in an animal tumor model. Subtle changes of the alkyl substituents on the bipyrrole moiety result in significant changes in activity. PCI-2050 and other derivatives that show in vivo efficacy will be further evaluated as possible anti-cancer agents.

129 POSTER

Biarylalanine inhibitors of histone deacetylase enhance radiation sensitivity in cancer cells

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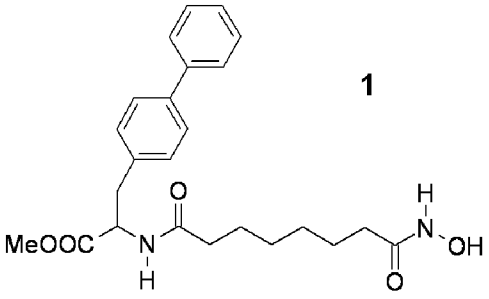
Background: We wanted to investigate the enhancement of radiation sensitivity in cancer cells by biarylalanine containing histone deacetylase inhibitors.
Material and Methods: The compounds are obtained from a suitably protected hydroxamic acid derivative of 4-bromophenylalanine by microwave assisted Suzuki coupling with arylboronic acids in reaction times of 5–10 minutes in good yields. Rat liver histone deacetylase and a fluorescent substrate are used for the determination of the IC_{50} values concerning in-vitro enzyme inhibition[1]. Human squamous carcinoma cells SQ-20B which had been shown previously to be intrinsically resistent to radiation were used for the investigation of the enhancement potential. Trichostatin A (TSA) and SAHA were used for comparison. IC_{50}-values for inhibition of proliferation were obtained and then cells were exposed to the compounds at their IC_{50}-value and graded doses of γ radiation according to standard protocols[2]. D_0-values as a measurement of the extent of enhancement were obtained for each compound.



Results: The parent biphenylalanine **1** which was reported previously as an HDAC inhibitor (IC_{50} = 290 nM) showed an IC_{50}-value in the SQ cells around 1 μM (TSA 200 nM, SAHA 3 μM). It proved to be an excellent enhancer of radiosensitivity with a D_0 of 1.45 at 1 μM. Control D_0 is 2.65, for TSA D_0 is 1.65 at 200 nM and for SAHA D_0 is 1.88 at 3 μM. We have synthesized several new substituted biphenylalanines as well as 4-heteroaryl phenylalanines. The most potent enzyme inhibitor so far is the 4-thienyl-phenylalanine analogue of **1** (IC_{50} = 190 nM, TSA: 10 nM, SAHA: 170 nM). The cellular investigation of the new analogues is currently under way.
Conclusion: Exchange of the anilide moiety of the histone deaceylase inhibitor SAHA that is currently undergoing clinical trials for the treatment of cancer by biarylalanines leads to compounds with similar enzyme inhibitory properties but an increased potency to enhance radiation sensitivity of cancer cells.

References

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130 POSTER

Structural features of texaphyrin metal complexes leading to altered metal cation response in cancer cells

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Motexafin gadolinium (MGd, Xcytrin®) selectively localizes in tumors and promotes stress by oxidizing intracellular reducing species. We recently showed by microarray analysis that treatment of A549 human lung carcinoma cells with MGd led to induction of metallothioneins (MT) and zinc transporter 1 (Hacia, Proc. AACR 43:3211, 2002). We have also reported that MGd at low concentrations modulates the cytotoxicity of the transition metal cations cadmium and zinc in cancer cells (Proc. AACR 45:1226, 2004). In the present study, we describe the effect of MGd and other metallotexaphyrins on the response of cancer cell lines to treatment with these ions. Human lymphoma (Ramos, DHL-4), lung carcinoma (A549), or prostate cancer cells (PC3) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. Zinc (0–100 μM) or cadmium (0–50 μM) and 0–25 μM MGd or texaphyrin congeners (**1-6**; M = Gd, Nd, Sm, Eu, Dy, Lu; R = OH; n = 2; **7-10**, M = Cd, Mn, Co, $FeO_{1/2}$; R = OH; n = 1) were added for 24 hr. Medium was exchanged and proliferation was assessed using a tetrazole (MTT) reduction assay at the end of 3 days. In other experiments, cells were treated with MGd and zinc or cadmium, and analyzed by flow cytometry using propidium iodide. RNA from treated cultures was harvested and metallothionein induction assessed by Northern blotting. Treatment with 6.25 μM or higher MGd raised the IC50 of cadmium, but lowered that of zinc, in all cell lines tested. Treatment with transition metal texaphyrins **7-10** at concentrations up to 25 μM did not alter the cytotoxic effect of zinc or cadmium, whereas early lanthanide series texaphyrin complexes **2-5** were as active as MGd. Late lanthanide series texaphyrin MLu, **6**, was inactive. This order of activity was found to correlate with MT induction. In order to evaluate whether the absence of activity of MLu was due to the lower solubility of this analogue, the more water-soluble diamine derivatives (**11-12**; M = Gd, Lu; R = NH_2; n = 2) were tested, and both found to be active. In summary, our findings suggest that texaphyrin lanthanide, but not transition metal complexes sensitize cancer cell lines to zinc and antagonize response to cadmium, provided these are sufficiently hydrophilic. These observations support the characterization of texaphyrins as a redox cycling agents that alter metal cation response by inducing the expression of metallothioneins and related genes.

Angiogenesis and metastasis inhibitors

131 POSTER

Pharmacodynamic analysis of apoptosis and anti-vascular activity in GIST patients treated with Imatinib

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Background: Most gastrointestinal stromal tumors (GISTs) contain activating mutations in the receptor tyrosine kinases c-Kit or platelet-derived growth factor-α (PDGFR-α) ε Imatinib mesylate (Gleevec) is a potent inhibitor of the c-Kit receptor tyrosine kinase. However, the mechanism(s) underlying its anti-tumor activity remains unknown. In an ongoing study we are investigating the mechanisms of early anti-tumor activity in GIST patients that achieve a response as measured by 18-FDG PET imaging. We hypothesize that imatinib's efficacy is due to both induction of GIST tumor cell apoptosis and anti-vascular activity via induction of tumor-associated endothelial cell apoptosis.
Material and Methods: We developed a clinical trial whereby patients with potentially resectable GIST were treated with imatinib (600 mg/day) for 3, 5, or 7 days before surgery. Perfusion CT and 18-FDG PET scans were performed before and after the initiation of imatinib therapy for 3, 5, or 7 days. All patients underwent pre-imatinib biopsy followed by surgical resection within 24 hours after completion of induction therapy. CT perfusion parameters acquired included blood flow (BF) and blood volume (BV). PET imaging was used to assess the standard uptake value (SUV) of FDG. Paired tumor biopsies and surgically resected tumors were examined using immunofluorescence coupled with laser laser scanning cytometry to quantify endothelial and tumor cell apoptosis, microvessel density (MVD), phosphorylated-c-Kit and phosphorylated-PDGR-α expression.
Results: Four out of five treated patients had a decrease in BF (avg. 40%, SD ±22.3, P=0.04) and BV (avg. 31%, SD ±22.8; P=0.05) in the solid portion of tumors corresponding to areas demonstrating a decrease in SUV (avg. 63%, SD ±19, P=0.05). One patient had little FDG uptake and displayed a 20% increase in BF/BV. The four responders to imatinib displayed a substantial decrease in phosphorylated-c-Kit expression in the tumor-associated endothelium (avg. 45%, SD ±12, P=0.07) and tumor cell compartment (avg. 52%, SD ±42%, P=0.13). These tumors displayed a 7-fold (P=0.08) and 2.8 fold (P=0.23) increase in endothelial and tumor cell death, respectively, and a 36% reduction in MVD. The tumor with the greatest reduction in BF (74%)/BV (61%) displayed the greatest increase in endothelial cell death (0.05% to 11%, $p<0.05$) after 3 days. The most significant reduction in MVD (78%, $P<0.05$) was observed in the tumor with the greatest reduction in FDG uptake (85%) after 7 days. Constitutive

expression of phosphorylated-PDGR-α was 7-fold higher in the tumor endothelium compared to the tumor cells. However levels of p-PDGFR within each compartment remained the same or were slightly increased after treatment.
Conclusions: GIST tumors that responded to imatinib therapy displayed lower overall levels of phosphorylated-c-Kit (most notably within the tumor endothelium), decreases in blood flow and volume, reductions in MVD, and increased levels of endothelial and tumor cell apoptosis. Imatinib may have anti-vascular effects on GIST tumors and this can be demonstrated at early time-points in therapy. We believe that inhibition of c-Kit activation in the tumor-associated endothelium by imatinib is a novel finding. The significance of these observations will be investigated in this ongoing study.

132 POSTER

A phase I and pharmacokinetic clinical trial of subcutaneous (sc) VEGF Trap in advanced solid tumor patients

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Background: VEGF Trap is a potent angiogenesis inhibitor comprised of portions of the human VEGF receptor VEGFR1 (Flt-1) and VEGFR2 (KDR) extracellular domains fused to the Fc portion of human IgG. VEGF Trap binds VEGF-A 100- to 1000-fold more tightly than monoclonal antibodies (Kd <1 pM) and neutralizes all circulating and tissue VEGF-A isoforms plus placental growth factor.
Methods: In this phase I trial, successive cohorts of pts with relapsed or refractory solid tumors received 1 (or 2) doses of sc VEGF Trap, followed 4 weeks later by 6 weekly (or twice weekly) doses. Pts without disease progression subsequently entered a long-term extension study. Study endpoints included safety, pharmacokinetics, and immunogenicity. Antitumor activity was assessed by CT scan.
Results: A total of 38 pts were treated across 7 dose levels: 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/kg weekly, and 0.8 mg/kg twice weekly. Potentially drug-related grade 3 or 4 adverse events (AEs) encountered included hypertension (n=2), proteinuria (n=1), afebrile neutropenia (n=1), and pulmonary embolism (n=1). Other than HTN, no dose-related pattern of AEs emerged. The maximum tolerated dose was not reached. No pts have developed anti-VEGF Trap antibodies, including those pts treated for ⩾6 mos. Plasma VEGF Trap levels associated with antitumor activity in animal models were approached in the 0.8 mg/kg once and twice weekly dose groups. Objective partial or complete responses were not achieved, but 17 of 35 evaluable pts, including 8 of 12 pts treated with ⩾0.8 mg/kg/week, maintained stable disease (SD) for at least 10 weeks and entered the extension study.
Conclusions: VEGF Trap has a favorable safety and tolerability profile. Consistent with previous findings with an anti-VEGF antibody, VEGF Trap may be associated with dose-dependent hypertension. Eight of 12 (67%) of evaluable pts treated with 0.8 mg/kg once or twice weekly, compared with 9 of 23 (39%) who received lower doses, maintained SD at the end of the 10-week study. Final results of the study will be presented.

134 POSTER

In vitro and in vivo characterization of exel-7647, a novel spectrum selective receptor tyrosine kinase inhibitior that modulates angiogenesis and tumor cell proliferation

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The receptor tyrosine kinases (RTKs) KDR, EGFR and ErbB2 have been implicated in tumor growth and angiogenesis and inhibitors of these RTKs have been validated in the clinic for oncology indications. We have embarked upon a drug discovery program to identify small molecules that simultaneously modulate the activity of the RTKs involved in both tumor growth and vascularization. This effort has identified EXEL-7647, which exhibits potent inhibitory activity *in vitro* against KDR, EGFR and ErbB2 and demonstrates potent anti-proliferative, anti-angiogenic activity and tumor growth inhibition (TGI).
Inhibition of KDR, EGFR and ErbB2 both enzymatically and in cellular assays of receptor phosphorylation by EXEL-7647 translates into potent inhibition of endothelial cell function in response to the key angiogenic factor, VEGF, and broad anti-proliferative activity against tumor cell lines.
In pharmacodynamic studies, oral administration of EXEL-7647 to athymic mice results in a dose dependent and sustained inhibition of KDR, EGFR, and ErbB2 phosphorylation with a single oral dose producing prolonged (>48 h) inhibition of EGFR and ErbB2 phosphorylation. Immunohistochemical analysis of MDA-MB-231 human breast carcinoma xenografts 3–7 days following a single oral dose of EXEL-7647 revealed a rapid and complete loss of microvessels (CD31) in the tumor, a significant decrease in the number of proliferating cells (Ki67) and an increase in tumor necrosis and hypoxia over time. *In vivo* efficacy studies in xenograft-bearing athymic mice demonstrate that EXEL-7647 exhibits broad anti-tumor activity, with a daily oral dose of 100 mg/kg of EXEL-7647 producing inhibition of tumor growth (85% or greater), completely halting growth in some models (A431). In MDA-MB-231 and PC-3 models, EXEL-7647 also induces regression of large established tumors (staged at 500 mg). In all xenograft studies, immunohistochemical analysis of tumors examined at the end of the dosing period, revealed dose dependent increases in tumor necrosis, decreases in tumor vascularity (CD31) and decreases in the number of cells in S-phase (Ki67), thus demonstrating that EXEL-7647 effects both tumor cell proliferation and angiogenesis.
EXEL-7647 is orally bioavailable in rodents and non-rodent species when dosed either as a solution or as a solid. EXEL-7647 exhibits moderate clearance, a half-life >8 h and a large volume of distribution.
In summary, EXEL-7647 is a potent anti-cancer agent that simultaneously modulates three clinically validated targets KDR, EGFR and ErbB2 and has the potential to act as an effective therapy for solid and metastatic tumors.

133 POSTER

A phase II study of oxaliplatin, capecitabine and bevacizumab in the treatment of metastatic colorectal cancer

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Background: Bevacizumab (BV, Avastin™) is a recombinant, humanized monoclonal antibody directed against vascular endothelial growth factor. Phase III results have demonstrated a survival advantage for the addition of BV to bolus IFL (irinotecan, 5-fluorouracil, leucovorin) as first line therapy for metastatic colorectal cancer. Recent data, however, suggest that the FOLFOX regimen (biweekly administration of oxaliplatin, leucovorin, and bolus plus continuous infusion of fluorouracil) is superior to bolus IFL with improved response rate, time to progression, and overall survival. The FOLFOX regimen requires the inconvenience of an ambulatory infusion pump which limits its use in many patients. We sought to investigate the combination of capecitabine, oxaliplatin and bevacizumab (XeloxA) as a more convenient and active regimen.
Methods: Patients with previously untreated metastatic colorectal cancer received oxaliplatin 85 mg/m^2 day 1, capecitabine 1000 mg/m^2 days 1–5 and 8–12, and BV 10 mg/kg day 1. Cycles were repeated every 2 weeks. Standard dose reductions for toxicity were permitted.
Results: Twenty patients received XeloxA therapy: 13 men, 7 women, median age 57 (range 24–76), median ECOG performance status 0 (range 0–1). Median follow-up is 5.6 months (range 1.2–7.3). 20 are fully evaluable for toxicity and 15 for efficacy. Therapy was generally well tolerated. Diarrhea was the most prominent adverse effect occurring in 11/20 (55%) patients, although only 7/20 (35%) had grade 3 diarrhea and no patient experienced grade 4 diarrhea. Mild (grade 1) hand-foot syndrome (HFS) was seen in most patients, 13/20 (65%); 9/20 (45%) developed grade 2 HFS, but no patient developed grade 3 HFS Other toxicities were minimal with one patient (5%) developing grade 3 neutropenia, and one patient (5%) developing grade 3 neuropathy. Fifteen patients (75%) required at least one dose reduction of capecitabine, and 6/20 (30%) required 2 dose reductions during treatment, typically for diarrhea and/or HFS. Of the 15 patients evaluable for efficacy, all were restaged every two months while on therapy. Nine patients experienced a partial response and one patient a complete response for an overall response rate of 67%. Responses were typically rapid with 7/10 (70%) responding patients achieving their partial or complete response at the first 2-month restaging. Stable disease as best response was seen in 5 patients (33%) with tumor reductions of 0–29% at follow-ups ranging from 2.7 to 6.2 months.
Conclusions: Preliminary evidence suggests that the XeloxA regimen is highly active. The alternative capecitabine dosing schedule appears to be well tolerated although many patients require an initial dose reduction of capecitabine. Consequently we have modified the current starting dose of capecitabine to 850 mg/m^2. Enrollment will continue to a planned accrual of 50 patients.