

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 30° 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.

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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.

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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≤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 ≥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 ≥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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POSTER

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

demonstrated greater activity than the SPDB conjugate against multidrug-resistant HCT-15 tumors in SCID mice. The advantage of the sulfo-SPDB linker was further demonstrated for anti-CanAg antibody conjugates against COLO 205^{MDR} cells, where the sulfo-SPDB linked conjugate was more active than the SPDB conjugate in arresting cells in the G2-M phase. Similar bystander killing activities were observed for sulfo-SPDB and SPDB conjugates: both conjugates eradicated mixed-cell populations of antigen-positive and antigen-negative cells in culture. Thus, we have developed a new linker that improves the potency of the disulfide-linked conjugate to multidrug resistant cancer cells, while preserving the conjugate activity towards non-resistant cells.

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POSTER

Antibody-maytansinoid conjugates targeting folate receptor 1 for cancer therapy

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Background: Folate receptor 1 (FOLR1) is highly expressed in ovarian cancers and several other epithelial malignancies. We wished to examine if conjugates of anti-FOLR1 antibodies with the highly cytotoxic maytansinoid derivative, DM4, would be effective in antigen-selective elimination of FOLR1-expressing cancer cell lines *in vitro* and in eradication of FOLR1-expressing xenograft tumors in mice.

Materials and Methods: A panel of anti-FOLR1 monoclonal antibodies was humanized using ImmunoGen's resurfacing technology. Affinities of these antibodies were examined on FOLR1-expressing cells by flow cytometry. Antibodies were conjugated to DM4 using the disulfide-containing SPDB linker by previously described methods. On the average, these conjugates contained 3.5 to 4 DM4 molecules per antibody. The cytotoxic activity of the conjugates *in vitro* and anti-tumor activity *in vivo* were analyzed on the cultured FOLR1-positive KB cell line and on KB-derived xenograft tumors in immunodeficient mice, respectively. Data for a representative conjugate huFR107-SPDB-DM4 are reported here. Immunohistochemistry (IHC) was performed on formalin fixed paraffin embedded ovarian carcinoma arrays with monoclonal antibody BN3.2 (Leica).

Results: The humanized FOLR1 antibody, huFR107, bound to FOLR1-expressing cells with a KD of 0.1 nM, and the huFR107-DM4 conjugate retained similar high affinity binding. The huFR107-SPDB-DM4 conjugate was potent in killing KB cells *in vitro* (IC50 of 70 pM). This activity was antigen-selective, since the cytotoxicity of huFR107-SPDB-DM4 for KB cells was at least 300-fold lower in the presence of an excess of huFR107 and also for FOLR1-negative cells. HuFR107-SPDB-DM4 was highly active in eradicating subcutaneous KB xenografts in mice. A single intravenous injection of the conjugate at 5 mg/kg completely eradicated the tumors, while tumor growth in mice treated with a non-targeting huAb-SPDB-DM4 conjugate was similar to that of PBS-treated control mice. IHC evaluation revealed that the expression of FOLR1 in KB xenografts was comparable to that found on 57% of ovarian clinical tumors (N=67).

Conclusions: Anti-FOLR1-DM4 conjugates were found to exhibit specific and highly potent activity against FOLR1-expressing cancer cells, both *in vitro* and *in vivo*. Our results suggest that antibody-maytansinoid conjugates targeting FOLR1 constitute a promising approach for the treatment of FOLR1-expressing tumors.

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POSTER

Mechanistic pharmacokinetic/pharmacodynamic (PK/PD) modeling of xenograft tumor response of Trastuzumab-DM1-antibody drug conjugates

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Trastuzumab-DM1 (T-DM1) is an antibody-drug conjugate (ADC) in development for the treatment of HER2+ metastatic breast cancer. Nonclinical studies of T-DM1, which employs a non-reducible thioether linker, showed slightly greater efficacy in mouse models of HER2+ breast

cancer than T-SPP-DM1, which employs a reducible disulfide linker. Previous studies of the two conjugates also found differences in the pharmacokinetics, uptake of conjugate into tumors, and the active products of intracellular catabolism of the conjugates, but similarities in the conjugate *in-vitro* potency, *in vitro* conjugate catabolism kinetics, and tumor catabolite concentrations. The objective of this study was to use PK/PD modeling to explore the differences in plasma and tumor conjugate and catabolite concentrations between these two ADCs and to better understand the mechanisms for their relative efficacy. A mechanistic PK/PD model was assembled which allowed prediction of tumor conjugate and catabolite concentrations from plasma PK data. The tumor catabolite concentrations, the presumed active agent, were then used to predict tumor response. This model is consistent with the proposed mechanism of action of ADCs.

The PK/PD model fit the data well, based on visual inspection and evaluation of estimate error. Tumor response was well-predicted from catabolite concentrations. Consistent with similarities seen in *in vitro* efficacy and tumor response with T-DM1 and T-SPP-DM1, the tumor PD based on catabolite concentrations were similar between T-DM1 and T-SPP-DM1 with a time to cell death of approximately 1-1.5 days, a maximal tumor kill rate of approximately 0.3 day⁻¹, and a 50% tumor kill concentration of approximately 180 pmol catabolite/gram of tumor. Tumor catabolism half-life was 1 day, similar to *in vitro* data. Differences in the catabolite efflux rate were found to explain the inconsistency between tumor conjugate concentrations and catabolite concentrations noted for these ADCs.

This study describes the use of a mechanistic model of ADC PK/PD, incorporating knowledge from tumor uptake and catabolism studies to improve the understanding of the pharmacologic behavior of these molecules.

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POSTER

Tumor penetration of therapeutic antibodies

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Background: It has been widely reported that therapeutic antibodies administered intravenously penetrate tumor tissue poorly from the tumor blood supply. Factors that can influence tumor penetration are: antibody size, concentration and affinity; antigen abundance; exposure time; interstitial tumor pressure, tumor vascularisation and vascular permeability. In this study we have examined the spatial-temporal distribution of two therapeutic antibodies, raised to an integrin target, in an *in vivo* tumour model to determine whether distribution might limit their efficacy.

Material and Methods: Two xenograft studies were performed in nude mice bearing established subcutaneous U87MG tumors. Firstly, a multi-dose efficacy study in which mice were dosed with two therapeutic antibodies raised to the same integrin (Ab-1 and Ab-2) at 20 mg/kg on days 1, 3 and 7 and tumors collected on day 8 and secondly a time-course study using a single dose of Ab-1 where tumors were collected 10 minutes - 24 hours post dose. Tumors from both studies were formalin-fixed, processed to paraffin blocks, sectioned and analyzed by immunohistochemistry and immunofluorescence for antibody distribution and endogenous expression of the integrin target and CD31.

Results: In the therapeutic study, both therapeutic antibodies, Ab-1 and Ab-2 were found to distribute evenly throughout U87MG tumors and mirrored the tumor cell membrane localization of their integrin target. In the time course study, Ab-1 was restricted to an intravascular distribution after 10 minutes. Within a further 50 minutes, Ab-1 had diffused from the vasculature and had reached at least two layers of tumor cells around the vasculature and exhibited a tumor cell membrane localization reflecting the membrane expression of the integrin target. Within 6 hours, Ab-1 had distributed evenly throughout the tumor. In contrast, the isotype control antibody was detected within vessels and multifocally within the tumor stroma and between tumor cells.

Conclusions: In summary, these data indicate that these therapeutic antibodies can rapidly diffuse from the tumor vasculature, penetrate the entire tumor mass to reach their target antigen and thus tumor penetration is unlikely to limit their anti-tumor activity in this model.