Thursday 30 September 08:00–09:45

PLENARY SESSION 4

Proteasome inhibitors, antichaperone drugs, stress pathway inhibitors

238 INVITED

Oncogenic aberrations of SCF ubiquitin ligases

M. Pagano. *New York University Medical Center, Dept. of Pathology (Experimental) MSB 5 548, New York, USA*

Temporally coordinated destruction of cell cycle regulatory proteins by the ubiquitin-proteasome pathway represents an important regulatory mechanism that drives progression through the cell division cycle in a unidirectional and irreversible manner. There is increasing evidence that in addition to genetic alterations, aberrant proteolysis of cell cycle regulators contributes significantly to tumorigenesis, and is indeed found in many types of human cancer. I will discuss the role played in cancer by the F-box protein components of the SCF ubiquitin ligases and why these represent drugable targets in the therapy of cancer and other proliferative diseases.

239 INVITED

The proteasome and proteasome pathway inhibitors in cancer

A.J. Rivett. *University of Bristol, Department of Biochemistry, Bristol, UK*

Proteasomes are multicatalytic endopeptidase complexes that are responsible for a large proportion of intracellular protein degradation in eukaryotic cells. Among other functions, they play an essential role in cell cycle regulation and in the activation of the transcription factor NFkappaB. Proteasomes have an unusual catalytic mechanism with threonine residues acting as the catalytic nucleophile at each of their catalytic sites. Proteasome inhibitors include peptide aldehydes, boronic acids and vinyl sulphones and a number of unrelated compounds such as lactacystin and epoxomicin. These inhibitors have been useful for the characterization of catalytic components and in the elucidation of proteasome functions in animal cells. Perhaps surprisingly, they have also been found to be effective anti-cancer agents. Potent small peptide boronic acid inhibitors of proteasomes have been shown to be effective in a variety of animal models, and one (VELCADE) has been used successfully in clinical trials. Transformed cells are generally more sensitive to apoptosis induced by proteasome inhibitors than non-transformed cells. Also treatment of normal cells with proteasome inhibitors can lead to induction of a non-proliferative senescent phenotype. Understanding the mechanisms that underlie the anti-cancer effects of proteasome inhibitors will aid the design of better and more selective treatments. In particular, since the regulatory role of proteasomes often involves ubiquitin-dependent degradation of key substrates, studies are also underway to evaluate E3 ubiquitin ligases as novel targets for cancer therapy.

240 INVITED

Hsp90 and molecular chaperones as targets in cancer therapy

N. Rosen. *USA*

Abstract not received.

241 INVITED

Targeting the myeloma cell in its bone marrow microenvironment

K.C. Anderson. *Dana-Farber Cancer Institute, Jerome Lipper Myeloma Center/Department of Adult Oncology, Boston, USA*

We have developed both in vitro systems and in vivo animal models to characterize MM cell homing to BM, as well as factors promoting MM cell growth, survival, drug resistance, and migration in the BM milieu. These model systems have allowed for development of promising biologically-based therapies targeting the MM cell and the BM microenvironment including (thalidomide/revlimid, velcade, VEGF receptor inhibitor PTK787, histone deacetylase inhibitors SAHA); those targeting MM cells including heat shock protein 90 inhibitor 17 AAG and insulin growth factor receptor inhibitor; and those which target only the BM microenvironment including I_ B kinase and p38MAPK inhibitors. We have translated our laboratory studies to phase I, II, and III clinical trials to evaluate their clinical utility and toxicity, and to move them rapidly from the bench to the bedside. Velcade and Revlimid have already demonstrated marked clinical anti-MM activity even in patients with refractory relapsed MM, confirming the utility of our preclinical models to identify and validate novel therapeutics. Importantly, gene array and proteomic studies have helped to identify in vivo mechanisms of action and drug resistance, as well as aiding in their clinical application. For example, gene microarray profiling of Velcade treated MM cells reveals induction of heat shock protein 90 stress response, providing the rationale for the combined clinical use of Velcade and 17-AAG to enhance anti-MM activity. Protein profiling of Velcade treated MM cells demonstrated cleavage of DNA repair enzymes, providing the rationale for combining Velcade with DNA damaging agents to enhance sensitivity or overcome resistance to these conventional therapies. Correlative microarray studies of ongoing clinical protocols have demonstrated mechanisms of resistance, i.e. hsp 27 conferring resistance to Velcade, and suggested strategies to overcome resistance, i.e. P38MAPK inhibitors. Ongoing studies are using gene and protein profiling both to select cocktails of targeted therapies for specific patients, and to define targets of sensitivity and resistance in order to develop next generation, more potent and less toxic, therapeutics. Our studies have therefore demonstrated the critical role of host BM-tumor cell interactions both in MM pathogenesis and as targets for novel therapies. They have provided the framework for a new treatment paradigm targeting MM cell-host BM stromal cell interactions and their sequelae in the BM milieu to overcome drug resistance and improve patient outcome in MM

Thursday 30 September 10:15–12:00

PLENARY SESSION 5

Hypoxia as a target

242 INVITED

Imaging hypoxia in tumours

P.L. Olive. *BC Cancer Research Centre, Medical Biophysics, Vancouver, BC, Canada*

Hypoxia that develops in solid tumours is associated with a more aggressive tumour phenotype and poorer response to treatment. This appreciation has stimulated efforts to develop methods that could be used routinely to assess pretreatment tumour hypoxia as well as rates of reoxygenation during therapy. Hypoxic tumour cells, although a limitation to response, are also a recognized target for new therapeutic agents. Methods that can detect hypoxic cells could be used to identify patients likely to benefit from hypoxia-directed bioreductive cytotoxins like tirapazamine or gene therapy approaches like GDEPT that rely on activation of pro-drugs by hypoxic cells. Although non-invasive imaging would be preferable for many reasons, the dynamics of tumour hypoxia, both spatially and temporally, need to be better understood before we can have confidence in the imaging results that typically provide much lower resolution. Towards this end, chemical hypoxia markers like pimonidazole and EF5 can be administered to patients and then detected in tissue sections or fine needle aspirates using simple antibody labeling procedures. These are robust methods that provide information on the fraction of hypoxic cells as well as the degree of hypoxia. A technically simpler approach is to measure expression of endogenous hypoxia markers like the transcription factor HIF-1α and its downstream targets, carbonic anhydrase 9 and glucose transporter 1. Although often criticized as providing only a snapshot in time of what are undoubtedly very dynamic processes, it is possible to obtain kinetic information by analysis of a single tumour section. Staggering the delivery of two hypoxia or perfusion markers before biopsy can be used to identify regions of transient perfusion. Alternatively, by making use of the variability in the half time of hypoxia-inducible protein formation or loss in response to specific microenvironmental changes, dynamic changes can be identified in a single tumour biopsy. Tumour models that are thoroughly characterized using these quantitative techniques will be more useful for experimental therapeutic studies. Many of these approaches are already being applied to clinical biopsies.

243 INVITED

Clinical significance of tumour hypoxia

M. Nordsmark[1], J. Eriksen[2], M. Horsman[2], J. Alsner[2], J. Overgaard[2]. *[1]Aarhus University Hospital, Oncology, Aarhus, Denmark; [2]Aarhus University Hospital, Experimental Clinical Oncology, Aarhus, Denmark*

Since the early seventies a number of clinical trials have been undertaken with the aim to modify tumor oxygenation. Some succeded and others

did not. Results from a meta-analysis showed that in particular head and neck tumors benefited from hypoxic modification and indeeed the DAHANCA 5 study evaluated the effect of the hypoxic radiosensitizer nimorazole (NIM) and found it to significantly improve the outcome of radiotherapy in supraglottic and pharynx tumours. Lately new assays have become available such as a) direct measurements of tumor oxygen tension b) exogenous nitroimidazole based assays and c) endogenous markers expressed under hypoxic conditions. Measurements of oxygen tension was the first way to characterise hypoxia in human tumors. More recently, basic hypoxia inducible factor 1 (HIF-1) was recognised as a key player of the transcriptional response to low oxygen tension. Carbonic anhydrase 9 was another indicator. Induction of hypoxia in-vitro relates to down-regulation of the tumor suppressor gene Von Hippel Lindau (VHL) and upregulation of osteopontin (OPN) while in human head and neck tumors plasma OPN inversely relates with low tumor pO_2 and indicates poor prognosis. A brief overview of these clinical studies will be given. Moreover, prospectively generated data from about 400 head and neck tumors showed that the percent of pO_2 values <2.5 mmHg was a strong marker for overall survival. Pretreatment OPN measured in 63 of these head and neck carcinomas using Elisa, immunohistochemical staining of HIF-1a and CA9 in archive paraffin material and measurements of tumor pO_2 using Eppendorf pO_2 electrodes were compared. For survival analysis patients were grouped into tertiles based on OPN values, the median tumor pO_2 and the fraction of pO_2 values £ 2.5 ($HP_{2.5}$). CA9 was scores as <1%, 1–30% and >30% staining, (n=54) and HIF-1alpha as <1%, 1–50% and >50% staining, (n=55). All patients received primary radiation therapy (RT). The median OPN was 625 ng/dl, range (168–3790). Overall median tumor pO_2 was 13 mmHg (range 0–54 mmHg) and $HP_{2.5}$ with a median of 27% (range 0–100). There was a statistical significant correlation between OPN and median tumor pO2 (p=0.02) not between OPN and $HP_{2.5}$(p=0.07), HIF–1alpha, (p=0.14) or CA9, (p=0.23), respectively. In Kaplan Meier analysis OPN, median tumor pO2 or $HP_{2.5}$ were prognostic for LC (p<0.002, p=0.05 and p=0.01, respectively) while there was a trend that HIF-1a was prognostic for LC (p=0.06) but CA9 was not (p=0.77). Using DSS as endpoint both OPN (p<0.01), median tumor pO2 (p=0.05) and HIF-1a (p=0.01) were statistical significant indicators for prognosis, while there was a trend that HP2.5 was prognostic (p=0.14) but CA9 was not (p=0.27). In all cases of statistical significance more hypoxia related with a poorer prognosis. Finally, stored plasma samples from 326 of the 414 patients from DAHANCA 5 was used to determine OPN and data was evaluated by 5-year actuarial univariate and Cox multivariate analyses. The 326 analyzed patients were representative of all 414 in the trial and did overall show a significant difference in loco-regional control in favour of NIM with 5-year values of 55% vs. 44%, p=0.05. Analyzing the odds ratio for the tertiles as a function of NIM treatment showed an odds ratio for patients with low OPN level of 1.0 (0.5–2.2, 95% cf.l.) and for intermediate of 0.9 (0.4–1.8), whereas for high OPN levels there was a significantly better outcome in the NIM treated patients 0.3 (0.1–0.6), p<0.01. Actuarial analysis confirmed that there was a significant benefit in 5-year loco-regional control (52% vs. 27%), p=0.01 and cancer specific survival (45% vs. 25%), p<0.05, if NIM was given to patients with high OPN level. The study is thus indicative of OPN as a predictor for clinical relevant hypoxia and may predict the patients who may benefit from hypoxic modification. OPN measurements should be included in clinical trials evaluating hypoxic modification in order to confirm this hypothesis. Supported by The Danish Cancer Society.

244 INVITED

Kidney cancer and the von Hippel-Lindau tumor suppressor protein: implications for therapy

W.G. Kaelin Jr[1,2], W. Kim[1], S. Lee[1], A. Reddy[1], M. Safran[1], Q. Yan[1], H. Yang[1]. [1]*Dana Farber Cancer Center, Boston, USA;* [2]*Howard Hughes Medical Institute, USA*

Germline inactivating mutations of the von Hippel-Lindau tumor suppressor gene (VHL) cause von Hippel-Lindau disease, which is characterized by increased risk of a variety of tumors including blood vessel tumors (hemangioblastomas), pheochromocytomas, and clear cell renal carcinomas. VHL inactivation, due to somatic mutations or hypermethylation, is also very common in sporadic clear cell renal carcinoma. The VHL gene product, pVHL, is the substrate recognition module of an E3 ubiquitin ligase complex. The best understood targets of this complex are the alpha subunits of the heterodimeric transcription factor called HIF (hypoxia-inducible factor). In the presence of oxygen HIF is polyubiquitylated and destroyed. Under low oxygen conditions, or in cells lacking functional pVHL, HIF accumulates and induces the transcription of a variety of genes important for tumorigenesis and angiogenesis. In nude mouse xenograft studies, inhibition of HIF is both necessary and sufficient for pVHL to suppress tumor growth. Accordingly, drugs that inhibit HIF or its downstream targets warrant testing in cancers such as renal cell carcinomas. A number of drugs indirectly lead to downregulation of HIF including rapamycin-like compounds and HSP90 inhibitors. Drugable HIF targets include VEGF, PDGF B, and TGFa, as well as their receptors. Notably, a neutralizing VEGF antibody (Avastin) was shown by Yang and colleagues to delay time to progression in a randomized Phase II study of patients with advanced renal cancer. To facilitate the testing of new drugs and new drug combinations, we are developing a series of mouse models based on VHL inactivation or HIF activation. In some cases we have also incorporated a bioluminescent reporter molecule that selectively accumulates in pVHL-defective cells, thereby allowing non-invasive imaging of pVHL-defective tumors in vivo

245 INVITED

HIF-1, hypoxia inducible factor-1 as a therapeutic target

I. Stratford, R. Cowen, K. Williams. *University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Manchester, UK*

HIF-1 is a heterodimeric transcription factor made up of the O2-sensitive component HIF-1(and the constitutively expressed HIF-1(, and HIF-1 plays the major role in controlling gene expression under hypoxic conditions. Hypoxia-regulated genes encode VEGF, glucose transporters and enzymes in the glycolytic pathway, all these being important in tumourigenesis and this is consistent with observations that cells with defective HIF-1 function show impaired ability to form tumours and generally show a slower rate of tumour growth. Other hypoxia regulated genes include those encoding the pro-apoptotic proteins bid, bad and bax (Erler et al Mol. Cell. Biol. (2004), 7, 2875–89), and this suggests that the resistance of hypoxic cells to drug treatment may have as its mechanistic basis hypoxia/HIF-1 mediated changes in the threshold for apoptosis following drug exposure. Evidence supporting this contention comes from studies of drug sensitivity where HIF-1 function has been attenuated in mouse embryonic fibroblasts and in human tumour cells when HIF-1 function is impaired by the use of dominant negative HIF-1(. Cells with compromised HIF-1 activity also show increased responsiveness to radiotherapy. Together, these results suggest HIF-1 may be a realistic therapeutic target. Recently, a whole range of small molecules have been identified that directly or indirectly modulate HIF-1 function and some of these also show anti-tumour activity. The majority of these agents interfere with cell signalling processes that influence the formation and stability of HIF-1(; e.g. inhibitors of the P13 kinase/akt pathway such as wortmannin, LY294002 or rapamycin, or inhibitors of the MEK/MAPK pathway such as PD98059. Alternatively, agents that interfere with translocation of HIF-1(to the nucleus such as geldanamycin and 2-MEZ can also down-regulate HIF-1 function.
In this presentation, we will review the current status of small molecule inhibitors of HIF-1 and tease-out whether these effects occur independent of non-specific effects on gene transcription and toxicity. Further, the impact of these and other novel HIF-1 inhibitors on tumour growth and response to therapy will be illustrated.

Thursday 30 September 15:00–16:00

PLENARY SESSION 6

Proffered papers

246 ORAL

Novel ATP-competitive Akt inhibitors slow the progression of tumors in vivo

V. Giranda[1], Y. Luo[1], Q. Li[1], A. Shoemaker[1], R. de Jong[2], X. Liu[1], E. Han[1], K. Woods[1], S. Thomas[1], S. Rosenberg[1]. [1]*Abbott Laboratories, Cancer Research, Abbott Park, USA;* [2]*IDUN Pharmaceuticals, Cancer Research, La Jolla, USA*

Akt (PKB) has been implicated in the generation and maintenance of the oncogenic phenotype in a wide variety of human tumors. Therefore, inhibition of Akt may be useful in cancer therapy. To test this hypothesis, a series of potent, selective, and novel ATP-competitive Akt inhibitors were synthesized. These compounds were examined for anti-cancer activities, both *in vitro* and *in vivo*. This series of compounds is exemplified by A-443654, which inhibits Akt1 with a Ki of 160 pM. *In vitro* evaluation of this series of compounds demonstrated that they inhibit Akt within cells. The phosphorylation of targets directly downstream of Akt, including GSK3α, β, FOXO3a, TSC-2, and mTOR, was diminished in the presence of the inhibitors. Also inhibited was the phosphorylation of targets further downstream in the signal transduction pathway, including $P70^{s6k}$ and the S6 protein. The Akt inhibitors induced apoptosis in tumor-derived cells, and this apoptosis correlated with the intracellular inhibition of