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Prediction of clinical response of rituximab containing chemotherapy using newly established live-cell-imaging procedure for estimating CDC susceptibility

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Background: Targeting malignant B cells using rituximab (anti-CD20) has greatly improved the efficacy of chemotherapy regimens used to treat patients with non-Hodgkin's lymphoma Rituximab activity has been reported to associated with complement-mediated cytotoxicity (CDC), Ab-dependent cellular cytotoxicity (ADCC), and induction of apoptosis. However, exact therapeutic functions of these mechanisms remain to be clarified. In addition, there is no established prognostic marker to predict an individual response. In this study, we aimed to verify the validity of ex vivo complement dependent cytotoxicity (CDC) susceptibility as predictors of pathologic tumor regression in patients undergoing rituximab containing chemotherapy.
Materials and Methods: A rapid assay system was established to evaluate the tumoricidal activity of rituximab using living-cell-imaging technique. We analyzed lymph node biopsies obtained from 234 patients with suspected lymphoma, and estimated association between the CDC susceptibility and the response to rituximab-containing combination chemotherapy in DLBCL (n = 41) and FL (n = 37).
Results: Promptness and minimal requirement of cell number of this assay system reduced a burden of biopsy and analysis of fresh lymphoma cells after collection was enabled. ROC curve analysis determined that a cutoff value of 18% had optimal sensitivity and specificity for CDC susceptibility index to distinguish clinical response to rituximab containing chemotherapy (AUC = 0.998, 95% CI: 0.95–1.00). In addition, correlation analysis confirmed that CDC susceptibility of the freshly obtained lymphoma cells from the patients was strongly associated to the response of rituximab containing chemotherapy in both DLBCL and FL. This correlation was not obvious in the cases that received the chemotherapy without rituximab.
Conclusions: The system that we have established allows a successful assessment of rituximab-induced CDC and made it possible to predict the therapy response. And the association between CDC susceptibility and therapy response suggested that CDC might play pivotal role in remission induction of rituximab concomitant chemotherapy. The advantages of imaging-based procedures include the minimal amount of a necessary specimen, promptness and traceability. All of these features are essential for the analysis of clinical specimen. Thus, live cell imaging may provide the possibility of prominent clinical evaluation.

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Improving the therapeutic window of antibody-drug conjugates through novel linker design

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We have previously demonstrated that conjugates consisting of monoclonal antibodies (mAbs) against tumor associated antigens and the highly potent antimitotic drugs monomethyl auristatin E and F (MMAE and MMAF) are highly active and lead to cures and regressions of established tumors in nude mice. One of the key features in these antibody-drug conjugates (ADCs) is a serum stable dipeptide linker between the auristatin drug and the mAb that is cleaved intracellularly by lysosomal enzymes once ADC internalization takes place within antigen positive cells. The linker consists of the dipeptide, valine-citrulline (vc), attached to MMAE or MMAF through a self-immolative p-aminobenzylcarbamate (PABC) spacer that facilitates proteolytic drug cleavage. Here, we explored a new approach toward attaching auristatins to mAbs utilizing the C-terminal carboxyl group present in auristatin F and several other novel variants such as auristatin M and auristatin W. A library of peptides was linked directly to the C-terminal positions of these drugs without utilizing the PABC spacer. ADCs were generated using the 1F6 mAb that recognizes the CD70 antigen on lymphomas and renal cell carcinomas. Highly potent ADCs were selected on the basis of in vitro cytotoxicity assays, and were tested in mice for tolerability and efficacy against human tumor xenografts. Sequences for the peptide linker were identified within the new conjugates that led to both improved tolerability and higher potency compared to corresponding 1F6-vc-PABC-auristatin ADCs. The results demonstrate that it is possible to significantly improve the therapeutic windows of ADCs through careful selection of the linker used for drug attachment.

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Supporting MetMAb entry into the clinic with nonclinical pharmacokinetic (PK) and pharmacodynamic (PD) information

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Background: MetMAb is a recombinant, humanized, aglycosylated, monovalent monoclonal antibody produced in *E. coli* that potently inhibits HGF binding to c-Met, blocking HGF-induced activation of c-Met. It is being evaluated as a potential therapy for cancer. The purpose of these studies was to provide nonclinical information to allow for MetMAb entry into the clinic, by (1) determining the driver(s) of efficacy of MetMAb in KP4 pancreatic tumor xenografts in athymic nude mice, (2) characterizing PK in mice, rats and cynomolgus monkeys, (3) calculating safety factors for starting dose in humans, (4) providing data to estimate the effective human equivalent dose (HED) and dose regimen through PK/PD modeling and simulation.
Materials and Methods: Single and multiple intravenous (IV) dose efficacy studies as well as an IV infusion efficacy study were conducted in KP4 xenograft models over the dose range of 0.825–120 mg/kg. Single dose PK studies were conducted in mice, rats and cynomolgus monkeys at a dose range of 0.5–30 mg/kg. A multiple dose toxicology study was conducted in cynomolgus monkeys to identify the highest non-severely toxic dose (HNSTD) by using a dose range of 3–100 mg/kg weekly for 13 doses.
Results: Our efficacy studies indicated that area under the serum concentration–time curve (AUC) is the PK driver of efficacy for MetMAb in the KP4 xenograft model. MetMAb clearance (CL) in the linear dose range was approximately 21, 19, and 13 mL/day/kg in mice, rats, and cynomolgus monkeys, respectively. Human CL of 5.5−10 mL/day/kg was estimated based on allometric and species-invariant time scaling methods. With 100 mg/kg observed as the HNSTD in the multiple dose cynomolgus toxicology study, safety factors of $\geqslant$32 based on body surface area, dose, AUC, maximal serum concentration were identified for a starting dose in humans of 1 mg/kg. Using the PK and PD data, clinical dose projections were made via PK/PD modeling and simulation. These results support MetMAb to be dosed every week to every three weeks, providing flexibility in the clinic.
Conclusions: This work demonstrated how to design and interpret nonclinical PK, PD, and toxicokinetic data to enable MetMAb entry into the clinic.

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Anti-tumor efficacy of the integrin-targeted immunoconjugate IMGN388 in preclinical models

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Background: IMGN388 is an immunoconjugate composed of an integrin-targeting monoclonal antibody with the maytansinoid DM4, a potent cytotoxic agent, covalently attached. IMGN388 is being developed for the treatment of solid tumors. Its integrin target has been found by immunohistochemical staining to be present on a wide range of human solid tumors, with high expression observed in lung carcinomas, renal cell carcinomas, thyroid carcinomas, bladder carcinomas, melanomas, and sarcomas.
Materials and Methods: The binding affinity of IMGN388 was in the range of 1 to 8 nM (EC50 values) for several human tumor cell lines, as determined by flow cytometry. The activity of IMGN388 has been evaluated in xenograft models in nude rats using a variety of human tumor cell lines. The antibody portion of IMGN388 does not cross-react with the murine integrin ortholog, but does bind to the rat ortholog, albeit at an affinity approximately 40-fold less than to the human integrin molecule.
Results: In one study, rats bearing established A549 human non-small cell lung tumors were treated with IMGN388 at 0.5, 1, 3, or 10 mg/kg, given weekly for six weeks. The response to IMGN388 was dose-dependent, with the minimum efficacious dose found to be 1 mg/kg. Tumor regressions were observed in animals treated at 1, 3 or 10 mg/kg with 5 complete