

CD3 ϵ antibody OKT-3 (muromab, Orthoclone®). Here we present evidence that two bispecific single chain antibodies of the BiTE class, targeting CD19 or EpCAM in addition to CD3, induce T-cell activation exclusively in the presence of target cells.

Material and Methods: Peripheral blood mononuclear cells from healthy donors were prepared by Ficoll density centrifugation. In a typical experiment, PBMC were incubated for 24 hours with bispecific single chain antibody in presence or absence of specific target cells. Target cell lysis was determined by measurement of adenylate kinase activity released from dead cells. De novo expression of activation markers CD69 and CD25 on T cells was assessed by flow cytometry using directly conjugated monoclonal antibodies, concentration of cytokines in the supernatant was determined by a commercial FACS based bead array.

Results: Two distinct bispecific single-chain antibody constructs of the BiTE class, called MT110 and MT103 (or MEDI-538), were analyzed for conditional T cell activation. In the presence of target-expressing cell lines, low picomolar concentrations of MT110 and MT103 were sufficient to stimulate a high percentage of peripheral human T cells to express cytokines and surface activation markers, enter into cell cycle and to induce redirected lysis of target cells. However, in the absence of target cells, the two BiTE molecules did no longer detectably activate human T cells even at concentrations exceeding the ED50 for redirected lysis and conditional T cell activation by more than five orders of magnitude. In the case of MT110, T-cell activation was no longer observed with a cell line harbouring a single amino acid substitution in human EpCAM inactivating the binding epitope for MT110.

Conclusion: Our data show that T cell activation by monomeric forms of MT110 and MT103 is highly conditional in that it is strictly dependent on the presence of cells expressing the proper target antigen. BiTE molecules therefore qualify for a highly controlled polyclonal T cell therapy of cancer.

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Denosumab safety, pharmacokinetics (PK), and pharmacodynamics (PD) in a phase 1 study of Japanese women with breast cancer-related bone metastasis

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Background: Receptor activator of NF- κ B ligand (RANKL) is an essential mediator of osteoclast formation, function, and survival. Denosumab is an investigational fully human monoclonal antibody that inhibits RANKL. This open label, dose escalation study evaluated the safety, PK, and PD of denosumab in Japanese women with breast cancer metastatic to bone.

Methods: Patients (n = 18; 6/cohort) received either a single 60- or 180-mg SC dose of denosumab, or denosumab 180 mg SC every 4 weeks (Q4W) for 3 doses. Patients could receive concurrent chemotherapy or hormonal therapy if the regimen was stable. Adverse events (AEs) were monitored for up to 20 weeks. Serum concentrations of denosumab and levels of the bone resorption markers serum C-telopeptide (sCTX) and urine N-telopeptide/creatinine (uNTX) were measured.

Results: The AE profile in these breast cancer patients receiving denosumab was similar to that of advanced breast cancer patients receiving systemic treatment. The most common AEs were fatigue, anorexia, headache, malaise, and nausea. One serious AE of myositis occurred in the 180-mg dose group and, per the investigator, was attributed to a paraneoplastic syndrome, although a possible relationship to study drug could not be excluded. No deaths occurred during the study, and no patients developed antibodies to denosumab. The increase in exposure to denosumab observed between the 60-mg and 180-mg doses was approximately proportional. In the 180-mg Q4W group, approximately 2-fold accumulation was observed by the third dose. Suppression of sCTX and uNTX was rapid (within 48 hours), extensive (to levels observed in healthy adults), and sustained (for at least 12 weeks) after dosing. Reductions were similar across the dose groups with overall median (interquartile range) reductions in sCTX of 84.5% (73.1%, 91.7%) and in uNTX of 71.2% (41.2%, 88.5%) 12 weeks after dosing.

Conclusions: In this group of patients, the safety profile of denosumab given as a single dose or as multiple doses (Q4W for 3 doses) was similar to that expected in treated advanced breast cancer patients. Suppression of bone resorption markers was rapid, extensive, and sustained in all cohorts tested. Both the PK and PD results were comparable to those observed in non-Japanese women treated with similar doses of denosumab (Body, Clin Can Res 2006). Further investigation of denosumab treatment in women with advanced cancer and bone metastases will be conducted in a phase 3 trial.

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Mapatumumab, a fully human agonistic monoclonal antibody that targets TRAIL-R1, in combination with gemcitabine and cisplatin: a phase 1 study in patients with advanced solid malignancies

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Background: Mapatumumab (HGS-ETR1, TRM-1) is a fully human monoclonal antibody that targets and activates the tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1). Adding mapatumumab to gemcitabine and cisplatin results in increased cytotoxicity in human tumor cell lines and mouse xenograft models. Prior phase 1 and 2 studies have shown that mapatumumab can be safely administered as a single agent. In this phase 1 study, the safety and tolerability of escalating doses of mapatumumab in combination with gemcitabine and cisplatin are being evaluated.

Methods: Patients with advanced solid malignancies received gemcitabine 1250 mg/m² IV on days 1 and 8, and cisplatin 80 mg/m² IV on day 1 every 21 days, for a maximum of 6 cycles. In case of clinical benefit, patients were allowed to continue on mapatumumab monotherapy. Following the first gemcitabine and cisplatin infusion, and concomitant in other cycles, mapatumumab was administered IV every 21 days. Planned dose escalation levels were 1, 3, 10 and 20 mg/kg (with extension of the 10 and 20 mg/kg cohorts to a maximum of 12 patients depending on toxicity). Toxicity was evaluated according to CTCv3.0. Tumor measurements were performed as appropriate. Pharmacokinetic analysis of plasma mapatumumab, gemcitabine, dFdU, unbound and total platinum was performed.

Results: To date, 26 patients have been enrolled at 1 mg/kg (n = 4), 3 mg/kg (n = 7), 10 mg/kg (n = 12) and 20 mg/kg (n = 3) mapatumumab. A median of 6 cycles (range 1–6 cycles) was administered. The adverse events most commonly observed reflect the toxicity profile of gemcitabine and cisplatin, with hematologic toxicity, nausea and vomiting, ototoxicity and liver function disturbances. To date, 4 patients have experienced transient grade 3 elevations of ALT (one in combination with AST elevation grade 3 and one with GGT elevation grade 3) and one additional patient has experienced a grade 3 elevation of AST. Gemcitabine was considered to be the most likely cause. In one patient with pancreatic cancer, bile duct obstruction resulted in ALT and GGT elevations. The 20 mg/kg cohort will be expanded to 12 patients. Eight patients have experienced a partial response thus far. Stable disease was seen in 12 patients. Pharmacokinetic analyses showed no signs of drug interactions.

Conclusions: The combination of mapatumumab with gemcitabine and cisplatin is safe in doses up to 20 mg/kg. Further phase 2 studies of this combination are warranted.

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Antitumor efficacy of DX-2400, a potent and selective human antibody MMP-14 inhibitor discovered using phage display technology

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Selective inhibition of the activity of matrix metalloproteinases (MMPs) could provide an attractive non-cytotoxic approach towards improving the therapy of aggressive, metastatic cancers. To date, no MMP inhibitors have been approved probably for reasons of insufficient specificity and/or dose-limiting side effects. The challenge in the MMP field is to design highly potent and selective inhibitors with optimal pharmacokinetics and minimal toxicity. This provides a unique opportunity for protein-based inhibitors. Utilizing our human Fab-displaying phage library and using a recombinant active catalytic domain of human MMP-14 as a target, we have discovered DX-2400. DX-2400 binds and specifically inhibits MMP-14 activity (K_i = 0.9 ± 0.3 nM) and does not inhibit activity of a panel of other MMPs tested. DX-2400 blocks pro-MMP2 activation on MMP-14/MMP-2 expressing cancer cells and therefore has the potential to inhibit extracellular matrix proteolysis. In addition, DX-2400 blocks in vitro invasion of select cancer cell lines through Matrigel. We evaluated the anti-cancer activity of DX-2400 in the orthotopic MDA-MB-231 breast cancer model. DX-2400 reduced tumor progression by 70% compared to an isotype control, with activity comparable to doxorubicin. Importantly, DX-2400 resulted in no body weight loss through the treatment. This specific