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