712 POSTER

In vivo efficacy of BI 2536, a potent and selective inhibitor of the mitotic kinase Plk1, in human hematopoietic cancers

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Background: Polo-like kinase 1 (Plk1), a key regulator of cell cycle progression and accurate spindle assembly, is an attractive target for cancer drug discovery. We have previously shown that BI 2536, a potent and selective small-molecule inhibitor of Plk1, induces a distinct mitotic arrest phenotype in prometaphase ("polo-arrest") with subsequent apoptosis in cell lines derived from a broad range of human epithelial cancers, irrespective of their oncogenome signatures. When used in vivo, BI 2536 given i.v. shows excellent activity in xenograft models of human epithelial cancers in nude mice at well-tolerated doses. BI 2536 is the first specific Plk1 inhibitor in clinical development and has demonstrated encouraging results in phase I trials. The present study was designed to extend the analysis of BI 2536 to human leukemias and lymphomas.
Material and Methods: A range of malignant human cell lines of hematopoietic and lymphoid derivation (Raji Burkitt's lymphoma; HL-60 acute myeloid leukemia; THP-1 acute monocytic leukemia; Granta-519 B-cell lymphoma; DOHH2 B-cell lymphoma) was used to evaluate the anti-tumor effects of BI 2536 in vitro using Alamar Blue™ assays. To assess the in vivo efficacy of BI 2536 in models of hematological cancers, C.B-17 SCID mice were inoculated intravenously with Granta-519 or DOHH2 B-cell lymphoma cells, respectively. Mice were treated with BI 2536 i.v. once weekly (50 mg/kg) or twice weekly on consecutive days (40 and 50 mg/kg). Body weight and clinical signs of progressive disease were monitored.
Results: BI 2536 potently inhibited proliferation in vitro with EC_{50} values of 6−14 nM. Cell cycle analysis by propidium iodide staining showed a G2/M arrest of BI 2536-treated leukemia and lymphoma cells at concentrations that matched closely the EC_{50} values in Alamar Blue™ assays. Immunocytochemical analysis of cytospin preparations of Granta-519 cells, using tubulin staining to visualize mitotic spindles and DAPI staining to visualize DNA, showed that cells arrest in mitosis with abnormal monopolar spindles.
In both lymphoma models tested, treatment with BI 2536 showed good efficacy with significant delay in the appearance of clinical signs of disease and prolonged survival compared to control animals.
Conclusion: BI 2536 is a potent inhibitor of leukemia and lymphoma cell proliferation in vitro and shows remarkable efficacy in two in vivo models of human B-cell lymphoma at well tolerated doses.

713 POSTER

Molecularly targeted immunochemotherapeutic formulation (SEVINA) composed of pegylated trispecific disulfide linked Fv (sdFv) targeting epitopes of EGFR, PTHrP and RANKL conjugated covalently with SATA to vinorelbine eradicated osteolytic metastases of multiple myeloma inhibiting proliferation of tumor associated and bone microvascular endothelial cells inducing ADCC, AMP and type I, II, III PCD

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Background: Bone metastasis is a common complication of multiple myeloma, and one for which only palliative treatment is available. We aim to inhibit the vicous cycle between tumor cells and the bone microenvironment, which results in increased tumor burden and bone destruction.
Materials and Methods: Multiple myeloma obtained from a metastatic patient were inoculated into the left cardia ventricle of nude mice. After osteoclastic metastases were observed radiographically, the animals were treated with pegylated trispecific disulfide linked Fv (sdFv) targeting epitopes of PTHrP, RANKL and EGFR conjugated covalently with SATA to vinorelbine-tartrate.
Results: Post-treatment, we observed autocrine inhibition of multiple myeloma cells, and paracrine inhibition of tumor associated endothelial cells, and bone microvascular endothelial cells. Downregulation of EGFR led to inhibition of expression of AP1 blocking transcription of VEGF, and its receptors KDR and Flt-1 exerting an angiocidal effect. EGFR downregulation led to reduced activity of osteoclasts, and inhibition of PI3K, MAPK and MMP-9. Downregulation of PTHrP and RANKL inhibited osteoclastogenesis. Subsequently, release of growth factors TGF-a and IGF from the bone matrix was blocked. Vinorelbine destabilised microtubule (MT) dynamics of tumor, and endothelial cells blocking cell cycle at G2/M. It also phosphorylated bcl-2 blocking uPAR and SP1 DNA binding activity through ERK signaling pathway. This led to inhibition of osteoclast proliferation by inhibiting hydrolysis of IGF-binding proteins, and downregulation of MMP-1, 3, 9. We observed antibody dependent cellular toxicity, and antibody mediated phagocytosis. In multiple myeloma cells, tumor associated and bone derived endothelial cells, we observed inhibition of DNA synthesis, and metabolic activity by BrdU and MTT analysis. Transmission electron microscopy exhibited nuclear PCD type III with pyknosis, and zeiosis leading to secondary necrosis. Type I PCD which is caspase-3 dept, and autophagic type II PCD led to a bystander killing effect of tumor and endothelial cells. There was not radiographic evidence of osteolytic bone metastases of multiple myeloma cells.
Conclusion: These findings suggest that this molecularly targeted immunochemotherapeutic formulation named SEVINA (under patent) may provide a novel therapeutic approach to inhibiting the processes involved in bone metastases from multiple myeloma.

714 POSTER

Phase I tolerability/safety of sunitinib in combination with capecitabine in patients (pts) with advanced solid tumors

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Background: Sunitinib malate (SU) is an oral, multitargeted tyrosine kinase inhibitor of VEGFR-1, -2 and -3, PDGFR-α and -β, KIT, RET, and FLT3, and is approved internationally for the treatment of advanced RCC and imatinib-resistant or -intolerant GIST. In a preclinical mouse xenograft model of human breast cancer, coadministration of sunitinib improved the antitumor activity of 5-FU. This phase I, dose-finding study assesses the maximum tolerated dose (MTD), tolerability, and safety of SU in combination with capecitabine (C) in pts with advanced solid tumors.
Materials and Methods: Entry criteria include diagnosis of an advanced solid tumor not amenable to curative therapy, previous treatment with $\leqslant$2 prior chemotherapy regimens, no prior antiangiogenic therapy, ECOG PS $\leqslant$1, and adequate organ function. C was always administered orally twice daily in 3 wk cycles (2 wks on treatment, 1 wk off). C and SU doses were alternately escalated in serial pt cohorts to determine the MTD of SU using a standard 3 + 3 design. In the first arm of the study, SU was administered once daily on a 6-wk cycle (4 wks on treatment, 2 wks off; 4/2 schedule). Following determination of the MTD on the 4/2 schedule, 2 additional arms (2/1 schedule [2 wks on treatment, 1 wk off] and a continuous dosing [CD] schedule) were assessed to determine the MTD of SU.
Results: As of Dec 2006, 48 pts have been enrolled: 28 on 4/2 schedule, 9 on 2/1 schedule, and 11 on CD schedule. Median cycles started (range) = 3 (1–9), 4 (2–9) and 2 (1–4), respectively. SU 50 mg + C 1000 mg/m^2 and SU 37.5 + C 1250 mg/m^2 on the 4/2 schedule exceeded the MTD. The MTD for the 4/2 schedule is SU 37.5 mg/d + C 1000 mg/m^2 bid; 1 dose limiting toxicity (DLT) was seen in 9 pts treated at the MTD (Grade 3 [G3] hand-foot syndrome $\geqslant$7 days). Across all doses on the 4/2 schedule, DLTs include G3 hand-foot syndrome (n = 3), G3 fatigue (n = 2), G3 myalgia (n = 1). 3 pts (thyroid, breast, NET cancers) treated on the 4/2 schedule (SU 37.5 mg/d + C 825, 1000 or 1250 mg/m^2 bid, respectively) achieved a confirmed partial response. Preliminary PK data do not indicate a drug-drug interaction. Pts continue to be accrued to the 2/1 and CD schedules.
Conclusions: In pts with advanced solid tumors, the MTD for the 4/2 schedule is SU 37.5 mg/d + C 1000 mg/m^2 bid. SU is being further studied on schedule 2/1 and CD. These preliminary data suggest that the SU + C combination is tolerable and further studies in breast cancer are warranted.

715 POSTER

Phase I dose-escalation study of brostallicin in combination with cisplatin (cDDP) in patients with advanced solid tumors

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Background: Brostallicin (B) is a new minor groove binder that interacts with DNA in presence of glutathione/glutathione transferase. Investigation of the combination is based on the novel mechanism of action of B, its

efficacy against cDDP resistant tumor cells, the synergy of the two drugs in preclinical models and their generally non-overlapping toxicity profiles.
Methods: Patients (pts) with advanced/metastatic solid tumors, relapsing after chemoradiotherapy or surgery plus radiotherapy (RT), were sequentially allotted to dose levels (DL) 1, 2 and 3 of B (5, 7 and 9 mg/m^2, respectively) and fixed dose of cDDP (75 mg/m^2) given IV every 3 weeks. Cohorts of 3 to 6 pts were treated. DLTs were defined as grade (G) 4 neutropenia for $\geqslant$7 days, febrile neutropenia (FN), neutropenic infection, G 4 thrombocytopenia for $\geqslant$7 days or associated with bleeding, any G 3/4 non-hematological toxicities, and 2-week delay in starting cycle 2 due to toxicity.
Results: 21 pts (11 males), median age 61 years [40−76], were treated. Primary tumor types included 15 squamous cell carcinoma (11 head and neck, 4 uterine cervix), 2 leiomyosarcoma, and 4 others. At study entry 8 pts had locally recurrent and 13 had metastatic disease. Median ECOG-PS was 0. All pts had at least one prior therapy: 1 pt had RT, 2 surgery plus RT, 4 surgery plus chemotherapy (CT), 2 RT and CT, 12 surgery plus RT and CT (most consisting of platinum-based combination therapy). Five pts were treated at DL1, 10 at DL2 and 6 at DL3. DLTs consisted of 1 FN, and 1 G 3 asthenia lasting 11 days in 1 pt each at DL3. DL2 was then expanded to 6 pts; none of them experienced DLTs. This cohort was again expanded to 10 pts for completing PK evaluations at the recommended dose. None of these pts experienced DLTs. G 3/4 treatment related toxicities at DL1 were neutropenia in 4 out of 5 pts, thrombocytopenia in 2 pts and FN in 1 pt; at DL2 they consisted of neutropenia in 8 out of 10 pts and in 1 pt vomiting and diarrhoea; at DL3 they were neutropenia in 5 out of 6 pts, thrombocytopenia in 4 pts, fatigue and FN in 2 pts each and anemia in 1 pt.
Conclusions: The recommended dose/schedule of 7 mg/m^2 of B and 75 mg/m^2 of cDDP/q3w is safe and all toxicities (essentially hematologic) were easily manageable. Further investigations of the combination in phase II trials should be warranted. To date 10 pts received 4 or more cycles and 5 of them are still under treatment. Complete results including PK will be presented.

716 POSTER

Sunitinib combined with modified (m) FOLFOX6 chemotherapy in patients with advanced solid tumors: a phase I study

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Background: Sunitinib malate (SUTENT®; SU) is an oral, multi-targeted tyrosine kinase inhibitor of VEGFR, PDGFR, KIT, FLT3 and RET. It is approved internationally for the treatment of advanced RCC and imatinib-resistant or -intolerant GIST. It inhibits angiogenesis pathways, which may improve antitumor activity when combined with FOLFOX. This phase I, open-label, dose-finding study investigated the safety, PK and efficacy of SU combined with mFOLFOX6 in patients (pts) with advanced solid tumors.
Patients and Methods: Successive cohorts of 3–6 pts received mFOLFOX6 in 2-wk cycles with escalating doses of SU (37.5 and 50mg/d) on 3 different dosing schedules: 2 wks on, 2 wks off (2/2); 4 wks on, 2 wks off (4/2); or continuously. The primary endpoint is the maximum tolerated dose (MTD) of SU in combination with mFOLFOX6 with each schedule of SU. Secondary endpoints include the antitumor activity and PK of this combination regimen.
Results: Twenty-one pts have been enrolled on the 3 SU dosing schedules, of whom 13 on the 2/2 schedule are evaluable (4 at 37.5 mg/d, 9 at 50 mg/d). Eight pts discontinued treatment due to disease progression; the remaining 5 completed 8 cycles of therapy and enrolled in a continuation study. Dose-limiting toxicities (DLTs) occurred in none of the pts at 37.5 mg/d and in 3 pts at 50 mg/d (1 grade 4 neutropenia, 2 grade 4 thrombocytopenia). As the 2 cases of thrombocytopenia occurred in heavily pretreated pts, the protocol was amended to limit prior chemotherapy. Four pts were enrolled under the amendment at 50 mg/d with no further DLTs reported. Based on these results, the MTD of SU on schedule 2/2 in combination with mFOLFOX6 was determined to be 50 mg/d. Two pts (1 with ovarian cancer and 1 with pancreatic cancer) achieved a confirmed PR. There were no PK-mediated drug–drug interactions for SU, its metabolite and oxaliplatin.
Conclusions: SU 50 mg/d on a 2/2 schedule with mFOLFOX6 in pts with advanced solid tumors who had not been heavily pretreated with chemotherapy was safe and well tolerated. Durable PRs were observed with this regimen. Patient enrollment continues at 50 mg/d 4/2 and 37.5 mg/d continuously, as well as at 50 mg/d 2/2 in pts with advanced colorectal cancer, to confirm the safety and antitumor efficacy of this combination regimen.

717 POSTER

Difluorodeoxyuridine (dFdU) plasma concentrations with weekly low dose gemcitabine during chemoradiation in head and neck cancer patients

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Background: Gemcitabine (dFdC) is an active antitumor agent with radiosensitizing properties. However, dFdC is rapidly metabolised by deoxycytidine deaminase to dFdU which has little antitumor activity on its own but is a potent radiosensitizer in vitro even at low concentrations ($\pm$2500 ng/ml for 24 hrs; Pauwels et al Cancer Cemother Pharmacol 2006, 58, 219). In contrast to dFdC, dFdU is detectable in plasma of patients treated with dFdC for a prolonged period of time (>24 hrs). In head and neck cancer (HNC) patients, chemoradiation with weekly dFdC results in excellent local control rates; however, it is associated with substantial mucosal and skin toxicities (Specenier et al; ASCO 2006, abstract 5547).
Aim: To investigate whether relevant plasma levels of dFdU can be detected during chemoradiation with weekly low dose of dFdC.
Methods: dFdC was administered weekly at three dose levels (10, 50 and 100 mg/m^2) along with conventional radiation therapy.
Plasma concentrations of dFdU were determined daily after the first administration (cycle 1) and before each weekly administration, thereafter. A high-performance liquid chromatographic method has been used and validated for the determination of dFdU in human plasma. Floxuridine (5-fl-2'-deoxyuridine) was used as an internal standard. Tetrahydrouridine was used to prevent the deamination of dFdC to dFdU after sampling. The limit of quantitation was about 50 ng/ml for dFdU. Within-run and between-run precisions were less than 10% and average accuracies were between 90% and 110%.
Results: Three patients were sampled at each dose level (only 2 presently available at 100 mg/m^2). dFdU AUCs, peak and trough concentrations are summarized in the table.

	Weekly dFdC dose (mg/m^2)			p-value
	10	50	100	
dFdU AUC day 1−5 (ng $\times$ min/ml), cycle 1	2.7×10^6	7.6×10^6	9.8×10^6	0.069
dFdU concentration (ng/ml) at 24 hrs, cycle 1	692	1819	2225	0.077
dFdU trough concentration (ng/ml) cycle 1	<50	455	694	0.034
dFdU trough concentrations (ng/ml) > cycle 1	458	549	658	0.101

All values are medians of available data.

Conclusion: During chemoradiation with weekly low dose dFdC, its potent radiosensitizing metabolite dFdU remains detectable at potentially radiosensitizing concentrations. A significant interpatient variation is observed.

718 POSTER

The pharmacokinetic and tolerability profile of once-daily oral ZD4054 in Japanese and Caucasian patients with hormone-refractory prostate cancer

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Background: ZD4054 is a specific endothelin A receptor antagonist in development for the treatment of cancer. To investigate potential