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

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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

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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

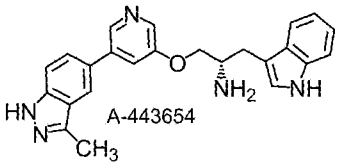
246 ORAL

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

Akt. These compounds displayed significant antitumor activity in multiple tumor models (xenograft, syngeneic, orthotopic, flank). Tumor growth was almost completely inhibited during compound administration, with tumor regrowth occurring upon cessation of dosing. These effects were dose dependent. The compounds were not only efficacious as monotherapy, but also when combined with other anti-tumor agents. Pharmacodynamic and pharmacokinetic studies demonstrate that Akt was inhibited within tumors at concentrations achieved during dosing. Although increased insulin secretion is observed concomitant with administration of these pan-Akt inhibitors, no significant changes were observed in blood glucose concentrations, whether measured randomly, or in oral glucose tolerance tests. In mouse tumor models, significant weight loss occurred at super-therapeutic doses (>MTD), and this was the dose limiting toxicity.



A-443654

247 ORAL

Silencing of survivin gene by small interfering RNAs (siRNAs) induces apoptosis in human prostate cancer cells and increases their sensitivity to 17-allylamino-17-demethoxygeldanamycin (17AAG)

F. Paduano, R. Villa, M. Binda, M. Folini, M. Pennati, M.G. Daidone, N. Zaffaroni. *Istituto Nazionale Tumori, Department of Experimental Oncology, Milano, Italy*

Background: Survivin is an anti-apoptotic gene overexpressed in most human tumors and also involved in mitotic checkpoint control. Recent evidence points to a role for HSP90 in survivin function regulation. While the survivin-HSP90 association may help tumor cells to increase their anti-apoptotic threshold and promote their growth, it may also suggest new opportunities for the design of rational anti-cancer strategies. In this context, we evaluated the effects of the inhibition of survivin expression, accomplished through RNA interference technology, on the proliferative potentials of human prostate cancer cells and their sensitivity profile to the HSP-90 inhibitor 17AAG.
Material and Methods: Five 21-mer double-stranded siRNAs directed against different portions of survivin mRNA were designed. DU145 human androgen-independent prostate cancer cells were transfected with each siRNA (100 nM) using Lipofectamine-2000 for 8 h. At different intervals after transfection, cells were collected and analyzed for survivin mRNA and protein expression, cell growth rate, ability to undergo apoptosis, and sensitivity to a 72-h exposure to 10–100 nM 17AAG.
Results: Transfection of DU145 cells with siRNAs induced a variable extent of inhibition of survivin mRNA levels, ranging from −35% to −85%, compared to Lipofectamine-treated samples, as a function of the different oligomers. Such an inhibition was paralleled by a reduction in the abundance of survivin protein, ranging from −30% to −72% of controls. The three siRNAs able to reduce survivin expression (mRNA and protein) by more than 50% also caused a time-dependent inhibition of DU145 cell growth and enhanced the rate of spontaneous apoptosis from 5% of the overall cell population detected in control samples to 25–40%, with a concomitant 2–3 fold increase in the catalytic activity of caspase-9 and caspase-3. Sequential treatment of DU145 cells with siRNA and 17AAG induced supra-additive growth suppression and enhanced caspase-9-dependent apoptotic response.
Conclusions: These findings suggest that strategies aimed at interfering with the survivin-HSP90 connection, which couples apoptosis resistance to the cellular stress response, may provide novel approaches for treatment of androgen-independent prostate cancer.

248 ORAL

Antitumor activity, pharmacodynamics and toxicity of PX-866 a novel inhibitor of phosphoinositide-3-kinase

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Background: Phosphoinositide-3-kinase (PI-3-K) activates an important cell survival signaling pathway and constitutive activation is seen in ovarian, head and neck, urinary tract, cervical and small cell lung cancer. PI-3-K signaling is attenuated by the phosphatase activity of the tumor suppressor PTEN that is absent in a number of human cancers. Inhibiting PI-3-K presents the opportunity to inhibit a major cancer cell survival signaling pathway and to overcome the action of an important deleted tumor suppressor, providing antitumor activity and increased sensitivity of tumors to a wide variety of cancer drugs. PX-866 was identified as a pan-PI-3-K inhibitor from a library of synthetic viridins and inhbits PI-3-K with IC_{50} of 0.1 nM and cancer cell PI-3-K, measured by phosphoSer473-Akt (pAkt), with an IC_{50} of 20 nM.
Methods: The study evaluated the antitumor activity of PX-866 against human tumor xenografts in scid mice, alone or in combination with taxol, gemcitabine, cisplatin, or Iressa. The studies were accompanied by the evaluation of toxicity and pharmacodynamic activity of PX-866.
Results: *Antitumor activity* PX-866 as a single agent administered Q2D×5 intravenously (iv) at 12 mg/kg provided OvCar-3 ovarian tumor growth inhibition (TGI = 100 − T/C%) of 58%, and at 4 mg/kg orally (po) of 53%. When given on same schedule with taxol 12 mg/kg ip, PX-866 iv 4 hr before, gave a TGI of 83% versus 58% for taxol alone. PX-866 po with taxol produced a TGI of 88%. Similar potentiation by PX-866 was observed for the antitumor activity of gemcitabine in Panc-1 pancreatic cancer and cisplatin in A-549 lung cancer. Against large (0.5 g) A-549 xenografts in combination with EGFR kinase inhibitor Iressa (75 mg/kg po 3 times a week × 5) PX-866 (12 mg/kg iv; 4 hr before) gave a TGI of 84% versus Iressa alone 46% or iv PX-866 alone 40%. The combination with po PX-866, 4 mg/kg, gave a TGI of 76% versus po PX-866 alone of 56%. There were no significant changes in blood WBC, NE, RBC, platelets, ALT, AST or glucose by PX-866 at doses that increased Iressa antitumor activity. *Pharmacodynmic activity*: PX-866 iv or po as a single dose inhibited tumor pAkt for up to 48 hr. Tumor pAkt was not inhibited by Iressa, while pEGFR was inhibited by Iressa but not by PX-866. Iressa and PX-866 combined inhibited pAkt and pEGFR.
Conclusions: The PI-3-K inhibitor PX-866 inhibits p-Akt survival signaling in human tumor xenografts, has antitumor activity as a single agent and potentiates the antitumor activity of a variety of cancer drugs including an EGFR-kinase inhibitor.

249 ORAL

Novel small molecule inhibitors of 3′-phosphoinositide-dependent kinase-1 (PDK-1)

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The PI-3 kinase/PDK-1/Akt pathway is highly activated in many cancers and plays a key role in regulating tumor cell survival, growth, metabolism and angiogenesis. From chemical screens using a coupled assay measuring PDK-1-activated AKT2 activity, we identified and optimized potent inhibitors of PDK-1 that display selectivity against a panel of related kinases. Here, we describe the biological characterization of three PDK-1 inhibitors (Compound 1, (*N*-(3-((5-iodo-4-((3-((2-thienylcarbonyl)-amino)propyl)amino)-2-pyrimidinyl)-amino)phenyl)-1-pyrrolidinecarboxamide); Compound 2, (*N*-(3-((5-bromo-4-((2-(*1H*-imidazol-4-yl)ethyl)amino)-2-pyrimidinyl)amino)phenyl)-1-pyrrolidinecarboxamide); and Compound 3, (N-[3-[[5-bromo-2-[[3-[(1-pyrrolidinylcarbonyl) amino] phenyl] amino]-4-pyrimidinyl] amino] propyl]-2,2-dimethyl-propanediamide). The compounds block PDK-1 and Akt signaling in PC-3 prostate cancer cells. Compound 1 (IC_{50}=6 nM) and Compound 3 (IC_{50}=22 nM) blocked both Thr308-Akt and Thr389-S6K1 phosphorylation with IC_{50} values of 0.3 μM and 1–3 μM, respectively. The compounds also blocked colony formation in soft agar by tumor cell lines. For example, Compound 3 displayed an average IC_{50} of 0.4 μM for inhibition of 9 tumor cell lines tested. Furthermore, PDK-1 inhibitors induced massive apoptosis by MDA-468 cells cultured on plastic. Other cells tested showed less dramatic or no apparent apoptotic response when cultured on plastic, although a large proportion of cell lines were effectively growth-inhibited by compounds (IC_{50} < 2 μM). After 18 h of treatment, compounds were found to delay in the G2/M phase of the cell cycle. In contrast, the PDK-1 inhibitors weakly inhibited growth of normal human primary mammary and prostate epithelial cells, thus showing selectivity for tumor cells. A number of cell lines displaying high levels of Akt activity (e.g., MDA-453, U87-MG and PC-3) were markedly more sensitive (>30 fold) to growth inhibition by Compound 3 in soft agar than in culture on plastic. These data suggest that tumor cells undergoing metastasis may be particularly vulnerable to PDK-1 inhibitors. Treatment of mice with Compound 3 inhibited a blood borne metastasis model in which tumor burden in the lung was measured 4 weeks subsequent to tail-vein injection of LOX melanoma cells. In conclusion, we report on the development of potent inhibitors of PDK-1 in the aminopyrimidine class that support further development of PDK-1 inhibitors as anticancer agents.