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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.

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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.

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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.

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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