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PL4 – PLENARY 4

ONE HUNDRED YEARS OF TARGET-DIRECTED THERAPY. "FROM PAUL EHRLICH'S MAGIC BULLET CONCEPT TO MULTI-TARGETED CANCER DRUGS"

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Cancer represents a disease prototype that is connected to defects in the cellular signaling network that controls proliferation, motility, survival and recognition by the immune system. The spectrum of genetic alterations identified in cancer cells includes mutations in various genes leading to structural and functional dysfunctions in signal transmission as well as over- or under-expression of positive or negative signal regulatory proteins.
For the past years we have investigated various aspects of signaling systems in tumor cells in order to identify critical switch points in the pathophysiological process that results in malignancy. These efforts aim at the selective blockade of abnormal, disease-promoting signaling mechanisms rather than the eradication of all growing cells in the body as in the case of currently used chemotherapeutic drugs. This strategic approach began with the cloning of the EGF receptor cDNA and the related receptor HER-2/neu. This work was initiated in 1983 and yielded the first specific oncogene-based FDA-approved (1998) therapeutic agent, "Herceptin", for the treatment of metastatic breast cancer. Analogous "target-driven drug development" efforts have led to the identification of the receptor tyrosine kinase Flk-1/VEGFR2 as a critical signaling element in tumor angiogenesis which served as basis for the development of anti-angiogenic small molecule drugs SU5416, SU6668 and SU11248 which block the phospho-transfer function of this receptor. The drug discovery process that led to SU11248 represents a prototypical example for the adaptation of cancer therapeutics from highly specific to multi-targeted drugs. In August 2005 Pfizer Inc. submitted an NDA to the FDA for approval of SU11248/SUTENT for the treatment of Gleevec-resistant GIST patients and on January 26, 2006 SUTENT was approved for the use of Renal Cell Carcinoma and GIST.
New developments and insights that were gained over the past twenty years of targeted cancer therapy development will be discussed.

GS5 – GENERAL SESSION 5
CLINICAL THERAPEUTICS

GS5.2
MTOR INHIBITORS : A REVIEW OF THEIR APPLICATION AND POTENTIAL IN BREAST CANCER

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The role and importance of the PI3K/PTEN/AKT signal transduction, survival pathway in the development and progression of breast cancer is of increasing interest to the oncology community. Indeed, aberration of signaling through this pathway can occur at many levels, including overexpression and mutation of pathway constituents, and is associated with the majority of breast cancers. For example, amplification/overexpression of the ErbB2 receptor tyrosine kinase is associated with a more aggressive tumor phenotype and worse patient prognosis. This is thought to be due in large part to deregulated coupling of the aberrantly expressed ErbB2-containing receptor complexes to the PI3K/AKT pathway. Consistently, resistance to ErbB-directed therapeutics has been associated with loss of the tumor suppressor PTEN and increased PI3K/AKT signaling in breast tumor cells. Intriguingly, activation of the PI3K/AKT pathway has also been associated with resistance to endocrine therapies in the preclinical setting, as well as a worse clinical outcome for endocrine-treated patients. An emerging mediator of PI3K/AKT activities relating to tumor cell growth and proliferation is the mammalian "Target Of Rapamycin" (mTOR) kinase. The mTOR

pathway is a central sensor for nutrient/energy availability; being further modulated by PI3K/AKT-dependent mechanisms. In the presence of mitogenic stimuli and sufficient nutrients and energy, mTOR relays a positive signal to the translational machinery facilitating events that drive cell growth. The importance of mTOR signaling in tumor biology is now widely accepted. Consequently, a number of agents that selectively target mTOR are being developed in the oncology indication. RAD001 (everolimus) is an orally bioavailable, mTOR inhibitor currently in phase II clinical trials in cancer patients. RAD001 potently inhibits tumor cell proliferation in vitro, exhibits antitumor activity in a range of animal models and has shown evidence of clinical activity in cancer patients. Breast cancer cell lines appear to be particularly sensitive to mTOR inhibition, with IC50 values for in vitro antiproliferative activity in the sub- to low nM range. As the PI3K/AKT pathway is heavily deregulated in breast cancer the application of RAD001 in this patient population is warranted.

Although in experimental models RAD001 displays potent antitumor activity as a mono-agent, data is emerging which suggests that the true potential of mTOR inhibitors may rather be in combination with other therapeutic agents. Indeed, positive interactions have been demonstrated between RAD001 and standard chemotherapeutics, as well as anticancer agents targeting growth-factor and signal transduction pathways (including ErbB receptor antagonists and aromatase inhibitors). Moreover, analysis for biomarkers which may be used as predictors of tumor response to mTOR inhibitors may aid future patient selection, particularly in the very heterogeneous tumor types typified by breast cancer. These points will be discussed in the light of pathway cross-talk, redundancy and potential resistance mechanisms.

GS5.3
IDENTIFICATION OF POTENTIAL THERAPEUTIC TARGETS INVOLVED ERBB-2 INDUCED TUMOR PROGRESSION

White D.E.[1], Rayment J.[1], Andrechek E.[1], Schade B.[1], Blaess S.[2], Mueller U.[2], Dedhar S.[3], Cardiff R.D.[4], St.Arnaud R.[5] and Muller W.J.[1]

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Whereas previous transgenic studies suggest that activation of *erbB-2* is a critical step in tumor progression, one limitation of the transgenic mouse models is that expression of *erbB-2* is driven by a strongviral promoter. In an attempt to more closely mimic the events involved in ErbB-2 induced mammary tumor progression, we have recently derived transgenic mice which carry a Cre inducible activated *erbB-2* under the transcriptional control of the endogenous erbB-2 promoter. In contrast to the rapid tumor progression observed in the MMTV activated *erbB-2* strains, focal mammary tumors arose only after an extended latency period. Tumor progression in these strains was further associated with a dramatic elevation of both ErbB-2 protein and transcript. Remarkably, the elevated expression of erbB-2 was further correlated with selective genomic amplification of the activated *erbB-2* allele. Thus like human breast cancers, amplification of *erbB-2* appears to be a critical event in mammary tumor progression in this unique transgenic mouse model.

Our laboratory has also discovered a naturally occurring human Her2 splice variant that lacks 16 amino acid region of the extracellular juxtamembrane domain (Her2_Ex16). This domain is slightly C-terminal to the region of Her2 that is targeted by Herceptin.

To compare Her2 and Her2_Ex16, normal murine mammary gland (NMuMG) cells were stably transfected with both the wild type and splice forms of Her2. A number of differences in the signalling outputs of the wild type and splice Her2 were observed. These differences may be linked to the inability of Her2_Ex16 shed its extracellular domain (ECD) or putative intracellular conformational changes. In addition to the signaling differences, we have also observed that NMuMG-Her2_Ex16 cells are more polarized in culture and less tumourigenic in nude mice than NMuMG-Her2 cells.

The regulated growth and development of the mammary epithelium is also dependent on the interaction between the epithelial cells with the adjacent extracellular matrix (ECM). This interaction is primarily mediated through the integrin receptor family. One of the primary signaling effectors on integrin class of receptors is the integrin-linked kinase (ILK). To further explore the importance ILK in mammary tumorigenesis, we have generated mammary specific knockouts of ILK using the Cre/LOXP recombination approach. Preliminary analyses of the

mammary ductal outgrowth in these strains has revealed that a functional ILK is required for normal mammary gland development Here we demonstrate that while ablation of ILK delays tumor progression in ErbB-2 induced tumor progression, tumors devoid of ILK eventually arise. Interestingly, the ErbB-2 ILK deficient tumors are completely devoid of Akt activity. These results suggest that ErbB-2 can induce mammary tumors in an ILK and Akt independent fashion These results may have important therapeutic implications for the treatment of HER2 positive breast cancer.

GS5.4
THERAPEUTIC EXPLOITATION OF THE DNA REPAIR DEFECT IN BRCA MUTANT TUMOURS

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About one in nine women in the Western world develop cancer of the breast and at least 5% of these cases are thought to result from a hereditary predisposition to the disease. Two breast cancer susceptibility (*BRCA*) genes have been identified and mutations in these genes account for most families with four or more cases of breast cancer diagnosed before the age of 60. Women who inherit loss-of-function mutations in either of these genes have an up to 85% risk of breast cancer by age 70. As well as breast cancer, carriers of mutations in *BRCA1* and *BRCA2* are at elevated risk of cancer of the ovary, prostate and pancreas. The genes are thought to be tumour suppressor genes as the wild-type allele of the gene is observed to be lost in tumours of heterozygous carriers. Both BRCA1 and BRCA2 have significant roles in the maintenance of genome integrity via roles in the repair of DNA damage via homologous recombination. The specific DNA repair defect in *BRCA*-mutant cells provides opportunities for novel therapeutic approaches based on selective inhibition of functionally interacting repair pathways. These approaches may also be applicable to sporadic cancers harbouring DNA repair defects. Progress towards developing these 'synthetic lethal' approaches will be discussed.

Selected publications:

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature **434**: 917-921

Turner N, Tutt A, Ashworth A (2004) Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer **4**: 814-819

Turner N, Tutt A, Ashworth A (2005) Targeting the DNA repair defect of BRCA tumours. Curr Opin Pharmaco **l5**: 388-393

Tutt A, Ashworth A (2002) The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. Trends Mol Med **8**: 571-576

GS5.5
CIRCULATING TUMOR CELLS IN BREAST CANCER

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Tumor cells in blood were first reported in cadavers in 1869 by Ashworth [1] Subsequently, several investigators have used a variety of methods to isolate, identify, and characterize circulating tumor cells (CTC) as markers of clinical behaviour [2]. Recently, an automated immunomagnetic system, designated CellSearch™ (Immunicon, Huntingdon Valley, PA), has been shown to be a highly reproducible and reliable predictor of clinical outcome in patients with metastatic breast cancer [3-5]. Approximately 50% of patients with metastatic breast cancer have ≥5 CTC/7.5 ml whole blood, and elevated CTC levels at baseline are associated with a significantly worse prognosis. Importantly, 30% of patients continue to have elevated CTC after only 3-4 weeks following the first treatment of a new chemotherapy regimen, and these patients have much shorter time to progression and survival than those patients whose CTC were not elevated at baseline or those whose CTC were elevated but returned to normal after treatment. A prospective randomized clinical trial is about to open in the Breast Cancer Intergroup (TBCI) of North America to test the clinical utility of this information. Recently published sub-studies indicate that CTC levels may be more accurate indicators of benefit than is clinical or radiographic response [6] and that elevated CTC levels are predictive of rapid progression at each time point during followup of patients with metastatic disease [7]. Subsequent studies have demonstrated that CTC can be characterized for various biological properties, including expression of HER-2 and bcl-2 as well as indications of apoptosis [8]. Clinical trials are now being designed to incorporate these findings into investigations of therapeutic efficacy, both in the metastatic and neo-adjuvant settings.

References:

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[6] Budd GT, Cristofanilli M, Terstappen LW, et al. Correlation of changes in circulating tumor cells and radiographic response to treatment in patients with metastatic breast cancer. *Clin Cancer Res*, in press.

[7] Hayes D, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression free and overall survival. *Clin Cancer Res*, in press.

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