ND, respectively; mean C_{max} and AUC_{inf} increased in a greater-than-dose-proportional manner, suggesting nonlinear IMC-A12 pharmacokinetics (C_{max} = 333, 306, 788 and 443 μg/mL; AUC_{inf}=55744, 57613, 156460 and 669562 hr*μg/mL, respectively); and mean clearance decreased (0.07–0.02 mL/hr/kg), suggesting the approach of IMC-A12 saturation. In patients receiving IMC-A12 at escalating doses of 6, 10 or 15 mg/kg on a q2w schedule, mean $t_{1/2}$ was 149 h, 139 h, and 211 h, respectively; mean C_{max} and AUC_{inf} increased in a greater-than-dose-proportional manner, suggesting nonlinear IMC-A12 pharmacokinetics (C_{max} = 554, 734, and 1193 μg/mL; AUC_{inf}=69679, 134067, and 191176 hr*μg/mL, respectively); and mean clearance was relatively constant (0.141, 0.122, and 0.078 mL/hr/kg), suggesting IMC-A12 saturation. In the weekly and q2w groups, increases in serum levels of IGF-I and IGFBP3 were observed in all dose groups suggesting that IMC-A12 was blocking IGF-IR binding to its receptor. Serum trough concentrations observed in both weekly and q2w studies exceed target concentrations associated with anti-cancer activity in preclinical models (target trough concentration 60 to 158 μg/mL), especially at the doses recommended for further evaluation.
Conclusions: At clinically tolerable doses, IMC-A12 can achieve and maintain plasma concentrations that are sufficient to inhibit ligand binding to the IGF-IR. IMC-A12 will be further evaluated in prostate, breast, and other cancers at a dose of 10 mg/kg q2w.

555 POSTER

The PARP inhibitor, ABT-888 overcomes resistance in temozolomide refractory breast and prostate xenograft tumors implanted in metastatic sites in vivo

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PARP's role in DNA damage recognition/repair makes PARP inhibition an attractive cancer therapeutic target. ABT-888 is a potent PARP inhibitor with excellent oral bioavailability that readily crosses the blood brain barrier and has entered Phase 1 clinical trials. We have shown ABT-888's robust ability to potentiate temozolomide (TMZ) in tumors from different histological types having differential TMZ sensitivity. In this study we explored the ability of ABT-888 to potentiate TMZ efficacy in the human breast carcinoma, MDA-231-LN-luc implanted brain and human prostate carcinoma, PC3M-luc both in orthotopic and intra-tibial models. Bioluminescent cells were injected into the brain striatum of female SCID mice (MDA-231-LN-luc) and into the proximal epiphysis of the tibia in male SCID mice (PC3M-luc osteolytic cells). Zoledronic acid (ZA, 0.25 mg/kg/d, bi-weekly), a biphosphonate shown to inhibit bone resorption was also used in the intra-tibial model, where the area of decreased decalcification was quantitated in x-rays. Mice were staged based on the tumor burden evaluated in vivo with bioluminescent images (BLI) and treated with monotherapy and combinations of TMZ (50 mg/kg/d, q.d.x5) +/− ABT-888 (25 mg/kg/d, b.i.d.x5) for two cycles (PC3M-luc with 22 days rest) or three cycles (MDA-231-LN-luc with 11 days rest). There were no significant health concerns observed in the prostate study, but weight loss was observed in the breast study after the 2nd and 3rd cycles. In the prostate model, all groups with ABT-888/TMZ combination showed profound efficacy compared to groups with TMZ alone (>77% tumor growth inhibition, TGI) after the 1st cycle. However, the TMZ sensitivity was lost and tumors became refractory to TMZ during a 2nd cycle of TMZ+ZA but not with ABT-888/TMZ combination (>81% TGI). While ZA significantly improved bone integrity, no reduction in tumor burden was observed in groups without the addition of ABT-888. In the breast model, ABT-888 showed significant potentiation of TMZ activity after the 1st cycle (66% increase in tumor from size-match compared to 54% regression in the TMZ+ABT-888 group). After a 2nd cycle, tumors in the TMZ group were refractory to TMZ compared to the combination group where regression was maintained until the end of study (>40 days). We show a profound potentiation of TMZ activity by ABT-888 in two metastases models. While tumors can become refractory to TMZ treatment, ABT-888 is able to sustain sensitivity at the 2nd or 3rd cycles of combination treatment. More importantly, TMZ resistance can be prevented by ABT-888 combination therapy in crossover treatments indicating that in these studies, resistance may be overcome by PARP inhibition. Altogether, these suggest that tumors refractory to TMZ do not preclude sensitivity to ABT-888 combination therapy. The underlying mechanisms are not completely understood but may involve mechanisms independent of MGMT.

556 POSTER

MetMAb significantly enhances anti-tumor activity of anti-VEGF and/or erlotinib in several animal tumor models

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Background: Crosstalk between the Met receptor tyrosine kinase (RTK) and other RTKs has been implicated in tumor cell growth and survival. For example, Met amplification leads to acquired resistance to epidermal growth factor receptor (EGFR) inhibitors in EGFR mutant NSCLC cell lines and primary tumors. We have shown that Met inhibition, via shRNA-knockdown or the anti-Met monovalent antibody, MetMAb, sensitizes EGFR wild-type NSCLC tumors to erlotinib (Tarceva®), possibly by modulation of HER3 levels. Here we further explore how Met interacts with the VEGF pathway and explore combination efficacy in xenograft tumor models.
Materials and Methods: HGF-induced human umbilical vein endothelial cell (HUVEC) sprouting assays were performed to investigate the impact of Met in endothelial cell function. To examine possible indirect effects on angiogenesis, mRNA and protein expression were analyzed in several Met-driven tumor cell lines and xenograft tumors after Met activation (HGF-treatment) or Met inactivation (shRNA or MetMAb). Anti-tumor efficacy studies were performed in multiple xenograft models, including Met amplified lines, HGF/Met autocrine lines, and paracrine models utilizing a human-HGF transgenic SCID model (hu-HGF-Tg-SCIDs). Met was targeted via shRNA against Met or treatment with MetMAb, in combination with anti-VEGF antibodies (B20−4.1).
Results: We show how Met can play both direct and indirect roles in modulating tumor angiogenesis and that combinations of MetMAb and anti-VEGF antibodies provide improved anti-tumor efficacy. First, MetMAb effectively blocked HGF induces HUVEC cell sprouting, highlighting how Met may directly modulate tumor endothelial cell organization and migration. Second, Met modulated angiogenesis in an indirect fashion by regulating tumor cell production of angiogenic factors, such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). Third, targeting Met, via shRNA or MetMAb, significantly enhanced efficacy of anti-VEGF antibodies in multiple Met-driven human xenograft tumor models. Anti-VEGF had similar efficacy to MetMAb in hu-HGF-Tg-SCID mice. MetMab plus anti-VEGF treatment resulted in additive effects that surpassed MetMab plus erlotinib. In contrast, erlotinib plus anti-VEGF antibodies were equivalent to anti-VEGF alone, highlighting the importance of Met in this model. Triple combination of MetMAb, anti-VEGF, and erlotinib showed much better activity than any two agents alone, with prolonged anti-tumor activity and 9/10 partial responses and 1/10 complete response.
Conclusions: These data indicate how Met can be a direct and indirect driver of tumor angiogenesis and highlight the potential therapeutic value of combining inhibitors of Met (such as MetMab) with those for VEGF (such as bevacizumab (Avastin®)) and/or EGFR (such as erlotinib (Tarceva®)).

557 POSTER

Pre-clinical activity of the PARP inhibitor AZD2281 in homologous recombination repair deficient triple negative breast cancer

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Background: Recently, the novel PARP inhibitor AZD2281 has been shown to have clinical activity in tumours from patients with hereditary BRCA mutations (Yap et al., 2007), supporting the concept of synthetic lethality previously described in pre-clinical models (Farmer et al. 2005; McCabe et al., 2005; Evers et al., 2008). The BRCA genes, which are associated with an increased incidence of breast and ovarian cancer, play key roles in the homologous recombination (HR) repair of DNA double strand breaks. Sensitivity to PARP inhibition has also been demonstrated in cells deficient in non-BRCA components of the HR repair pathway (McCabe et al., 2006), suggesting the broader clinical potential of PARP inhibitors in tumours that are HR deficient (HRD). Triple negative (ER−, PR−, HER2−) breast cancers have been associated with HRD, however, the extent and make-up of deficiency in this tumour type is currently not well defined. We have undertaken pre-clinical studies to assess both the sensitivity of triple negative (TN) breast cancers to AZD2281 and the nature of any HRD associated with response.
Materials and Methods: In vitro sensitivity to AZD2281 in a panel of TN cancer cell lines (including a number with defined BRCA1 mutations)

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

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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.
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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

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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.
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560 POSTER

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

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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.