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**

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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 GSK3$\alpha$, $\beta$, 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