was determined through clonogenic assays (both in soft agar and on plastic). Characterization of HR status was determined by both RAD51 foci formation assays and by an analysis of multiple components of the HR pathway including BRCA1, BRCA2, ATM, CHK2 and MDC1. A number of these TN cell lines were also assessed with xenograft tumours implanted sub-cutaneously in the flanks of nude mice.
Results: A high proportion of TN cancer cells lines demonstrated sensitivity to AZD2281. Responsive cell lines included but were not limited to BRCA1 germline mutations. In addition, sensitivity to the PARP inhibitor also correlated with HRD such as low levels of ATM expression.
Conclusion: AZD2281 demonstrates pre-clinical activity in TN breast cancer cell lines including those with non-BRCA HRD. These data therefore support the further assessment of this PARP inhibitor in TN breast cancer clinical trials.

558 POSTER

Pediatric Preclinical Testing Program (PPTP) evaluation of the fully human anti-IGF-1R antibody IMC-A12

E.A. Kolb[1], C. Morton[2], P.J. Houghton[2], J.M. Maris[3], H.S. Friedman[4], S.T. Kier[4], R.G. Gorlick[5], M.H. Kang[6], C.P. Reynolds[6], M.A. Smith[7]. *[1]Alfred I duPont Institute, Hematology and Oncology, Wilmington, DE, USA; [2]St. Jude Children's Research Hospital, Molecular Pharmacology, Memphis, TN, USA; [3]Children's Hospital of Philadelphia, Hematology and Oncology, Philadelphia, USA; [4]Duke University, Neuro-Oncology, Durham, USA; [5]Albert Einstein College of Medicine, Hematology and Oncology, Bronx, USA; [6]Children's Hospital of Los Angeles, Hematology and Oncology, Los Angeles, CA, USA; [7]CTEP/NCI, Pediatric Section, Bethesda, USA*

Background: IMC-A12 is a fully human antibody targeted to the insulin-like growth factor 1 receptor (IGF-1R). IGF-1R signaling may be especially important in the childhood cancer setting, with preclinical data supporting its role in the growth and survival of multiple pediatric cancers. The activity of IMC-A12 was evaluated against the in vitro and in vivo panels of the PPTP.
Methods: The PPTP includes a molecularly characterized in vitro panel of cell lines (n = 27) and in vivo panel of xenografts (n = 61) representing most of the common types of childhood solid tumors and childhood ALL. IMC-A12 was tested against the PPTP in vitro panel at concentrations from 0.01 nM to 100 nM using culture medium supplemented with 20% FBS. It was tested against the PPTP in vivo panels at a dose of 1 mg per mouse administered twice weekly for six weeks via I.P. injection. IMC-A12 was not evaluated against the ALL in vivo panel. Three measures of antitumor activity were used: (1) response criteria modeled after the clinical setting; (2) treated to control (T/C) tumor volume at day 21; and (3) a time to event (4-fold increase in tumor volume) 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: IMC-A12 induced 50% or greater in vitro growth inhibition in 3 of 23 cell lines (1 rhabdomyosarcoma and 2 Ewing sarcoma cell lines). IMC-A12 significantly increased event-free survival in 24 of 34 (71%) solid tumor xenograft models with tumor regressions in one rhabdomyosarcoma (RMS) model (maintained complete response). Although objective responses were not noted in the remaining RMS or osteosarcoma panels, tumor progression was significantly delayed with EFS T/C values >2 for 9 out of 11 (82%) models. Using the time to event activity measure, IMC-A12 had intermediate (n = 13) or high (n = 1) activity against 33 evaluable xenografts, including xenografts from the rhabdoid tumor (1 of 3), Ewing (1 of 5), rhabdomyosarcoma (6 of 6), glioblastoma (1 of 4), neuroblastoma (2 of 5), and osteosarcoma panels (3 of 5).
Conclusions: IMC-A12 demonstrated broad antitumor activity against the PPTP's in vivo solid tumor panels. Further studies characterizing molecular predictors of response, as well as the activity of combinations of IMC-A12 with other agents are anticipated.
(Supported by NCI NO1CM42216).

559 POSTER

The role of the ErbB3/PI3K/AKT pathway in determining breast cancer cell sensitivity against the irreversible dual EGFR/ErbB2 inhibitor EKB-569

W. Shabbir[1], C. Brünner-Kubarth[1], M. Grusch[2], W. Berger[2], B. Marian[2], R. Wagner[1], D. Lötsch[2], C.C. Zielinski[3], T.W. Grunt[3]. *[1]Division of Oncology, Medicine I, Vienna, Austria; [2]Institute of Cancer Research, Medicine I, Vienna, Austria; [3]Division of Oncology, Medicine I & Ludwig Boltzmann Cluster Oncology, Vienna, Austria*

ErbB transmembrane proteins belong to the family of tyrosine kinase receptors. Four members have been described: ErbB1 (EGFR), ErbB2, ErbB3, and ErbB4. ErbB1 and 2 are overexpressed/hyperactivated in many tumors, including ovarian and breast cancer. They stimulate carcinogenesis and malignant progression, and confer unfavorable prognosis. Clinical success has recently been obtained by targeting ErbB2 in ErbB2+ breast cancer. However, only 30% of ErbB2+ breast cancers respond to targeted ErbB2 blockade and most of the responders develop secondary resistance. The situation is even worse, when ovarian cancer is considered. Unfortunately, predictive markers for assessing ErbB inhibitor sensitivity/resistance are still widely lacking. Using MTT assay and Western blotting we examined the effects of the novel irreversible ErbB inhibitor pelitinib (EKB-569, Wyeth) on the growth activity and on ErbB-triggered signaling in 11 human breast and 11 human ovarian cancer cell lines. SKBR3 and T47D were identified as most sensitive and most resistant breast cancer cell lines, respectively. In contrast, the sensitivity of the ovarian cancer cell lines did not vary as much. Interestingly, the antiproliferative activity of the drug did not correlate with EGFR and ErbB2 protein levels. Moreover, drug-dependent inhibition of EGFR, of ErbB2 and of ERK1/2 phosphorylation was seen in both pelitinib-sensitive and pelitinib-resistant cells indicating that inhibition of ERK1/2 downstream signaling is not sufficient for drug-dependent growth arrest. In contrast, phosphorylation of ErbB3 at Tyr1289, of AKT at Ser473 and at Thr308, and of GSK3beta at Ser9 was blocked only in the sensitive, but not in the resistant cells. Moreover, ectopic expression of constitutively active AKT induced resistance to pelitinib in SKBR-3 cells. Conversely, pelitinib rapidly induced phosphorylation of PTEN at Ser380 in sensitive, but not in resistant cells. Taken together, our data suggest that ErbB3/PI3K/AKT, but not ERK1/2 signaling plays crucial roles in determining sensitivity/resistance of the cells against the irreversible dual EGFR/ErbB2 inhibitor pelitinib. Therefore, we propose that drug-mediated downregulation of phospho-AKT and phospho-ErbB3 levels might be useful surrogate markers for ErbB drug efficacy in breast cancer.
Supported by "Initiative Krebsforschung", Vienna, Austria.

560 POSTER

Discovery and preclinical characterization of BMS-777607: a potent, small molecule inhibitor of Met receptor tyrosine kinase

R. Borzilleri[1], J. Fargnoli[2]. *[1]Bristol-Myers Squibb R&D, Oncology Chemistry, Princeton, NJ, USA; [2]Bristol-Myers Squibb R&D, Oncology Drug Discovery, Princeton, NJ, USA*

Met receptor tyrosine kinase (RTK), also known as hepatocyte growth factor receptor (HGFR) is expressed predominantly in epithelial and endothelial cells, and serves as the only known high-affinity receptor for the mesenchyme-derived ligand, hepatocyte growth factor (HGF). Ligand-dependent activation of the Met receptor and subsequent signal transduction triggers complex biological responses, such as cellular proliferation, motility, migration, invasion, survival, morphogenesis and angiogenesis. While these pleiotropic effects are essential for mammalian development and tissue homeostasis, dysregulated Met-HGF signaling has been implicated in the pathogenesis of a wide array of human malignancies. Through the use of structure-based drug design, a novel series of substituted 2-aminopyridines, exemplified by BMS-777607, was identified. These compounds demonstrated nanomolar biochemical activity against Met and potent antiproliferative effects against Met-dependent solid tumor cell lines. X-ray crystal structure analysis of the BMS-777607/Met kinase domain complex confirmed that the compound binds in the ATP-binding site. In preliminary kinase screening, BMS-777607 was found to be a potent inhibitor of Ron (Met family member) and Axl (member of the phylogenetically related Axl/Tyro3/Mer subfamily). BMS-777607 exhibited excellent selectivity versus a panel of >200 additional RTKs, non-RTKs and serine/threonine kinases in either biochemical or Ambit binding assays. In cell culture, BMS-777607 inhibited the proliferation of human tumor cell lines containing constitutively activated Met receptor due to gene amplification (GTL-16 gastric carcinoma). The concentrations required for antiproliferative activity correlated with those necessary to inhibit Met phosphorylation in the same cell line. Tumor cell lines whose growth is stimulated by HGF (U87 glioblastoma) were also effectively inhibited by BMS-777607. *In vivo*, BMS-777607 demonstrated dose-dependent tumor growth inhibition following oral administration in the human tumor xenograft model derived from the GTL-16 cell line. On the basis of its desirable pharmacological profile, acceptable *in vitro* ADME and safety characteristics, and favorable pharmacokinetic properties in multiple species, BMS-777607 was advanced into clinical trials. The design, synthesis and structure–activity relationships leading to the identification of BMS-777607 will be presented along with a summary of the preclinical profile.