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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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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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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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.