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

between antitumor effects (T/C%) and biomarker measurements made on tumor samples collected prior to treatment. Although biomarker data is still accumulating, significant correlations have been detected for monotherapies, but not with the cocktail due to its more consistent antitumor effects. Among these correlations is reduced efficacy of DC101 in xenograft models with higher human VEGF expression evaluated by ELISA ($r^2 = 0.46$, $p = 0.03$). Related to this, cetuximab significantly reduced tumor HIF-1 activity and VEGF concentration. Moreover when given in combination with DC101, cetuximab prevented the increase in tumor HIF-1 activity and VEGF production induced by DC101 monotherapy in multiple xenograft models. Cetuximab, therefore, prevented HIF-1 activity and VEGF production from overcoming or weakening the effects of VEGFR2 targeted therapy. Thus in the preclinical models tested, inhibition of pathways including HIF-1, results in antitumor effects of combination targeted therapy that are more consistent than monotherapy effects. This point is further illustrated in an orthotopic HT-29 colon cancer model using in vivo imaging, where DC101 monotherapy only inhibited primary tumor growth, cetuximab monotherapy only inhibited lymph node metastasis, and the cocktail treatment inhibited both. In conclusion, combination targeted inhibition of EGFR, VEGFR2, and IGF-IR, and in particular EGFR and VEGFR2, results in greater and more consistent tumor growth inhibition than monotherapies in preclinical cancer models, demonstrating the potential of this strategy in multiple cancer indications.

208 POSTER

Pharmacokinetic (PK), pharmacodynamic (PD) modeling and simulation analysis of PRO132365, a HER2 antibody-drug conjugate

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Objectives: PRO132365 is an antibody-drug conjugate targeting the HER2/neu receptor. Modeling and simulation approaches were used to integrate mouse xenograft model exposure-anti-tumor activity relationships and cynomolgus monkey pharmacokinetics to determine an efficacious dosing regimen for PRO132365 in the clinic.
Methods: Doses identified to provide maximum anti-tumor activity using a Q3W dosing schedule in an athymic mouse xenograft model were subsequently fractionated and tested using Q1W and Q2W regimens. A population tumor-kill PK/PD model was developed from the composite individual animal data using NONMEM. A transit compartment model with a non-linear tumor-cell kill function, which is dependent on PRO132365 serum concentrations, was implemented to describe the PK/PD relationship. A two-compartment model from separate PK studies was used as a forcing function for modeling these tumor-volume data. PRO132365 exposure-anti-tumor clinical projections were enabled by first predicting human PRO132365 PK disposition from monkey data (PRO132365 has been shown to bind the human and cynomolgus monkey HER2 receptor, but does not cross-react with the corresponding rodent receptor neu), and subsequently utilizing the predicted human PK to simulate the predicted PRO132365 anti-tumor activity derived from the tumor-kill model. The optimal regimen was determined as the dose and dose regimen providing a probability of achieving a target treatment outcome defined as a $\geqslant$30% reduction in tumor volume from baseline in the majority of simulated subjects.
Results: Classification and regression tree analysis (CART) demonstrates that the probability of predicting successful treatment outcome is greatly increased by achieving an exposure/minimum tumoricidal concentration (AUC/MTC) ratio $\geqslant$40 [days mg/L]/1 [mg/L] in an individual subject. This AUC/MTC ratio is predicted to be achieved in the majority of subjects with a dose schedule of $\geqslant$10 mg/kg PRO132365 dosed once every 3 weeks.
Conclusions: For the antibody-drug conjugate PRO132365, population modeling and simulation methodologies were employed to achieve the integration of preclinical PK and efficacy data with desired clinical outcome. This allowed the estimation of an optimal clinical dose and dose regimen that was useful in guiding decision-making as this novel therapeutic enters clinical trials for the treatment of HER2/neu positive breast cancer.

209 POSTER

Cancer therapy with antibodies conjugated to radionuclides emitting low-energy electrons

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Background: To kill a cell with radioactive decays on the cell surface or in the cytoplasm, the optimal electron energy is 20–25 keV. In contrast, the beta-particles generally used for radioimmunotherapy have tissue path lengths at least 50 times longer than a cell diameter. These studies were intended to evaluate the potential of radionuclides emitting low-energy electrons (LEEs) for single-cell kill in vitro and for tumor therapy in vivo. LEEs include both Auger and conversion electrons, which are emitted by most photon-emitters and by other radionuclides.
Materials and Methods: Antibodies (Abs) were conjugated to 111-In using the chelator benzyl-DTPA, to a specific activity of 40–80 mCi/mg. The Abs tested included Abs to CD20, CD74 and HLA-DR, for B-lymphoma target cells; and Abs to EGFr and HER-2, for carcinomas. In vitro, cells were incubated with the Abs for 2 days in vitro, then evaluated in clonogenic assays. Immunodeficient mice, both nude and scid, bearing human tumor xenografts were treated with radiolabeled Abs, injected i.v., at various times after tumor inoculation. Non-reactive control Abs labeled in the same way were tested similarly.
Results: Tumor cells were killed effectively and specifically with these Ab conjugates. Essentially 100% kill could be obtained (>5 logs). The radiation dose delivered to the nucleus was estimated from subcellular S values (for decays occurring on the cell surface or in the cytoplasm), and was consistent with the level of toxicity observed. In vivo, therapy of microscopic tumors was effective, with many cures, but effective therapy of macroscopic tumors has not yet been achieved.
Conclusions: For high-density antigens, which allow the delivery of large amounts of radioactivity per cell, these conjugates are potent and specific toxic agents. They are effective from the cell surface or the endosomal/lysosomal compartment, and do not require delivery to the cytosol or nucleus, as do drug- or toxin-Ab conjugates. Although treating macroscopic tumors is more difficult, this approach was effective in therapy of micrometastases, and thus is applicable to patients with minimal residual disease.

210 POSTER

Development of drug-conjugated monoclonal antibodies against MUC16 for the treatment of epithelial ovarian cancers

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The MUC16 glycoprotein is highly expressed on the surface of epithelial ovarian cancer cells, particularly of the serous subtype, and the shed extracellular sequence (CA125) is widely used as a marker for disease progression and response to therapy. We have generated antibodies against the extracellular mucin repeats of MUC16, such that each copy of the protein is bound simultaneously by multiple antibodies. As expected, these antibodies generate larger flow cytometry shifts on human ovarian cancer cell lines as compared with antibodies that recognize unique sites on MUC16. The repeat-binding antibodies are specific for MUC16 and do not bind to cells or tissues that lack MUC16 expression. One such antibody (Ab1) was conjugated to cytotoxic small molecules of the auristatin class using stable and labile linkers. The antibody-drug conjugates are potent anti-proliferative agents in vitro (IC50 < 10 ng/mL) and in vivo against human ovarian cancer models. For example, a single dose of one such conjugate at 6 mg/kg was sufficient to eliminate established OVCAR-3 mammary fat pad tumors in 8/10 mice. While in vitro activities were comparable among conjugates, in vivo studies revealed differences in efficacy and safety depending on the cytotoxin and linker. Importantly, efficacious doses of the conjugated antibodies do not elicit significant toxicity in rats or cynomolgous monkeys, including rats bearing xenograft tumors that express the target antigen and carry the MUC16 extracellular domain (CA125) in circulation. We believe that these drug-conjugated antibodies are promising therapeutics for ovarian cancer.

211 POSTER

Design of an anti MUC1 DNA aptamer as novel radiopharmaceutical for the diagnostic imaging and targeted radiotherapy of tumours

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Aptamers are novel oligonucleotide-based recognition molecules that can bind to almost any target, including extracellular receptor proteins, antibodies, peptides and small molecules. Aptamers can be rapidly generated and offer reduced immunogenicity, good tumour penetration, rapid uptake and clearance, which favour their application as effective vehicles for cytotoxic agents or radioisotopes. Thus, these molecules can be used as alternatives to monoclonal antibodies in molecular targeted radiotherapy and diagnostic imaging applications and overcome some of the problems associated with the latter.