reduces total c-Met in various tumor cell lines and inhibits proliferation of tumor cells that depend on high c-Met expression. In this poster, we further explore LA480 mechanism of action. Our data demonstrate that LA480 can block HGF-induced p-Met, p-Akt and p-Erk in multiple tumor cell lines. In ligand independent MKN45 cells (a model of cancer cells with high c-Met amplification), LA480 treatment *in vitro* results in G1 cell cycle arrest, but not apoptosis. In mouse xenograft studies, LA480 significantly inhibits MKN45 tumor growth by 34% at day 15 and 91% at day 35. Consistent with cell culture data, the percentage of mitotic proliferating cells is decreased in the treated tumors by 71%. Tumors treated with LA480 are less hypoxic and have a decreased percent total apoptotic area. These findings suggest that LA480 may be a promising therapy for treatment of cancers driven by ligand-dependent and ligand-independent c-Met activation.

229 POSTER

Anti-IGF1R therapy with dalotuzumab is efficacious in a sub-set of KRAS mutant cetuximab refractory CRC models

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The monoclonal antibody targeting EGFR, cetuximab is currently used in the treatment of advanced colorectal cancer. Recent studies indicate that cetuximab is ineffective in the treatment of patients with colorectal cancers harboring activating mutants in KRAS or BRAF, components of the RAS-MAPK pathway. Cross-talk between EGFR and insulin like growth factor receptor (IGF1R) has been reported. MK-0646 (dalotuzumab), a monoclonal antibody targeting IGF1R is currently being developed for the treatment of various cancers. Here we have investigated activity of MK-0646 in KRAS or BRAF mutant, cetuximab refractory pre-clinical colon cancer models. A subset of cetuximab-refractory CRC cell lines (3/12) were responsive to MK-0646 treatment. Addition of cetuximab did not further potentiate MK-0646 mediated growth inhibition in KRAS/BRAF mutant CRC models. Strikingly, MK-0646-mediated inhibition of IGF1R signaling enhanced the sensitivity to irinotecan. In xenograft models that expressed high levels of IGF1R, MK-0646 significantly enhanced irinotecan-mediated growth inhibition. The combination of MK-0646 with irinotecan produced lasting tumor growth inhibition that persisted even after treatment withdrawal, indicating a durable response to this combination. In contrast, in xenograft tumors with low levels of IGF1R expression, the combination of MK-0646 and irinotecan failed to enhance irinotecan-mediated growth inhibition. In this study a molecular rationale for the combination benefit with MK-0646 and irinotecan was established. Irinotecan treatment resulted in the activation of IGF1R and PI3K signaling pathways, representing as a possible tumor survival mechanism. Combined treatment with MK-0646 and irinotecan prevented the activation of these survival signals, leading to increased anti-tumor activity. These studies suggest that MK-0646 in combination with irinotecan may have utility in the treatment of KRAS or BRAF mutant colorectal cancer patients. This hypothesis is currently being tested in the clinic. Preliminary data, from early Phase 1 clinical studies have shown activity of MK-0646 based therapy in KRAS mutant patients.

230 POSTER

Essential role of fibroblast growth factor receptor 2 (FGFR2) in tumorigenesis of human cancers harboring FGFR2 amplification demonstrated by a functional blocking antibody

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Fibroblast growth factors (FGFs) play important roles in regulating many fundamental biological processes including embryogenesis, tissue homeostasis, metabolism, angiogenesis, and wound healing. Dysregulated FGF signaling has been implicated in the pathogenesis of human cancers. We generated monoclonal antibodies (mAbs) against the extracellular ligand binding domain of fibroblast growth factor receptor 2 (FGFR2) to address the role of FGFR2 in tumorigenesis and to explore the potential of FGFR2 as a novel therapeutic target. Human gastric and breast cancer cell lines harboring *FGFR2* amplification predominantly express the IIIb-isoform of FGFR2. Therefore, we used an FGFR2-IIIb specific antibody, GP369, to investigate the importance of FGFR2 signaling in such cell lines *in vitro* and *in vivo*. GP369 specifically and potently suppressed ligand-induced phosphorylation of FGFR2-IIIb and downstream signaling *in vitro*. The administration of GP369 in mice significantly inhibited the growth of *FGFR2*-amplified human cancer xenografts. Our findings strongly support an essential role of FGFR2 in the initiation and/or maintenance of human cancers harboring *FGFR2* amplification. Cancer patients with activated/amplified FGFR2 signaling could potentially benefit from therapeutic intervention with FGFR2-targeting antibodies.

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Efficacy of VEGFR2 targeted mAb therapy in preclinical cancer models resistant to antiangiogenic therapy

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Several antiangiogenic agents are approved for the treatment of cancer, including the anti-VEGF mAb bevacizumab (metastatic breast and colorectal cancers) and the multi-targeted kinase inhibitor sorafenib (advanced hepatocellular carcinoma). These therapies do not confer tumor control in some patients, and the majority of tumors develop resistance. Identification of alternative treatment options that enable disease control in the setting of resistance is essential for improved patient outcomes. A monoclonal antibody targeting VEGFR2, IMC-1121B, has been associated with preliminary efficacy in TKI-refractory renal cancer and is currently being evaluated in bevacizumab and sorafenib resistant colorectal and hepatocellular cancers, respectively. We evaluated an antibody specific to murine VEGFR2, DC101, as monotherapy or in combination with chemotherapy in preclinical models of cancer resistant to sorafenib or anti-VEGF therapies.

In HuH-7 hepatocellular carcinoma xenografts, tumors that had grown on average by 100% over 8 days of sorafenib therapy (30 mg/kg, PO, daily), grew 20% over 15 days of therapy with the anti-VEGFR2 mAb DC101 (40 mg/kg, IP, 3×/week; $p < 0.0001$ versus rat IgG).

To mimic bevacizumab activity and resistance in preclinical cancer models, we developed a human antibody, S12, that specifically targets both human and mouse VEGF-A. S12 (40 mg/kg, IP, 3×/week) was combined with paclitaxel (10 mg/kg, IP, q7d) to develop refractory or non-responsive breast cancer models. In MDA-MB-231LP breast carcinoma xenografts, tumors that had grown on average by 100% over 8 days of paclitaxel + S12 therapy, grew 11% over 17 days of therapy with DC101 + 5-FU/LV (125/62 mg/kg) ($p < 0.0001$ versus saline). Antitumor benefits with the combination were significant compared to DC101 ($p = 0.0005$) or 5-FU/LV ($p = 0.0009$) alone. Similarly, DU4475 tumors that had grown on average by 100% over 17 days of paclitaxel + S12 therapy, regressed on average by 22% over 10 days of therapy with the DC101 + 5-FU/LV combination ($p < 0.0001$ versus saline), although the benefits did not reach statistical significance compared to 5-FU/LV.

These results support the conclusion that VEGFR2 targeted antibody therapy may be efficacious in breast and hepatocellular cancers that are refractory to anti-VEGF antibody based therapy or anti-VEGFR2 targeted TKIs.

232 POSTER

Unique molecular recognition of CD20 by the type II CD20 antibody GA101

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Background: CD20 is a specific cell surface marker found on normal and malignant B cells. Therapeutic anti-CD20 antibodies can be classified as type I and type II CD20 antibodies differing significantly in their mode of action. Rituximab is a type I CD20 antibody that has had a major impact on the treatment of malignant lymphomas. GA101 is a novel type II glycoengineered CD20 antibody. The molecular basis of the type I and type II classification of CD20 antibodies is incompletely understood.

Material and Methods: We used data from epitope mapping, point mutagenesis, co-crystallization, and protein tomography to precisely map the epitopes and characterize the molecular interactions of different anti-CD20 antibodies.

Results: The binding site of the monoclonal antibody GA101 on CD20 was found on the cyclic loop formed by the amino acids Cys167-Cys183 of human CD20. The CD20 epitope of GA101 and different type I and

type II CD20 antibodies was determined via Pepscan technology. Site directed mutagenesis confirmed the findings and showed that single amino acid exchanges in CD20 affect binding of rituximab and GA101 differently. Although the epitope regions of GA101 and rituximab largely overlap the epitope recognized by the type II antibodies GA101 and B1 is shifted slightly to the right. The scheme below shows the epitope as determined. The crystal structure of a GA101 Fab fragment in complex with a cyclic CD20 peptide (representing the large extracellular loop of CD20) shows that due to this shift GA101 binds the CD20 peptide in a completely different orientation from rituximab and that the binding region covers a larger surface area. Moreover, the elbow angle of GA101 is almost 30º wider than that of rituximab. As a result of this, the spatial arrangement of two CD20 molecules bound to a single GA101 molecule is predicted to differ substantially from those in the corresponding rituximab complex.
Conclusions: Our data suggest that engagement by type II versus type I antibodies favors different conformations and spatial arrangements of CD20. This offers a molecular explanation for the different cellular responses they elicit.

$_{167}$ CEPANPSEKNSPST $_{180}$

233 POSTER

Engineering and characterization of a monovalent c-Met receptor Anticalin® antagonist with potent in vivo activity

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The c-met protooncogene encodes a transmembrane receptor tyrosine kinase expressed on many epithelial cells. The MET ligand is known as Hepatocyte Growth Factor or Scatter Factor (HGF/SF), based on the observation that it causes migration/dispersion of epithelial cells and acts as a strong mitogen for primary hepatocytes. While both receptor and ligand have non-redundant roles in embryonic development, activation of the MET pathway in human malignancies correlates with poor prognosis. Preclinical and early clinical work suggest an anti-tumor activity of MET receptor tyrosine kinase inhibitors or specific biologics approaches directed against the extracellular domain or the ligand.
Anticalins are a novel class of therapeutic proteins based on the human lipocalin scaffold. In the current project, human tear lipocalin was used as a protein scaffold to engineer an Anticalin that specifically binds and antagonizes the function of MET. In contrast to monoclonal antibodies, Anticalins are monovalent and thus cannot activate receptors by dimerization. Starting from a naïve combinatorial library where residues forming the natural ligand binding site of Tlc were randomized, followed by multiple cycles of affinity maturation, the Anticalin was selected to bind to MET with a K_D of 7 nM. The Anticalin was found to cross react with the cynomolgus orthologue. The Anticalin efficiently antagonizes the interaction of HGF/SF with MET in biochemical and cell-based assays with IC50 values in the low nanomolar range. To allow persistent systemic inhibition of MET function, the plasma half-life of the Anticalin was extended by site-directed PEGylation. The modified Anticalin efficiently blocks tumor xenograft growth in *nude* mice in a model that relies on autocrine production of the ligand with significant activity at 1.9 mg/kg/day. The Anticalin activity will be further explored in combination settings with either chemotherapy or other targeted agents such as Angiocal®, the anti-VEGF Anticalin that is currently undergoing safety evaluation in a phase I clinical program.
The newly developed c-met Anticalin provides a novel small protein antagonist that may open unique therapeutic opportunities for oncology and indications.

234 POSTER

A phase 1, open-label, dose-finding study to assess the safety and tolerability of U3 1287 (AMG 888), a human monoclonal antibody targeting HER3 in patients with advanced solid tumors

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Background: HER3 is a key dimerization partner of HER family members and activates oncogenic signaling pathways. Overexpression of HER3 occurs in many solid tumors and is associated with poor prognosis and decreased survival. U3–1287 (AMG 888) is a fully-human anti-HER3 monoclonal antibody that has demonstrated anticancer activity in preclinical models. We report results from the first clinical study of U3–1287 (AMG 888) in cancer patients (pts). There were 2 Parts; dose escalation (Part 1) and dose expansion (Part 2). Data from Part 1 as of March 9, 2010 are presented.
Material and Methods: To be eligible, pts had refractory solid tumors assumed to express HER3 (eg, breast, colon, lung, prostate, ovarian, cervical, endometrial, gastric, pancreatic, bladder, head and neck, liver, or esophageal cancer). In Part 1 of the study, pts enrolled into sequential dose cohorts and received U3–1287 (AMG 888) by IV infusion. The second dose of U3–1287 (AMG 888) was administered 3 weeks after the first, then every 2 weeks thereafter. Dose escalation followed a typical 3 + 3 design with the DLT window encompassing the first 21 days of therapy. Study endpoints included: adverse event (AE) incidence, pharmacokinetics (PK), tumor response (per modified RECIST), anti-U3–1287 (AMG 888) antibody formation, and the maximum tolerated dose (MTD).
Results: Twenty-six pts enrolled into the following dose cohorts: 0.3, 1, 3, 6, 9, 14, or 20 mg/kg (N = 3, 3, 5, 4, 4, 4, 3, respectively). Pts had received a median (range) of 6 (2−13) prior chemotherapy regimens; 19 (73%) pts were male and 7 (27%) were female, 24 (92%) had ECOG PS$\leqslant$1, and median (range) age was 57 (39−75) years. Primary tumor types were: CRC (18 pts), breast cancer (3 pts), NSCLC (3 pts), SCLC (1 pt) and ovarian cancer (1 pt). Pts received a median (range) of 2 (1−8) doses. Twenty-four pts (92%) discontinued treatment: 22 (85%) due to disease progression, 1 (4%) due to AE, and 1 (4%) withdrew consent. AEs grade $\geqslant$3 occurred in 12 pts (46%); of these, 1 was considered drug-related (grade 3 hypophosphatemia in 1 pt from the 3 mg/kg cohort on day 23). Two grade 5 AEs were reported as unrelated to study drug (respiratory failure and disease progression). No treatment-related serious AEs and no DLTs were observed; the MTD was not reached. There were no infusion reactions, and no neutralizing antibodies to U3-1287 (AMG 888) were detected. The PK of U3-1287 (AMG 888) was non-linear, and preliminary data indicated that in pts treated with 9, 14 or 20 mg/kg Q2W, the steady-state minimum serum concentration was 10-fold greater than the threshold concentration required for 90% inhibition of xenograft tumor growth (EC90 C_{min} = 3 mcg/ml). Three pts had a best response of stable disease for $\geqslant$8 weeks (in the 1, 6, and 9 mg/kg cohorts), and 2 pts in the 20 mg/kg cohort received 2 doses of drug and remained on therapy at the data cutoff. Updated response data and FDG-PET imaging data will be presented.
Conclusions: U3–1287 (AMG 888) was well-tolerated in pts with advanced solid tumors. Based on the observed tolerability up to 20 mg/kg and the preliminary PK profile, dose levels of 9, 14, and 20 mg/kg Q2W were selected for Part 2 of the study.

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Negatively-charged sulfonate group in linker improves potency of antibody–maytansinoid conjugates against multidrug-resistant cancer cells

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Antibody–maytansinoid conjugates (AMCs) targeting cancer cell-surface antigens are in clinical trials against several cancers. The AMCs in the clinic employ disulfide- or thioether-bonds to link cytotoxic maytansinoid molecules to the antibody. In pre-clinical models, disulfide-linked AMCs demonstrate superior *in vivo* activity compared to thioether-linked conjugates for several reasons including bystander killing. Cancer cells can be multi-drug resistant due to overexpression of drug efflux transporters such as MDR1. A goal of this study was to enhance the potency of disulfide-linked conjugates against multidrug-resistant cancer cells. Since MDR1 favors neutral substrates, we hypothesized that the incorporation of a negatively-charged sulfonate group in the disulfide linker would improve retention of the polar metabolite inside the cell and enhance conjugate potency to MDR1-expressing cells. We compared the cytotoxic potencies of disulfide-linked anti-EpCAM AMCs with a neutral linker (SPDB) and a negatively-charged linker (sulfo-SPDB) in several EpCAM-expressing cells with different levels of MDR1. The conjugates had similar cytotoxicities toward MDR1-negative cells, but the sulfo-SPDB conjugate was 10- to 30-fold more potent than the SPDB conjugate toward MDR1-positive COLO 205^{MDR} and HCT-15 cells. An MDR1 inhibitor enhanced the cytotoxic potency of the SPDB conjugate to a level similar to that of the sulfo-SPDB conjugate. The sulfo-SPDB linker allowed preparation of AMCs with high numbers of maytansinoid molecules per antibody (6−8), which showed increased cytotoxic potency over conjugates with the usual level of payload (3−4) against the MDR1 cells. Importantly, the sulfo-SPDB conjugate