## Wednesday, 22 March 2006 11:15–12:45

PLENARY KEYNOTE

# Research

1 Invited

### Hunting for breast cancer genes

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First degree relatives of women affected by breast cancer have an approximately two-fold risk of developing the disease. This excess familial risk is predominantly attributable to inherited susceptibility to the disease. Mutations in *BRCA1* and *BRCA2* account for approximately 20% of the familial risk of breast cancer, therefore 80% remains to be explained. The hunt for these additional breast cancer susceptibility genes is being conducted in three ways: genetic linkage analyses; association studies; and mutational screens of candidate genes. The progress and results of these strategies will be reviewed.

2 Invited

### Stem cells in breast development and cancer: implications for prevention and therapy

M. Wicha. *University of Michigan Comprehensive Cancer Center, Ann Arbor, USA*

The mammary gland epithelial components are thought to arise from stem cells which have a unique capacity for self-renewal as well as for differentiation into the lineages which comprise the lobular alveolar structure of the adult gland. Furthermore, there is increasing evidence that these stem and early progenitor cells may be targets for transformation during breast carcinogenesis. Normal stem cells and their transformed counterparts share many characteristics, including the ability to under self-renew, to differentiate, albeit abberantly in cancer, telomerase expression, resistance to apoptosis, and ability to metastasize. Thus, we and others have postulated that a key event in transformation may be the disregulation of pathways that regulate normal stem cell self-renewal.

We have described an in vitro system for the propagation of human mammary stem and progenitor cells in suspension culture. We demonstrated that human mammary cells isolated from reduction mammoplasties generates spherical colonies in suspension that we have termed "mammospheres" which are highly enriched in mammary stem and progenitor cells capable of both self-renewal and multi-lineage differentiation.

We have utilized this culture system to investigate the molecular pathways that regulate stem cell self-renewal including Hedgehog, Notch, and Bmi-1. Utilizing real time PCR, we demonstrated that components of both Hedgehog and Notch signaling are highly activated in mammospheres compared to cells cultured under differentiating conditions. We demonstrate bi-directional cross-talk between Hedgehog and Notch signaling, with both signal transduction pathways resulting in the regulation of the polycomb gene Bmi-1. Since Bmi-1 has been found to regulate the self-renewal of normal hematopoietic and neuronal stem cells, we investigated whether the effects of Notch and Hedgehog were mediated by a Bmi-1. Expression of siRNA to Bmi-1 abrogated the stimulatory effects of Hedgehog and Notch signaling on mammosphere formation. In order to further investigate the effects of disregulation of these self-renewal pathways on mammary development, we utilized an in vivo system in which mammospheres are transplanted into the fat pads of NOD-SCID mice, humanized by introduction of irradiated human mammary fibroblasts. We demonstrate that over-expression of the Hedgehog target gene Gli2 in mammosphere initiating cells produces ductal hyperplasia.

We have previously found that breast cancers are driven by a subcomponent of cells which are CD44+ CD24 low lin- that demonstrate stem cell properties. As few as 200 cells with this phenotype are capable of transferring tumors in NOD-SCID mice, whereas 20,000 cells that do not bear this phenotype are nontumorigenic. Furthermore, the tumors generated by these tumor initiating cells, recapitulate the phenotypic heterogeneity of the original tumor as would be predicted from a stem cell model. Tumor stem cells display increased Hedgehog signaling and Bmi-1 expression compared to non-tumorigenic cells isolated from the same tumor. Together these studies support the tumor stem cell hypothesis in which transformation of mammary stem and/or progenitor cells produces mammary tumors driven by a cancer stem cell component. The cancer stem cell model has important implications for developing effective strategies for cancer prevention and treatment.

3 Invited

### Local and systemic effects that promote human breast tumorigenesis: insights on heterotype interactions from xenograft models

C. Kuperwasser, M. Wu, D. Proia, J. Weremowicz, A. Richardson, S. Naber. *Tufts University School of Medicine, Molecular Oncology Research Institute (MORI), USA*

The study of normal breast epithelial morphogenesis and carcinogenesis in vivo has largely utilized rodent models. Efforts at studying mammary morphogenesis and cancer with xenotransplanted human epithelial cells have failed to recapitulate the full extent of development seen in the human breast. We have developed an orthotopic xenograft model in which both the stromal and epithelial components of the reconstructed mammary gland are of human origin. Genetic modification of the human stromal cells prior to the implantation of ostensibly normal human mammary epithelial cells resulted in the outgrowth of benign and malignant lesions. We have extended this tissue reconstitution model and combined it with defined genetic manipulation of human breast epithelial tissues to create an in vivo model of human breast cancer in mice. Invasive human breast adenocarcinomas developed as early as 5 weeks post implantation when the expression of dominant oncogenes was combined with a specialized stromal microenvironment. The development of these tumors was dependent on specialized stromal fibroblasts, as tumors rarely developed in the absence of this microenvironment. This experimental model allows for studies of human epithelial morphogenesis and differentiation in vivo and underscores the critical role of heterotypic interactions in human breast development and carcinogenesis.

## Wednesday, 22 March 2006 14:15–16:00

SCIENTIFIC SESSION

# Specific issues in metastatic disease

4 Invited

### Has first-line therapy had an impact on general outcome in metastatic breast cancer?

C. Hudis. *Memorial Sloan Kettering Cancer Center, New York, USA*

Conventionally, clinically detected metastatic growths of breast cancer are not considered curable but are, instead treatable. Usual options include systemic treatment with a sequence of hormonal therapies and manipulations if appropriate, single agent and/or combination chemotherapy regimens alone or combined with newer targeted antibodies (trastuzumab and bevacizumab as indicated), and supportive care. Ultimately, most patients with metastatic breast cancer die of this disease although some have competing causes of mortality.

Despite many clinical trials and a long list of active drugs, proof that first line therapy actually extends life has been historically difficult to obtain. Indeed, support for systemic treatment, and chemotherapy in particular, came from studies suggesting that it improved quality of life even if it did not extend life. Recently, however, several lines of evidence clearly suggest that first line therapy improves general outcomes including overall survival.

Untreated metastatic breast cancer is or can be painful and unpleasant and the immediate beneficial effects of active therapy can be observed in concrete fashion in terms of objective responses in measurable lesions, diminished pain (and use of pain medication), increased performance status, and improved overall quality of life. In many cases tumor response and progression-free survival can serve as surrogates for these endpoints. The challenge, given the sometimes long natural history of metastatic breast cancer and the multiple lines of active therapy available, has been to demonstrate that this evidence of activity in the first-line setting translates to improved overall survival.

In the use of hormone therapy, demonstration of improved overall survival remains challenging as appropriate tumors often have an indolent course, can be treated with multiple active interventions, and occur in older patients for whom other causes of mortality are operative. This was true for the past few decades and remains true today. Yet "general outcomes" are improved by the use of safer and more active drugs (ie, selective aromatase inhibitors) even if overall survival can not be shown to be consistently improved.

For chemotherapy there is evidence from multiple trials suggesting improved survival as well as quality of life with modern drugs and regimens.

As single agents, the taxanes were associated with improved overall survival compared to historically accepted standard regimens. In some cases, combinations of chemotherapy agents have also been associated with improved survival although this is a complex body of data. In many cases, a combination of drugs compared to the same agents given in sequence yields less "activity" but the same overall survival. On the other hand, the fact that, for example, a combination of docetaxel and capecitabine, or a combination of gemcitabine and paditaxel can improve overall survival compared to the taxane alone, supports the hypothesis that overall survival is in fact changeable. The active biological agents now available (trastuzumab and bevacizumab) also appear capable of improving overall survival.

For breast cancer the overall death rate appears to be declining and this is certainly multifactorial in origin. However, it is increasingly likely that some of the fall in mortality rates may be attributable to improved systemic therapy for metastatic disease even if these treatments are not "curative". This lecture will review this data in detail and suggest optimal strategies for the use of available agents as well as possible studies for the near future.

**5** Invited

**Efficient use of bisphosphonates in metastatic bone disease**

R.E. Coleman. *Weston Park Hospital, Academic Unit of Clinical Oncology, Sheffield, United Kingdom*

In recent years the treatment of bone metastases by radiotherapy and systemic endocrine and cytotoxic drugs has been supplemented by the co-administration of BP. These drugs are potent inhibitors of tumour-induced bone resorption that can relieve metastatic bone pain and improve the structural integrity of bone. Zoledronic acid and ibandronate are the most potent agents available and prevent 40–50% of the expected skeletal morbidity from advanced breast cancer [1]. Zoledronic acid also significantly reduces the risk of a skeletal complication in endocrine resistant prostate cancer (hazard ratio [HR] 0.64) and in a broad range of solid tumours other than breast and prostate cancers (HR 0.69). Thus BP should now be considered for any patient with symptomatic bone metastases, especially when bone is the dominant site of metastasis. However, for economic reasons selection or prioritisation of patients for BP may in some situations be necessary and more cost effective use of BP is desired in view of the long term nature of treatment.

It is now clear that the risk of a skeletal complication is related to the rate of bone resorption. Patients with rapid bone resorption, as measured by type I collagen fragments, are at significantly greater risk of an event and thus have potentially more to gain from the administration of a BP. Additionally, suppression of bone resorption should be the aim of treatment. Patients failing to normalise bone resorption are at much higher risk of future skeletal complications, progression of disease and death [2]. Attention is now turning to the development of more rational treatment schedules using biochemical markers of bone metabolism to guide treatment in individual patients. BISMARK, a large phase III trial comparing a standard schedule of zoledronic acid to marker directed therapy has recently commenced in the UK and is expected to become an international study during 2006.

**References**

[1] Coleman RE. Ann Oncol 16: 687–695, 2005.
[2] Coleman RE, Major P, Lipton A, et al. J Clin Oncol 23: 4925–35, 2005.

**6** Invited

**Need for new drugs in metastatic breast cancer (MBC)**

P. Fumoleau, B. Coudert, F. Mayer, E. Ferrand. *Centre Georges-François Leclerc, Medical Oncology, Dijon, France*

Patients who present with metastatic disease, either de novo or following surgery, are treated in the vast majority of cases with palliative intent, since progression and ultimately death from breast cancer, are almost inevitable. Most patients can be shown to have objective responses, many times associated with palliation of symptoms, but complete response (CR) are uncommon and responses are short-lived. The median survival for patients with metastases at diagnosis is around 2–3 year with less than 20% still alive at years. Although the treatment of MBC is usually palliative in intent, this malignancy readily responds to systemic agents, and prolongation of survival and symptom palliation are now possible with modern medical management. Systemic treatments are in continual evolution as more active chemotherapeutic agents become available and biological factors have been incorporated into treatment.

Hormonal treatment is the treatment of choice for hormone-sensitive, non life threatening MBC. It has the advantage of being efficacious, easy to administer and well tolerated. For women who are not candidates for hormonal therapy, cytotoxic chemotherapy is currently the treatment of choice. There are many agents that are available for the treatment of MBC which are used alone or in combination according to the clinical situation. The most active drugs are the anthracyclines, tubulin-interacting agents, alkylating agents and antimetabolites. Used as single agents they produce response rates of 20–70%. The now common use of anthracyclines in the adjuvant/neoadjuvant of early breast cancer has both increased the incidence of anthracycline resistant MBC, and restricted the use of the anthracyclines in later stages of the disease in order to avoid the cardiac cumulative dose-limiting toxicity. The introduction of taxanes in the 1990s has led to additional improvement in the management of MBC and there is an increasing trend towards using taxanes earlier in the management of breast cancer. With the growing understanding of the biology of breast cancer, multiple new targets for anti-cancer therapies are being identified. Trastuzumab which targets the HER2 receptor is approved for use, either in monotherapy or in combination with chemotherapy in HER2 positive MBC following the publication of positive survival data. Its approval has allowed clinicians to tailor treatment according to HER2 status and has highlighted the different prognoses of the HER2 positive and negative population.

Improvement in survival is an important treatment goal. Developing, for the control of advanced, relapsed or refractory breast cancer, new well-tolerated agents with novel mechanisms of action, and non-overlapping toxicity, that could be combined with established treatment, is a justified endeavour and a continuous challenge.

**1. New cytotoxic agents:** The new drug development is mainly focused towards tubulin inhibitors. Microtubular structures are required for cell division and vital interphase processes, and their disruption causes cell death. Tubulin, a structural subunit of microtubules, is a clinically validated target for anti-cancer therapy. Two classes of tubulin-interacting agents, taxanes (paclitaxel and docetaxel, microtubule stabilizers) and vinca alkaloids (vinorelbine, tubulin polymerization inhibitor) are on the market.

Several new taxanes derivatives including paclitaxel conjugates, new formulations and 2nd generation taxanes (XRP 9881, XRP 6258) are under clinical development (phase II and phase III trials). These 2nd generation taxanes are active in preclinical models of paclitaxel/docetaxel resistance and further potential benefits include activity against MDR-1 expressing tumors and the ability to cross the blood-brain barrier. Epothilones, (ixabepilone, epothilone D) a new class of microtubule stabilizers, have very promising efficacy in heavily and taxanes-naive treated patients. E7389, a structurally simplified analog of Halichondrin B with a novel anti-tubulin activity, characterized by sequestration of tubulin into nonfunctional aggregates and prevention of microtubule growth seems safe and effective in patients with refractory breast cancer.

Vinflunine (Javlor) is a novel tubulin polymerization inhibitor obtained by semi-synthetic process from vinca alkaloid base showing higher antitumour activity compared with parent compounds. Vinflunine showed definite (high or moderate) antitumour activity in 64% of xenografts tested, versus moderate activity only with vinorelbine in 27%. Against the human MX-1 breast xenografts, vinflunine produced an overall growth inhibition of 61% whereas vinorelbine did not result in any significant inhibition under the same experimental conditions. The results of a recent phase II trial demonstrated that vinflunine is an active, well tolerated drug in the treatment of metastatic breast cancer patients previously treated with anthracycline and taxane-based regimens. Other polymerization inhibitors as new dolastatin are currently being investigated.

**2. Targeted therapies:** Bevacizumab is a promising new therapy with a novel mechanism of action that targets angiogenesis. Bevacizumab improved response rate, PFS in patients with chemotherapy naive MBC when added to paditaxel in a large phase III trial. SU11248 is an oral multitargeted tyrosine kinase receptor inhibitor (TKI) with antitumor and antiangiogenic activity, inhibiting VEGFR, PDGFR, KIT and FLT3 TKs. Results of a recent a phase II study reported that SU11248 has significant single-agent activity in patients with refractory MBC. Lapatinib is a selective, reversible, oral small molecule inhibitor of both ErbB1 (EGFR) and ErbB2 (HER-2/neu) kinase activity. From phase II studies, lapatinib appeared well tolerated and showed evidence of activity as first-line and after trastuzumab failure for women with HER-2-amplified advanced breast cancer. In addition, lapatinib has the ability to cross the blood-brain barrier.

Other targeted therapies as farnesyl transferase inhibitors, mTOR inhibitors are currently being investigated.

New cytotoxic agents and new targeted therapies are currently developed. Some demonstrated promising activity in MBC and can be combined with established treatments for breast cancer.