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 $_{180}$

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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.

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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