CD3ε 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.

201 POSTER

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-kB 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.

202 POSTER

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/m^2 IV on days 1 and 8, and cisplatin 80 mg/m^2 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.

203 POSTER

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 (Ki = 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

fully human antibody represents an innovative approach for the inhibition of MMP activity and a candidate for therapeutic development.

204 POSTER

Discovery and validation of a promising new target for therapeutic monoclonal antibodies: a type II transmembrane serine protease overexpressed in human ovarian and pancreatic cancers

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Transmembrane and GPI-linked serine proteases represent a family of cell surface proteins that play interesting and important roles in a variety of key physiological processes. A number of these membrane anchored serine proteases, with catalytic domains displayed on the external plasma membrane of cells, have been reported to function in cell growth and development as well as in tumor invasion and metastasis. Other proteases of this family exhibit regulated expression in endothelial cells during differentiation and morphogenesis and may function in physiologic as well as pathologic vasculogenesis and angiogenesis or participate in the regulation of blood pressure.
To identify new therapeutic antibody targets we utilized a variety of genomic approaches to discover sequences upregulated in human cancers. These efforts yielded many membrane proteins, including several cell surface serine proteases. We identified a type II transmembrane serine protease, DD-O115, whose mRNA is overexpressed in human ovarian and pancreatic cancer tissue with low or no expression in normal tissues. Monoclonal antibodies recognizing DD-O115 were generated and used to identify and characterize the DD-O115 protein. Western blot analysis showed the DD-O115 glycoprotein to be expressed on the surface of human tumor cell lines and ovarian or pancreatic tumor tissues but not other normal tissues tested. Immunohistochemical studies with monoclonal antibodies against DD-O115 also revealed strong plasma membrane staining of human cancers with little or no normal tissue expression. siRNA-mediated knockdown of DD-O115 expression in cultured human tumor cells inhibited cell migration suggesting that DD-O115 protein may play a role in promoting tumor growth by facilitating tumor invasion or metastasis.
We next developed and characterized monoclonal antibodies against DD-O115 protein which bind strongly by FACS and immunofluorescence to DD-O115 on the surface of live tumor cell lines. Some of these monoclonal antibodies are capable of inhibiting the enzymatic activity of DD-O115 on the surface of live cells. The tumor-specific over-expression of DD-O115 and its functional role in promoting malignant transformation make this cell surface antigen an ideal target for a monoclonal antibody therapeutic strategy; a variety of mouse tumor xenograft efficacy studies are in progress with our monoclonal antibodies.

205 POSTER

AMG 479, a fully human anti IGF-1 receptor monoclonal antibody, enhances the response of established colon and pancreatic xenografts to chemotherapeutic agents

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Binding of IGF-1 or IGF-2 to the insulin-like growth factor receptor (IGF-1R) results in the activation of its intracellular kinase activity and the induction of proliferation and survival signals critical in transformation and tumorigenesis. We have generated a fully human anti-IGF-1R monoclonal antibody, AMG 479, that binds IGF-1R (Kd = 0.3 nM), blocks ligand binding and receptor phosphorylation, and arrests the growth of engineered, IGF-1 dependent, 32D cells. Treatment of Colo-205, BxPC-3 and MiaPaCa xenografts with AMG 479 (2×/wk, i.p. 30–571 ug/dose) resulted in significant and dose dependent maximal tumor growth inhibition of 60 %. In mouse studies, AMG 479 serum concentrations reached the steady-state after 6 doses and increased approximately dose proportionally. The mean AMG 479 concentrations at 2 hrs post dose were 22, 58 and 330 ug/ml for the 30, 100 and 571 ug dose, respectively. Efficacious treatment of xenografts with AMG 479 did not result in body weight loss or changes in glucose/insulin level. Platelets, lymphocytes and red blood cells were also unaffected. In contrast, a statistically significant, dose dependent reduction (50%) in peripheral blood neutrophils was observed. This effect was reversible and murine specific. The anti-apoptotic and survival signals driven by IGF-1R have been shown to play a critical role in the emergence of resistance to conventional chemotherapeutics. Therefore, we tested the potential of AMG 479 to enhance the response of tumor cells to chemotherapeutic agents *in vivo*. Results showed that simultaneous treatment of established Colo-205 xenografts with AMG 479 (300 ug/dose twice/week) in combination with 35 mg/kg of irinotecan was significantly more effective than either agent alone reaching more than 80% growth inhibition. Similarly, simultaneous combination of AMG 479 with 80 mg/kg of gemcitabine resulted in better than 80% growth inhibition of established BxPC-3 and MiaPaCa xenografts, demonstrating greater efficacy than either agent alone. No changes in body weight or other observable negative effects were recorded as a result of these combination regimens. Taken together these results show that blockade of IGF-1R signaling with AMG 479 results in single agent efficacy as well as enhancement of standard chemotherapeutic activity while displaying few effects on normal cell compartments. This data strongly suggest that AMG 479 should be evaluated clinically in combination with standard chemotherapeutics.

206 POSTER

EGFR and PDGFR crosstalk may dictate the resistance to EGFR therapy in bladder cancer

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Overexpression of receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR) and platelet derived growth factor receptor (PDGFR) have been associated with tumor progression. Recently we have discovered that human bladder carcinomas often co-express these receptors.
Our objective was to determine whether the co-expression of EGFR and PDGFRβ is redundant or if there is a functional crosstalk between the two RTKs in regulating various biological functions.
The UM-UC5 bladder carcinoma cells which express the EGFR but not PDGFR-β were stably transfected with a PDGFR-β construct. We assessed DNA synthesis and cell invasion potential in vitro under anti-EGFR (C225), anti-PDGFRβ (2C5) or combination therapy. Tumorigenicity and metastatic potential of bladder cancer cells were assessed using orthotopic mouse models and tail-vein injections. Tumor growth was assessed using a Luciferase-based bioluminescence system.
The EGFR receptor expression levels did not correlate with the sensitivity to EGFR therapy in bladder cancer cells. However PDGFRβ expression was identified in cells resistant to anti-EGFR therapy. Forced expression of PDGFRβ in EGFR-sensitive UMUC5 cells (IC50 < 10 nM) significantly reduced their responsiveness to the EGFR inhibitor (IC50 < 100 nM). The PDGFR-expressing cells were five times more invasive than the parental lines and demonstrated evidence of tumorigenicity and increased metastatic potential. Confocal microscopy analysis of PDGFRβ-expressing cells co-stained for EGFR and PDGFRβ proteins, demonstrated cytoplasmic internalization of both RTKs with cytoplasmic colocalization. Biochemical analyses demonstrated the existence of EGFR/PDGFRβ heterodimers with increased activation of the downstream signaling pathway MAPKinase and increased phosphorylation (inactivation) of GSK-3β. These modifications were associated with a significant decrease in E-cadherin expression. Dual inhibition of the EGFR and PDGFR-β receptors blocked cell invasion, reduced cell proliferation and rescued the E-cadherin expression to levels comparable to those found in parental UMUC5 cells. Finally, reduction of tumor growth was associated with increased E-cadherin expression after intraperitoneal administration of combination therapy that specifically targeted EGFR and PDGFRβ.
In EGFR-expressing urothelial carcinomas, co-expression of PDGFRβ and its impact on cell proliferation, invasion and tumorigenicity requires to be considered as a therapeutic target.

207 POSTER

Combined antibody mediated inhibition of IGF-IR, EGFR, and VEGFR2 for more consistent and greater antitumor effects

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To maintain the limited toxicity profile and increase the efficacy of targeted antitumor agents, combination targeted therapies are being developed. We have studied a combination strategy targeting three receptor tyrosine kinases important in malignancy – EGFR, VEGFR2, and IGF-IR using the monoclonal antibodies cetuximab, DC101 and IMC-A12, respectively, that specifically block the function of these receptors. Eleven subcutaneous xenograft models using a variety of human cancer cell types were utilized. In all of these models, the antitumor effects of a cocktail of DC101+cetuximab+IMC-A12 (40/10/10 mg/kg, respectively, M-W-F), were greater than that achieved with high dose monotherapy (40 mg/kg, M-W-F). In the models tested, the effects of the cocktail were dominated by the effects of DC101 and cetuximab. Biomarker studies tested for correlations