41 POSTER

Clinical and biomarker responses in a phase I study of BAY 57 9352 – a VEGFR-2 inhibitor – administered as continuous dosing in patients with advanced solid tumors

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Background: BAY 57–9352 (telatinib) (BAY) inhibits the VEGFR-2 and VEGFR-3 tyrosine kinases, in addition to PDGFR-β and c-Kit. BAY showed anti-tumor activity in colon, breast, pancreatic, and NSCLC preclinical models.
Methods: This study investigated the safety, pharmacokinetics (PK), and pharmacodynamics of oral BAY administered as continuous dosing until discontinuation due to toxicity or progression. PK was assessed on Days 1 and 14. Dynamic contrast-enhanced MRI (DCE-MRI) was performed at baseline, and on Days 2, 14, 35, and 56. Plasma biomarkers [VEGF, soluble (s)VEGFR-2] were assessed at each 21-day cycle.
Results: Twenty-five patients (pts) were enrolled at doses of BAY from 600 mg twice daily (bid) to 1500 mg bid. Common tumor types were CRC (n = 8) and RCC (n = 6). Frequent drug-related adverse events (⩾5% pts) were hypertension (6 pts [24%], all grade 3), diarrhea (6 pts [24%], all grades; 3 pts [12%], grade 3), anorexia (5 pts [20%], grade 1–2), flatulence (5 pts [20%], all grade 1), nausea (4 pts [16%], grade 1–2), dizziness (4 pts [16%], all grade 1), hoarseness (3 pts [12%], all grade 1), dyspepsia (3 pts [12%], grade 1–2), palpitations (2 pts [8%], both grade 1), and abdominal pain (2 pts [8%], grade 1–2). Study treatment-related adverse events leading to a dose reduction or interruption were diarrhea (n = 2) and hypertension (n = 1). BAY AUC plateaued at doses above 900 mg bid and exhibited moderate to high variability. Evaluable DCE-MRI measurements (defined by at least two consecutive measurements beyond baseline) were available from 22 patients. Eleven patients showed a significant DCE-MRI response defined by an at least 30% decrease in $iAUC_{60}$ of Gd-DTPA at two consecutive timepoints. Disease stabilization as indicated by treatment with BAY for ⩾90 days was achieved in 13 patients. Biomarker responses (increase of VEGF and decrease of sVEGFR-2) increased in a dose-dependent manner up to 900 mg bid. A final analysis correlating the pharmacodynamic data (DCE-MRI, VEGF, sVEGFR-2) to the clinical outcome will be presented at the meeting.
Conclusions: The maximum tolerated dose was not reached for BAY up to 1500 mg bid administered continuously. BAY had a favorable safety profile. Disease stabilization was shown for a significant number of patients. The pharmacodynamic effect of BAY was demonstrated by DCE-MRI and plasma biomarkers. The recommended dose for Phase II evaluations is 900 mg bid continuously administered.

42 POSTER

Potent inhibition of glioblastoma growth and angiogenesis by melanotransferrin

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Background: Glioblastomas are the most common brain tumor and the most invasive type of astrocytomas. Despite the considerable work done on malignant glioblastoma, no treatment is known to be totally successful and reappearance of the tumor is recurrent. Thus, novel treatment strategies are needed to improve the clinical management of this disease. Recombinant soluble melanotransferrin (sMTf) was shown to exert *in vitro* antiangiogenic properties by the inhibition of endothelial cell migration and capillary-like formation. In this study, we investigated the effects of sMTf on glioma invasion.
Materials and Methods: Glioblastoma tumors were established by subcutaneous injection of human multiform glioblastoma cells (U-87 MG) in severe combined immunodeficient mice nu/nu. sMTf treatment was either administered by single subcutaneous (s.c.) injection or continuously by Alzet mini-osmotic pump.
Results: Here we show that endothelial cells (EC) treated with sMTf show a reduction in LRP and u-PAR expression with a concomitant inhibition of EC migration. Additionally, our *in vivo* studies demonstrate that sMTf treatment leads to the inhibition by 50% of the neovascularization in Matrigel™ implants stimulated by vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF). Using a glioblastoma xenograft model, we investigated whether treatment with sMTf could inhibit U-87 MG derived tumor growth and angiogenesis. Although U-87 MG invasive capacities were unaffected by the presence of sMTf, results obtained *in vivo* reveal that sMTf treatment reduces tumor growth by 20% and 80% at 2.5 and 10 mg/kg/day respectively. Compared to initial volume, U-87 derived tumors were also reported to regress for a 15 days period when treated with 10 mg/kg/day of sMTf. In order to evaluate the angiogenic development in glioblastoma tumors, the content of hemoglobin and endoglin (CD105) were monitored. In association with a reduction of endoglin mRNA expression, the hemoglobin content is decreased by 50% in treated tumors.
Conclusion: Altogether, our results demonstrate that sMTf exert an antiangiogenic activity *in vivo* and strongly suggest that its s.c. administration may provide a novel therapeutic strategy for the treatment of angiogenesis-related disorders.

43 POSTER

CD44 regulates the expression of the cysteine protease Cathepsin K: implications for bone metastasis of breast cancer

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The hyaluronan (HA) receptor CD44 has been implicated in the tumorigenicity and metastasis of breast cancer. We previously highlighted the significance of CD44 in mediating cancer cell adhesion to bone marrow endothelial cells (BMECs). Our recent experiments have also determined that the expression of CD44 is elevated in a bone homing breast cancer subline MDA-MB-231BO relative to that detected in the parental MDA-MB-231 cells. To further understand the potential significance of CD44 signaling to breast cancer metastasis, we established a tetracycline-regulated CD44 expression system which was utilized to detect differential gene expression by microarray analysis. Expression and activation of CD44 was associated with increased expression of a subset of genes with known function in promoting cell motility, invasion and bone metastasis. Our microarray data predicted that the cysteine protease, cathepsin K was upregulated upon CD44 expression and activation. This protease targets collagen I, a major component of the bone matrix whose degradation is a major consequence of osteolytic metastasis of breast cancer. Consistent with their respective metastatic potential, immunoblotting and ELISA-based experiments have confirmed that cathepsin K expression is elevated in MDA-MB-231BO bone homing cells relative to parental MDA-MB-231 cells. Furthermore, the expression of cathepsin K in MDA-MB-231BO cells was significantly decreased upon RNAi-mediated suppression of CD44. Quantitative RT-PCR, immunoblotting and ELISA-based experiments have also demonstrated that the transcript and protein expression of cathepsin K increase in response to CD44-HA signaling in a panel of CD44-expressing breast cancer cell lines. We are currently (i) investigating the mechanistic basis underpinning the transcription of cathepsin K, (ii) determining the functional significance of its overexpression in enabling breast cancer cells to degrade a collagen I matrix and (iii) investigating mechanisms of cathepsin K inhibition and potential effects on the degradation of collagen I. The long term objective of our research will be to determine whether CD44 expression and that of its transcriptional targets may be predictive for those breast cancer patients at higher risk of developing skeletal disease and/or potentially lead to the development of novel and more effective therapeutic strategies to attenuate bone metastasis.

44 POSTER

Regulating hypoxia-driven VEGF by inhibiting IGF-1 signaling in childhood cancer cells

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Background: VEGF is one of the most potent angiogenic factors that plays an essential role in cellular adaptation to hypoxia. VEGF is rapidly and significantly upregulated in response to hypoxia via activation of the hypoxia-inducible factor 1 alpha (HIF-1a), leading to angiogenesis and restoration of tissue oxygenation. We are investigating the insulin-like growth factor (IGF) axis in control of VEGF in cell lines derived from pediatric rhabdomyosarcoma (RMS) and neuroblastoma (NB).
Materials and Methods: VEGF was determined using ELISA assay in the panel of 7 RMS and 7 NB cell lines after 24hr exposure to hypoxia (1% O2) with or without addition of drugs. Cell numbers were determined by counting nuclei following cell lysis. Levels of HIF-1a, Akt, pS6, GSK-3b, IGF-IR and b-actin were detected by Western blot analysis, and normalized to b-actin expression.
Results: RMSs and NBs are characterized by expression of type II IGF, a ligand for the type 1 receptor (IGF-1R). We examined the effect of rapamycin, as it has previously been reported that translation of HIF-1a and VEGF production is regulated by mTOR signaling. Rapamycin, at concentrations that ablated mTOR signaling, poorly inhibited hypoxia-induced increases in VEGF in 6/7 NB and 6/7 RMS lines, suggesting

that mTOR only partially regulates VEGF under hypoxia. To determine whether VEGF production was controlled by Akt signaling, independent of mTOR, we exposed cells to the Akt-selective inhibitor A443654 for 24 hr and VEGF secreted into medium determined. A443654 completely inhibited signaling through Akt as judged by loss of detectable phospho-S6 protein, and hypophosphorylation of GSK-3b. Of note inhibition of Akt was more effective than rapamycin in blocking hypoxia-driven VEGF in 3/3 RMS and 2/4 NB cell lines. Combination of A443654 with rapamycin was additive or synergistic and completely blocked hypoxia-driven increases in VEGF. Because inhibition of Akt may result in unacceptable toxicity (hyperglycemia and hyperinsuliemia), we have explored the effect of blocking the IGF-1R using an antibody (CP-751871) as an alternative strategy. Administration of 0.25 mg to tumor bearing mice resulted in dramatic downregulation of IGF-1R in 4/5 sarcoma xenograft models, associated with a dramatic decrease in pAkt and pS6 levels.
Conclusions: These preliminary results suggest that direct inhibition of IGF-1R may be an interesting approach to modulating VEGF in pediatric sarcoma, and other solid tumors.

45 POSTER

Focal adhesion kinase is a key signalling intermediate in interleukin-8 promoted chemotaxis and adhesion of prostate cancer cells to bone marrow endothelium

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Purpose: To characterize the biochemical and functional relationship between interleukin-8 (IL-8) signaling and focal adhesion kinase (FAK) activation and to define their importance to prostate cancer metastasis.
Experimental design: Immunohistochemistry (IHC) conducted on human prostate biopsy tissue was used to determine the phosphorylation status of FAK in tumour cells relative to IL-8 expression. Experiments using metastatic prostate cancer PC3 cells established the biochemical, molecular and functional importance of FAK to IL-8 promoted cell motility and adhesion to bone marrow endothelial cells (BMECs).
Results: IHC demonstrated normal prostate epithelium to be devoid of FAK expression/activation but expression and autophosphorylation of FAK was detected in tumour cells of locally-invasive and hormone-independent prostate tissue. Statistical analysis confirmed that IL-8 expression correlated with increased autophosphorylation of FAK on Tyr^{397} in prostate cancer cells ($p < 0.001$). Stimulation of PC3 cells with IL-8 induced cell polarization and promoted the redistribution of FAK to sites of focal adhesion. Immunoblotting confirmed that IL-8 induced time-dependent phosphorylation of FAK on Tyr^{397}, Tyr^{576} and Tyr^{925}, that was mediated by a complex signaling cascade downstream of CXCR1 and CXCR2 receptors. Inhibition of FAK activity, using the dominant-negative FRNK construct or through RNAi-mediated depletion of FAK, attenuated IL-8-promoted activation of Rac-GTPase in pull-down assays, abrogated IL-8 promoted chemotaxis and attenuated IL-8 potentiated adhesion of PC-3 cells to BMECs, respectively.
Conclusions: IL-8 signaling regulates FAK activation in prostate cancer cells and is functionally important in mediating IL-8 promoted cell motility and adhesion, consistent with the metastasis promoting function of this chemokine. Our results describe a novel molecular basis to IL-8 promoted metastasis of prostate cancer and indicate the potential therapeutic significance of attenuating IL-8 expression in prostate cancer.

46 POSTER

Genetically engineered PAI-1 in anti-angiogenic and anti-metastatic therapy

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Abnormal proteolytic activity of urokinase (uPA) is one of the factors causing metastasis and angiogenesis. Thus eradication of uPA activity might result in inhibition of these processes. The small molecular inhibitors of uPA and plasminogen activator inhibitor (PAI-1) have been successfully used to reduce the angiogenesis and tumor growth. While uPA inhibitors were used we have observed reduction of sprout formation of human vascular endothelial cells, lowering blood vessel density in chick embryo chorioallantoic membrane (CAM) assay and reduction of tumor size of xenografts of human prostate LnCAP and DU145 cancer cells.
Plasminogen activator inhibitor (PAI-1) that inhibits the uPA could be used as an inhibitor of angiogenesis. However, wild PAI-1 is not stable and converts into the latent form in t1/2 $\approx$ 2 hours. This conversion is associated with partial insertion of the reactive loop (P4−P10′) into the PAI-1 molecule. In such conformation, P1−P1′ are not accessible for reaction with uPA. By 2 to 6 point mutations that could form disulfide bridges in PAI-1s in proximity of A3, A5 strands, we have extended half-life of this protein up to ~650 h. This PAI-1 is called VLHL PAI-1 and is fully functional as demonstrated by uPA inhibition. Additional, genetically engineered clone was produced by deleting part of this protein and lowering its size to create molecule that is more therapeutically desired. Mutant of Arg346 $\rightarrow$ Ala produced VLHLns PAI-1 that do not react with uPA and will be used as negative control.
Using baculovirus expression system PAI-1s were expressed in Sf9 insect cells and purified using affinity tag (6His). In single step purification we achieve +95% purity. The identity of PAI-1 was conformed by tandem liquid chromatography-mass spectroscopy. Disulfide bridge of VLHL PAI-1 could be reduced by DTT and reduced cysteine can't keep A3 and A5 strands together that is prerequisite of extending PAI-1 activity. Reduced form of VLHL PAI-1s convert into latent form as wPAI-1 does, and do not inhibit uPA. These conform our assumption of importance of the disulfide bridges in extending the half life of PAI-1.
Novel PAI-1 was fully functional against uPA and showed anti-angiogenic activity in the in vitro and in vivo models. Such prolonged serpin activity, which is therapeutically desired in cancer treatment could launch a new class of novel anti-cancer agents based on Cys mutated PAI-1s.

47 POSTER

A phase II study of Sorafenib (BAY 43–9006) in recurrent and/or metastatic squamous cell carcinoma of the head and neck (SCCHN) and nasopharyngeal cancer (NPC): final results

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Background: Sorafenib is an oral multi-kinase inhibitor targeting Raf kinase and VEGFR-2 among others. As the Ras-Raf-MAPK-ERK signaling pathway and angiogenesis are thought to play a significant role in the pathogenesis of head and neck cancers, we conducted a phase II study of sorafenib in recurrent and/or metastatic SCCHN and NPC to determine its efficacy and safety in this patient population.
Patients and Methods: This is a single arm, two-stage phase II trial. Sorafenib was administered orally at 400 mg BID continuously. Patients had $\leqslant$1 line of chemo for recurrent disease, performance status (PS) ECOG 0–2, and adequate organ function. Response was evaluated every 8 weeks according to RECIST criteria. At the end of stage one, efficacy criteria for further accrual were not met but the study was amended to enroll an additional 5 patients for pharmacodynamic evaluations. The biologic effects of sorafenib on tumors were assessed before and 4 weeks after treatment initiation.
Results: We enrolled 28 patients, of whom 27 and 26 were eligible for toxicity and efficacy evaluations. Median age was 53 years (range 37−77); 63% patients were male; 89% had PS 0 or 1; 74% SCCHN and 26% NPC; 70% of patients received prior chemotherapy, 48% had prior first-line chemotherapy for their recurrent and/or metastatic disease. In total, 72 cycles have been administered with a median of 2 cycles per patient (range 1–7). Most common adverse events (AE), at least possibly related to sorafenib, were fatigue in 79%, lymphopenia in 42%, mucositis in 42%, anemia in 35%, hand-foot skin reaction in 29% and hypertension in 28% of cycles. Most common grade 3 AEs were lymphopenia and fatigue in 17% and 7% of cycles. No grade 4 AEs were observed, 2 deaths on study were unlikely related to sorafenib. One patient with SCCHN (3.7%) had a confirmed partial response, 10 (37%) had stable disease ranging from 2 to 6 cycles and 15 patients (55.6%) had progressive disease. Median time to progression was 1.8 months (95% CI: 1.6–3.4) and median overall survival was 4.2 months (95% CI: 3.6–8.7). Results of the PD analysis will be presented at the meeting.
Conclusions: Sorafenib was well tolerated in this group of patients. Although the criteria for the second stage were not met, single-agent sorafenib has modest anti-tumor activity, comparable to single-agent erlotinib and gefitinib. Further evaluation of sorafenib in combination with other agents may be warranted in SCCHN and NPC.

48 POSTER

Distinct gene expression profiles and cell death pathways in clear-cell renal cell carcinoma (CCRCC) and colorectal carcinoma (CRC) cells:r elationship to hypoxia, von Hippel Lindau protein (pVHL) expression and anti-tumor activity of sorafenib

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Background: The interaction between pVHL and hypoxia is important in regulating target genes in CCRCC cells. Sorafenib, a multi-kinase inhibitor