322 POSTER

Pediatric Preclinical Testing Program (PPTP) evaluation of the Src-Abl inhibitor dasatinib (BMS-354825)

M.A. Smith[1], J.M. Maris[2], S.T. Keir[3], H.S. Friedman[3], R.B. Lock[4], E.A. Kolb[5], N. Keshelava[6], C.P. Reynolds[6], C. Morton[7], P.J. Houghton[7]. *[1]National Cancer Institute, US, Bethesda, MD, USA; [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA; [3]Duke University, Durham, NC, USA; [4]Children's Cancer Institute, Randwick, Australia; [5]Albert Einstein College of Medicine, New York, NY, USA; [6]Children's Hospital of Los Angeles, Los Angeles, CA, USA; [7]St. Jude Children's Research Hospital, Memphis, TN, USA*

Background: Dasatinib is a dual Src/Bcr-Abl kinase inhibitor that maintains activity against most imatinib-resistant Bcr-Abl mutants and that is active in many patients with imatinib-resistant Bcr-Abl leukemias. Dasatinib induced complete regressions in Bcr-Abl preclinical models and primarily caused tumor growth delay in adult solid tumor preclinical models. The COG Phase 1 Consortium has initiated a phase 1 trial of dasatinib for children with refractory solid tumors and Bcr-Abl leukemias.
Methods: The PPTP includes an *in vitro* panel (23 lines) as well as panels of xenografts (n = 61) representing most of the common types of childhood solid tumors and childhood ALL. Dasatinib was administered orally twice-daily (solid tumors) or once-daily (ALL), for four weeks (5-days on, 2-days off) at a dose of 50 mg/kg/dose. Three measures of antitumor activity were used: (1) response criteria modeled after the clinical setting [e.g., partial response (PR), complete response (CR), etc.]; (2) treated to control (T/C) tumor volume at day 21; and (3) a time to event measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: Dasatinib induced significant tumor growth delay in 26% of the 27 evaluable solid tumor xenografts tested. Using a time to event measure of efficacy, dasatinib had intermediate activity against 1 of 25 (4%) of the solid tumor xenografts evaluable for this measure (a rhabdoid tumor line with median EFS T/C = 2.3). Dasatinib did not induce objective responses (PR/CR) against the solid tumor xenografts, but induced a CR against the PPTP's only ALL xenograft harboring a Bcr-Abl translocation. One of 4 evaluable non-Bcr-Abl ALL xenografts achieved a PR and 2 of 4 showed significant growth delay. Ten of 22 tested lines in the *in vitro* panel had EC_{50} < 1 μm. Kasumi-1, the most sensitive line with an EC_{50} of 11 nM, is known to have a gain-of-function $KIT^{Asn822Lys}$ mutation. Other lines with EC_{50} < 100 nM were the rhabdoid tumor line CHLA-266 (15 nM) and the T-cell ALL line MOLT-4 (45 nM).
Conclusions: Dasatinib was highly active against the PPTP's Bcr-Abl ALL xenograft, but had limited activity against the PPTP's solid tumor xenografts. Several lines in the *in vitro* panel were particularly sensitive to dasatinib (EC_{50} < 50 nM), and further work is needed to define the molecular basis of this activity.
Supported by NCI NO1CM42216.

323 POSTER

In vitro evaluation of potential thioredoxin inhibitors in breast cancer and endothelial cells

L. Zhang[1], H. Evans[1], K. Huber[1], M.F.G. Stevens[2], T. Bradshaw[2], A. Westwell[3], P. Patel[1], S. Martin[1]. *[1]Academic Unit of Clinical Oncology, University of Nottingham, Nottingham, United Kingdom; [2]School of Pharmacy, University of Nottingham, Nottingham, United Kingdom; [3]Welsh School of Pharmacy, University of Cardif, Cardiff, United Kingdom*

The thioredoxin (Trx) system represents a novel target for anticancer therapy. Two putative inhibitors (PMX464 and 290) have recently been developed by the School of Pharmacy, University of Nottingham. The activity of these benzathiazole substituted quinol compounds have been compared against IV-2, an alkyl-2-imidazolyl disulfide, that has been shown to inhibit the Trx system. Drugs were evaluated on four breast cancer cell lines including MCF-7 and MDA-MB-231, and on human umbilical vein endothelial cells (HUVEC) cultured under normoxic and hypoxic conditions (1% O_2) using proliferation, clonogenic survival, cell cycle progression, and VEGF production as endpoints. Assays of enzyme function were also investigated. In addition to Trx and thioredoxin reductase (TrxR), assessment of protein disulfide isomerase (PDI) function, as this protein contains two thioredoxin motifs, was also performed.
Results on proliferating cells, with all three drugs, show a cytotoxic mechanism of action, under both normoxic and hypoxic conditions, with the quinol compounds showing greater potency than IV-2 (IC_{50} 0.5−1 uM vs. 5−10 uM respectively). Quiescent HUVEC, cultured under normoxic conditions, show resistance to all drugs (quinols IC_{50} > 15 uM, IV-2 > 50 uM), even though the level of Trx function is similar between quiescent and proliferating cells. All drugs inhibit VEGF production by tumour cells under both normoxic and hypoxic conditions (quinols IC_{50} 0.5−1 uM, IV-2 5−10 uM). Quiescent HUVEC, incubated under hypoxic conditions, showed hyper-sensitivity to quinol compounds (IC_{50} < 0.001 uM), but not to IV-2 ($IC_{50} \approx$ 15 uM). In addition, IV-2 induced a G2/M block, with no significant block being observed with quinol compounds. These results suggest different mechanisms of action between both classes of compounds and, potentially, additional targets for the quinol compounds, eg PDI. In terms of enzyme function, the quinol compounds and IV-2 inhibit Trx (IC_{50} 5−10 uM vs. 25−50 uM respectively), however the former, unlike IV-2, also inhibits PDI (IC_{50} 400–600 uM similar to the known PDI inhibitor bacitracin). IV-2 in addition to inhibiting Trx, also inhibits TrxR (IC_{50} 100−500 uM).
In summary, PMX 464 and 290, and IV-2 are cytotoxic to breast cancer and endothelial cells with little hypoxic sensitivity evident except quiescent HUVEC. They exhibit both direct and indirect antiangiogenic effects with quinol compounds appearing to be more potent, but perhaps less specific.

Genomic, proteomic biomarkers, functional imaging

324 POSTER

Expression of TNF-alpha or BCL-3 is significantly associated with Helicobacter pylori-independent status of early-stage high-grade transformed gastric MALT lymphoma

K. Sung-Hsin[1], Y. Pei-Yen[2], C. Li-Tzong[3], K. Kuan-Ting[4], L. Jaw-Town[5], C. Ann-Lii[6]. *[1]National Taiwan University Hospital, Department of Oncology, Taiwan; [2]National Health Research Institutes, Division of Cancer Research, Taiwan; [3]National Taiwan University Hospital, Department of Pathology, Taiwan; [4]National Taiwan University Hospital, Department of Internal Medicine, Taiwan*

Background: We have recently reported that nuclear translocation of BCL10 and NF-κB are two molecular determinants of H. pylori-independence of early-stage high-grade transformed gastric mucosa-associated lymphoid tissue (MALT) lymphoma (J Clin Oncol 2004;22:3491–7). Furthermore, we have clarified that Akt, activated by TNF-α, phosphorylates BCL10 at Ser218 and Ser231 and that phosphorylated BCL10 subsequently complexes with BCL3 to enter the nucleus (J Biol Chem 2006;281;167–175). This study examined whether TNF-α and BCL3 are involved in the molecular mechanisms of H. pylori-independence of high-grade transformed gastric MALT lymphoma.
Materials and Methods: Pfeiffer (diffuse large cell lymphoma), and Ramos (EBV-negative Burkitt's lymphoma) cell lines were used in this study to mimic the in-vitro environment of high-grade transformed MALT lymphoma. Twenty-six patients who had participated in a prospective study of H. pylori eradication for stage IE high-grade transformed gastric MALT lymphomas were studied. Expression of pAkt (Ser 473), TNF-α, BCL-3, BCL10, and NF-κB in lymphoma cells and tumor cells of patients was measured by immunoblotting, and immunohistochemistry, respectively.
Results: We found that TNFα induced partial BCL10 nuclear translocation in these two lymphoma cell lines. Further, TNF-α induced a very quick but transient activation of Akt, and that LY294002 (TNF-α inhibitor) suppressed this effect. Concurrently, LY294002 also blocked TNF-α induced BCL10 nuclear translocation. Furthermore, BCL10 was phosphorylated by Akt and then forms complex with BCL3, which contains a nucleus localization signal (NLS), to translocate into the nuclei. The intranuclear BCL10/BCL3 complex thereby affected the transactivating activity of NF-κB. In lymphoma samples, aberrant nuclear expression of BCL3 was detected in 8 (80.0%) of 10 H. pylori-independent and in 3 (18.8%) of 16 H. pylori-dependent high-grade transformed gastric MALT lymphomas (P = 0.004). Similarly, the frequency of nuclear expression of TNF-α was also significantly higher in H. pylori-independent tumors than in H. pylori-dependent tumors (7 of 10 [70.0%] vs 4 of 16 [25.0%]; P = 0.043). Furthermore, the nuclear expression of BCL3 was significantly associated with nuclear expression of BCL10 (P = 0.004); and the nuclear expression of TNF-α was significantly associated with nuclear expression of NF-κB (P = 0.014).
Conclusion: These findings suggest that TNF-α produced by tumor cells and microenvironments can result in unremitting NF-κB activation by forming a positive feed-back loop pathway and thereby contribute to the proliferation and survival of H. pylori-independent lymphoma cells. Since nuclear expression of BCL3 or TNF-α is closely associated with H pylori-independent status of high-grade gastric transformed MALT lymphoma, anti-BCL3 or anti-TNF-α agents may be a useful adjunct in the treatment of this group of tumors.