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

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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

J. Papkoff[1], A. Strelow[1], S. Lee[1], A. Nguyen[2], G. Diedrich[2], S. Zhuo[2], W. Liu[1]. *[1]CFD Therapeutics, Inc., California, USA; [2]diaDexus, Inc., California, USA*

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.

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

P.J. Beltran, J. Lu, E. Cajulis, Y. Chung, M. Tsai, C. Chen, R. Deshpande, R.L. Kendall, R. Radinsky, F.J. Calzone. *Amgen Inc, Oncology Research, Thousand Oaks, USA*

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.

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

L. Adam[1], P. Black[1], G. Brown[1], M. Munsell[2], W. Kassouf[1], P. Nicola[1], C. Dinney[1], D. McConkey[3], M. Bar-Eli[3]. *[1]The UT-MDACC, Urology, Houston, USA; [2]The UT-MDACC, Biostatistics, Houston, USA; [3]The UT-MDACC, Cancer Biology, Houston, USA*

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.

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

J. Tonra, D. Deevi, E. Corcoran, H. Li, P. Moeljadi, J. Huber, D. Ludwig, D. Surguladze, D. Hicklin. *ImClone Systems Inc., Experimental Therapeutics, New York, USA*

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