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Symposium I: Molecular genetics, phenotypes for prognosis and response to treatments

S1

The role of gene expression profiling by microarray analysis for prognostic classification of breast cancer

MJ van de Vijver

Department of Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands

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Introduction Prognostic and predictive factors play important roles in the treatment of breast cancer. Genome-wide monitoring of gene expression using DNA microarrays makes it possible to study thousands of genes in a tumour sample in a single experiment. By looking for an association between the gene expression pattern and tumour behaviour, it should be possible to identify new prognostic and predictive factors.

Method We used gene expression profiling using two different microarray platforms: one containing 25,000 oligonucleotide probes and one containing 18,000 cDNA probes. To obtain prognostic gene expression profiles, we isolated RNA from tumours from a series of 295 patients younger than 53 years presenting with stage I and II breast cancer treated at our institute between 1984 and 1993. The expression of 25,000 genes was assessed, and using various statistical approaches correlation of gene expression with distant metastasis-free probability and overall survival was assessed [1-3]. In addition, we started studies to obtain gene expression profiles predicting response to specific chemotherapy regimens. Within a single-institution, randomized phase II trial, patients with locally advanced breast cancer received six courses of either AC ($n = 24$) or AD ($n = 24$) containing neoadjuvant chemotherapy. Gene expression profiles for 18,000 genes were generated from core needle biopsies obtained before treatment and correlated with the response of the primary tumour to the chemotherapy administered [4]. Additionally, pretreatment gene expression profiles were compared with those in tumours remaining after chemotherapy.

Results We previously identified a 70-gene expression profile associated with increased risk for developing distant metastases within 5 years [1,2]. More recently, we studied a Wound Signature in these same tumors [3]. By combining the 70-gene expression profile to subdivide the tumours into 'good prognosis' and 'poor prognosis' tumours, and the Wound signature to subdivide tumours into 'activated' and 'quiescent' tumours, subgroups of patients with markedly different prognosis can be identified. Additional gene expression signatures are being tested in this series of tumours to arrive at an optimal prognostic classifier and to obtain improved insight into breast cancer biology.

In the study to identify predictive profiles, 10 (20%) of the 48 patients showed (near) pathological complete remission of the primary tumour after treatment [4]. No gene expression pattern correlating with response could be identified for all patients, or for the AC or AD treated groups separately.

Conclusion Various gene expression profiles in breast cancer are associated with the propensity of the tumour to develop distant metastases. Gene expression profile predicting the response of primary breast carcinomas to AC or AD based neoadjuvant chemotherapy are most likely to be very subtle and cannot be detected when small series of patients are studied. Genetic tests derived from gene expression profiling studies are likely to become useful as prognostic and predictive tests to guide clinical decision making in the treatment of primary breast cancer.

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S2

Gene expression profiles and molecular classification to predict distant metastasis and tamoxifen-resistant breast cancer

JGM Klijn[1], EMJJ Berns[1], J Martens[1], MPHM Jansen[1], D Atkins[2], JA Foekens[1], Y Wang[2]

[1]Daniel den Hoed Cancer Center/Erasmus MC, Rotterdam, The Netherlands; [2]Veridex LLC, Johnson and Johnson, Molecular Diagnostics, San Diego, California, USA

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Introduction Genome-wide measures of gene expression can identify patterns of gene activity that subclassify tumours and might provide better means than are currently available for individual risk assessment in patients with primary breast cancer and for prediction of tamoxifen resistance.

Methods We analyzed, with Affymetrix Human U133a GeneChips, the expression of 22,000 transcripts from total RNA of frozen tumour samples from 286 lymph node negative (LNN) patients who had not received adjuvant systemic treatment. In a separate second study conducted in 112 estrogen receptor (ER)-positive primary breast carcinomas from patients with metastatic disease and clearly defined types of response to first-line treatment with tamoxifen, a 18,000 human cDNA microarray was used to discover gene expression profiles predictive of tamoxifen resistance.

Results In the first single-center study, in a training set of 115 tumors (80 ER^+ and 35 ER^- tumors) we identified a 76-gene signature (60 genes for ER^+ and 16 for ER^-) for predicting the occurrence of distant metastasis within 5 years. This signature was successfully validated with 93% sensitivity in an independent test set of 171 LNN patients as a whole, irrespective of age or ER status. The 76-gene profile was strongly predictive of those patients who will develop a distant metastasis within 5 years or will remain recurrence free during that period (hazard ratio [HR] 5.67; $P < 0.00002$) and in multivariate analysis when corrected for traditional prognostic factors including grade (HR 5.55; $P < 0.00003$). Analogously, the 76-gene expression profile strongly predicted overall survival (HR 8.62; $P < 0.00002$). The 76-gene profile was also a strong prognostic factor in the subgroup of 79 patients with a tumor size ranging from 10 to 20 mm (HR 14.1; $P < 0.00003$) and in 84 premenopausal patients (HR 9.60; $P < 0.0002$) and 87 postmenopausal patients (HR 4.04; $P = 0.0017$). In the subgroup of 42 ER^- patients in the validation set, even a profile of only 16 genes appeared to have a strong prognostic value (HR 8.74; $P = 0.012$). Recently, our 76-gene expression signature was successfully validated in a separate multicenter European study of 180 patients from four institutions (Nijmegen, Munich, Bari, Ljubljana) (HR 7.41; $P < 0.0001$) with similar sensitivity and specificity.

In the second study, conducted in 112 patients with metastatic disease, using a training set of 46 breast cancers 81 genes were found to be differentially expressed between tamoxifen-responsive and -resistant tumors. From the 81 genes, a predictive signature of 44 genes was extracted and validated in an independent set of 66 tumors. This 44-gene signature is significantly superior (odds ratio [OR] 3.16; $P = 0.03$) to traditional predictive factors in univariate analysis and significantly related to longer progression-free survival in univariate as well as in multivariate analyses ($P = 0.03$). The predictive value of the 44-gene signature was recently confirmed in an extended series of 280 patients with advanced disease.

Conclusion In the first study, the identified 76-gene signature provides a powerful tool for identification of patients at high or low risk for distant recurrence or death due to breast cancer, allowing clinicians to adapt choices of adjuvant systemic therapy. In the second study, the 44-gene signature predicts tamoxifen resistance more accurately than do traditional predictive factors. Interestingly, in a third study DNA methylation status also appeared to be useful in predicting tamoxifen resistance.

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S3

Methods for gene expression profiling in clinical trials for breast cancer

S Paik

Division of Pathology, National Surgical Adjuvant Breast and Bowel Project, Pittsburgh, Philadelphia, USA

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High throughput gene expression profiling provides a powerful tool for discovery of prognostic and predictive markers for breast cancer [1-4]. The main limitation to this approach is the requirement for high-quality RNA, which is difficult in the multicenter clinical trial setting. One solution is to use RNAlater, which allows procurement and shipping of tissue specimens at room temperature [5,6]. The National Surgical Adjuvant Breast and Bowel Project (NSABP) has conducted a pilot study to procure pretreatement core biopsy specimens in a neoadjuvant study. Most of the samples in this study provided high-quality RNA, as determined by Bioanalyzer and Affymetrix GeneChip analyses. When combined with a RNA amplification method, quality data could be obtained from 10 ng of total RNA as starting material. NSABP currently has two neoadjuvant trials in which pretreatment specimens are procured in RNAlater. However, the typical practice pattern in the USA makes it difficult to procure tissue in the adjuvant setting even with the use of RNAlater. Therefore, methods that permit high throughput gene expression profiling of formalin-fixed, paraffin-embedded materials are in great need. Such methods will also allow interrogation of archived tissue banks with annotation established from previously finished trials and will therefore shorten the time for marker development and validation. Chemical modification by formalin and degradation during storage make RNA extracted from paraffin a poor substrate for gene expression profiling [7]. We have examined both microarray and RT-PCR platforms for this purpose. In general microarray analysis using the Arcturus Paradise system has been a disappointment in our hands, with high rate for assay failure for materials older than 3 years. However, there are RNA amplification and labeling methods in development that are not dependent on oligo-dT priming for cDNA synthesis and may provide better results. In collaboration with Genomic Health, Inc., we have explored the use of high-throughput real time RT-PCR for discovery and validation of prognostic markers for node negative and estrogen receptor positive breast cancer [8]. This has resulted in development of the OncotypeDx assay, which is offered as a commercial reference laboratory test. The disadvantage of real-time RT-PCR assays is relatively low throughput (less than 1000 genes, even at industrial scale). DASL assay from Illumina is a kind of hybrid between PCR and microarray platforms, and may provide relatively cost-efficient means by which to assay many candidate genes using degraded RNA obtainable from paraffin blocks [9].

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S4

Gene expression profile-based predictors of response to chemotherapy

L Pusztai

Anderson Cancer Center, Houston, Texas, USA

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A molecular test that could help in selecting the most effective chemotherapy for a particular individual could save patients from unnecessary toxicity, and the right choice of drugs may save lives, particularly in the adjuvant treatment of breast cancer. Administration of chemotherapy before surgery provides an attractive opportunity to discover predictors of response [1]. Pathologic complete eradication of cancer from the breast and lymph nodes (pCR) represents an extreme form of chemotherapy sensitivity and invariably heralds excellent long-term survival. We adopted pCR as an early surrogate of clinically meaningful benefit from therapy and as an outcome that is worth predicting. There are simple clinical and histological parameters, including grade, estrogen receptor status and tumor size, that can be combined into powerful prediction scores. However, these clinical variables do not yield treatment regimen specific predictions, and they cannot be used to select one therapy over another. Assessment of traditional single gene markers of chemotherapy sensitivity has not yet resulted in clinically useful tests. Gene expression profiling, which enables simultaneous measurement of thousands of genes, represents a promising new tool that may be applied to this clinical problem. It is currently unknown what the best strategy is to discover response predictors from high dimensional gene expression data. The simplest approach may be to search for the single most informative gene that is differentially expressed between responders and nonresponders. This may lead to new mechanistic insights into the biology of chemotherapy response and could yield easy-to-use but moderately powerful single gene predictive markers [2]. Another approach is to identify gene expression signatures that are predictive of response, assuming that the combined information provided by multiple genes would result in more accurate predictions than any single gene can do. Several small studies have suggested that this is feasible [3]. Large-scale validation of these results is needed and is currently underway. Yet another approach is to recognize the different molecular subtypes of breast cancer and attempt to develop distinct predictors for each subtype [4]. This approach assumes that, by focusing on the molecularly more homogenous subgroups, more accurate predictors could be developed than by analyzing all breast cancers together. We shall present results from our own research program, illustrating the successes and limitations of each of these approaches.

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Lecture

S5

Present situation and future of genetic profiling for prognosis and treatment

GN Hortobagyi

Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

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Classification and staging systems are important in oncology to predict clinical behavior and determine prognosis. In addition, they may contribute to the selection of optimal treatment strategies. Much clinical and translational research over the past 30 years was directed at establishing or refining prognostic and predictive factors for breast cancer. Initially, tumor related factors such as size, grade, lymph node involvement, and hormone receptor status were considered in the determination of prognosis. Patient characteristics, such as age, menopausal status and performance status, also contributed to these estimates. Some factors such as estrogen receptor (ER) status were shown to be better predictive factors than prognostic factors. Thus, although ER-positive tumors have a slightly better prognosis during the early years of follow up than do ER-negative ones, the major application of ER status is to predict response to endocrine therapy. A variety of biochemical and molecular factors were reported to have prognostic or predictive ability over the past 20 years. These included cathepsin D, HER2, EGFR, p53, UPA, PAI, and many others. Of these, only HER2 was consistently validated as a prognostic factor, as well as a predictor of response to the monoclonal antibody trastuzumab (Herceptin). Developing, assessing, and discarding these various putative prognostic and/or predictive factors was the result of an enormous investment of time and effort of many scientists from many countries around the world. Considering that only one new prognostic/predictive factor was universally adopted over the past 25 years (HER2 status), it must be concluded that this is an enormously inefficient process.

The Human Genome Project was a major milestone in the history of medicine. Both the genetic information obtained and the technological advances that took place during this large multicenter effort have had enormous influence over all fields of medicine. For the field of prognostication and prediction in breast cancer, the major consequence was the development of technology that led to the simultaneous evaluation of gene expression for hundreds and, more recently, thousands of genes. In fact, recently launched gene arrays include the entire human genome. Thus, we have the opportunity to assess, in a small tumor sample, the expression profile of all known human genes. There are multiple technological platforms under evaluation for this purpose, and the results obtained with one cannot automatically be substituted for results obtained with another platform. Nevertheless, on the basis of several reports, it can be stated that gene expression profiling of human breast cancer provides valuable information in the following areas:

1. Molecular classification of primary breast cancer
2. Identification of multiple distinct prognostic subgroups
3. Determination of expression level of several genes of interest (ER, PR, HER2, etc.)
4. Identification of genetic networks
5. Prediction of response to chemotherapy

The initial reports were based on small patient numbers that presented substantial statistical challenges for adequate estimation of end-points and to prevent frequent false-positive or false-negative results. More recent analyses have included several dozen and up to a few hundred patients. These reports provide greater statistical power and greater reliability. However, these reports still represent retrospective analyses of subsets of patients, and prospective validation is still sorely needed. Reports are beginning to appear comparing the performance of different platforms on the same tumor samples and considering the same end-points. The source of tumor material, the manner in which it was handled before testing, and the amount of tissue needed for reliable testing are all under intense scrutiny. Gene profiling with currently available platforms includes a number of genes or gene

segments of uncertain function (ESTs). These provide an excellent opportunity to assess the functional value of these genes and enrich our understanding of their biological function. Many centers and groups are assessing the potential of molecular profiling in the prediction of response to therapy. As technology evolves, this type of information will transform the way we think of breast cancer, the way we assess and stage primary and metastatic breast cancer, and the manner in which we select the best combination and sequence of therapies to obtain optimal therapeutic results. Today's costs, while substantial, are rapidly falling and newer technology will make these assays much more accessible. Furthermore, because multiple relevant markers can be determined using a single assay, it is likely that gene expression profiling will be more cost-effective than currently used diagnostic and prognostic tests.

The major challenges in gene profiling are still in developing and using the most appropriate statistical methods for data analysis. The need for handling tens of thousands or hundreds of thousands of data points, especially originating from a much smaller number of tumors, is daunting and mistaken conclusions might be reached in the absence of optimal analytical techniques.

Finally, prospective validation of the clinical utility of gene profiling for classification, determination of prognosis, and selection of optimal therapies for individual patients will require large, prospective, multicenter, controlled clinical trials. If successful, these will take us one step closer to individualized medicine.

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Symposium II: Advances in diagnosis and staging

S6

The role of magnetic resonance imaging as an imaging tool to assess disease status and residual disease in locally advanced breast cancer

M Cristofanilli

Department of Breast Medical Oncology, The University of Texas, MD Anderson Cancer Center, Houston, Texas, USA

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Magnetic resonance imaging (MRI) is a very sensitive diagnostic tool for the evaluation of breast cancer. A MRI is more frequently utilized in conjunction with other diagnostic modalities, particularly mammogram, to better assess a breast abnormality or biopsy-proven cancer, including ductal carcinoma *in situ* (DCIS) [1]. Most recently it has also been demonstrated to be a better screening tool for women at high risk for developing breast cancer, including women with documented genetic predisposition. Because MRI has the advantages of providing a three-dimensional view of the breast, it has been shown to be more precise than standard imaging in determining the initial staging and evaluation of the extension of invasive disease [2]. This information is of particular value in patients with locally advanced disease, including inflammatory breast cancer (IBC) and in classic lobular histology, which may exhibit diffuse involvement of the breast at initial presentation and therefore are frequently treated with primary systemic therapy (PST) [3,4].

The management of primary breast cancer has evolved significantly in the past decade, with the increasing use of preoperative or primary chemotherapy (PST), and most recently also primary hormonal therapy for both early and locally advanced breast cancers (LABCs). The advantages of the early use of systemic therapy are considered: the feasibility of a more conservative surgery, and the possibility of true *in vivo* testing of the tumor's drug sensitivity. The amount of residual disease found following surgical excision represents the pathological response to the preoperative treatment and remains the most important prognostic factor.

The high staging accuracy of breast MRI makes it an attractive method for assessing tumor response to PST. MRI can contribute in several ways to the management of patients receiving preoperative chemotherapy, including the initial determination of extent of disease for proper staging (baseline evaluation), early identification of poor responders (intermediate evaluation during treatment), and identification and description of the presence and extent of residual disease for surgical planning (preoperative imaging study) [3,4]. MRI measurements of tumor response may have predictive value for disease recurrence and responsiveness to novel therapeutics. Comparison of dynamic parameters (e.g. signal enhancement ratio) at baseline and at subsequent evaluation time points can also contribute information on the response to treatment and predict residual disease [4]. A series of prospective trials has been conducted in patients with LABC, including IBC. Most recently a large multicenter trial sponsored jointly by the American College of Radiology Imaging Network, the Cancer and Leukemia Group B, and the National Cancer institute is integrating serial MRI tumor measurements with serial collection of tissue for evaluation of biomarkers (expression, genomic, protein arrays, as well as specific immunohistochemical markers and fluorescence *in situ* hybridization). The goal of such studies is to compare tissue assessment of biomarkers with imaging to identify the most appropriate tool for prediction of pathological response to PST. This study could impact on the present management of primary breast cancer by allowing the early introduction of novel therapeutics in patients with early demonstration of poor response to treatment.

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S7

The roles of PET and CT/PET as preoperative studies

RC Delgado-Bolton[1], JL Carreras Delgado[2]

[1]Instituto PET Focuscan, Madrid, Spain; [2]Nuclear Medicine Department, Hospital Clínico San Carlos, Madrid, Spain

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Introduction Positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (FDG) is a functional imaging technique that has demonstrated advantages over anatomically based imaging modalities

in oncology in the detection of malignant lesions. The recent introduction of combined computed tomography (CT)/PET systems allows the coregistration of functional PET and anatomical CT images, which will very likely improve accuracy. The role of PET and CT/PET in breast cancer is reviewed.

Methods In breast cancer, FDG-PET has been used for detection, staging, and response monitoring; one of its main clinical applications is defining the extent of recurrent or metastatic disease [1]. We have reviewed the evidence regarding the roles of PET and CT/PET as preoperative studies in breast cancer.

Results The review of the evidence shows that PET and CT/PET can contribute to patient diagnosis and management as preoperative studies in breast cancer in the following situations:

1. Detection of primary breast cancer. When evaluating suspicious breast abnormalities, FDG-PET has a sensitivity of 80–100% and specificity of 75–100%. However, its role in primary tumor detection is not clear when compared with conventional imaging methods and remains to be determined [1].
2. Prognostic value of FDG uptake in primary tumor. Most studies suggest that the level of FDG uptake in primary breast tumors carries clinical and biological information, and that a higher FDG uptake is correlated with more clinically aggressive tumors [1]. This information may help to stratify patients according to prognosis and risk for recurrence, and may help to tailor treatments to the individual patient.
3. Axillary node staging. In this situation, FDG-PET had a sensitivity of 57–100% and specificity of 66–100%. FDG-PET underestimates the number of tumor-involved nodes compared with pathologic evaluation from conventional dissection. Therefore, FDG-PET should not replace axillary node sampling for routine staging of the axilla because even microscopic nodal involvement may be important for prognosis and treatment planning [1]. However, FDG-PET may be complementary to sentinel lymph node mapping and other standard axillary procedures in patients with more advanced tumors or equivocally palpable axillary nodes.
4. Detection of locoregional and distant metastases. Functional imaging with FDG-PET is more accurate than CT for the detection of nodal involvement in the mediastinum; the sensitivity of FDG-PET was significantly higher (85%) than CT (50%), with nearly the same specificity (90% for FDG-PET versus 83% for CT). Regarding the detection of distant metastases, FDG-PET can accurately detect sites of distant disease with a sensitivity of 80–97% and specificity of 75–94% [1].
5. Evaluation of therapy response. In locally advanced breast cancer (LABC), the assessment of response to neoadjuvant chemotherapy with conventional imaging methods is often inaccurate or slow because it depends on morphological criteria. Initial studies have shown the utility of FDG-PET in the evaluation of treatment response, specifically in its ability to discriminate responders from nonresponders more accurately and earlier than conventional imaging methods [1]. Changes in FDG uptake after a single course of chemotherapy can predict pathological response in primary LABC tumors [2,3]. Histopathological response could be predicted with an accuracy of 88–91% after the first and second course of chemotherapy [3]. Other PET tracers may be used in the evaluation of the primary tumor; preliminary results suggest that applying PET in this way may help to identify physiologic manifestations of drug resistance, which would help to tailor systemic therapy [1]. Preliminary data also suggest that FDG-PET may be useful in the assessment of sites of disease other than the primary tumor for monitoring response to chemotherapy in advanced breast cancer. Initial studies suggest other possible applications of FDG-PET, such as evaluation of the response of skeletal metastases to therapy, and prediction of the response to antiestrogen therapy in patients with advanced estrogen receptor positive breast cancer [1]. Regarding CT/PET, to date only few studies have been reported, but the advantages of CT/PET compared with PET alone may be taken to indicate that CT/PET may improve the accuracy in the evaluation of treatment response by directly defining metabolic and morphological changes [4].
6. Future applications. FDG is the most important radiotracer for PET in breast cancer and therefore it is analyzed in most studies. However, in the near future more specific PET radiopharmaceuticals may help to guide treatment, individualizing therapies to a particular patient depending on the tumor's biologic characteristics [1]. PET may help in management decisions by quantifying the therapeutic target, identifying resistance factors, and measuring early response to therapy.

Conclusion The clinical application of PET and CT/PET in breast cancer will help to predict clinical behavior, and allow one to choose the appropriate treatment and to tailor local treatment options to the individual patient. PET and CT/PET are also likely to play key roles in monitoring systemic therapy and evaluating the response to therapy at an earlier stage than conventional methods. In the future, PET may be applied with other tracers in addition to FDG, to improve characterization of tumor biology and more effectively measure response to therapy.

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S8

Minimal residual disease (MRD) in the bone marrow in ER-α-positive primary breast cancer patients

RC Coombes[1,4], MT De Bella[1], G Tripuraneni[1], A Zaidi[1], S Anwer[1], DA Stephens[2], B Ward[1], HD Sinnett[3], MJ Slade[1,4,5]

[1]Cancer Research UK Laboratories, Department of Medicine, Imperial College, London, UK; [2]Department of Mathematics, Imperial College, London, UK; [3]Breast Clinic, Charing Cross Hospital, Faculty of Medicine, Imperial College, London, UK; [4,5]Joint senior authors

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Introduction We conducted a study to determine whether a group of estrogen-induced genes could be used to detect and monitor for micrometastases in the bone marrow of patients with breast cancer.

Methods We data-mined for potential markers of estrogen action, verified their relationship to ER in cell lines and purified cells from patient biopsies, and checked their estrogen-inducibility after developing a real-time quantitative PCR assay for each. We then examined 99 bone marrow samples obtained over 2 years during the follow up of good (n = 7) or poor (n = 19) prognosis patients to determine the expression frequency.

Results We discovered that the expression of eight out of 23 genes, identified by data-mining, were estrogen-regulated. We developed real-time quantitative PCR (QPCR) assays for measurement of the genes for which ESTs were available (ER-α, PR and GATA-3, EEIG-1, EP-3, PS2). We examined their expression in purified breast cancer cells from primary cancers and also from metastases from endocrine-resistant cancers and confirmed that these genes were still expressed. Of these, three were expressed in peripheral blood, excluding them as candidate markers. We then examined 79 samples of bone marrow from 19 poor prognosis patients and 20 from seven good prognosis patients. We found that GATA-3 and ER expressions were significantly higher in the bone marrow of poor-prognosis patients.

Conclusion GATA-3 and ER appear to be potentially useful markers, in addition to CK19, for monitoring the effects of treatment in the bone marrow of patients with ER-positive breast cancer.

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S9

Circulating tumor cells and novel biomarkers for prognostic and biological of breast cancer

M Cristofanilli

Department of Breast Medical Oncology, The University of Texas, MD Anderson Cancer Center, Houston, Texas, USA

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The detection of microscopic disease in breast cancer has been evaluated in lymph nodes, bone marrow (primary breast cancer), and peripheral blood (metastatic disease) [1,2]. Most of these studies demonstrated that the detection of microscopic disease in breast cancer patients contributes prognostic information and, in selected cases, can predict the efficacy of treatments [1,2]. In primary breast cancer, the detection of microscopic disease in lymph nodes and bone marrow has led to a better understanding of the role of minimal residual disease (MRD). In metastatic breast cancer (MBC) reliable detection of circulating tumor cells (CTCs) had been obtained by using immunomagnetic separation and subsequent analysis by the CellSpotter™ analyzer (Veridex LLC, a Johnson & Johnson company, Warren, NJ, USA). This technology is becoming a standard tool for the 'real-time' assessment of prognosis and response to treatment. This is particularly important in the context of advanced disease management, considering the incurable status of the disease and the increasing therapeutic options available that could at least contribute to improve palliation and impact on overall survival.

In fact, despite years of clinical research, the odds of achieving complete response, and hence major survival benefit, for patients with MBC remain extremely low. Only a few patients who achieve a complete response after chemotherapy remain in this state for prolonged periods of time, with some remaining in remission beyond 20 years. There are presently no reliable biological markers that can predict prognosis and monitor therapy effects in MBC.

The detection of CTCs in patients with MBC about to start a new line of treatment has been shown to predict progression-free survival (PFS) and overall survival (OS). This prognostic value was independent of the line of therapy (e.g. first-line versus second-line or more) [2,3]. Moreover, in multivariate analysis CTCs demonstrated superior value compared with site of metastasis (e.g. visceral versus soft tissue/bone), type of therapy, and length of time to recurrence after definitive primary surgery. In recent analysis, detection of CTCs has also been found to be prognostic in patients with bone-only disease (not measurable disease). CTCs have been shown to be superior to standard tumor markers (e.g. Ca27-29) in predicting prognosis. Furthermore, the efficacy or benefit to systemic therapy could be predicted by the level of CTCs as early as 3-4 weeks after initiation of therapy. Patients with persistent of ≥5 CTCs demonstrated lack of response or progressive disease at the time of restaging by standard imaging modalities. Conversely, patients with < 5CTCs showed objective remission. These data clearly suggest that CTCs can be used as an early predictor of treatment efficacy and be extremely useful in sparing patients from futile therapy early in the course of their treatment.

Prospective clinical trials are presently being conducted in MBC to validate further the prognostic value of CTCs, possibly to use this diagnostic tool to better stratify patients with metastatic disease, eventually modifying the current staging system (International Stage IV Stratification Study [ISSS]). Patients with metastatic disease could be divided into the subcategories IV_A and IV_B, depending on the presence or absence of CTCs. Additional studies are presently assessing the survival benefit of early change in treatment based on the persistence of CTCs and the possibility of collecting the cells, after sorting for evaluation of biomarkers (RT-PCR, gene profiling). Exploratory studies in PBC are also being conducted.

This technology could be integrated with other new investigation tools to develop blood-based integrated platforms that will facilitate screening, diagnosis, prognosis and target discovery. A recent acquisition is represented by the use of glycan arrays [4].

Malignant transformation and tumor progression are associated with the specific changes in the complex surface carbohydrates known as tumor-associated carbohydrate antigens (TACAs). Production of autoantibodies against these abnormal carbohydrates during cancer progression is expected. A robust printed glycan array was recently fabricated that employs a library of over 200 well defined structures comprising carbohydrate sequences of *N*-glycans, *O*-glycans, glycolipids, and glycoproteins. This printed glycan array was used to simultaneously detect multiple specific antiglycan autoantibodies in sera from breast cancer patients.

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Symposium III: Advances in local treatment

S10

Simultaneous reconstructive surgery for radical mastectomy

G Robb

Division of Plastic Surgery, Department of Surgery, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

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Breast reconstruction following radical mastectomy, if desired, is considered vital to the patient's rehabilitation and is an intrinsic part of her breast cancer treatment. Immediate reconstruction – especially immediate reconstruction using autologous tissues – has become more established since the introduction of the skin-sparing mastectomy in the early 1990s. Now, as the more current therapeutic armamentarium has been expanded to feature preoperative tumor shrinking with chemotherapy, accelerated or partial breast radiotherapy, and, in particular, the increased use of breast conservation surgery for larger tumors, immediate breast reconstruction techniques have also further evolved to address the radical mastectomy defect with newer microsurgical techniques and autologous flap tissues, such as the IGAP, gracilis [1], and SIEA flaps, as well as improved silicone and anatomic saline implant designs [2] with post-operative adjustment capabilities designed to facilitate longer term symmetrical breast reconstruction outcomes.

The increased use of postmastectomy radiation therapy in patients with early-stage breast cancer has increased the complexity of planning for immediate breast reconstruction. Studies have evaluated the outcomes of breast reconstruction performed before radiation therapy, revealing a high incidence of complications and poor aesthetic outcomes [3]. Moreover, immediate breast reconstruction can interfere with the delivery of postmastectomy radiation therapy. Multidisciplinary breast conference identification of early breast cancer patients at high risk for radiation therapy has evolved a unique and highly successful 'delayed immediate' reconstruction [4] approach that preserves the aesthetic outcomes of immediate reconstruction and avoids radiation injury to the reconstructive tissues. This is accomplished by utilizing a filled subpectoral tissue expander to temporarily preserve the breast skin envelope until the final tissue pathology is confirmed and the patient either goes on to definitive reconstruction or to radiation therapy with the expander deflated. A total of 28 high-risk early breast cancer patients have undergone the delayed immediate approach with 20 patients (71%) not ultimately requiring radiation therapy. Nineteen

patients in the non-radiated group (95%) have now completed definitive reconstruction, primarily with the use of autologous tissues. The eight patients who required radiation have completed the radiation therapy and six (75%) have undergone tissue re-expansion and skin-preserving delayed reconstruction designed to be as similar in outcome to immediate reconstruction as possible. The complication rate for the initial expander placement at the time of mastectomy was 18% for all patients. Five nonradiated patients (25%) had complications in the second stage of definitive reconstruction and one patient (17%) following radiation therapy had complications in the skin-preserving delayed reconstruction.

Finally, following the successful experience of the delayed immediate approach for early breast cancer patients, 17 advanced stage patients with planned postoperative radiation therapy also had the opportunity for skin-preserving tissue expansion prior to radiation therapy upon multidisciplinary approval. All the patients received neoadjuvant chemotherapy. Five of the patients (29%) had complications in the first stage of expander placement but two patients (12%) have now completed definitive reconstruction following radiation therapy with re-expansion of preserved breast skin and have experienced no complications.

Immediate reconstruction minimizes incisional scars on the breast and improves overall breast contour, shape, and appearance. The improved aesthetic outcomes over delayed reconstruction, achieved as well by these diverse skin-preserving 'delayed immediate' approaches without significant incidents of complications, has convinced many breast cancer patients to view mastectomy with reconstruction as a viable and positive treatment choice.

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S11

Sentinel node biopsy versus conventional axillary dissection in clinically node-negative breast cancer patients

HD Bear

Division of Surgical Oncology and the Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia, USA

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Introduction Lymphatic mapping and biopsy of the sentinel lymph nodes (SLNs) as a method for pathologically staging breast cancer patients has been extensively evaluated over the past 10 years. The goal of this approach is to stage patients accurately in order to make appropriate decisions about adjuvant treatment, but also to avoid the potential morbidity of conventional axillary lymph node dissection (ALND). A large number of single center and multicenter trials have been reported that indicate the accuracy of several different methods, and the largest prospective randomized trial of SLN biopsy versus ALND, conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP), completed accrual last year. Other trials with different designs and objectives have also been completed. A great deal of information is now available on the use of this approach to breast cancer staging, but many questions remain controversial, including technical issues and patient selection parameters.

Methods A review of the literature was performed, with particular attention to recently reported results from the NSABP's B-32 trial and the UK ALMANAC trial.

Results Single center and multicenter validation trials of sentinel node biopsy for breast cancer have demonstrated success rates varying from under 70% to 100%, accuracy rates from 95% to 100%, and false-negative (FN) rates from 0% to 19% [1]. The NSABP B-32 study is a randomized trial comparing SLN biopsy alone versus SLN biopsy plus ALND. Patients with positive SLN by routine histology (without immunohistochemical staining) underwent completion axillary node dissection. A total of 5611 patients were accrued to this trial, and the technical results and accuracy of SLN biopsy were recently reported [2]. At least one SLN was identified in over 97% of the evaluable subjects, and the SLN was positive for metastases in 26%. The FN rate in the group who also had an ALND was 9.7%. The SLN was the only positive node in 61.5% of patients, and only 0.6% of patients had a positive SLN outside of the axilla. SLN identification improved with increasing surgeon experience, and the FN rate was higher after surgical biopsy of the breast versus needle biopsy. In the ALMANAC trial, patients were randomly assigned to SLN biopsy or ALND. Analysis of morbidity demonstrated markedly decreased functional sequelae after SLN biopsy versus ALND, especially in the incidence of sensory loss and arm edema [3].

Issues that are controversial include technical parameters, such as the use of a radionuclide or visible dye alone versus the combination, the sites of injection (subareolar, intradermal, or intraparenchymal), and timing of injection. Several patient selection factors, such as age, obesity, tumor size, and multicentricity, may also impact on the success rate and accuracy of SLN biopsy. Some have advocated routine use of SLN biopsy in patients with ductal carcinoma *in situ* (DCIS), but it is not clear that this impacts on treatment decisions. It is appropriate to consider SLN biopsy in patients with extensive DCIS diagnosed by needle biopsy, especially if there is a high risk for finding invasive cancer on definitive excision or if the patient is undergoing a total mastectomy. The prognostic significance of 'occult' micrometastases found in SLN by immunohistochemistry is uncertain, but will hopefully be resolved by the NSABP B-32 trial and the American College of Surgeons Oncology Group (ACOSOG) Z0010 study. There is also great interest in being able to predict accurately which patients with a positive SLN have no other nodes involved and could therefore avoid completion ALND. Finally, there is disagreement about the role and timing of SLN biopsy in breast cancer patients receiving neoadjuvant chemotherapy. The FN rates for SLN after chemotherapy have been extremely varied, but in the largest series of patients who underwent SLN biopsy and ALND after chemotherapy (in the NSABP B-27 trial) the FN rate was 10.7% and was not affected by clinical nodal status prior to treatment [4].

Conclusion SLN biopsy, in experienced hands, is a very accurate method for assessing lymph node status in women with breast cancer and clinically negative nodes. A surprising array of techniques and patients selected for the procedure appear to be successful. SLN biopsy has the potential to reduce drastically the incidence of morbidity related to surgical staging of the regional lymph nodes in women with breast cancer.

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S12

Partial breast irradiation: why and how

FA Calvo[1], JA Díaz[2], Á Montero[3], A Álvarez González[1]

[1]Hospital General Universitario Gregorio Marañón, Madrid, Spain; [2]Clínica Universitaria de Navarra, Madrid, Spain; [3]Hospital Ramón y Cajal, Madrid, Spain

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Introduction Successful treatment of early breast cancer, with high cure rates and excellent cosmetic results, is a reality that has been achieved in the past 25 years due in part to the use of post-tumorectomy whole breast radiotherapy [1]. The EORTC randomized trial questioned the need for radiation boost to the post-tumorectomy surgical bed, with an evident age-related local control effect [2]. Furthermore, examination of the topography of breast cancer recurrences after breast conservation, whether or not a radiotherapy treatment component was included, revealed that recurrences developed in the operative area in 90% of cases [1]. These factors have stimulated emerging interest in exploring partial breast irradiation (PBI) in early breast cancer. Intraoperative radiation therapy (IORT) is an appropriate technical alternative to delivering PBI, together with high dose rate brachytherapy and/or external irradiation precision techniques (3DCRT, IMRT).

Method IORT implies delivery of a high, single dose of radiation to a limited intrasurgical anatomic area. In the case of post-tumorectomy early breast cancer, the target volume is the tumor bed, maintaining a safety margin in depth (thickness of tissue to be treated) and laterally. Dosimetrically, electrons and high dose rate brachytherapy are well suited to these requirements. Intrabeam (soft X-rays at 50 kV), mammosite 3DCRT and IMRT are alternative technologies that have been adopted into clinical radiotherapy practice and have theoretically favourable dose–gradient effects. Target size, normal tissues included in the radiation fields, and operative/treatment time are variables that differ for each individual patient. The optimal PBI dose is under investigation based upon radiobiological dose-effects models. An efficient therapeutic index in IORT trials has been identified, with boost doses in the range 10–12.5 Gy (maximum 15 Gy). For IORT single radiation component, clinical information is scarce [3,4].

Clínical trials Limited institutional experience and pilot studies are available in the literature describing results with IORT as a boost, hypofractionated HDRB, or external irradiation. There are two ongoing randomized trials that have been recruiting patients since 2000 using Intrabeam system (active in UK, Europe, USA and Australia) and Novac-7 (electrons; Milan). Both trials are exploring single doses around 20 Gy. In 2005 a multi-institutional randomized trial including PBI HDR brachytherapy was initiated. Selection criteria for inclusion are strict in these trials, and a highly selected group of breast cancer patients with good prognosis are apparently being investigated. An extensive review of clinical research considerations, radiobiological implications, pathology and surgical methodological requirements, physics specifications, and summary of the available literature was recently published after a group expert meeting to define the state of the art and science of PBI, including all available techniques [5].

Discussion Recent randomized trials have questioned the need for systematic use of whole breast irradiation after lumpectomy in the context of selection by age, tumor size, or tamoxifen treatment [6]. While the data in PBI consolidate and mature, there is solid evidence to support moderation in clinical practice modification. Professor Bartelink [7] has summarized arguments to question the potential contribution of PBI, in particular IORT, to change clínical practice in the treatment of early breast cancer. The most relevant issues to be addressed, for an sceptical or conservative opinion regarding PBI, are as follows:

1. The omission of external beam irradiation without validated tools for selection of patients according to biological risk might compromise local control and survival.
2. The biological effects (both in tumor control and normal tissue toxicity) of a high single radiation dose, as is used in IORT, or altered fractionation as is used in other PBI techniques trials are speculative, with a significant risk for unpredictable late damage to normal tissue.
3. Target volume definition and dosimetric characteristics of the two ongoing randomized clinical trials have major methodological and technical differences, which will make local results uncomparable.

Some additional topics will be introduced for discussion in the presentation, such as influence of PBI in the radiotherapy management of metastatic axia, modification of scales for cosmetic assessment, treatment planning availability, dosimetric disturbances with the use of shielding material, and opportunities for prospective testing of biological predictive factors on tolerance of normal tissues.

The mentioned arguments seem valid and should be influential in the scientific development of PBI for breast cancer. Experts in PBI and precision radiotherapy for human cancer have been particularly meticulous in analyzing local effects and topography of recurrences. If PBI successfully contributes to the treatment of breast cancer, then surgeons and radiation oncologists should be open minded and change their clinical practice. Health authorities should facilitate the appropriate technology to ensure that this particular population breast cancer patients receives quality treatment.

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Clinical case discussion: interactive session

S13

Simultaneous breast reconstruction

F Gómez Bravo

Clinica Ruber, Madrid and Erasmus University Medical Center, Rotterdam, The Netherlands

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The timing of breast reconstruction following mastectomy has been an area of controversy. Simultaneous or immediate breast reconstruction (IBR) allows the patient to adjust to the loss of a breast by restoring her body image. It also reduces the surgical stages involved and provides an enhanced aesthetic outcome when combined with skin sparing mastectomy techniques. Still, IBR has not been widely accepted due to concerns about interference with locoregional recurrence control, possible delay in adjuvant chemotherapy application and technical difficulties in postmastectomy radiation therapy delivery.

During this presentation a clinical case discussion will serve as the introduction to a thorough literature review regarding the oncological safety and convenience of IBR both for partial mastectomy defects after breast conservation surgery as well as for skin sparing mastectomies. Special attention will be given to the main factors involved in the decision making process, including type, stage, and

location of the tumour, the necessity for adjuvant therapy, and the techniques used for breast reconstruction.

All cases should be individually discussed in a multidisciplinary breast team including pathologists, radiologists, oncologic breast surgeons, reconstructive plastic surgeons, and medical and radiation oncologists, with active participation of the patient.

S14

Radiotherapy in early stage invasive breast cancer: current tendencies

JA Diaz-Gonzalez[1], KH Shin[1], R Martinez-Monge[2], M Zelefsky[3]

[1]Sloan-Kettering Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, New York, New York, USA; [2]Clinica Universitaria, University of Navarra, Pamplona, Spain; [3]Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

Breast Cancer Research 2005, **7(Suppl 1):**S14 (DOI 10.1186/bcr1218)

Introduction Breast-conserving treatment (BCT) constitutes the predominant approach to local treatment in early stage invasive breast cancer. This modality of treatment includes breast-conserving surgery (BCS), radiotherapy, and systemic treatment. These three pillars of BCT have been intensively studied during the past few decades to determine their role and possible variations in their application.

Method This panel discusses the state of the art and the current developments in radiotherapy for patients with early stage invasive breast cancer treated with BCT. Different radiotherapy options are systematically presented according to particular clinical scenarios and available scientific data.

Results Whole breast irradiation (WBI) represents the radiation 'gold standard' after BCS in breast cancer stages I–II. Several randomized trials, enrolling thousands of patients and with long-term follow up, have shown a clear improvement in local control when WBI is used after BCS. A recent meta-analysis has confirmed a threefold increase in local control rates. In addition, an 8.6% decrease in the risk for death was demonstrated [1].

Adjuvant chemotherapy and hormone therapy also contribute to increase local control rates in this group of patients [2]. Substantial efforts have been made to identify a low-risk subgroup of patients who do not benefit from radiotherapy after BCS. However, this subgroup has not been yet identified because even low-risk patients (T <2 cm, margin negative, EIC negative, age >70 years) do benefit from adjuvant WBI [3]. Nevertheless, several well known clinical and pathological factors define a profile of lower risk for local relapse in which more conservative radiotherapy modalities are being explored.

In this context accelerated partial breast irradiation (APBI) appears to be a promising alternative to WBI in selected patients, with possible similar efficacy, a considerable reduction in the treatment length with a resultant improved quality of life, and potential decreased toxicity. Different APBI techniques can be used, such as intraoperative electrons, catheter-based interstitial brachytherapy, MammoSite Balloon brachytherapy, or external-beam partial irradiation. Encouraging results with adequate recruitment and medium term follow up have been published in terms of local control and tolerability, the majority of them with the use of catheter-based interstitial brachytherapy [4]. However, some concerns remain, particularly regarding potential late adverse effects and potential differences among techniques. Patient selection, expertise, and high quality technology and assurance are key elements to the success of this emerging approach. Current multicentric randomized trials are ongoing and hopefully will help to define the ideal criteria for patient selection, the most satisfactory treatment modality, and the exact role of APBI in terms of outcome and toxicity.

Conclusion Although WBI remains the radiation standard of care in early-stage invasive breast cancer after BCS, APBI emerges as promising approach for treating selected patients.

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Lecture

S15

Chemoprevention: beyond tamoxifen

J Cuzick

Cancer Research UK Centre for Epidemiology, Mathematics and Statistics, Wolfson Institute of Preventive Medicine, Queen Mary, University of London, London, UK

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Four trials have now reported on the use of tamoxifen for the prevention of breast cancer and one trial on the use of raloxifene. Overall, more than 28,000 women have participated in tamoxifen prevention trials and more than 140,000 women-years of follow up have accrued. Although early reports on the ability of tamoxifen to prevent breast cancer were apparently contradictory, with further follow up a consensus is now emerging indicating that 30–40% of breast cancers can be prevented by tamoxifen [1]. The benefit is restricted to oestrogen receptor positive tumours, where it is about 50%, but no reduction in receptor negative tumours has been found. Thromboembolic events are emerging as the most important side effects, and endometrial cancers are increased about twofold, although these are almost all low/intermediate grade, stage I cancers.

Raloxifene does not have the gynaecologic problems of tamoxifen, but still leads to an increase in thromboembolic events. Recent data from CORE/MORE [2] suggests that this selective oestrogen receptor modulator (SERM) may be more effective in prevention than tamoxifen.

Six adjuvant trials have reported on the use of aromatase inhibitors for early breast cancer. All of them show a marked reduction in contralateral tumours compared with tamoxifen [3]. The drugs are also better tolerated and have fewer side effects than tamoxifen, suggesting that they are very promising agents for breast cancer prevention. These data will be reviewed and ongoing chemoprevention trials discussed.

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Symposium IV: Primary systemic treatment

S16

Preoperative hormonal therapy: results and implications

M Dowsett

Academic Department of Biochemistry, Royal Marsden Hospital, London, UK

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The development of presurgical endocrine strategies for the treatment of primary breast cancer was developed initially from the application of such treatments in elderly patients to try to avoid the potential complications of surgery. Although there are few data available, one study has indicated that in oestrogen receptor (ER)-positive tumours clinical responses are as frequent with endocrine therapy as with cytotoxic chemotherapy. The optimal duration of treatment is not established, although most trials range between 3 and 4 months. A randomized trial indicated that the aromatase inhibitor letrozole was significantly better than tamoxifen when given as neoadjuvant therapy to patients ineligible for breast conserving surgery (BCS). A similar result was obtained for anastrozole in another randomized trial (IMPACT) but no greater efficacy than tamoxifen was seen in tumours in which BCS was possible. These two studies have provided an indication that aromatase inhibitors may be significantly more effective than tamoxifen in HER2-positive tumours. In the IMPACT trial the changes in the proliferation marker Ki67 were predictive of outcome in the large ATAC adjuvant trial, supporting the concept of using the neoadjuvant scenario to assess new therapeutic agents/ideas prior to initiating large phase 3 studies. The relatively easy availability of tissue samples during before and during neoadjuvant trials makes this a particularly valuable arena for translational research studies with new targeted agents in combination with hormonal treatment.

S17

Primary chemotherapy for operable breast cancer: the NSABP experience

HD Bear

Division of Surgical Oncology and the Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia, USA

Breast Cancer Research 2005, **7(Suppl 1):**S17 (DOI 10.1186/bcr1221)

Introduction Primary or neoadjuvant systemic chemotherapy, initially described for patients with locally advanced or borderline inoperable breast cancer, has been increasingly utilized for patients with less advanced or operable breast cancer. Theoretically, primary systemic therapy could inhibit the rapid growth of metastases after surgery and may decrease the emergence of chemoresistant clones of cells. On a practical level, primary systemic therapy has the potential to increase the use of breast conservation by decreasing tumor size. Beginning in 1988, the National Surgical Adjuvant Breast and Bowel Project (NSABP) cooperative group conducted two sequential trials to test the value of neoadjuvant chemotherapy and to optimize the treatment regimen for operable breast cancers.

Methods NSABP Protocol B-18 was designed to compare preoperative chemotherapy with doxorubicin (adriamycin) and cyclophosphamide (AC) given every 3 weeks for four cycles versus the same chemotherapy treatment given in the adjuvant setting. In protocol B-18, 1523 women with operable breast cancer were randomized to receive four cycles of AC followed by surgery or surgery followed by four cycles of AC. Women 50 years of age or older also received tamoxifen for 5 years, starting after chemotherapy.

Subsequently, NSABP Protocol B-27 was conducted with the intent to determine the effect of adding docetaxel (taxotere [T]) after four cycles of preoperative AC on disease-free survival (DFS) and overall survival (OS) of women with operable breast cancer. A total of 2411 women with operable primary breast cancer were randomized to receive either four cycles of preoperative AC followed by surgery (group I) or four cycles of AC followed by four cycles of T, followed by surgery (group II), or four cycles of AC followed by surgery and then four cycles of T (group III). Tamoxifen was given to all patients, starting concurrently with chemotherapy.

Results In protocol B-18, mean tumor size was 3.5 cm. Preoperative AC produced objective clinical responses in 79% of the treated patients and clinical complete responses (cCR) in 36%. Pathologic complete responses (pCR, defined as no invasive cancer in the breast) were observed in 13%. OS and DFS were similar in the two randomized treatment groups. Preoperative chemotherapy resulted in a statistically significant increase in the rate of breast conserving therapy (BCT), from 60% to 68%. This was particularly notable in the patients with tumors >5 cm, in whom BCT was increased from 8% to 22% [1]. Although there was a trend toward increased ipsilateral breast tumor recurrence (IBTR) in preoperative chemotherapy patients who were downstaged to lumpectomy compared with patients treated preoperatively who were considered to be candidates for BCT at the outset (15.9% versus 9.9%), this difference was not statistically significant after controlling for patient age and tumor size [2]. Patients in the preoperative chemotherapy group who experienced a pCR had significantly improved DFS and OS compared with all other patients in the preoperative chemotherapy group ($P < 0.0001$). Clinical response was also associated with improved outcomes with long-term follow up [2,3].

For protocol B-27, mean tumor size was 4.5 cm; this and other key characteristics were evenly balanced among the three treatment arms. The addition of docetaxel preoperatively resulted in significant increases in cCR and pCR at the time of surgery compared with AC alone (63.6% versus 40.1% and 26.1% versus 13.7%, respectively) [4]. Despite this, addition of docetaxel to AC did not significantly impact on survival in this cohort of patients [5]. There was a trend toward improved DFS in group II patients who received preoperative T, but this was not statistically significant (72% versus 67% DFS at 5 years; HR = 0.86, $P = 0.10$). In an analysis of relapse-free survival (RFS), which did not include second primary cancers, group II had a significantly better outcome compared with group I (74% versus 69% RFS at 5 years; HR = 0.81, $P = 0.03$). Group III RFS was not significantly different from group I (71% at 5 years; HR = 0.91, $P = 0.32$). Addition of docetaxel significantly reduced the incidence of local recurrences as first events, including IBTR in patients treated with breast conservation. There were no significant interactions between treatment and estrogen receptor status, age, tumor size, or clinical nodal status. An exploratory analysis of treatment effects in subsets of patients according to clinical response to AC suggests that preoperative T, but not postoperative T, significantly increased DFS in patients who had a partial clinical response after four cycles of AC (63%, 74%, 65% at 5 years for groups I, II, and III; HR = 0.68 for group II versus group I, $P = 0.003$). Addition of T did not appear to be beneficial in patients who were nonresponders after AC nor in those patients who had a cCR after AC. Pathologic complete response was a highly significant predictor of DFS and OS in all treatment groups (HR = 0.45, $P < 0.0001$, and HR = 0.33, $P < 0.0001$, respectively). In addition, pathologic nodal status after chemotherapy was a significant prognostic factor for survival, independent of pathologic response in the breast.

Conclusion The B-18 trial did not demonstrate superiority of neoadjuvant over adjuvant chemotherapy for operable breast cancer, but with equivalent survival and increased BCT, the neoadjuvant approach can safely be used to offer BCT to more women with breast cancer. This trial also demonstrated a strong association between pCR and improved patient outcomes. In B-27, however, despite a doubling of the pathologic complete response rate in the breast with the addition of T preoperatively, we have not yet observed a significant improvement in DFS or OS for the study as a whole. Addition of preoperative or postoperative docetaxel decreased the incidence of local recurrences. There was a decrease in relapses with the addition of preoperative T, particularly in a subset of patients who had partial clinical responses to AC alone. Post-treatment pathologic response in the breast and nodal status remained powerful predictors of patient outcomes. Future studies will examine the value of additional drugs given with doctaxel after AC preoperatively and will also be designed to

assess the ability of genomic and molecular profiles of pretreatment tumor to predict responsiveness to chemotherapy.

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S18

Neoadjuvant treatment: the MD Anderson experience

GN Hortobagyi

Department of Breast Medical Oncology, The University of Texas, MD Anderson Cancer Center, Houston, Texas, USA

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Adjuvant chemotherapy has traditionally been administered in the postoperative setting. However, numerous studies have evaluated its use preoperatively. The potential benefits of neoadjuvant chemotherapy (NACT) include downstaging the primary tumor to allow breast-conserving surgery and assessment of a tumor's *in vivo* sensitivity to individual chemotherapeutic regimens. Our group at the MD Anderson Cancer Center initiated clinical trials with neoadjuvant chemotherapy in 1974, and over the past three decades we have treated several thousand patients in prospective clinical trials. Initially, NACT was reserved for the treatment of patients with locally advanced and/or inflammatory breast cancers. These studies clearly demonstrated that most patients had a marked reduction in tumor volume with anthracycline–cyclophosphamide–fluorouracil based regimens. Inoperable tumors became operable for most patients, and later studies indicated that even large tumors became candidates for breast-conserving therapies. We described 20 years ago the prognostic value of pathological complete remission (pCR), and subsequent studies included pCR as a surrogate end-point for long-term efficacy. These findings were later confirmed by the largest study evaluating the impact of neoadjuvant chemotherapy, the NSABP B-18. In this study, 1523 women were randomized to receive four cycles of doxorubicin and cyclophosphamide either prior to or after surgical resection. Another large study of similar design was performed by the EORTC, with similar results. The timing of chemotherapy did not affect the disease-free or overall survival for the entire cohort, although more patients who received preoperative therapy were able to undergo breast conservation rather than mastectomy in comparison to those treated postoperatively. These studies confirmed the clear correlation of pathological complete response (pCR) in the breast (absence of invasive cancer cells) with survival. Using a single, anthracycline-containing chemotherapy regimen, a pCR rate of about 10–13% can be obtained. The definition of pCR used by our group includes the absence of lymph node involvement, in contrast to the definitions used by NSABP and other groups. The pCR rate has become one of the most important intermediate trial endpoints in assessing the efficacy of new adjuvant chemotherapy regimens.

Published studies of anthracycline-based preoperative chemotherapy demonstrate pCR rates of up to 17%. Several recently reported studies including the sequential use of anthracycline-based regimens and taxanes have achieved significantly higher pathologic responses, ranging from 25% to 34%. Our studies focused on the sequential use of anthracyclines and taxanes, showing excellent tolerance and efficacy of this strategy. In addition, we demonstrated the therapeutic superiority of weekly paclitaxel in this setting. These findings were subsequently confirmed by much larger, randomized trials conducted by another cooperative group. We used the neoadjuvant strategy for the initial evaluation of trastuzumab in patients with primary breast cancer. That small randomized trial indicated an almost threefold increase in pCR with the addition of trastuzumab to chemotherapy.

Currently, we conduct studies with gene profiling in the neoadjuvant setting to determine predictors of pCR, and therefore long-term prognosis, and to develop individualized medicine for patients with primary breast cancer.

There are multiple remaining questions related to the use of this strategy, however. Some pertain to optimal local–regional therapies: when should axillary assessment be performed in relation to NACT, what should be the criteria for administration of postmastectomy radiation therapy following NACT, and how to optimally perform breast-conserving surgery following NACT. The role and relative timing of neoadjuvant hormone therapy (NAHT) is also under intensive evaluation at this time. This is solely relevant to the group of patients with hormone receptor-positive tumors, but has potential impact on the type and sequence of local, regional and systemic therapies.

Symposium V: Advances in adjuvant treatment

S19

Tamoxifen resistance and adjuvant hormone therapy

A Howell

CRUK Department of Medical Oncology, University of Manchester, Manchester, UK

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The Oxford Overview of adjuvant endocrine trials [1] indicates that 5 years of adjuvant tamoxifen reduces recurrence by 41% and deaths by 34% in women with oestrogen receptor (ER)-positive breast cancers. At 5 years, in all patients studied, the recurrence rate was 25.8% in controls but 13.9% on tamoxifen. There was a substantial 'carry over' effect of tamoxifen such that even after 15 years of follow up mortality was about 30% less in tamoxifen-treated patients. The effect of tamoxifen was greater in patients with ER-positive, PR-positive as compared with ER-positive PR-negative tumours. These data indicate a substantial effect of tamoxifen but it is clear that approximately half of patients are resistant to tamoxifen *de novo* (early relapses) or acquire resistance if we assume that women who relapsed later had an initial response to tamoxifen. The potential reasons for resistance include activated growth factor pathways overriding the inhibitory effects of the drug either via nuclear or membrane ER. Of ER-positive PR-negative tumours, 30% are HER1/2-positive, as compared with about 10% of ER-positive PR-positive tumours, and this difference may account for their lower activity of tamoxifen in PR-negative tumours. Modern aromatase inhibitors (AIs) are more effective in reducing relapse compared with tamoxifen whether AI treatment is initiated after surgery (ATAC and BIG1-98 trials) or after 2–3 years of tamoxifen (ITA, ARNO/ABCSG). At present, it is difficult to distinguish any differences in effectiveness between the three agents used in these trials (anastrozole, letrozole and exemestane) but small differences in toxicity patterns are beginning to emerge. The reason for the greater effectiveness of AIs is not clear. In randomized studies of neoadjuvant endocrine therapy [2] and in the anastrozole adjuvant trials (ATAC and ARNO/ABCSG) [3], the AIs used were particularly more active than tamoxifen in the ER-positive PR-negative subgroup of tumours, but this was not seen in the BIG1-98 and IES trials. Studies on letrozole

resistant human mammary tumour cell lines show that growth factor pathways such as MAPK (mitogen-activated protein kinase) are activated, and sensitivity to the AI can be restored by growth factor pathway inhibitors [4]. Also, AI resistance can be reduced by combined treatment with fulvestrant in animal models. These data suggest mechanisms whereby AI resistance may be circumvented in patients and point to new approaches to adjuvant treatment.

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S20

Classical versus new prognostic factors for adjuvant treatment selection based on line software to estimate risk and benefit

PM Ravdin

University of Texas Health Sciences Center, San Antonio, Texas, USA

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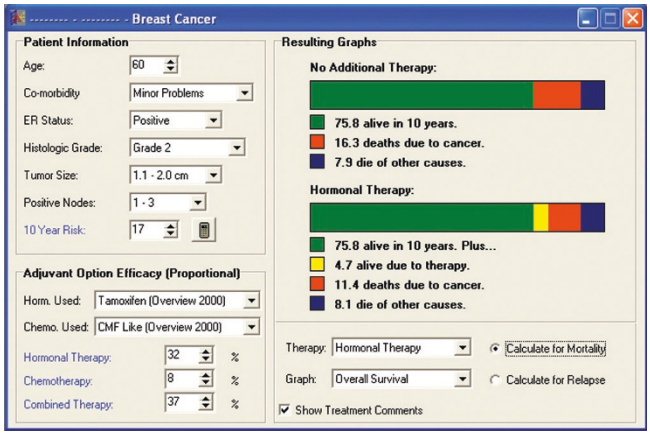
The decision about whether to receive systemic adjuvant therapy for cancer depends on weighing the benefit (in terms of increased relapse-free or overall survival) against the cost and risk with such therapy. As the use of adjuvant therapy has been extended into node-negative breast cancer, the decisions have become less obvious because the benefit is smaller, particularly as compared with the risks associated with the therapy and nonbreast cancer mortality. Guidelines can be helpful in these situations, but a limitation of guidelines is that they usually are compendiums of expert opinion and provide little quantitative guidance. In addition, it can be difficult to state guidelines that can be patient specific when multiple parameters might be used in the decision (e.g. age, comorbidity state, actual number of nodes, tumor size, hormone receptor status, histologic grade, and additional pathologic laboratory evidence).

To address this problem the decision tool Adjuvant! (Fig. 1) was created [1,2]. It uses data from national databases and other sources to make estimates of a patient's baseline prognoses. It uses data from the Overview, and individual clinical trials to make estimates of treatment efficacy. It uses national data about age-specific competing mortality to make estimates of competing mortality. Although the program provides these estimates, it has the flexibility to allow the user to modify the estimates as they think appropriate. Over 200 pages of help files allow the user to review the data on which the program is based, and the methods used by the program and the assumptions which it makes. That for the most part these estimates are reasonable is supported by a validation of Adjuvant!'s estimates in a large independent database [3]. Specific sheets describing the toxicity and safety issues of different adjuvant treatment options are included.

The presentation will discuss the strengths and limitations of this approach. The major strength is that this tool allows the doctor and patient to review in a quantitative sense the benefits and risk of different options. The limitations are that for many therapies we have limited knowledge about their long-term efficacy (and Adjuvant! makes specific assumptions to deal with this), and undoubtedly we will learn more about how some tumors' characters may affect their sensitivity to therapy. How new prognostic factors and genomic information may be included will be discussed.

Figure 1



Main screen of Adjuvant!

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S21

Adjuvant chemotherapy for operable breast cancer: old vs new schema

P Valagussa, L Gianni, G Bonadonna

Istituto Nazionale Tumori, Milano, Italy

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In 1975 we presented our first report on the efficacy of cyclophosphamide, methotrexate and fluorouracil (CMF) as adjuvant treatment for node-positive breast cancer. Thirty years later, the results of this study demonstrate that the significant advantage in both relapse-free (reduction in relative risk for relapse of 29%) and overall survival (reduction in relative risk for death of 21%) persists over the years and that adjuvant CMF can exert a moderate but worthwhile suppression of micrometastases, regardless of anatomical sites [1].

With the aim of further improving the prognosis of operable breast cancer patients, in the early 1980s many research groups designed and carried out new randomized trials including anthracyclines. Despite the fact that many individual trials failed to observe a true benefit for the tested anthracycline regimen, the arithmetic construction on which the international overview is based (i.e. the summing up of many individual trials to increase the statistical power) allowed it to be estimated that there was a reduction in the risk for disease relapse and death of approximately 10%, corresponding to an absolute difference of approximately 3% [2].

At the Milan Cancer Institute we designed two different studies to test the effectiveness of sequential non-cross-resistant regimens containing anthracyclines. Briefly, in patients with moderate risk for relapse the sequential delivery of CMF followed by adriamycin was compared with CMF alone, whereas in a second study conducted in high-risk patient the inverse sequence (adriamycin followed by CMF) was tested. The rationale behind these studies was that switching to another regimen early in the administration of chemotherapy could overcome drug resistance. The updated 20-year results confirmed that the relative merits of anthracycline-containing adjuvant programs can also depend on the modality of administration and must be assessed in properly

designed trials in which the magnitude of the benefits can be weighted against potential risks [3].

The treatment results observed after sequential adriamycin followed by CMF in a poor risk subset could probably be explained by an increased density of the anthracycline, which was delivered at full dose within the first 9 weeks of treatment. The value of dose density was recently confirmed by the National Cancer Institute's Breast Intergroup INT C9741 trial, in which patients who received the dose-dense regimens had significantly improved early treatment outcome compared with their counterparts who did not receive these regimens.

The role of sequential non-cross-resistant regimens was tested in many other trials, both in the adjuvant and neoadjuvant settings. Of note, the 3-year joint efficacy analysis of the National Epirubicin Adjuvant Trial (NEAT) and of the Scottish Cancer Trials Breast Group reported a highly significant benefit in favor of the sequential regimen, supporting the hypothesis that the sequential administration of single-agent anthracyclines given upfront before CMF can indeed improve treatment outcome. Also, the addition of taxanes after delivery of adriamycin and cyclophosphamide contributed to improving therapeutic results over the nontaxane regimen both in the adjuvant and neoadjuvant settings. By contrast, in the INT C9741 trial no difference was detected in treatment outcome between the concurrent or sequential schedules of adriamycin, cyclophosphamide and paclitaxel. Similarly, the MD Anderson randomized study failed to detect superiority of the sequential arm.

Thirty years ago, treating patients who were free of identifiable metastatic disease with systemic adjuvant therapy because some of them might eventually develop distant disease was a revolutionary departure from prior treatment approaches [4]. It has been estimated that improvements since the 1970s in the way in which breast cancer is managed must have prevented about 25–30% of the breast cancer deaths in middle-aged women that would otherwise have occurred in the year 2000.

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Controversial panel discussion

S22

Prognostic studies in patients with high-risk primary breast cancer (HRPBC) receiving high-dose chemotherapy (HDC)

Y Nieto

Clínica Universitaria de Navarra, Pamplona, Spain

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Introduction After standard-dose chemotherapy (SDC), more than 50% of patients with HRPBC (defined as extensive axillary node involvement or inflammatory disease) experience relapse. Controversy has surrounded the use of HDC for HRPBC for over a decade. Current results from at least 15 randomized trials comparing diverse forms of HDC and SDC for HRPBC appear contradictory at this point [1]. In addition, some studies suggest that younger women might benefit selectively from HDC. An NCI-sponsored meta-analysis is in progress.

Regardless of this unsettled issue, it is clear that a substantial percentage of HRPBC patients still relapse after HDC. Extensive prognostic studies are required to improve their outcome. Such investigations may allow us to identify adequate patient subsets and new therapeutic targets for trials of developmental therapeutics. Combinations of HDC, capable of ample cytoreduction, with novel agents that target specific tumor features responsible for post-transplant relapse may hold promise.

Methods We analyzed clinical variables in HRPBC patients enrolled at the University of Colorado in clinical trials of HDC targeting 4–9+, ≥10+ nodes, or inflammatory disease. First, we developed a prognostic model among 176 patients treated from 1990 to 1997, and validated it in an external sample. Subsequently, the model was validated prospectively in a second cohort of 88 patients treated at Colorado since 1997.

We hypothesized that intrinsic biologic differences, insurmountable by HDC, existed between the two risk categories identified by the clinical model. Through immunohistochemical analyses of paraffin-embedded tumor blocks collected from the referring institutions, we studied a series of putative molecular candidates, related to signal transduction pathways or an angiogenic phenotype, which could be responsible, at least in part, for those differences.

Results At median follow up of more than 7 years, the relapse-free survival (RFS) and overall survival (OS) rates for the whole group of 264 patients treated at Colorado were 69.8% and 73%, respectively. The median time to relapse was 14 months (63.5% relapses within the first 2 years, 6.7% after the 5th year).

We identified three clinical variables independently associated with outcome: nodal ratio (number of involved nodes /number of dissected nodes), pathological tumor size, and hormone receptors [2]. A scoring system was constructed with those variables: score = (nodal ratio × 3.05) + (tumor size × 0.15) – (ER/PR × 1.15). In this formula, size is entered in cm, and ER/PR is assigned '1' if positive (estrogen receptor [ER] and/or progesterone receptor [PR] positive), or '0' if negative (both negative). A cutoff score of 2.41 yields the best sensitivity and specificity. Thus, patients with low (<2.41) and high (≥2.41) scores before transplant presented significant differences in outcome. This model was validated in an external sample of 225 HRPBC patients treated at Duke University with the same HDC. It was subsequently validated prospectively in our second patient cohort [3].

Overexpression of HER2, identified as an independent predictor of outcome, complemented the clinical model, establishing the following risk groups: low risk (low score, HER2-negative; 44% patients; 87% RFS), intermediate risk (low score, HER2-positive; 29% patients; 68% RFS), high risk (high score, any HER2; 27% patients; 49% RFS) [4].

We detected an independent prognostic effect of EGFR (epidermal growth factor receptor), particularly among HER2-positive patients [5], which suggests a synergistic effect through heterodimerization of both receptors. In contrast, we did not observe a prognostic effect of p53 status [4].

Tumor angiogenesis, assessed through CD31-stained microvessel count, was an independent adverse predictor of outcome [6]. In contrast, tumor VEGF (vascular endothelial growth factor) expression lacked prognostic significance in our population with locoregionally advanced tumors, in contrast to multiple prior observations in patients with earlier disease [6].

Finally, we observed that the presence of tumor cells contaminating the apheresis product, detected through immunocytochemistry for cytokeratins, was independently associated with post-transplant relapse [7].

Conclusions We can now predict which HRPBC patients are most likely to remain long-term disease free after HDC. Additionally, we identified important prognostic molecular markers that could constitute relevant targets for studies combining novel therapeutics with HDC in HRPBC.

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S23

Efficacy of high-dose alkylating chemotherapy in the adjuvant treatment of HER2/neu-negative primary breast cancer: update of the Dutch randomized trial

S Rodenhuis[1], M Bontenbal[2], LVAM Beex[3], WM Smit[4], MA Nooij[5], EE Voest[6], E van der Wall[7], P Hupperets[8], Harm van Tinteren[1], HL Peterse[1], MJ van de Vijver[1], EGE de Vries[9], for the Netherlands Working Party on Autologous Transplantation in Solid Tumors

[1]Netherlands Cancer Institute, Amsterdam, The Netherlands; [2]Erasmus Medical Center/Daniel den Hoed Cancer Center, Rotterdam, The Netherlands; [3]University Hospital Nijmegen, The Netherlands; [4]Medical Hospital Twente, Enschede, The Netherlands; [5]University Medical Center, Leiden, The Netherlands; [6]University Medical Center, Utrecht, The Netherlands; [7]Free University Hospital, Amsterdam, The Netherlands; [8]University Hospital, Maastricht, The Netherlands; [9]University Hospital Groningen, Groningen, The Netherlands

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Introduction The role of high-dose chemotherapy in the adjuvant treatment of high-dose breast cancer has not been established. Results have been reported from six randomized studies with a symmetrical study design (Table 1). All show a lower relapse rate in the high-dose arm, but in only one study was this result statistically significant.

Methods Patients below 56 years of age who had undergone surgery for stage II or III breast cancer were eligible if they had at least four tumor-positive axillary lymph nodes. Patients in the conventional dose (CD) arm received five courses of FEC (fluorouracil 500 mg/m^2, epirubicin 90 mg/m^2 and cyclophosphamide 500 mg/m^2; every 3 weeks) followed by radiation therapy and tamoxifen. The high-dose (HD) arm was identical, except that high-dose chemotherapy (CTC [cyclophosphamide 6 g/m^2, thiotepa 480 mg/m^2 and carboplatin 1600 mg/m^2]) with peripheral blood progenitor cell reinfusion was given instead of the fifth FEC course.

Results Between August 1993 and July 1999, 885 patients with primary breast cancer and four or more tumor-positive lymph nodes were randomized in 10 Dutch centers in a study of high-dose chemotherapy. The results of this study at 57 months of follow-up have now been updated at 87 months. In a pathology review, 621 tumor samples were shown to be HER2/neu-negative (either 0+ at immunohistochemistry or negative at *in situ* hybridization). Patients with HER2/neu-negative disease had a 5-year RFS of 72% following HD and of 59% after CD ($P = 0.002$). Overall survival in the HD group was 78% at 5 years versus 71% for the CD group ($P = 0.02$). Young age and low malignancy grade were associated with a relative benefit for HD (tests for interactions: $P = 0.04$ and $P = 0.0057$, respectively). The treatment-related mortality in the high-dose chemotherapy arm was 1%. An equal number of second malignancies were observed in both arms.

Conclusion Although the subgroup analysis of HER2/neu-negative disease was not planned in the original protocol, these findings are consistent with findings from other studies [4]. The marked efficacy of HD therapy in HER2/neu-negative breast cancer may have been masked in this and in other studies by its disadvantage in the HER2/neu-positive group, which may have benefited from a higher dose of anthracycline-dose in the control arm. High-dose alkylating chemotherapy is a viable option for high-risk breast cancer patients with HER2/neu-negative disease.

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Table 1 (abstract S23)

Randomized studies evaluating the role of high-dose chemotherapy in high-risk breast cancer

Author	Patients	Selection	Conventional arm	High-dose arm	RFS analysis
Rodenhuis [1]	885	4$^+$ nodes	5×FEC	4×FEC – CTC	HD better ($P = 0.08$)
Peters [2]	785	10$^+$ nodes	4×CAF + ID-CPB	4×CAF + HD-CPB	No difference, HD fewer relapses
Tallman [3]	540	10$^+$ nodes	6×CAF	6×CAF + CT	No difference, HD fewer relapses
Roché	314	8$^+$ nodes	4×FEC	4×FEC + CMA	HD better ($P = 0.002$)
Tokuda	97	10$^+$ nodes	6×CAF	6×CAF + CT	HD better (NS)
Rodenhuis	81	Infraclav biopsy	4×FEC	4×FEC + CTC	No difference, HD less relapses

CAF, cyclophosphamide, doxorubicin, fluorouracil; CMA, cyclophosphamide, mitoxantrone and melphalan; CT, cyclophosphamide and thiotepa; CTC, cyclophosphamide, thiotepa and carboplatin; FEC, fluorouracil, epirubicin, cyclophosphamide; nodes, tumor-positive axillary lymph nodes; HD-CPB, high-dose cyclophosphamide, cisplatin and BCNU; ID-CPB, intermediate-dose cyclophosphamide, cisplatin and BCNU.

S24

SOLTI (Solid Tumor Intensification) Group experience with high-dose chemotherapy treatment for early breast carcinoma

H Cortés Funes[1], A Lluch[2], M Climent[3], JJ López[4], B Ojeda[4], J Hornedo[1], E Ciruelos[1], J Baselga[5], on behalf of the SOLTI Group

[1]Medical Oncology Dpt.,Hospital Universitario 12 de Octubre, Madrid, Spain; [2]Medical Oncology Dpt., Hospital Clínico Universitario, Valencia, Spain; [3]Medical Oncology Dpt., Instituto Valenciano de Oncología, Valencia, Spain; [4]Medical Oncology Dpt., Hospital Santa Creu i Sant Pau, Barcelona, Spain; [5]Medical Oncology Dpt., Hospital Universitario Vall d' Hebron, Barcelona, Spain.

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Introduction Primary carcinoma of the breast is a worldwide public-health problem; despite conventional treatment, long-term prognosis is poor, especially for large tumors or in cases with axillary involvement. In an attempt to improve these results, phase II high-dose chemotherapy trials were performed by the SOLTI Group.

Method A total of 416 patients were included in three high-dose chemotherapy trials in the adjuvant setting, as follows. The 9301 trial included 297 patients with stage II/III breast carcinoma with more than 10 axillary lymph nodes involved. After conventional adjuvant chemotherapy (FEC regimen), high-dose chemotherapy (STAMP V regimen) with peripheral stem cell support was performed. The 9302 trial included 66 patients with inflamatory breast carcinoma treated with three to six cycles of FEC neoadjuvant therapy. Responding patients were treated with high-dose chemotherapy after surgery with STAMP V regimen and blood stem cell support. The 9702 trial included 53 patients with stage III breast cancer treated with neoadjuvant doxorubicin and paclitaxel. Patients with pathologic axillary involvement in the surgical specimen were treated with adjuvant STAMP V high-dose chemotherapy.

Results In trial 9301, with a median follow up of 63 months, 5-year disease-free survival (DFS) was 59% and overall survival (OS) was 80%. In trial 9302, median DFS was 30 months and median OS 75 months, with 55.3% of patients alive at 5 years. In trial 9702, with a median follow up of 31 months, the median DFS and OS have not yet been determined. No toxic deaths were reported. Most common nonhematological toxicities were emesis, mucositis, hepatic and alopecia. Neutropenia was easily resolved with G-CSF support; and anemia and trombopenia were frequent (50–60% patients).

Conclusion The toxicity of treatment with high-dose chemotherapy is acceptable and similar to that described in other series. Although the results obtained are promising, their comparison with historic controls and the information derived from other reported trials do not enable us to recommend high-dose chemotherapy with bone-marrow rescue as a routine treatment in high-risk breast cancer. It nevertheless remains a valid investigational strategy.

Symposium VI: Molecular targeted agents

Symposium VII: Advances in metastatic disease

S25

Oral vinorelbine in metastatic breast cancer

PF Conte, S Giovannelli

Division of Medical Oncology, Department of Oncology and Haematology, University Hospital, University of Modena and Reggio Emilia, Modena, Italy

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Introduction A new oral formulation of vinorelbine has been introduced in clinical studies since 1994, following increasing interest in the development of oral chemotherapy, driven by pharmacoeconomic issues, patient convenience and the potential for improved quality of life. A dose-finding study [1] established that 100 mg/m^2 was the MTD dose, limiting toxicities being neutropenia, nausea and vomiting, and neuroconstipation; the recommended dose was then defined at 80 mg/m^2 per week. The first phase II studies conducted in chemotherapy-naive NSCLC and as first-line chemotherapy in advanced breast cancer (ABC) showed an excessive rate of complicated neutropenia. This led to the formulation of a new schedule in which a lower dose of 60 mg/m^2 per week was administered for the first three courses with escalation to 80 mg/m^2, with a safety profile qualitatively comparable to that of intravenous vinorelbine at standard doses [2]. Equivalent blood concentrations were demonstrated between 80 mg/m^2 oral and 30 mg/m^2 intravenous, and between 60 mg/m^2 oral and 25 mg/m^2 intravenous [3].

Studies in metastatic breast cancer (MBC): single agent A multicenter phase II trial assessed the activity, safety and pharmacokinetic profile of oral navelbine in ABC. Sixty-four patients were entered to receive oral NVB on a weekly basis for a total of 8 weeks unless progression or toxicity occurred. Oral vinorelbine was given at 60 mg/m^2 weekly for the first three administrations and was increased to 80 mg/m^2 for the subsequent administrations if there was no grade 4 neutropenia or no more than one episode of grade 3 neutropenia. Patients with objective response or stable disease continued treatment up to a total of 12 weeks or more. Fifty-eight evaluable patients were included. Four patients (6.9%) had complete responses and 14 (24.1%) had partial responses, for an overall response rate of 31% (95% confidence interval 19–43%). The median progression-free survival was 17.4 weeks, and the median overall survival was 22.9 months. There were no treatment-related deaths. The main toxicity was neutropenia: grade 4 in 17.2% of the patients, and 1.8% of administrations and associated clinical serious events in four patients (6.2%). Grade 3 and 4 nausea and/or vomiting were noted in 3.1% and 4.6% of the patients, respectively. Only one patient developed grade 3 neuroconstipation. An analysis of Quality of Life Questionnaire C30 forms revealed no significant alteration between baseline and weeks 8 and 16 in global quality of life. Oral navelbine as single agent in first-line MBC has the same efficacy of intravenous vinorelbine in phase II studies in terms of OR, duration of response, progression-free survival and overall survival, and is well tolerated with a manageable gastrointestinal toxicity (8% of G3-4 N/V without prophylactic antiemetic treatment) [5].

A second phase II trial is still ongoing. An interim analysis on the first 72 patients (median age 63 years) showed a similar toxicity profile with a RR of 30%, a median progression free-survival of 4.6 months and a median survival of 20.7 months.

Studies in MBC: combinations The increasing prevalence of antracycline and taxane treatment in adjuvant setting led to an exploration of new combinations of non-cross-resistant therapies, in particular for those patients for which a polychemotherapy might offer greater benefits than single agents. In this setting the results of intravenous navelbine in combination with other drugs suggested new models in which to introduce the oral formulation.

Several phase II studies had investigated intravenous vinorelbine in association with capecitabine with a RR in second line >50% and with a mild toxicity. Only one phase II trial with this combination has been conducted as first-line chemotherapy, and it confirmed a good toxicity profile. The availability of oral formulations of both of these drugs led to investigation of their attractive combination, and preliminary results are now available from an ongoing phase II trial of vinorelbine oral plus capecitabine ± trastuzumab in MBC as first-line chemotherapy. Capecitabine is administered at the dose of 2000 mg/m^2 per day given days 1 to 14, and vinorelbine at the dose of 60 mg/m^2 on days 1 and 8 every 3 weeks for the first course, then escalated to 80 mg/m^2 from the second course. After 81 courses the incidence of G3 nausea/vomiting was 2.5%, G3 diarrhea 3.7%, grade 3 HFS 1.2%, and G3/4 neutropenia 13.5%. After 2 cycles the combination has revealed 4/16 CR-PR, 10/16 SD and 1/16 PD; and after 4 cycles 7/15 CR-PR and 7/15 SD. From these preliminary results this combination seems to be effective in the treatment of MBC previously treated with an anthracycline and/or a taxane with low toxicity.

Several studies have investigated the combination of intravenous navelbine and trastuzumab with an OR of 61–75%, demonstrating that this can be an effective and well tolerated option for HER2$^+$ MBC. It is

an important regimen, given the increasing prevalence of antracycline and taxane treatment in adjuvant setting.

Other sequential combinations of oral vinorelbine with paclitaxel or docetaxel or epirubicin are being explored, suggesting a possible role for the oral formulation in a sequential prolonged treatment in MBC.

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S26

The role of Xeloda in metastasic breast cancer

H Cortés-Funes

Hospital Universitario '12 de Octubre', Jefe de Servicio de Oncología, Madrid, Spain

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Currently, breast cancer is the primary reason for death due to cancer in Spanish women aged between 35 and 64 years. Nevertheless, despite detecting an increased incidence, it is observed a decrease on mortality rates, in agreement with it is observed in other European countries. This issue is basically due to the development of programs to sift and the use of widespread systemic treatments, both on early and advanced stages of the disease. During the past decade breast cancer has benefited from incorporation of new antitumor drugs to treat this kind of cancer. These new drugs are able to control, in an effective way, the progress of metastatic disease and slow down or eradicate micrometastasis in early stages.

Patients with metastatic disease have been useful in the study of new active agents and their combinations subsequently used in adjuvant regimens. Breast cancer treatment often achieves important objective responses with different durations of treatment, but these durations have an impact on patient survival. The median of survival in this group varies between 18 and 24 months, and the main objective for the treatment of metastatic breast cancer is palliation by means of the control of disease-related symptoms, with an adequate profile of toxicity, which provides an improvement in quality of life.

There are currently several lines of work on the development of breast cancer treatments at different stages. These are expected to improve survival and quality of life parameters. These lines of development range from the incorporation of new active agents (cytotoxic and hormonal) to optimization of current schemes with active agents (old and new), by means of new combinations used for metastatic disease and use in early stages, even before surgery.

Breast cancer is one of the most chemo sensible solid tumours, that respond to almost every cytotoxic drug used alone or in combination. These active agents include alkylating drugs (e.g. cyclophosphamide and the cisplatins) and antimetabolites (e.g. fluorouracil and methotrexate), which were used for the design of the first-line polychemotherapy schemes in solid tumours such as CMF. After this, the anthracyclines (doxorubicin and epirubicin) were substituted for methotrexate in the latter combination, giving us the FAC and FEC schemes, which were classic treatments until relatively recently. During the past decade, many new agents have been incorporated that have improved both response rates and patient survival, such as the taxanes (paclitaxel and docetaxel), vinorelbine, caelyx and, recently, gemcitabine; there have been used in new combinations or have even been used as isolated agents as second- and third-line treatments for with advanced disease.

The main problem with these agents and their combinations is the complexity of administration, at day hospitals and through intravenous injections, which seriously impairs patient quality of life. However, the benefit in terms of survival parameters and symptom reduction offset these difficulties.

Capecitabine (Xeloda), one of the most recently introduced active agents into treatment for metastatic breast cancer, has the same antitumoral activity as current agents but without many of their inconveniences, which is the reason why its incorporation into standard treatment for breast cancer patients is becoming increasingly common. It is orally administered, avoiding the difficulties associated with intravenous injection. Its mode of action is similar to that of 5-fluorouracyl in continuous infusion, without the need for infuser or central catheters. On the other hand, it has a synergistic action with the majority of cytotoxic drugs, in particular with docetaxel, leading to a better survival rates. The results of studies conducted in recent years have confirmed the important role of capecitabine in the treatment of advanced disease, and it have been the base for the studies with capecitabine on the adjuvant and neoadjuvant setting.

S27

Targeting the right chemotherapy for the right patient

PF Conte, V Guarneri

Department of Oncology and Hematology, University of Modena and Reggio Emilia, Modena, Italy

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In the past few years breast cancer mortality has been declining in most Western countries as a consequence of better education, implementation of screening programs and more effective therapies. However, a small proportion of patients are metastatic at initial diagnosis (about 5–7%), and 25–30% of patients develop metastases following primary treatment. At this stage, the disease is considered incurable, the median survival ranges from 2 to 4 years, and a limited proportion of patients (about 20%) survive more than 5 years. In this scenario, it is important to identify the aims of treatment on the basis of individual patient needs. Data from clinical trials, meta-analyses, databases of large institutions, and cancer registries indicate that chemotherapy can prolong survival, and survival prolongation is associated with the activity of drugs [1-5]. Moreover, those patients who achieve a complete response have about 20% chance of surviving beyond 5 years [6]. Finally, although there are few trials specifically addressing symptomatic control and quality of life, it is generally agreed that tumor shrinkage is associated with better control of symptoms, and that quality of life results from the balance between activity and tolerability of treatments.

Metastatic breast cancer patients represent a very heterogeneous population, and several factors are important in determining prognosis: patient characteristics such as motivation, compliance with treatment, age, performance status and comorbidities; tumor characteristics such as hormonal receptor status and expression of HER2-neu; prior adjuvant therapies and disease-free interval; and site and extension of metastatic spread.

Treatment options include locoregional treatments, endocrine agents, monoclonal antibodies, bisphosphonates and cytotoxic agents. The aims of these treatments include symptomatic control, maintenance of quality of life, tumor shrinkage, prolonging time to progression, and survival prolongation.

Although the role of radiation therapy in controlling locoregional relapses, and of endocrine therapy in the upfront treatment of

endocrine-sensitive tumors is undebatable, the optimal use of cytotoxic chemotherapy remains controversial. The main reasons for the lack of general consensus are the heterogeneity of the patient population and the availability of several effective options. In the following paragraphs we discuss the different treatment options in the most common clinical scenarios.

Treatment options based on patient characteristics The majority of metastatic breast cancer patients are over 65 years of age, and therefore a significant proportion are affected by comorbidities such as hypertension, diabetes and respiratory disease [7]. Tolerability of chemotherapy can be profoundly influenced by these comorbidities as well as by their specific treatments.

In the case of elderly patients with significant comorbidities and declining general condition, the aim of treatment is symptomatic control with improvement in quality of life (QoL). To date, the availability of oral agents such as capecitabine and navelbine allows for a good balance between activity, tolerability, compliance and dosage flexibility. Furthermore, weekly administration allows maintenance of the activity of important cytotoxic agents such as taxanes and platinum salts with substantial decrease in toxicity. Finally, new antimetabolites (gemcitabine) or new formulations of old drugs (liposome-encapsulated doxorubicin) are generally associated with reduced toxicity. For these patients it is clear that the best choice is the use of single agents that can be sequentially administered on the basis of tolerability and disease control.

About 20% of patients present with locoregional relapses (regional lymph nodes, skin metastases). Moreover more sensitive imaging techniques (Multislice computed tomography [CT], magnetic resonance imaging [MRI], positron emission tomography [PET] scan) and the development of methods to detect micrometastatic disease (i.e. circulating tumor cells) permit diagnosis of an increasing proportion of patients with oligometastatic disease. A reasonable percentage of these patients can be cured or at least rendered disease free for prolonged period of time; a chemotherapy regimen to induce rapid and important tumor shrinkage followed by locoregional treatments (radiotherapy, surgery, radiofrequency ablation) is necessary to achieve this goal. In this setting, it is clear that combination regimens should be the preferred option.

Another clinical scenario is represented by younger patients, without comorbid conditions, and massive visceral involvement. Here again, rapid tumor shrinkage is important in preventing life-threatening organ failure. In this setting, combination regimens ensure a higher percentage of objective responses [8,9] and a shorter time to response [10].

Treatment options based on tumor characteristics Breast cancer tumors are at least as heterogeneous as breast cancer patients. However, the only tumor-related parameters currently utilized in the decision making process are the disease course in the individual patient, hormone receptor status, and HER2-neu overexpression.

In the case of patients with slowly-growing hormone receptor-negative tumors and predominant bone disease, main aims of treatment are maintenance of QoL and prolonging time to progression. In these cases, single-agent chemotherapy provides a better balance between activity and tolerability. More frequently, patients with hormone-sensitive tumors receive several lines of endocrine therapy until development of hormone resistance. Here again, when chemotherapy is required, the preferred choice is the sequential administration of single agents.

Another important tumor characteristic is the HER2-neu status; HER2-neu is overexpressed in 25–30% of breast cancers. As a single agent trastuzumab, a monoclonal antibody directed against the extra-membrane portion of HER2 receptor, can induce a 30% response rate in HER2-overexpressing tumors. The addition of trastuzumab to chemotherapy as compared with chemotherapy alone is associated with a significant improvement in objective response rate, duration of response and overall survival [11].

Several cytotoxic agents showed synergism or additive effect when combined with trastuzumab; clinical trials have shown that trastuzumab can successfully be combined with both single agent and combination chemotherapy.

Treatment options based on prior adjuvant therapy Anthracyclines represent the most active agents, and anthracycline-containing regimens are more effective in terms of response rates, complete remission rates, remission duration and survival. However, anthracycline regimens are increasingly used in the adjuvant setting, and therefore retreatment with anthracyclines, even if effective, is limited to patients exposed to low cumulative anthracycline doses and with a relapse-free survival after adjuvant chemotherapy longer than 12 months. The main limitation to anthracyclines is their dose-dependent cardiac toxicity; patients should not exceed the cumulative dose of 450–550 mg/m^2 for doxorubicin and 800-900 mg/m^2 for epirubicin. However, few data are available on the efficacy of anthracycline rechallenge after prior exposure to adjuvant anthracycline. A recent report from our group [12] has shown that anthracycline–taxane combinations as first-line treatment for metastatic breast cancer are effective, regardless of previous adjuvant chemotherapy.

As more and more patients are receiving taxanes as a component of their adjuvant program, it will be important also to have data on the efficacy of taxane rechallenge in metastatic patients.

Conclusion Survival prolongation must be the primary goal of treatment, and this aim can be achieved with the incorporation of new active agents in the treatment strategy. Both combined and sequential single agents are acceptable options; however, if not contraindicated by the conditions of the patient and if feasible with an acceptable toxicity profile, there is no reason to delay the upfront use of active agents.

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Symposium VIII: Other advances in metastatic disease

S28

Role of translational studies in optimizing palliative chemotherapy

R Kramer

Breast Care Center, Baylor College of Medicine, Houston, Texas, USA, and Visiting Professor, Hospital Universitario '12 de Octubre', Madrid, Spain

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Introduction Patients with metastatic breast cancer have a highly variable clinical course. Systemic chemotherapy may decrease symptoms and prolong survival for some patients. However, others experience significant toxicity and achieve little benefit. Predictive markers are needed to determine which patients will benefit from particular chemotherapy agents and which should be offered novel therapeutics.

Methods The English literature related to predictive markers for metastatic cancer was reviewed. Translational studies examining the potential role of tumor profiles in the selection of optimal chemotherapy agents were identified. Particular attention was paid to studies examining sensitivity and resistance to anthracyclines, taxanes, vinorelbine and capecitabine.

Conclusion This paper will summarize the rapidly expanding data published to data and will discuss clinical trial designs that will facilitate future studies.

S29

Letrozole efficacy is related to human aromatase CYP19 single nucleotide polymorphisms (SNPs) in metastatic breast cancer

R Colomer[1,2], M Monzo[3], I Tusquets[4], J Rifa[5], JM Baena[6], A Barnadas[7], L Calvo[8], F Carabantes[9], C Crespo[10], M Muñoz[11], A Llombart[12], A Plazaola[13], E Alba[14], D Fuster[3], M Gilabert[15], B Lloveras[16]

[1]Institut Catala d'Oncologia, Girona, Spain; [2]Hospital 12 de Octubre, Madrid, Spain; [3]Universitat de Barcelona, Barcelona, Spain; [4]Hospital del Mar, Barcelona, Spain; [5]Hospital Son Dureta, Palma de Mallorca, Spain; [6]Hospital Puerta del Mar, Cadiz, Spain; [7]Institut Catala d'Oncologia, Badalona, Spain; [8]Hospital Juan Canalejo, Coruña, Spain; [9]Hospital carlos Haya, Malaga, Spain; [10]Hospital Ramon y Cajal, Madrid, Spain; [12]Hospital Clinic, Barcelona, Spain; [13]Instituto Valenciano de Oncologia, Valencia, Spain; [14]Instituto Oncológico de Guipuzcoa, San Sebastian, Spain; [15]Hospital Virgen de la Victoria, Malaga, Spain; [16]Novartis, Barcelona, Spain; [17]Institut Catala d'Oncologia, Barcelona, Spain

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Introduction The efficacy of aromatase inhibitors varies widely among postmenopausal breast cancer patients, but it is not associated with aromatase gene expression. We evaluated whether polymorphisms of the aromatase gene CYP19 are related to the efficacy of the aromatase inhibitor letrozole.

Methods PCR allelic discrimination was used to examine single nucleotide polymorphisms (SNPs) in DNA obtained from 67 breast carcinomas. Postmenopausal patients with hormone receptor positive metastatic breast cancer were treated with the aromatase inhibitor letrozole. All patients in the study had documented disease progression before receiving letrozole, and had been treated previously with tamoxifen or another selective estrogen receptor modulator (SERM). Sixty-five patients were evaluable for efficacy. Three regions of the aromatase CYP19 gene were examined: two were localized in the 3' untranslated region (UTR; rs10046 and rs4646) and one in the intronic region (rs727479). Presence of variant gene polymorphisms was correlated with the efficacy end-point of the study, which was time to treatment progression (TTP).

Results The median age of patients was 62 years, and the median number of metastatic sites was two. Median TTP was 12.1 months. Percentage of cases with allelic SNP variation of rs10046 was 69%, of rs4646 was 48%, and of rs727479 was 63%. TTP was significantly longer in patients with the rs4646 variant of CYP19 when compared with normal CYP19 (17.2 months versus 6.4 months; $P = 0.02$). A relationship of TTP with the rs10046 or rs727479 variant was not observed.

Conclusion In hormone receptor positive metastatic breast cancer patients treated with the aromatase inhibitor letrozole, the presence of a SNP on the 3'-UTR of the CYP19 aromatase gene is associated with improved treatment efficacy, and may help in the future to select patients for antiaromatase therapy.

S30

New agents for bone metastasis

GN Hortobagyi

Department of Breast Medical Oncology, The University of Texas, MD Anderson Cancer Center, Houston, Texas, USA

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Metastases to osseous structures represent the most common metastatic destination for human breast carcinoma. Conversely, breast cancer is one of the most common sources of bone metastases. Bone metastases produce multiple complications, including pain, pathological fractures, hypercalcemia, and spinal cord compression. Some of these complications are potentially catastrophic and all reduce the quality of life of patients with metastatic breast cancer.

Analgesics, orthopedic surgery, and radiotherapy have been considered the only successful approaches to the management of complications of bone metastases prior to the therapeutic development of bisphosphonates. Research over the past decade expanded our understanding of the metastatic process, including the pathophysiology of bone metastases. Cancer cells play a minor role in the direct process of bone metastases. Rather, cancer cells produce and secrete a variety of growth factors (transforming growth factors ∀ and ∃, epidermal growth factor, granulocyte–macrophage colony-stimulating factor) as well as a number of cytokines (tumor necrosis factor, IL-1 and IL-6, prostaglandins) and parathyroid hormone-related protein (PTHrP), which recruit osteoclast precursors and activate mature osteoclasts. It is the excessive osteoclastic activity, uncoupled from matched osteoblastic activity, that results in excessive bone resorption and bone metastases. Therefore, efforts at interfering with osteoclast activation, maturation and the recruitment of osteoclast precursors have become the main focuses of therapeutic research in the area of bone metastases.

A variety of substances have been shown to inhibit osteoclast activity; calcitonins, gallium nitrate and bisphosphonates have marked therapeutic effects in the management of hypercalcemia of malignancy, Paget's disease of bone, and bone metastases. Bisphosphonates are considered the standard of care for all these indications. Clodronate, pamidronate, ibandronate, and zoledronic acid have all been shown to reduce bone-related events in randomized trials, including fractures, pain, hypercalcemia, and spinal cord compression. In addition, the systematic use of bisphosphonates, especially IV aminobisphosphonates (pamidronate, zoledronic acid), was also shown in placebo-controlled trials to reduce the need for radiotherapy and orthopedic surgery. Therefore, current practice guidelines indicate initiation of monthly intravenous bisphosphonate therapy upon the identification of bone metastases by imaging, regardless of other anticancer treatment being administered. Bisphosphonate therapy should continue indefinitely, with periodic monitoring of renal function.

In patients with primary breast cancer, the use of bisphosphonate therapy for prevention of bone metastases is not indicated outside of a clinical trial. There are conflicting results from three, relatively small, randomized trials regarding the clinical utility of adjuvant bisphosphonate therapy. Ongoing and planned randomized trials in the adjuvant setting should determine the contribution, if any, of bisphosphonates to the management of primary breast cancer. In this

regard, NSABP B-34 has completed accrual, and results should be available within the next 1–2 years.

A different indication for bisphosphonates, independent of the presence or absence of breast cancer, is for management of osteopenia or osteoporosis. National and international guidelines exist for postmenopausal patients, and patients with breast cancer should be monitored and treated for osteoporosis in the same manner. Because some of the anticancer treatments used to manage breast cancer result in premature ovarian ablation or suppression resulting in rapid bone loss, early assessment of bone density in these patients is necessary to determine the role of bisphosphonate therapy to preserve bone strength and density.

A clearer understanding of signaling pathways involved in osteoclast activation and uncoupled and unmatched bone resorption associated with malignant tumors has led to the development of several new strategies to manage metastatic bone disease. Osteoprotegerin, and antibodies directed to the ligand or the receptor activator of nuclear factor-κB, have demonstrated substantial activity in inhibiting osteoclast activation, recruitment and differentiation. Phase II and III trials with some of these agents are progressing rapidly.

PTHrP is a critical initiating factor in the process of bone resorption and it is produced by a number of malignant tumor cells, including breast cancer. Antibodies against PTHrP are being explored as therapeutic agents in advanced clinical trials.

Src inhibitors might have an important role to play in inhibiting osteoclast activation, and several lead compounds are initiating their clinical evaluation alone and in combination with other relevant treatments.

The management of bone metastases has benefited enormously by our expanded understanding of basic biological processes related to osteoclast and osteoblast function. These advances will result in improve management of established bone metastases and possibly effective preventive interventions.

Closing lecture

Posters

P1

Cyclo-oxygenase-2 expression is associated with poor clinical outcome after doxorubicin-based chemotherapy in node-positive breast cancer: integration of tissue microarray

SH Han[1], JS Kim[2], SS Jung[2], BJ Song[2]

[1]Department of Surgery, Inje University Sanggye Paik Hospital, Seoul, Korea; [2]Department of Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea

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Introduction We performed this study to evaluate the frequency and clinical implications of cyclo-oxygenase (COX)-2 expression in clinical breast cancer.

Method COX-2 expression was analyzed on tissue microarray (TMA) of 178 node-positive patients treated with doxorubicin-based adjuvant chemotherapy by immunohistochemistry (IHC).

Results COX-2 was over-expressed in 70 (39.3%) out of 178 invasive breast cancers. COX-2 expression was significantly increased in undifferentiated tumor with high S-phase fraction. COX-2 expression appeared to be increased in HER2-amplified tumors but the difference was not statistically significant. There was no significant association between COX-2 over-expression and other clinical and biologic profiles such as tumor size, histologic grade, and oestrogen receptor (ER) expression. Disease-free survival (DFS) and overall survival (OS) of the patients with COX-2 expressing tumor was significantly decreased compared with the patients with COX-2 negative tumor ($P = 0.009$ for DFS, $P = 0.011$ for OS). Cox-2 expression and histologic grade were significant prognostic factors for DFS and OS in multivariate analysis.

Conclusion The intimate association of COX-2 expression with increased S-phase and high histologic grade, together with poor clinical outcomes for COX-2 expressing tumors, indicates that COX-2 expression represents a highly aggressive phenotype of breast cancer.

P2

Predictors of positive axillary lymph nodes in breast cancer patients with metastatic sentinel lymph node

IP Callejo[1], J Brito[2], R Alves[1], P Dias[1], M Limbert[1], N Abecasis[1], J Faria[1], J Weinholtz[1], S André[3], C Costa[1], O Almeida[3], M e Sousa[1]

[1]Department of Surgery, Portuguese Institute of Cancer, Lisbon, Portugal; [2]Department of Mathematics, Physics and Computer Science, Ryerson University, Toronto, Canada; [3]Department of Pathology, Portuguese Institute of Cancer, Lisbon, Portugal

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Introduction Breast cancer with metastatic sentinel lymph nodes (SLN) may have clinicopathologic factors associated with the presence of positive nonsentinel axillary nodes (NSLN). The aim of the present study was to determine factors that predict involvement of NSLN in breast cancer patients with positive SLN.

Method A prospective database search identified 80 patients who underwent SLN biopsy for invasive breast cancer between January 1999 and August 2002. Clinicopathologic data were analyzed to determine factors that predicted additional positive axillary nodes.

Results A total of 23 patients had positive SLN and underwent conventional axillary lymph node dissection. Statistical analysis revealed that lymphovascular invasion ($P \sim 0.00000$), SLN metastasis >2 mm ($P = 0.002$), and the presence of extranodal involvement ($P = 0.002$), were positive predictors of the metastatic involvement of NSLN.

Conclusion The likelihood of positive NSLN correlates with pathologic parameters such as the presence of lymphovascular invasion, size of the SLN metastasis, and extranodal involvement. These data may be helpful with the regard to the decision to undertake axillary dissection in breast cancer patients with metastatic SLN.

P3

Fibroadenoma of breast in Iranian women between 1994 and 2004

A Abdollahi[1], S Shalbaf[2]

[1]Consultant Surgeon and Dean of Department and [2]General Physician, Surgery Department, Tehran Medical School, Islamic Azad University, Tehran, Iran

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Introduction Fibroadenomas are the most common benign tumors of the female breast and are associated with a slight increase in risk for subsequent breast cancer. The aim of this study was to make a methodical inventory of risk factors for fibroadenoma in women.

Materials and method This study was conducted between 1994 and 2004 in cross-section in 4000 women hospitalized in some Iranian hospitals for fibroadenoma. In the study the following factors were examined: age, menstrual cycle pattern, age of menarche, menopause after 50 years, oral contraceptive use, mastalgia, marriage, number of full-term pregnancies, first pregnancy over age 35 years, history of breastfeeding, cigar smoking, the kind of radiology for diagnosis, the place of tumor, tumor location in breast, diameter of tumor, multiple tumor, patient background and family history of fibroadenoma, and pathology report.

Results The common age was between 26 and 30 years; 42% had bleeding for more than 35 days; 57.8% had menarche before 12 years old; 2.6% had menopause after 50 years old; 67.3% of patients used oral contraceptive; 57% had premenstrual mastalgia; 11.9% were single; the risk for fibroadenoma decreased with increasing number of full-term pregnancies; 10.2% had first pregnancy at age over 35 years; 36.4% had breastfed; 7.9% had smoked cigarettes; 36.9% of patients had only mammography, 82.1% only had sonography examination, and

28.9% had both; the left breast was affected slightly more than the right; the most frequent location was the upper-outer quadrant; the most frequent size was between 4 and 5.9 cm; 24.7% had multiple tumors; 12.2% had background and 62.9% had family history of fibroadenoma; 17.6% had malignancy in their pathology report.

Conclusion The results for the studied risk factors for fibroadenoma are similar to those of studies conducted in other countries. In our study, however, we observed some differences in breastfeeding, cigarette smoking, patient background, and malignancy in the pathology report.

P4

Nanoparticulate paclitaxel loaded into sterically stabilized mixed phospholipid micelles to improve chemotherapy of breast cancer

I Rubinstein, A Krishnadas, LR Peddakota, H Önyüksel

Departments of Biopharmaceutical Sciences and Medicine, Colleges of Pharmacy and Medicine, University of Illinois at Chicago, Chicago, Illinois, USA

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Active targeting of water-insoluble chemotherapeutic drugs, such as paclitaxel, to breast cancer is a highly desirable because of its associated increase in anticancer efficacy coupled with reduced systemic drug toxicity. However, rational design of these drug delivery platforms should take into account both pathobiological attributes of breast cancer, such as enhanced permeability and retention phenomenon and overexpression of vasoactive intestinal peptide (VIP) receptors, as well as biophysical properties of its ingredients, including ease of preparation, water insoluble drug loading capacity, steric hindrance, nanosize, and scale-up production and storage. To this end, we developed and tested a novel biocompatible and biodegradable nanoparticulate formulation of VIP-conjugated sterically stabilized phospholipid mixed micelles (SSMM-VIP; size ~14 nm) composed of disteraroyl phosphatidylethanolamnine-poly(ethylenglycol-2000) and egg yolk phosphatidylcholine. This construct solubilized 1 mg/ml paclitaxel (P-SSMM-VIP) and retained its biophysical properties upon lyophylization and reconstitution in saline. Moreover, it exhibited a twofold increase in cytotoxicity to MCF-7 breast cancer cells in comparison with P-SSMM and paclitaxel in DMSO ($P < 0.05$). In addition, the construct targeted VIP receptors overexpressed in methyl nitrosurea (MNU)-induced *in situ* rat breast cancer tissues. There was a twofold increase in accumulation of intravenously administered P-SSMM-VIP (1 mg/kg) in MNU-induced rat breast cancer, coupled with a significantly greater regression of breast cancer in comparison with P-SSMM and Taxol ($P < 0.05$). At the same time there was a significant reduction in P-SSMM-VIP accumulation in bone marrow, spleen and other organs in comparison with P-SSMM and Taxol ($P < 0.05$). There was no significant change in systemic arterial pressure during administration of P-SSMM-VIP. Collectively, these data indicate that actively targeting paclitaxel passively loaded into biocompatible, biodegradable, long-circulating SSMM to breast cancer through VIP receptors improves drug efficacy and reduces its uptake in injury-prone normal tissues. We suggest that P-SSMM-VIP is an efficacious and safe, actively targeted drug delivery platform to treat breast cancer.

P5

6-(1-oxobutyl)-5,8-dimetoxy-1,4-naphthoquinone inhibits the growth of MCF-7 cells via inhibition of angiogenesis and hypoxia inducible factor alpha

S-H Kim

Department of Oncology, Graduate School of East-West Medical Science, Kyunghee University, Yongin, South Korea

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Introduction Hypoxia induces the transcription of various genes that are involved in angiogenesis and anaerobic metabolism necessary for the growth of tumor cells. Hypoxia-inducible factor (HIF)-1α regulates genes that are involved in the response to hypoxia and promotes neoangiogenesis in cancer. Thus, 6-(1-oxobutyl)-5,8-dimetoxy-1,4-naphthoquinone (OXO) was synthesized, to develop an anticancer agent with antiangiogenic activity in hypoxic cancer cells.

Method The XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) assay for cytotoxicity, ELISA (enzyme linked immunosorbent assay), RT-PCR and Western blotting analysis were employed in MCF-7 human breast cancer cells under hypoxic conditions.

Results OXO exhibited cytotoxicity against MCF-7 cells, human breast cancer cells with an IC_{50} of 20 μmol/l. OXO also reduced the levels of vascular endothelial cell growth factor (VEGF) and HIF-1α in MCF cells exposed to hypoxia. Similarly, OXO downregulated the expression of HIF-1 and VEGF by western blotting and RT-PCR. In addition, OXO inhibited the basic fibroblast growth factor (bFGF) induced proliferation, tube formation of human umbilical vein endothelial cells, and disrupted the neovasularization in bFGF treated Matrigel *in vivo*.

Conclusion Taken together, OXO may exert antitumor and antiangiogenic activity against MCF-7 cells via regulation of HIF-1α and VEGF.

P6

Clinical profile and management of bilateral breast cancer

SV Suryanarayana Deo[1], D Shridhar[1], NK Shukla[1], S Kumar[1], J Purkayastha[1], V Raina[2], GK Rath[3]

[1]Department of Surgical Oncology, Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India; [2]Department of Medical Oncology, Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India; [3]Department of Radiation Oncology, Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India

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Bilateral breast cancer (BBC) is a rare clinical entity. Unlike unilateral breast cancer there are no clear treatment guidelines for BBC. There are several controversial issues regarding BBC pertaining to the diagnostic criteria, nomenclature, and management policies. To address these issues, a retrospective analysis of breast cancer database at a tertiary care cancer center was performed and the clinical profile, treatment patterns and outcome of patients with BBC were analyzed.

Thirty out of 1100 (2.7%) patients with breast cancer treated between 1993 and 2003 had BBC, of whom 20 patients had metachronous and 10 patients had synchronous BBC. Family history of breast cancer was present in five patients (16%) only. Contralateral breast cancer (CBC) was detected mammographically in three and by clinical examination in 27 patients. Most CBC patients had early-stage disease compared with the index side (73% versus 27%). Fifty-six out of 60 tumors were found to be invasive ductal carcinoma, and none of the patients had lobular carcinoma. Twenty-three patients had bilateral mastectomy, three had unilateral mastectomy and four had a combination of breast conservation and mastectomy. Sixteen patients had unilateral and six had bilateral adjuvant radiotherapy. All patients received adjuvant chemotherapy and/or hormonal therapy both for index and CBC based on the stage and hormone receptor status. At a median follow up of 31.5 months (3–142 months), 23 (76%) patients were disease free and seven (24%) patients had disease relapse. Mean overall survival of patients with MBBC was significantly longer than those with SBBC (30.4 months versus 19.2 months; $P = 0.045$).

BBC is an uncommon clinical entity. These patients require individualized treatment planning based on the tumor factors and treatment factors of the index lesion. Optimal results can be obtained by using a logical multimodality treatment approach for BBC.

P7

Adjuvant radiotherapy after surgery in breast cancer: evaluation of acute toxicity

MA Molinaro, C Vaccaro

UO Radiotherapy and Radiobiology Hospital Pugliese-Ciaccio, Catanzaro, Italy

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Introduction Postoperative irradiation of conservatively operated breast carcinoma is one of the most common practice in radiation oncology. In this job we analyze the acute toxicity during treatment.

Materials and method From 1999 to January 2003, 220 patients aged 31–71 (median, 55.8) with breast cancer in stage I and II after conservative surgery were studied. Menopause was induced in 75 of the patients with hormonal therapy. Radiation therapy of the breast after quadrantectomy is based on the use of lateral and medial tangential portals. With the advent of conformal 3D-treatment planning precise physical dose localization can be achieved optimizing the dose distribution. The definition of the volume target and the elaboration of the treatment plan were executed on computed tomography scans. A total dose of 50 Gy is given to the whole breast and followed by a 10 Gy boost. In the patients with neoplastic involvement of the lymph nodes, chemotherapy treatment and/or endocrine therapy was instituted. The analysis of the acute toxicity was valuated with scale EORTC RTOG.

Results and conclusion Postoperative radiotherapy was well tolerated on its own and concomitant with chemotherapy. The cutaneous acute toxicity in patients managed with single radiotherapy was as follows: G0 = 5.6%, G1 = 17.6%, G2 = 4.8% and G3 = 0.6%. For patients managed with OT the cutaneous acute toxicity was as follows: G0 = 4.8%, G1 = 22.2%, G2 = 5.8% and G3 = 0.6%. For patients managed with CT the cutaneous acute toxicity was as follows: G0 = 4.7%, G1 = 23.6%, G2 = 0.8% and G3 = 1.6%. Differences in acute cutaneous toxicity between different outlines of CT did not emerge. Pulmonary and/or cardiac acute reactions were not found.

P8

Breast cancer-screening programme amongst unpriviledged women in Charleroi (Hainaut), Belgium

F Bastin[1], M Lejeune[2]

[1]Cancer Screening Centre – CPAS Charleroi, Oncology Department CHU Charleroi, Belgium; [2]Carolo Prévention Santé, Charleroi, Belgium

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Introduction Cancer is the second cause of mortality in Belgium and in Hainaut. For women, breast cancer is highly prevalent (33% of cancers). Several studies have shown that there is an increase in certain cancers in disadvantaged populations because of risk factors that favour the disease, and there is cancer-screening reticence in this population. It should be noted that there is less detection breast cancer screening in Charleroi (48% in Hainaut versus 56% in Belgium).

There are high levels of precarious lifestyle because of low level of education, high female unemployment (33% in Charleroi versus 15% in Belgium), and lower average income compared with the Belgian average.

Methods The project has three axes: to study the barriers and potential levers in systematic screening; to promote awareness sessions/to encourage underprivileged populations; and to incite GPs to promote cancer detection screening.

Results More than 1000 women have been reached. Qualitative and quantitative evaluation have been done for each axis. Principal barriers for screening were fear, budget, lack of time, lack of information and negligence. Possible levers were information, free testing, proximity of screening centres and systematic contact with GPs. Local GPs have been sensitized about their central role in breast cancer prevention.

Conclusion Lack of knowledge is a major barrier to breast cancer screening. Negative consequences of breast cancer are more important in underprivileged women, which is why our project is pertinent and can help women not to dramatize and perhaps undertake a screening test.

P9

A performance study of different soft-computing procedures for automatic detection of breast cancer malignancy

M Ortega-Moral[1], V Gómez-Verdejo[1], J Arenas-García[1], LA Azpicueta-Ruiz[1], M Lázaro-Gredilla[1], J Madrid-Sánchez[1], E López-Beltrán[2], AR Figueiras-Vidal[1]

[1]Department of Signal Theory and Communications, Universidad Carlos III de Madrid, Madrid, Spain; [2]Department of Electronics and Systems, Universidad Alfonso X El Sabio, Madrid, Spain

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Breast cancer is one of the most common cancers in women, and its early detection increase the patient's rate of survival. Several studies have shown that computer assisted diagnosis can improve detection of breast cancer, or at least detect those suspicious cases that are worth studying in detail by an expert.

In this paper we analyze the performance of different neural classifiers that are designed for identifying malignant microcalcifications on mammograms. Specifically, we have used different techniques, such as Multi Layer Perceptrons, Radial Basis Functions and Support Vector Machines, comparing the capabilities of the resulting systems with other approaches that can be found in the literature.

We have used information collected from mammograms of 210 patients in which 229 clustered microcalcifications were detected. This information has been automatically extracted, and is related to characteristics of the cluster and to the individual microcalcifications. Additionally, biopsy results from each patient determined that 46% of the cases were malignant and 54% were benign tumors.

Analysis of performance is based on receiver operating characteristic (ROC) curves, showing that these general purpose classifiers achieve results that are similar to those of previous automatic designs. Hopefully, *ad hoc* neural designs could improve these results even further. Finally, a comparison with the opinion of three human experts shows that these technologies can be of great help to assist doctors in the clinical decision process.

P10

A phase II randomized trial of doxorubicin (DXR) and gemcitabine (GMZ) administered in patients with metastatic breast cancer (MBC)

A Bensalem, K Bouzid

Medical Oncology, Chu Dr Benbardis, Constantine, Algérie

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Introduction We conducted a phase II trial to define the safety, the efficacy, the pathological response rate and survival associated with four cycles DXR–GMZ administered every 3 weeks followed by surgery, then four cycles of FAC50 as a primary therapy in MBC.

Method Patients with histologically or cytologically confirmed MBC, ECOG PS: 2 and adequate hepatic, renal and cardiac functions were eligible. Prior chemotherapy was not allowed.

Results Fifty-one patients were included, after signing an informed consent. Median age was 47.07 years, and 100% had stage IV. A total of 373 cycles was administrated. Main grade 3/4 toxicities were neutropenia in 1.1 %, anaemia in 0.5% and thrombopenia grade 2 in 1.1%. Nausea and vomiting grade 2–3 occurred in 17.4%. Regarding efficacy, 49 out of 51 patients achieved four cycles. The overall response rate was in 84.1%, with complete response in 58.8% and partial response in 25.3%. Progression of disease occurred in 2.4%. Surgery was performed in 30 patients, and 13 had histological response (43.2%), with complete histological response in 36.6% and partial histological response in 6.6 %. The median time to progression was 13.3 months.

Conclusion: The combination of DXR with GMZ in MBC appears to be an active regimen with a favourable toxicity profile. It is well tolerated and achieved encouraging pathological response rates.

P11

The axillary study with ultrasound and cytological puncture with fine needle in invasive breast cancer

M Izquierdo, M Cusido, D Dexeus, J Feu, F Tresserra, C Ara, B Navarro, L Lopez Marin, R Fabregas

Department of Obstetric Gynecology, Institut Universitari Dexeus, Barcelona, Spain

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Introduction Axillary study with ultrasound and cytological puncture with fine needle aspirate (FNA) in patients with invasive breast cancer is a diagnostic method included in protocols.

Method Fifty-four patients with invasive breast cancer treated in 2004 underwent axillary ultrasound and cytological puncture with fine needle of suspicious nodes before surgery. Suspicious nodes were those with at least one of the following signs: long-to-short axis ratio less than 1.5, absence of hilius and cortical disruption. If the results were compatible with metastasis then we performed axillary lymphadenectomy; if it was found to be benign then we conducted sentinel node study.

Results In 10 patients cytological puncture with fine needle was positive. When we conducted axillary lymphadenectomy, two patients (20%) were found to have one positive node, one patient (10%) two positive nodes, four patients (40%) three positive nodes, and three patients (30%) more than three positive nodes. In the 44 patients who had axillary ultrasound and were FNA negative, we conducted sentinel node study: 36 patients (81.8%) were $pN0i^-$, three (6.8%) were $pN0i^+$, one patient (2.2%) had a micrometastases, and three patients (9%) had macrometastases (pN1a).

Conclusion Axillary study with ultrasound and FNA before surgery allows excluding a group of patients to make the sentinel node.

P12

Expression of c-met gene in invasive ductal carcinomas

CH Park[1], MH Lee[2], ES Chang[3], BJ Song[4], SS Jung[4]

[1]Department of Surgery, Hallym University College of Medicine, Seoul, Korea; [2]Department of Surgery, College of Medicine, Soonchunhyang University, Cheonan, Korea; [3]Department of Surgery, College of Medicine, Chungnam National University, Daejeon, Korea; [4]Department of Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea

Breast Cancer Research 2005, **7(Suppl 1):**P12 (DOI 10.1186/bcr1246)

Introduction The c-Met protein, known as the hepatocyte growth factor (HGF) receptor, is a transmembrane 190 kDa heterodimer with tyrosine kinase activity, encoded by the c-met oncogene. The HGF/c-Met signalling pathway has been shown to demonstrate various cellular responses, including mitogenic, proliferative, morphogenic and angiogenic activities. Although HGF and c-Met are known to be expressed in a variety of organs and play important roles in signal transduction, studies of its expression correlated with clinico-pathological parameters in breast cancer are rare.

Method In this study we examined c-met mRNA and c-Met protein expression by means of RT-PCR and immunohistochemical methods in 50 cases of invasive ductal carcinomas and 20 normal breast tissue samples.

Results c-met mRNA amplification was detected in 35 cases (70%), but not in normal tissues. c-Met protein overexpression was detected in 27 cases (54%) and two cases (10%), respectively. Both mRNA amplification and protein overexpression rates were significantly higher in tumor than in normal tissue. The c-met mRNA amplification exhibited an increased tendency according to tumor invasiveness and nodal metastasis. The c-Met protein overexpression was significantly correlated with well differentiated grade and showed decreased tendency in metastatic tumor. The concordance between both mRNA amplification and protein expressions were not recognized.

Conclusion These results suggest that HGF/c-Met signal pathway may be associated with breast cancer development. c-met mRNA amplification may play an important role in tumor progression and metastasis. c-Met protein overexpression may contribute to the morphogenesis of well differentiated tumor.

Proffered papers

P13

[ASCRIT] Antisense chemoradioimmunotherapy consisting of anti-POEM (Arg-Gly-Asp) scFv linked onto high-energy radioisotopes, vinorelbine-tartrate and 21-nucleotide double-stranded siRNA targeted to DNMT1 induce apoptosis in metastatic breast cancer (MBC) characterized by hypermethylated p53, p16, RASSF1A, RAR-b2, BRCA2, H1C1, ESRI1, CDH1, Trbeta1, GSTP1, CCND2 and overexpression of bcl-2, cdc25c, Raf-1 and $\alpha_8\beta1$ integrin

J Giannios[1,3], P Lambrinos[2], E Maragudakis[3], N Alexandropoulos[4]

[1]Department of Radiotherapeutic Oncology,IASO Hospital, Athens, Greece; [2]Department of Oncology, PF-Research Center, Athens, Greece; [3]Department of Radiotherapeutic Oncology, IASO, Athens, Greece; [4]Department of Clinical Biochemistry, Ippokration Hospital, Athens, Greece

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Introduction Metastatic breast cancer (MBC) is resistant to almost all cytotoxic drugs and radiation, making it one of the most aggressive malignancies in humans, with the worst mortality. The failure of tumour cells to undergo apoptosis causes resistance to chemoradiological therapies due to overexpression of oncogenes and transcriptionally repressed apoptotic tumour suppressor genes due to aberrant methylation ($CIMP^+$). Also, upregulation of the ECM gene POEM is associated with adhesion, migration and invasion of highly aggressive and MBC.

Materials and method We obtained surgically a total of 168 MBC specimens from lymph nodes and lungs of patients. Genomic DNA of tumours was analyzed for CpG island hypermethylation by using methylation specific PCR. All of the tumours showed hypermethylation of tumour suppressor genes with the following frequencies: p16 89%, RASSF1A 84%, RARb2 76%, BRCA2 54%, p53 52%, HIC1 47%, ESR1 43%, CDH1 37% and below 35% for TRbeta1, GSTP1 and CCND2. Quantitative IHC, WB, SB and RT-PCR revealed overexpression of DNMT1, $\alpha_8\beta_1$ integrin, bcl-2, Raf-1 and cdc25c. We treated the MBC with anti-POEM (Arg-Gly-Asp) scFv attached onto high-energy radioisotopes, vinorelbine-tartrate and 21-nucleotide double-stranded siRNA segment generated against DNMT1.

Results Post-treatment, we detected re-expression of tumour suppressor genes p53, p16, RASSF1A, RARb2, BRCA2, HIC1, ESR1, CDH1, TRbeta1, GSTP1 and CCND2 after inhibition of DNMT1mRNA. There was downregulation of metastatic ECM gene POEM (Arg-Gly-Asp) due to targeted scFv blocking binding to $\alpha_8\beta_1$ integrin receptor with subsequent inhibition of adhesion, spreading and survival of metastatic tumour cells. There was inactivation of bcl-2,Raf-1 and cdc25c due to phosphorylation by vinorelbine. Furthermore, we detected upregulation of p21waf1, p27kip, Bid and Bak. The high energy radioisotopes induced DNA double-strand breaks in MBC cells and with MT depolymerizer agent vinorelbine they arrested their growth at the G2/M transition according to flow cytometry analysis. We detected externalization of PS, depolarization of mitochondrial transmembrane potential ($\Delta\psi M$), activation of caspase-3, -7, -8 and -9 and bax, cleavage of poly(ADP-ribose)polymerase and DNA fragmentation. TEM exhibited irreversible D2 apoptotic signs, forming apoptotic bodies that were phagocytosed by adjacent tumor cells, leading to a bystander killing effect (BKE). BrdU and MTT exhibited inhibition of DNA synthesis and metabolic activity of treated MBC cells compared with untreated controls.

Conclusion We were able to eradicate MBC cells with combined chemoradioimmunotherapy after circumvention of chemoresistance and radioresistance mechanisms such as hypermethylation of p53, p16, RASSF1A, RARb2, BRCA2, HIC1, ESR1, CDH1, TRbeta1, GSTP1 and CCND2, and overexpression of bcl-2, POEM, $\alpha8\beta1$, Raf-1 and cdc25c.