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Regulation of tumour angiogenesis and cell survival by integrin-linked kinase (ILK): pre-clinical evaluation of novel small molecule inhibitors

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Integrin-linked kinase(ILK) is a PI-3Kinase-dependent focal adhesion serine/threonine protein kinase and couples integrins and growth factor receptors to the actin cytoskeleton and the Akt/PKB signaling pathway. ILK is overexpressed in many types of tumours and its expression is often correlated with disease grade and inversely correlated with patient outcome. ILK activity is also constitutively activated in PTEN-null tumour cells, and inhibition of ILK activity in such cells results in the inhibition of Akt/PKB phosphorylation and induction of apoptosis. We now demonstrate that ILK stimulates the expression of HIF-1alpha and VEGF in a Akt/PKB and mTOR-dependent manner. ILK is also required for VEGF stimulated endothelial cell migration and proliferation. We have identified highly selective small molecule inhibitors of ILK activity. We will provide data with KP-074728, a pre-clinical lead candidate, demonstrating inhibition of Akt/PKB phosphorylation on Serine-473, HIF-1alpha, and VEGF expression in tumour cells. The inhibitor also causes marked inhibition of VEGF stimulated endothelial cell morphogenesis as well as tumor growth and angiogenesis in human prostate and glioblastoma xenograft models in vivo. This compound is also highly effective in inducing apoptosis and growth arrest in several human breast cancer cell lines in vitro and in vivo. ILK is thus an important therapeutic target for the control of tumour angiogenesis and tumour progression.

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A clinical phase I dose escalation, pharmacokinetic (PK) and pharmacodynamic (PD) study of BIBF 1120 in advanced cancer patients

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Background: BIBF 1120 is a potent, orally available triple kinase inhibitor (VEGFRs, PDGFRs, FGFRs) that suppresses tumour growth by inhibition of tumour angiogenesis. A first-in-man study was carried out with a once daily schedule in chemotherapy refractory advanced cancer patients.
Methods: Treatment cycles consisted of 28 days continuous administration of fixed oral BIBF 1120 doses starting at 50 mg/d followed by one week rest. The dose was escalated until dose limiting toxicity (DLT) was observed. Consecutive treatment cycles were allowed in the absence of progressive disease and persistent toxicity. Full PK profiles were obtained at the beginning and at the end of the 1st treatment cycle. Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) measurements were performed at baseline, at day 2, day 28 and after each further cycle. Toxicity was graded according to the Common Toxicity Criteria (CTC). Tumour assessment was done using the RECIST criteria.
Results: 25 pts (10 f, 15 m) were included, 23 received at least one treatment cycle, 3 patients were excluded during the first cycle due to early progression (2) or non compliance (1). The dose was escalated following an accelerated titration scheme from 50 to 450 mg/d: 50 mg (n=2); 100 mg (n=1); 200 mg (n=8); 250 mg (n= 6); 300 mg (n=5); 450 mg (n=3). Predominant drug-related adverse events were nausea, vomiting, diarrhoea, abdominal pain, and elevation of hepatic enzymes (AST, ALT, and GGT). Liver enzyme elevations were dose limiting (=CTC Grade 3) in 1/8 patients at 200 mg/d, in 2/5 patients at 300 mg/d, and in 2/3 patients at 450 mg/d. 13/25 patients were treated for more than 2 cycles. Stable disease (SD) was observed for 2 months (n=1), 3 months (n=5), 4 months (n=2), 5 months (n=1), and 7 months (n=1). 3 patients are still on treatment (+7, +8, +14 months). One patient (renal cancer) treated with 200 mg/d showed a complete regression of pulmonary metastases. In patients with SD or tumour regression DCE-MRI measurements reflected this result by a reduction of permeability and blood flow as judged by $IAUC_{60}$ evaluation. PK evaluations showed that BIBF 1120 exposure (AUC) increased with dose with moderate to high variability. Maximum measured plasma concentrations were reached approximately 3 h after intake. BIBF 1120 was distributed out of the blood and showed a high clearance resulting in a mean terminal half-life of around 13 h. Steady state was reached within 9 days.
Conclusion: BIBF 1120 was well tolerated in this study. Adverse events were mainly of gastrointestinal nature with mild to moderate intensity. Asymptomatic elevations of liver enzymes constituted dose limiting toxicitiy. The dose level of 250 mg/d BIBF 1120 administered once daily is considered the maximum tolerated dose in this study. A significant number of patients reached durable disease stabilization. BIBF 1120 is a promising compound that warrants further clinical evaluation.

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A phase I study of an oral vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase inhibitor, CP-547,632, in patients with advanced solid tumors

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VEGF over-expression occurs in a broad spectrum of common tumors, mediates tumor neovascularization, and predicts for a poor clinical prognosis. CP-547,632 is an oral, anti-angiogenic small molecule inhibitor of VEGFR-2 tyrosine kinase activity. It is selective vs EGFR, PDGFR-beta and other related tyrosine kinase receptors. This study examined the feasibility of continuous once-daily oral administration of CP-547,632 in patients with advanced solid tumors and the relationships of CP-547,632 pharmacokinetic parameters with toxicity and biologic activity. Seventy-two patients have received a median of 2 courses (range 1–9) over dose levels ranging from 35 mg po for 14 days to 400 mg po per day continuously. The maximally administered dose was 400mg/d with 2 of 2 patients experiencing dose-limiting headache. Bleeding events, both non-tumor related (eg. epistaxis, gingival bleed, hematuria, hematochezia, melena) and tumor-related have been observed across dose levels. Evaluations of causality have been confounded by progressive bulky disease, prior surgery and concomitant medications such as nonsteroidal anti-inflammatory agents; however, serious non-tumor related events have been limited to doses =300 mg. CTC Gr. 3 hypertension occurred at doses of 300 mg/d in 2 of 6 patients. Other CP-547,632 related adverse events include: mild to moderate diarrhea, fatigue after several cycles of treatment, transient maculopapular or pruritic rash, mild to moderate nausea and anorexia and rare, mild emesis. Of the 62 patients evaluable for tumor response, 12 patients had stable disease for 2 cycles (8 weeks), 1 patient had stable disease for 4 cycles (16 weeks), and 4 patients had stable disease for 6 cycles (24 weeks), 2 of whom had evidence of disease cavitation. One patient had an unconfirmed partial response at cycle 4 (16 weeks). At doses >160 mg QD, the plasma concentrations throughout the dosing interval exceed concentrations associated with anti-angiogenesis in certain preclinical models. The maximally tolerated dose is 250 mg/d for disease directed studies. An encouraging safety profile, evidence of anti-tumor effect and pharmacokinetic parameters that portend angiogenesis inhibition in preclinical models provide further impetus for study in the Phase II setting.

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Enhancement of the action of the antivascular drug 5,6-dimethylxanthenone-4-acetic acid (DMXAA) by co-administration of non-steroidal anti-inflammatory drugs

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Background: DMXAA, a low molecular weight antivascular drug that is currently in clinical trial for the treatment of a variety of cancer types, acts both directly and indirectly to damage tumour vascular endothelial cells and selectively inhibit tumour blood flow. Prostaglandins are often released in response to tissue injury and are likely to increase in response to vascular endothelial injury. Several prostaglandins, particularly PGE1, may have a protective role. We wished to determine whether non-steroidal anti-inflammatory drugs (NSAIDs) modulated the antitumour and antivascular effects of DMXAA in mice. The plasma concentration of serotonin, which is released by platelets in response to a number of antivascular drugs, was used as a surrogate marker for antivascular effects.
Methods: Antitumour effects were measured in Colon 38 murine carcinomas growing in C57Bl mice. Plasma concentrations of DMXAA and of the serotonin metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) were measured by high performance liquid chromatography.
Results: Administration of DMXAA alone as a single sub-optimal dose (25 mg/kg i.p.) provided growth delays of 4 and 6 days in two independent experiments. Administration of diclofenac alone (5 mg/kg) caused no significant growth delay. Co-administration of diclofenac and DMXAA

provided growth delays of 10 and >28 days, respectively in the two above experiments. Plasma DMXAA concentrations were measured up to 6 h after DMXAA administration, with and without diclofenac, and demonstrated no significant change in DMXAA pharmacokinetics. Tumour tissue DMXAA concentrations were also unchanged for up to 4.5 h. Plasma 5-HIAA concentrations, measured after 4 h, were proportional to DMXAA dose. Administration of diclofenac alone caused a dose-dependent increase in 5-HIAA, and co-administration with DMXAA provided an additive effect on 5-HIAA concentration.
Conclusions: Administration of diclofenac at a pharmacological dose caused large increase in the antitumour activity of DMXAA in a murine tumour model. Similar increases in activity have been observed for salicylate and rofecoxib (data not shown). The ability of NSAIDs to prevent local protective effects of prostaglandins released in response to vascular injury may explain this effect. Co-administration of NSAIDs may have general utility in therapies targeting the tumour vasculature.
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BAY 57-9352: an inhibitor of VEGFR-2 and PDGFR receptor tyrosine kinases that demonstrates anti-angiogenic activity in vitro and in vivo

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Tumor angiogenesis depends on proliferation, maturation and survival of endothelial cells along with key components of the supporting stroma such as smooth muscle cells. Endothelial cell proliferation and survival is stimulated via VEGFR-2 while PDGFR activation results in smooth muscle cell proliferation. Blockade of VEGFR-2 kinase activity has been shown to inhibit tumor growth in a variety of preclinical models. The present studies describe a novel, small molecule, BAY 57-9352, that inhibits both VEGFR-2 and PDGFR tyrosine kinases. In a biochemical assay, this compound inhibits VEGFR-2 and PDGFR with IC_{50}s of 6 nM and 15 nM, respectively. VEGF-dependent receptor autophosphorylation in mouse fibroblasts that express human VEGFR-2 is inhibited *in vitro* by BAY 57-9352 with an IC_{50} of 19 nM. Similar results are observed with VEGF-stimulated human endothelial cells (ECs) *in vitro*: BAY 57-9352 inhibits EC proliferation with an IC_{50} of 26 nM and Western blot analysis of treated cells confirmed the dose-dependent inhibition of VEGFR-2 autophosphorylation. *In vitro* treatment with BAY 57-9352 of human aortic smooth muscle cells (SMCs) that respond to PDGF inhibited SMC proliferation and inhibition of receptor autophosphorylation after treatment was confirmed by Western blotting. The proliferation of many epithelial-derived tumor cells is independent of VEGFR-2 or PDGFR *in vitro*, and consistent with this, BAY 57-9352 up to 20 μM exhibited no effect on the proliferation *in vitro* of a panel of human tumor cell lines. By contrast, *in vivo* administration of BAY 57-9352 results in inhibition of tumor growth in human tumor xenograft models. Based on the favorable *in vitro* and *in vivo* profile, BAY 57-9352 has advanced to Phase 1 clinical trials as an anti-angiogenic agent.

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A two-stage phase II study of the matrix metalloproteinase inhibitor (MMPI) Col-3 in patients with advanced soft tissue sarcoma (ASTS) – report of Stage I data

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Background: Col-3 (Metastat; Collagenex Pharmaceuticals, Newton, PA) is a tetracycline analog that specifically inhibits the production and activation of MMP-2 and MMP-9. A phase I study has established the tolerability of continuous uninterrupted dosing of Col-3, with photosensitivity and malaise as principal toxicities. Col-3 is exceptional amongst MMPIs, having demonstrated clinical benefit in pts with ASTS, as well as a 44% overall response in patients with AIDs-related KS. Phase II studies of active agents in ASTS, in patients previously treated with anthracyclines and ifosfamide, show that » 40% of pts have progression of disease (PD) at first evaluation. With this in mind, and the demonstrated potential for Col-3 to delay tumor progression, we designed a two-stage Phase II study to determine the proportion of ASTS pts with PD at 8 weeks following Col-3 therapy. Applying the two-stage design, with multinomial stopping rules, 15 pts are evaluated in stage I. If >4 of the first 15 pts develop PD on first evaluation, the likelihood that the true proportion of pts with early PD is <40% is <10% and the trial will be terminated. Otherwise if <13 of 30 pts develop PD, the drug will be considered of interest to pursue phase III evaluation.
Patients and Methods: Pts with ASTS meeting inclusion criteria and willing to minimize sun exposure are eligible. COL-3 is administered by continuous uninterrupted oral dosing at 50 mg/m2/d in 28 day cycles until PD.
Results: Twelve pts (5M/7F), median age 52 yrs (range 38–84) and PS0–2 with ASTS (leiomyosarcoma n=5, liposarcoma n=2, other n=5) have enrolled. With one exception, all pts have failed at least 2 prior chemotherapy regimens. Median treatment cycles administered is 3 (range 1–6). Grade 3 toxicities include photosensitivity (1), transaminitis (2) and reversible anemia (4) requiring transfusion in 2 pts. Most common < Grade 2 toxicities include fatigue, photosensitivity, anemia and transaminitis. Assessment of initial response at 8 weeks showed 8 of 11 (73%) evaluable pts with stable disease. Two pts had PD and 1 pt with clinically SD had discontinued therapy early by choice. Median duration of SD in evaluable pts is 14 weeks (range 11–24), median TTP has not been reached and six pts continue on therapy.
Conclusion: With stage I of this Phase II trial nearing completion, COL-3 appears to delay tumor progression with an encouraging 73% of ASTS pts maintaining SD beyond 8 weeks. Accrual to this study continues.

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The spectrum-selective kinase inhibitor EXEL-0999 inhibits mitogenic and angiogenic kinases, and causes rapid tumor vasculature destruction and regression in mouse xenograft models

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Receptor tyrosine kinases (RTKs) such as VEGFRs, FGFRs, PDGFRs, KIT and FLT3 play roles in tumor angiogenesis and/or tumor cell proliferation. EXEL-0999 is a potent, orally-available small molecule inhibitor of these RTKs, with low nanomolar potency in biochemical enzyme assays for VEGFRs 1–3, PDGFR-α and β, FGFRs 1 and 3, KIT, and FLT3. EXEL-0999 also inhibits RTK autophosphorylation in cell-based assays, with high potency against VEGFR2, VEGFR3, FGFR1, PDGFR-β, FLT3, and KIT. In functional angiogenesis assays in vitro, EXEL-0999 inhibits tubule formation and migration of endothelial cells in culture in response to VEGF or bFGF. EXEL-0999 also displays potent anti-proliferative activity against a variety of tumor cell lines in vitro.
In pharmacodynamic studies in nude mice, EXEL-0999 exhibits potent inhibition of VEGFR2, PDGFR-β, FGFR1, FLT3, and KIT, and shows sustained duration of action after a single oral dose. To determine the effect of EXEL-0999 in vivo on tumor cell proliferation and tumor angiogenesis, the compound was administered daily to nude mice bearing MDA-MB-231 human breast carcinoma xenografts. Tumors were harvested 4h to 96h after initiation of treatment, and analyzed histologically for vessel density, tumor cell proliferation, and cell death. EXEL-0999 caused a rapid destruction of the tumor vasculature, with tumor and endothelial cell death evident 2h to 4h after administration of the first dose. Longer exposure to the drug (24h to 96h) resulted in large decreases in vessel density and proliferating cells, and large increases in tumor necrosis. EXEL-0999 targets endothelial cells selectively in the tumor vasculature, as effects on endothelial cells were not observed in normal tissues such as liver, kidney, lung, intestines, and brain. These acute effects of EXEL-0999 translate to potent anti-tumor activity in efficacy studies, with once-daily oral administration causing substantial tumor growth inhibition of MDA-MB-231, PC-3, Calu6, HT-29, and A431 human tumor xenografts, as well as regression of larger, well established MDA-MB-231 xenografts. In a model of FLT3-driven leukemia, EXEL-0999 substantially increased the survival of nude mice injected intravenously with cells expressing human FLT3-ITD. Overall, these data indicate that targeting a spectrum of kinases including VEGFRs, FGFRs, PDGFRs, KIT and FLT3 with EXEL-0999 causes dramatic vascular destruction and shrinkage of solid tumors, and increased survival of leukemic mice, and provide a rational basis for clinical development of EXEL-0999 for treatment of solid tumors and FLT3-driven leukemia.

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Impact of scheduling on combined ZD6474 and radiotherapy in head and neck tumor xenografts

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Background: ZD6474 is a novel, orally available inhibitor of vascular endothelial growth factor receptor-2 tyrosine kinase activity with additional activity against epidermal growth factor receptor tyrosine kinase. ZD6474 has demonstrated enhanced efficacy in combination with radiation therapy (RT) in human tumor models and this study aimed to identify the optimal scheduling for this treatment regimen.