breast cancer development where breast tumor stromal cells have been shown to also display altered gene expression. Similar to the molecular signatures of breast cancer cells, the stromal cell and immune response signatures have been correlated with clinical outcome. Thus, gene expression profiling is an important evolving tool not only for refining prognostication, for improving patient selection but also for understanding the tumor microenvironment and the processes underlying dissemination.

193 Invited

Targeted imaging

Abstract not received.

194 Invited

Targeting breast cancer stem cells

K. Polyak[1], M. Shipitsin[1], N. Qimron[1], L. Campbell[1], J. Yao[1]. [1]*Dana-Farber Cancer Center, Department of Medical Oncology, Boston, USA*

Background: Breast cancer is a heterogeneous disease including multiple tumor subtypes associated with distinct clinical outcomes. Besides the high degree of inter-tumoral variability significant intra-tumoral heterogeneity also exists that likely contribute to therapeutic resistance and recurrence. Understanding the molecular basis of breast tumor heterogeneity is key for the development of targeted cancer preventative and therapeutic interventions. Current hypotheses explaining breast cancer diversity are the cancer stem cell and the clonal evolution models.

Material and Methods: To characterize cells with stem-like characteristics, we determined the gene expression, genetic, and epigenetic

profiles of distinct cell populations purified from breast carcinomas and normal breast tissue using cell surface markers CD24 and CD44 that have been associated with stem cell-like properties. Gene expression profiles were analyzed using SAGE (Serial Analysis of Gene Expression), genetic alterations were investigated using SNP (Single Nucleotide Polymorphism) arrays and FISH (Fluorescence In Situ Hybridization), and DNA methylation patterns were analyzed using MSDK (Methylation-Specific Digital Karyotyping).

Results: The CD24+ more differentiated and the CD44+ stem cell-like populations from the same tumor were clonally related but not always identical and epigenetically distinct. A gene signature specific for CD44+ cells was enriched for known stem cell markers and was associated with decreased overall and distant metastasis free survival in lymph node negative breast cancer patients. Systemic network analyses determined that the TGF-β pathway is specifically active in CD44+ breast cancer cells and its inhibition induces their epithelial differentiation. CD24+ and CD44+ also demonstrated distinct responses to various therapeutic agents.

Conclusions: Our study demonstrates that cancer cell phenotype is subject to dynamic regulation by genetic and epigenetic mechanisms as well as by the tumor microenvironment. Thus, tumor progression is a dynamic and complex process that is influenced strongly by the intrinsic level of genetic instability in a given tumor at a given time and location. Understanding the molecular mechanisms responsible for breast tumor heterogeneity and specific targeting of each cell types within tumors will facilitate the development of more effective ways to treat and prevent breast cancer.

Thursday, 17 April 2008 11:00–12:30

KEYNOTE SYMPOSIUM

Tailoring local regional therapy

195 Invited

Arrays – are they helpful?

D.S.A. Nuyten[1], M.J. Van de Vijver[2]. [1]*The Netherlands Cancer Institute, Radiation Oncology, Amsterdam, The Netherlands;* [2]*Amsterdam University Medical Center, Pathology, Amsterdam, The Netherlands*

Since the late 1990's microarray analysis has been increasingly used to study breast cancer. Most studies so far have either looked at overall gene expression and have tried to subcategorize tumors based on differences in gene expression (unsupervised analysis) [1−3], or have focused on developing of prognostic assay's that predict changes of developing metastasis by linking gene expression data to clinical outcome data (supervised analysis) [4,5]. Clinically, these assays can be used for treatment discussion making, e.g. adjuvant systemic treatment advice. Two assays are currently being tested prospectively in their ability to better predict the need for adjuvant treatment in node-negative breast cancer (respectively the 70-gene prognosis profile in the MINDACT Trial [6] (microarray based) and the 21-gene recurrence score in the TailorX Trial [7] (PCR-based assay)). Another interesting possible clinical application is therapy response. A few studies haven been published on chemotherapy response [8−10].

For breast cancer radiation oncologists the question is: can I expect clinical assays to help guide decision making for post-mastectomy radiotherapy (yes/ or no in clinically intermediate risk patients) and selecting patients that might not be offered breast conservation because of a very high risk of local recurrence or perhaps patients that might benefit from a higher dose (boost). Last but not least, identification of patients with a very low risk of local recurrence that might be offered partial breast irradiation.

Cheng et al. applied a supervised learning approach on a breast cancer patient cohort all treated by mastectomy without post-mastectomy radiation [11]. The identified a 34-gene classifier that could predict local recurrence in the validation set with a sensitivity of 67% and specificity of 83%. Looking at the biology behind this list processes like cell cycle regulation, cell death and proliferation can be found.

In patients treated by breast conserving therapy, age is an independent predictive factor for local recurrence, even after compensating for margin status, radiotherapy dose and estrogen receptor status [12]. By hypothesizing that in optimally treated patients, young age represents specific poor prognosis biology, Kreike et al looked at patients under 51 years of age treated by breast conserving therapy and by definition all patients received radiotherapy. They applied different methods and found one robust list that was presumably driven by ER-status. After correction for ER status, no robust classifier was found.

We applied a different supervised approach on a young patient cohort (<53) treated by breast conserving therapy [13]. A group of 161 patients were divided in a training and validation set. After a standard supervised approach had failed to segregate groups into a low and high risk group for local recurrence, we tested different signatures that previously had been found to predict metastasis free and overall survival in breast cancer patients [4,14,15]. These signatures (the 70-gene prognosis profile, the wound signature and the hypoxia signature) were optimized towards the clinical end point of local recurrence after breast conservation and subsequently validated. One of the three signatures tested, the wound signature, could segregate groups of patients at low or high risk of a local recurrence. In the validation set a large difference in 10-year local recurrence rate (5% vs 29%) was found with a sensitivity of 88% and a specificity of 75%. The classifier was an independent predictor in multivariate analysis.

Assays that are currently being tested in clinical trials have shown the amount of time, money and effort it takes to bring molecular assays into the clinic. For local recurrence prediction the first step now is validation on archived material. Two large trails that are currently being conducted are collecting tumors samples and potentially allow for validation of these assays.

196 Invited

New imaging techniques for tailoring therapy

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In the patient with breast cancer, there are three different scenarios in which imaging studies are used to guide therapy: In the neoadjuvant setting, functional imaging is used to yield surrogate markers to predict and monitor response to therapy of a given tumor. In the pre-operative setting, structural imaging studies are needed to delineate the extent of disease as accurately as possible. For neoadjuvant treatment, many different concepts have been established or are being developed that target at different molecular pathways. Ideally, an imaging tool should be able to provide information on response to specific molecular pathways and it should help predict response and identify response as early as possible. PET has traditionally been perceived as a "functional" imaging tool for response prediction and assessment. New, specific molecular targets are being developed and evaluated in translational research that target at anti-angiogenetic and growth factor receptor signalling pathways. Moreover, functional MR-based technologies like dynamic contrast enhanced MRI, Diffusion weighted MRI, Perfusion MRI, MR based oxygen mapping, and MR spectroscopy have been introduced that appear to exhibit a similar sensitivity for the pathophysiological processes associated with response. For the second scenario, i.e. pre-operative staging of primary or residual breast cancer, MRI has been an established role to guide surgical treatment. The importance of an accurate staging will be even increase with the increasing use of more regional therapies such as intra-operative radiotherapy. This lecture will review recent results and controversies around imaging for tailoring neoadjuvant and surgical therapy.

197 Invited

Tailoring radiotherapy

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Adjuvant radiotherapy (RT) in breast cancer is now widely accepted as the standard treatment after breast-conserving surgery and in many cases after mastectomy.

Nevertheless, several factors may influence both indications and modalities. For example and in case of conservative breast management, the impact of the boost is still under debate in the elderly. In addition, in hormone receptor positive-cancer patients older than 70 years old, one has recently advocated the equivalent benefit of the sole hormonotherapy after surgery. Finally, new molecular surrogate markers are under extensive researches to identify which patients will or not benefit from RT.

In case of mastectomy, only large primitive tumors and node positive patients (>4 N+) received adjuvant RT. The updated meta-analysis has proposed to extend this indication to the 1−3 N+ patients. In addition, specific genes have recently been identified to help tailoring RT treatment.

In all cases, the survival impact of internal mammary chain RT has not been yet proven and its indication is extremely debated.

Concerning prediction of late toxicities, evidence has accumulated in recent years suggestive of a genetic basis for a susceptibility to the development of radiation injury following cancer radiotherapy. We assessed whether patients with severe radiation-induced sequelae (RIS) display both a low capacity of initial normal tissue apoptosis and possess certain single