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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

responses and one partial response in the 7 animals treated at 10 mg/kg. IMGN388 also demonstrated efficacy against established human tumors of colon (HT-29), large cell lung (H460), pancreatic (AsPC-1), ovarian (A2780, SKOV-3), and breast (MDA-MB-231.OT.F2) carcinomas in nude rat models. Additionally, IMGN388 has been found to inhibit angiogenesis using an in vivo model of basic fibroblast growth factor-induced angiogenesis in nude rats. Thus, the anti-tumor effects of IMGN388 can be attributed to two distinct mechanisms of action: direct tumor-cell killing and anti angiogenic activity.
Conclusion: The broad expression of the target integrin among solid tumors and the observed anti-tumor efficacy of IMGN388 in xenograft models of pancreatic, colon, lung, breast, and ovarian carcinomas in rats support the clinical evaluation of IMGN388 for the treatment of solid tumors.

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Use the humanized anti-EGFR MAb (nimotuzumab) and radiotherapy for the treatment of high grade glioma patients

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Background: The incidence of brain tumors is worldwide increasing and despite advances in neurosurgery and radiotherapy, limited progress has been made in the treatment of patients with high-grade gliomas. Amplification and rearrangement of the Epidermal Growth Factor Receptor (EGFR) have been found in primary high-grade astrocytomas. For primary brain tumors, over-expression of EGFR has been associated with poor survival due to growth advantages. Nimotuzumab is a humanized monoclonal antibody that recognizes EGFR with high affinity, inhibiting tyrosine kinase activation.
Material and Methods: A Phase II/III clinical trial was conducted to evaluate the efficacy and safety of nimotuzumab in combination with radiotherapy in newly diagnosed high-grade glioma patients. It was a multicentric, controlled, double blinded trial where 80 patients bearing high grade glioma were randomized to receive radiotherapy and nimotuzumab or irradiation plus a placebo. Patients received 6 weekly infusions of the placebo or nimotuzumab (200 mg) while they were receiving radiotherapy. After irradiation, patients received a maintenance dose of the investigational drug, every 21 days until completing a year of treatment.
Results: So far, 65 patients have been enrolled, 30 patients bearing glioblastoma and 35 bearing anaplastic astrocytomas. Fifteen additional anaplastic astrocytoma patients should be enrolled to finish the trial. All patients had surgery (biopsy, partial or total resection) before the inclusion in the trial. Both groups were very well balanced in relation to the factors that predict the outcome of the disease: Karnofsky index, previous surgery and age. Since the enrollment of the glioblastoma stratum is finished, a preliminary evaluation of safety and survival was done. The antibody was very well tolerated. Adverse events were more frequent in the placebo arm as compared to the nimotuzumab arm. The antibody did not provoke skin rash or allergic reactions. A preliminary survival analysis was done for all subjects bearing glioblastoma that received curative intent radiotherapy. The mean and median survival time for the patients treated with nimotuzumab plus radiotherapy was 14.31 and 16.43 months, respectively, while the mean and median survival time for the placebo arm was 8.67 and 10.49 months. The median survival time is similar to the one reached in the previous single arm study in patients bearing glioblastoma treated with nimotuzumab and radiotherapy (17.43 months) and compares favorably with the overall survival after irradiation and temozolomide (14.6 months). The evaluation of human anti-humanized antibody response (HAHA) is ongoing.
Conclusions: For the subgroup of glioblastoma multiforme patients, nimotuzumab combined with radiotherapy was safe and showed a trend toward a survival benefit as compared to the placebo arm.

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Junctional complexes as a factor limiting the extravascular penetration of trastuzumab

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The tumor microenvironment presents many barriers to drug penetration, including abnormal microvessel structure and function, deficient or absent lymphatics and variable extracellular matrix composition. Previously, using immunohistochemical mapping of MDA-435/LCC6^{HER2} xenografts we found the extravascular distribution of trastuzumab (generic, Herceptin®), to be incomplete and highly heterogeneous. To characterize properties of the tumor microenvironment that govern trastuzumab penetration, we extended these studies using HER2 over-expressing MCF-7 breast cancer cells (MCF-7^{HER2}) and a tight junction marker, ZO-1. Additionally, we used multilayered cell cultures (MCCs) in conjunction with transmission electron microscopy (TEM) to assess trastuzumab penetration through MCF-7^{HER2} tumor cells in vitro.
In mapping studies, mice bearing MCF-7^{HER2} tumors were given single doses of 4 mg/kg trastuzumab with tumor harvest at various time points thereafter; bound trastuzumab was imaged in tumor cryosections using fluorescent anti-human secondary antibodies. Combinations of additional markers, including HER2, 5-bromo-2-deoxyuridine, CD31, DioC7, and ZO-1 were also mapped on the same tumor sections. MCF-7^{HER2} MCCs were exposed from both sides to 60ug/mL trastuzumab for 1−24 h before drug removal, washing, and freezing. MCCs were cryosectioned and immunostained for trastuzumab, HER2, and ZO-1. For TEM studies untreated MCCs were fixed in gluteraldehyde, treated with osmium tetroxide and embedded in epoxy resin; ultra-thin 60 nm sections were imaged.
Similar to the MDA-435/LCC6^{HER2} model, 4 mg/kg trastuzumab did not saturate MCF-7^{HER2} tumors even after 72 h following administration. Trastuzumab exposure to both sides of MCF-7^{HER2} MCCs revealed an interesting phenomena wherein trastuzmab penetrated from only one surface of the discoid culture, despite ubiquitous HER2 expression. Staining for ZO-1 revealed the presence of continuous tight junctions along the surface of the culture disallowing trastuzumab penetration. TEM images confirmed the existence of tight junctions along the surface of MCF-7 MCCs. This suggests paracellular transport is required for trastuzumab penetration and implicates the need for structurally aberrant vasculature within tumors to facilitate the extravascular distribution of trastuzumab. This observation warrants further investigation into the junctional complexes of tumor tissue and endothelium as a factor limiting the penetration of anti-cancer agents.

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A chimerized anti-CD4 monoclonal antibody for the treatment of T cell lymphomas acts through activation of membrane acid sphingomyelinase leading to increased ceramide release and CD4/ZAP-70 protein redistribution in membrane rafts

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Background: recombinant IgG1 antibody 13B8.2 (rIgG1 13B8.2) binds to the CDR3-like loop on the D1 domain of CD4, and both inhibits proliferation and induces complement- and antibody-dependent cell cytotoxicity of T lymphoma cells. The biological effects of rIgG1 13B8.2 are partly due to signals that prevent NF-κB nuclear translocation, but precise mechanisms of action, particularly at the level of membrane proximal-signalling, remains obscure.
Materials and Methods: upon crosslinking of Jurkat T lymphoma cells with rIgG1 13B8.2, membrane rafts were extracted using Brij98 as detergent at 37ºC and subsequently separated by sucrose gradient centrifugation. Protein analysis was performed by western blot using appropriate antibodies. Lipid composition was measured by using Amplex red kits for cholesterol and acid sphingomyelinase (Invitrogen), and thin-layer chromatography.
Results: rIgG1 13B8.2 was found to induce an accumulation/retention of the CD4 molecule inside Brij 98 detergent-resistant membrane rafts, together with recruitment of TCR, CD3, p56 Lck, Lyn and Syk p70 kinases, LAT and Cbp/PAG adaptor proteins, and PKCtheta, but excluded ZAP-70 and its downstream targets SLP-76, PLCgamma1, and Vav-1. Analysis of key upstream events such as ZAP-70 phosphorylation showed that modulation of Tyr292 and Tyr319 phosphorylation occurred concomitantly with 13B8.2-induced ZAP-70 exclusion from the membrane rafts. rIgG1 13B8.2 did not affect membrane cholesterol but increased ceramide synthesis in membrane raft, in correlation with enhanced acid