carcinomas of colon, breast, lung, ovary and prostate while its low expression on normal tissue is restricted to the gastric mucosa, small intestine and pancreas. A calicheamicin conjugate that targets LeY could therefore provide a treatment option for a variety of cancers. The humanized IgG1 antibody, Hu3S193, that recognizes LeY was conjugated to a CM analog. FACS analysis showed that this conjugate (Hu3S193-CM) bound LeY-positive cells with similar selectivity and avidity as Hu3S193 indicating that conjugation did not influence these parameters. When tested on monolayer cultures of various human LeY-positive carcinoma cells, Hu3S193-CM was consistently more cytotoxic than a control conjugate. This difference in efficacy was not noted on LeY-negative cells. The efficacy of Hu3S193-CM depended qualitatively on the expression of LeY and on the sensitivity to CM. Quantitatively, the efficacy was neither directly proportional to the CM-sensitivity of tumor cells nor to their amount of LeY. Hence, other factors such as the nature of the surface proteins that carry LeY can co-determine the sensitivity to Hu3S193-CM. *In vivo*, Hu3S193-CM inhibited tumor growth of xenografted human gastric (N87), colon (LOVO) and prostate carcinomas (LNCaP) more effectively than control conjugates. When used against the N87, Hu3S193-CM (160 ug/kg, q4dx3) was curative. Our data show that Hu3S193-CM can specifically eliminate tumors that express LeY and therefore encourage development of this conjugate as an option for the treatment of carcinomas.

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Inhibition of tumor metastasis by ING-1(heMab), a human-engineered(TM) monoclonal antibody targeting the epithelial cell adhesion molecule

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Tumor metastasis is one of the hallmarks of malignancy and remains the main cause of death in cancer patients. Despite this, very few treatment strategies are effective in preventing metastasis from primary tumors. Therapeutic monoclonal antibodies, either alone or conjugated with cytotoxic agents, have shown promise in inhibiting tumor invasion and metastasis. Here we demonstrate that a Human-Engineered(TM) monoclonal antibody, ING-1(heMAb), which recognizes a 40-KDa glycoprotein, Epithelial Cell Adhesion Molecule (Ep-CAM), significantly inhibits tumor metastasis in a murine model of human cancer. To investigate the efficacy of ING-1(heMAb), we established an *in vivo* adenocarcinoma-lung metastasis model, in which athymic nude mice received an intravenous (IV) injection of human HT-29 colon adenocarcinoma cells via their tail veins. All mice were pre-treated with human interleukin-1beta to augment tumor metastasis. In all studies, mice were observed for 8 weeks to allow for lung metastasis development. Mice (10 per group) received IV dosing of 1 mg/kg ING-1(heMAb) twice weekly for 3 weeks starting on day 2 or day 5. A negative control group received human IgG with the same dosing schedule starting on day 2. In the positive control group, 100 mg/kg 5-FU/leucovorin was injected intraperitoneally once weekly for 4 weeks. At the end of the study, the mice were sacrificed for gross necropsy and tissue collection. Three different methods were used for data analysis. First, visible tumor nodules (>3 mm) were counted throughout animal body cavities. Second, tumor nodules on lung surfaces were counted under a dissecting microscope. Last, micrometastatic foci in sections of lung tissues were counted after detection with hemotoxylin/eosin staining. ING-1(heMAb) treatment starting on day 2 significantly reduced the number of tumor nodules visible during necropsy ($P<0.01$, vs IgG group). When nodules on lung surfaces were examined, a significant reduction was again observed in the ING-1 (heMAb)/day 2-treated group ($P<0.005$, vs IgG group). Lastly, ING-1(heMAb) treatment starting on day 2 resulted in 91% reduction of micrometastases in lung tissues ($P<0.005$, vs IgG group). Delaying ING-1 treatment until day 5 still led to 54% reduction in micrometastases ($P<0.005$, vs IgG group). The above results have been confirmed in another independent study. In conclusion, ING-1(heMAb) effectively reduced tumor metastasis in a murine model of human cancer. While ING-1(heMAb) was particularly effective when administered early in the study on day 2, day 5 treatment was also effective in reducing micrometastases. These data suggest that ING-1 (heMAb) may show promise in treating metastatic diseases in humans.

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Development of monoclonal antibodies targeting the uPA system for diagnosis and therapy

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The urokinase plasminogen activator system, comprised of the serine protease urokinase (uPA), its cell surface receptor uPAR and plasminogen activator inhibitor-1 (PAI-1), has been implicated in the neo-vascularization, invasion and metastasis of many solid tumors. uPAR plays an essential role in the regulated degradation and remodeling of the extracellular matrix by tumor cells and angiogenic endothelial cells. uPA-uPAR dependent cascades also result in the activation of proMMP-9 and the activation and release of growth factors and angiogenic factors including HGF, VEGF and TGFbeta. The important role of uPA-uPAR in tumor growth and its abundant expression within tumor, but not normal tissue, makes it an attractive diagnostic and therapeutic target. We previously described the production and characterization of a panel of monoclonal antibodies against the amino terminal fragment (ATF) of uPA. We have tested the ability of two of these antibodies, ATN-291 and ATN-292, to inhibit tumor growth *in vivo*. Briefly, 7x105 MDA MB 231 cells were injected into the mammary fat pad of Balb/c nu/nu mice. Tumors were staged to 35 mm3 before the start of treatment with anti-ATF or isotype matched control antibodies (10 mg/kg, 3 × week, IP). Treatment with ATN-291 or ATN-292 significantly inhibited tumor growth in this model. In addition, we have generated a panel of monoclonal antibodies against domains 2 and 3 (D2D3) of uPAR conjugated to KLH. Initial characterization of the antibodies indicates that they bind suPAR with affinities of 3-80 nM in direct binding assays and recognize suPAR, D2D3 but not domain 1 (D1) of uPAR, as determined by western blot. In functional assays a D2D3 antibody, as well as a polyclonal antibody against D2D3, effectively inhibited adhesion of integrin alpha5beta1 expressing CHO cells to suPAR. Furthermore, the antibody also inhibited uPA induced cell migration. Preliminary experiments indicate that the antibodies can be efficiently labeled with I-125 with no significant change in the binding characteristics of the antibodies. We will present results of internalization studies using these labeled antibodies and assess their potential to deliver cytotoxic agents to tumor cells.

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Characterization of the intracellular mechanisms behind the cytotoxic effect of the immunotoxin, 425.3-PE in two breast cancer cell lines

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The aim of the study was to investigate the mechanisms behind immunotoxin (425.3-PE)-induced cell death. In addition, the combinational effect of 425.3-PE and cycloheximide (CHX) in cell culture was evaluated. Our immunotoxin consists of antibody 425.3, recognizing the EGF receptor, chemically conjugated to the bacterial toxin Pseudomonas exotoxin (PE). The two different breast cancer cell lines used, MA11 and MT1, respond differently to 425.3-PE treatment in several aspects, thereby giving us opportunity to evaluate mechanistic events in 425.3-PE induced cell death. The immunotoxin treatment of these two cell lines do not only inhibit protein synthesis (PS) (IC_{50}=0.5-2 ng/ml) by ADP-ribosylation of EF-2, it also induces caspase activation, cleavage of poly(ADP)-ribose polymerase (PARP) and DNA fragmentation, characteristic events of apoptosis. Western blot demonstrated immunotoxin-induced PARP cleavage, more rapidly induced in MA11 cells (maximal 5h) contra MT1 cells (maximal 10h). The broad-spectrum caspase inhibitor (z-VADfmk) totally inhibited PPAR cleavage and improved the cell viability (approx. 3-fold) without altering inhibition of PS in both MA11 and MT1 cells treated with 425.3-PE. The specific caspase-9 inhibitor (z-LEDHfmk) had no effect on 425.3-PE induced cell death in MT1 cells. However, in MA11 cells this specific inhibitor made them 1.6 times more resistant to 425.3-PE, suggesting activation of caspase-3 by caspase-9, mediated by mitochondria alterations. By combination of two PS inhibitors, CHX and 425.3-PE, the cell viability decreased in both MA11 and MT1 cells to a level corresponding to that of a 10 times higher concentration of 425.3-PE alone. The CHX concentration used gave by itself minimal effects on PS inhibition and cell viability. For MA11 cells the combination of CHX+425.3-PE increased the PS inhibition effect further, whereas MT1 cells were insensitive to the combination. Notably, 425.3-PE also decreased the p53 protein level in MA11 cells to 30% of control. The increased activity of 425.3-PE when combined with CHX will be further examined. In conclusion, the apoptotic program which 425.3-PE starts in MA11 and in MT1

cells are differently regulated, in time and in activation of caspases. They also respond dissimilarly to the co-treatment with 425.3-PE+CHX. Thus, MA11 and MT1 cells give us an opportunity to further elucidate essential pathways involved in immunotoxin-induced cell death.

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Biological and therapeutic properties of a novel, fully human monoclonal antibody targeting prostate specific membrane antigen

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There is an urgent need for effective therapies for recurrent, hormone-refractory prostate cancer, which is largely resistant to conventional chemotherapeutic agents. Accordingly, we have developed a novel panel of fully human monoclonal antibodies (mAbs) to prostate specific membrane antigen (PSMA), which is widely regarded as compelling target for immunotherapy of prostate as well as other cancers. Due to the differential expression of mRNA splice variants, PSMA is found in normal prostate as a cytoplasmic protein and in prostate cancer as a type II membrane glycoprotein whose surface expression increases with disease progression. Interestingly, PSMA is also expressed in the neovasculature of most other solid tumors. PSMA is both rapidly internalized upon antibody binding and enzymatically active. Collectively, the expression profile and biological properties of PSMA make this molecule a highly attractive target for cancer therapy. Using novel recombinant forms of PSMA and XenoMouse® technology (Abgenix, Fremont, CA), we have generated a panel of high affinity and fully human mAbs against PSMA. Using a battery of Biacore, ELISA and cell-binding assays, we demonstrated that these human mAbs specifically recognize conformational epitopes on PSMA with sub-nanomolar affinity. Strikingly, the affinity of the human mAbs is greater than that of a similarly generated panel of conventional mouse mAbs. The mAbs were further compared for internalization and for inhibition of PSMA's folate hydrolase and NAALADase activities. A final series of studies examined the mAbs' intrinsic cytotoxic/signaling effects as well as their ability to specifically deliver cytotoxic agents and radioisotopes to PSMA-expressing tumor cells *in vitro* and *in vivo*. Based on these studies, a lead fully human mAb candidate has been selected for human clinical testing. *PSMA Development Company LLC is a joint venture between Progenics Pharmaceuticals, Inc. and Cytogen Corporation.

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Novel recombinant Fab fragments of the TAG-72 monoclonal antibody cc49 containing an integrated radiometal binding site for radioimmunoguided surgery of DCIS

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Surgical treatment of DCIS is currently inadequate due to the inability to define accurately disease margins. Radioimmunoguided surgery (RIGS) employing radionuclide-conjugated monoclonal antibodies (mAbs) against breast cancer antigens and a sensitive gamma-detecting probe may improve the surgical management of DCIS by more clearly identifying malignant tissue. Tumor-associated antigen-72 (TAG-72) is overexpressed in 81% of DCIS by immunohistochemical staining with mAb CC49. Our objective was to construct novel recombinant Fab fragments of mAb CC49 containing an integrated radiometal binding site that can be directly labeled with Tc-99m through the C-terminal hexahistidine (6xHis) tag* for RIGS of DCIS. Recombinant Fab (rFab) consists of the entire light chain (L) and the Fd portion of the heavy chain of CC49. L and Fd chains were cloned from CC49 hybridoma cells by RT-PCR into TA cloning vectors, then individually subcloned into a yeast secretion vector pPICZalphaA using primers to incorporate the affinity tags 6xHis at the C-terminus of Fd and FLAG at the N-terminus of L. The coexpression vector was constructed in which L and Fd were placed in a pPICZalphaA vector, but under separate control of the promoter and transcriptional terminator. L and Fd were coexpressed in KM71H Pichia pastoris and secreted into the culture medium as correctly folded Fab. The expression was optimized by induction with 0.5% methanol at 30oC for 72 h. rFab was purified by Ni-NTA affinity chromatography. SDS-PAGE showed one major band at ~53kDa (77%) and one minor band at ~27kDa (23%) under nonreducing conditions. The ~53kDa product dissociated into ~27kDa proteins under reducing conditions. Both bands (~53kDa and ~27kDa) were reactive with goat anti-mouse Fab by Western blot indicating that the ~53kDa band corresponded to rFab. The purity of rFab was 77% after this single step purification. rFab purified by Ni-NTA affinity column was immunoreactive with bovine submaxillary mucin (a TAG-72 source) by ELISA assay. We concluded that immunoreactive Fab fragments of CC49 were expressed in Pichia pastoris and purified. Currently, we are conducting a larger scale purification using Ni-NTA and anti-FLAG affinity chromatography to produce rFab with higher purity for protein assay and also for determination of its antigen binding affinity. *Waibel R. et al. Nat. Biotechnol., 17:897-901, 1999

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Monoclonal antibody against VEGFR-1 directly inhibits flt1-positive breast tumor growth

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Vascular endothelial growth factor receptor-1 (VEGFR-1) is a key regulator of angiogenesis and tumor growth that is activated by the ligands VEGF-A, VEGF-B and placental growth factor (PlGF). VEGFR-1 is expressed in endothelial cells, smooth muscle cells, monocytes, and some tumor types. While studies have shown that inhibition of VEGFR-1 function in endothelial cells suppresses angiogenesis and angiogenesis-depended tumor growth, the role of VEGFR-1 expression on tumor cell growth is yet to be established. Previously, we reported on the expression of VEGFR-1 in human and murine breast cancers and the inhibitory effect of an anti-VEGFR-1 neutralizing monoclonal antibody (mAb) on growth of VEGFR-1-positive breast tumors. Here, we report on the further validation of VEGFR-1 in breast tumorigenesis. Treatment of breast tumor cells with an anti-VEGFR-1 neutralizing mAb blocked PlGF-stimulated downstream signaling to p42/44 MAP kinase and prevented growth of breast tumor cells *in vitro*. Treatment with anti-human VEGFR-1 mAb significantly suppressed growth of a number of estrogen-dependent and -independent human breast xenograft tumors in nude mice. Histological examination of anti-VEGFR-1-treated tumors showed reduced proliferation of tumor cells, tumor cell apoptosis and necrosis. Since this antibody does not crossreact with murine VEGFR-1 present on mouse vasculature, these data demonstrate a direct effect of blocking VEGFR-1 on human breast tumor cells. These data confirm that VEGFR-1 plays a functional role in the growth of human breast tumors and that an anti-VEGFR-1 neutralizing mAb can inhibit growth of these tumors in pre-clinical models.

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A phase I and pharmacokinetic study of BB10901, a maytansinoid immunoconjugate, in CD56 expressing tumors

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BB10901 (huN901-DM1) is a humanized monoclonal antibody (huN901) linked to approximately 4 molecules of the extremely potent maytansinoid, DM1, and targeted to the CD56 antigen that is present in high prevalence on small cell lung carcinomas, neuroblastomas and neuroendocrine tumors. In preclinical investigations, treatment with BB10901 led to cures in nude mice bearing human N417 and SW2 small cell lung carcinoma xenograft tumors. The objectives of this phase I study were to determine the feasibility of administrating BB10901 intravenously once weekly for 4 weeks every 6 weeks, to quantitatively and qualitatively define the toxicities of this therapy, to characterize the pharmacokinetics of BB10901, and to preliminarily determine antitumor activity. To date, 24 patients have been treated at doses ranging from 5 mg/m^2 to 75 mg/m^2. Patient demographics include; 15 patients had SCLC and 9 patients neuroendocrine tumors, who had received a median number of 2 prior chemotherapy regimens (range 0-3). No moderate or severe (Grade $>$ 2) hematologic toxicity has been observed. Mild sensory neuropathy has been observed in 2 patients, however no changes in conduction velocity have been observed on serial nerve conduction studies. One patient experienced dose-limiting pancreatitis possibly related to treatment at 60 mg/m^2. No human antihuman Ig or human anti-DM1 antibodies have been detected nor allergic reactions observed. The average T1/2