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

is 23 hours, and clearance 49 ml/h/m^2. BB10901 clearance is non-dose-proportional with greater clearance observed at lower dose levels perhaps secondary to NK cell binding. Two minor responses have been observed (1 neuroendocrine, 1 SCLC patient). BB10901 can be administered safely to patients with CD 56 expressing tumors with encouraging preliminary biologic activity observed. Patient accrual continues at 75 mg/m^2 weekly.

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Human Prostate Specific Membrane Antigen (PSMA) is expressed as a non-covalent dimer and provides an attractive target for cancer immunotherapy

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Prostate Specific Membrane Antigen (PSMA) is a type-2 membrane protein that is expressed abundantly on the surface of prostate cancer cells but not on normal human tissues. PSMA is also expressed on the neovasculature of a variety of other solid tumors. An antibody to the intracellular portion of PSMA is currently in use for *in vivo* imaging of prostate cancer, and the large extracellular portion of PSMA (707 of 750 amino acids) provides an attractive target for therapeutic vaccines. We have produced a recombinant soluble PSMA (rsPSMA) protein that comprises the entire ectodomain for use as a candidate vaccine. To compare the oligomeric nature of membrane-bound PSMA and the rsPSMA protein, we used gel filtration and Blue Native polyacrylamide gel electrophoresis, a novel high-resolution molecular sizing assay. The analyses indicate that PSMA is expressed as a non-covalent homodimer on the surface of LNCaP cells as well as on 3T3 cells stably transfected with full-length PSMA. In addition, rsPSMA was secreted as a non-covalent dimer from stably transfected Chinese hamster ovary cells, indicating that the extracellular residues of PSMA are sufficient for dimerization. Both, cell surface PSMA and rsPSMA, possess folate hydrolase and NAALADase activity and display similar patterns of reactivity with a panel of conformation-specific monoclonal antibodies. In contrast, the monomeric form of the protein exhibited only minimal enzymatic activity. In summary, PSMA is naturally expressed as a dimeric protein on the surface of prostate cancer cells. Only the homodimer form of PSMA is enzymatically active, may play an important role in tumor progression, and as such provides an attractive target for prostate cancer immunotherapy. To this end, we have developed rsPSMA, which faithfully mimics native PSMA in terms of tertiary and quaternary structure as well as enzymatic function. Thus, rsPSMA represents a promising candidate for evaluation as a therapeutic vaccine in combination with potent immunostimulatory adjuvants. *PSMA Development Company LLC is a joint venture between Progenics Pharmaceuticals Inc. and Cytogen Corp.

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A Phase II Study of Erbitux (IMC-C225), an Epidermal Growth Factor Receptor (EGFR) blocking ntibody, in combination with docetaxel in chemotherapy refractory/resistant patients with advanced Non-Small Cell Lung Cancer (NSCLC)

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EGFR has become an important target in cancer therapy as it is over-expressed in many solid tumors including NSCLC. IMC-C225 is a monoclonal antibody to EGFR that has demonstrated activity and synergy with chemotherapy in both preclinical and clinical settings. Docetaxel is the FDA approved 2nd line therapy for NSCLC. We investigated the combination of IMC-C225 and docetaxel as second-line therapy in chemotherapy refractory/resistant patients (pts) with advanced NSCLC. The objectives were to determine the tumor response rate, duration of response, survival, safety and toxicity, and pharmacokinetics (PK) of this combination therapy. Eligibility criteria included pts with advanced NSCLC who had progressive disease during or disease recurrence within 3 months after chemotherapy and tumor EGFR expression of at least 1+ by immunohistochemistry. IMC-C225 was administered as 400 mg/m^2 IV during the first week only followed by 250 mg/m^2 IV weekly. Docetaxel was administered at 75 mg/m^2 IV every 3 weeks.
Since May 8, 2001, we have enrolled 50 patients, 30 of which are evaluable for response and toxicity. Patient characteristics: 15 males and 15 females; median age 57 years (range 31-76); median ECOG PS 1 (0-2), and EGFR expression 3+ (25 pts), 2+ (3), 1+ (2). Thus far, 8 pts have achieved a partial response (5 confirmed and 3 unconfirmed) and 8 pts have stable disease. Median number of cycles is 3 (range, 1-10). Preliminary pharmacokinetic analysis shows no interaction of IMC-C225 with docetaxel. This regimen is very well tolerated with minimal toxicities including acneiform rash 7 pts (grade III/IV) and febrile neutropenia 3 pts (grade III/IV). IMC-C225 in combination with docetaxel appears to be well tolerated and the response rate suggests clinical activity in the second-line setting. Trial accrual has been completed. Final response data, duration of response and survival are still being collected.

Natural products

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A novel mechanism of potentiation of trail-induced apoptosis: resveratrol sensitizes resistant tumor cells for TRAIL by p21-mediated G1 arrest

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Since resistance of many tumors to current treatments protocols remains a major concern in oncology, novel strategies are necessary to target resistance. Here, we identify the chemopreventive agent resveratrol, a polyphenolic phytoalexin found in grapes and wine, as a potent sensitizer for TRAIL-induced apoptosis. Resveratrol strongly enhanced TRAIL-induced apoptosis through p21-mediated G1 cell cycle arrest indicating for the first time that sensitivity for TRAIL may be linked to cell cycle regulation. Also, resveratrol sensitized for apoptosis induced by CD95 triggering or by cytotoxic drug, e.g. doxorubicin, etoposide or cisplatin. Resveratrol-induced sensitization for TRAIL was mediated by rapid induction of p21 protein and G1 cell cycle arrest, since pretreatment with p21 antisense oligonucleotides abrogated the synergistic effect. Likewise, ectopic expression of p21 or pretreatment with the G1 cell cycle inhibitor mimosine strongly enhanced TRAIL-induced apoptosis. Induction of p21 by Resveratrol was mediated through p38 kinase-dependent p53 phosphorylation, since p53 phosphorylation and p21 induction was blocked by the p38 kinase specific inhibitor SB202190. Importantly, Resveratrol induced p21 expression also independent of wild-type p53 function, since p21 induction and sensitization for TRAIL treatment was found in p53 null Saos osteosarcoma cells or in p53-deficient HCT116 colon carcinoma cells. Resveratrol-induced G1 arrest resulted in rapid downregulation of Survivin protein expression, which was prevented by the proteasome inhibitor lactacystine, with no changes in Survivin mRNA expression. Likewise, Survivin antisense oligonucleotides enhanced TRAIL-induced apoptosis indicating that Resveratrol-induced potentiation of apoptosis was mediated by proteasomal degradation of Survivin at G1. Most importantly, synergy between resveratrol and TRAIL was found in a variety of different tumor types derived from neuroblastoma, medulloblastoma, glioblastoma, Ewing tumor, melanoma, pancreatic carcinoma, colon carcinoma, breast carcinoma or leukemia, and even in TRAIL-resistant cells and in patients' derived primary tumor cells. Therefore, by demonstrating that TRAIL-induced apoptosis is strongly enhanced by resveratrol through p21-mediated G1 arrest in a variety of tumors, our findings may have important clinical implication. Thus, the combination of TRAIL and resveratrol may be a novel strategy to overcome resistance of various tumors.

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Development of high-throughput *in vitro* and *in vivo* testing strategies for the discovery of novel anticancer agents of natural origin

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Natural products have proven to be a rich source of novel drugs. Their structural diversity offers excellent opportunities for finding low molecular weight compounds. The majority of anticancer agents which successfully completed clinical trials were of natural origin e.g. taxol or CPT-11. With the continuing need for novel lead structures against defined molecular targets, natural products will remain important to the future of anticancer drug discovery. We have opted to test a collection of over 5.000 chemically defined, pure natural products mainly from German Universities, and have developed high-throughput *in vitro* and *in vivo* testing strategies. The major obstacle in using isolated versus synthetic material is its limited availability - often only a few milligrams can be provided. We developped a cellular screen using 10 xenograft-derived human cell lines comprised of 7 slow or