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

intermediate growing and sensitive as well as chemoresistant solid tumor types. The cell lines resemble the original patient tumor and xenograft histology after s.c. injection into nude mice. Each drug was tested in 2 concentrations (0.3, 3 μg/ml). Of 10 registered anticancer agents within the natural product pool, 9 were found active applying our screening criteria. 1.5 % of the total number of novel compounds screened, possessed potent and differential *in vitro* cytotoxicity profiles. Eleven of these hits were forwarded for *in vivo* testing. To save precious compound, we developed a model system, which would give a hint of *in vivo* antitumor activity and enable estimation of the maximal tolerated dose. Two or four different xenografts with similar doubling times were implanted s.c. between the fore and hind flanks of nude mice. They were validated for stable growth, response to clinical agents and maintaining the morphology. The xenograft doublet was composed of mammary (MAXF 401) and lung cancer models (LXFL 529) which had also been used for *in vitro* screening. Against these tumors, leads were tested i.p. on days 1,5, and 9 in a single mouse and subsequently in tumor quartets composed of fast growing cancers (RXF 944, MX-1, OVXF 899, XF 575), or slower growing tumors (LXFL 529, MAXF 401, MEXF 989, CXF 280). Six of 11 compounds showed a reproducible *in vivo* activity. They are candidates for preclinical development. By using this approach, clinical candidate agents will be discovered on an economical and fast track.

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The silybin-phospholipid complex IdB 1016 inhibits human ovarian cancer growth in athymic nude mice

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We have recently reported that the flavonoid silybin, and its more bioavailable derivative IdB 1016, are able to potentiate, both in *in vitro* and *in vivo* experimental models, the antiproliferative activity of optimal or sub-optimal doses of cisplatin against human ovarian cancer cells (A2780); we also showed for the first time, an antiangiogenic effect of IdB 1016 in an *in vivo* experimental model. In the present study we provide evidences for the growth inhibitory activity of IdB 1016 when used as single agent against human ovarian cancer. IdB 1016 (450 mg/kg/day) was administered by oral route for 20 days to athymic nude mice bearing A2780 xenografts, starting from the day of tumour cell inoculation. This treatment significantly inhibited tumour growth (TWI=78% and LCK=1.1) and, importantly, treated animals did not show any signs of toxicity such as weight loss or reduced food consumption. Bioavailability of silybin after administration of a pharmacologically active dose of IdB 1016 was also assessed: free plasma silybin levels were found to be in the range of 0.2 to 13.4 mcg/ml. Tumour samples were also taken from treated animals and analysed to determine silybin tissue levels and to evaluate the modulation exerted by IdB 1016 on angiogenesis gene expression profile. Furthermore, the effect of IdB 1016 on secretion of VEGF by human ovarian cancer cells has been studied by analysing human VEGF levels in serum from athymic mice bearing A2780 xenografts. Due to this interesting pre-clinical anti-tumour profile, a clinical trial is currently undergoing in order to evaluate the efficacy of the drug in patients with serological recurrence of ovarian cancer.

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The intravenous administration of rViscumin induces cytokine release and antibody formation. Results of EORTC New Drug Development Group trial 16002

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Background: rViscumin (proposed INN: aviscumine) is a recombinant E.coli-derived type II ribosome-inactivating protein with potent antitumor activity *in vitro* and *in vivo*. The drug is currently being investigated in the first intravenous dose-escalating phase I clinical trial performed by the EORTC New Drug Development Group (NDDG) in Hannover and Nantes (EORTC 16002). rViscumin is given twice weekly as a 1 hour i.v. central line infusion in patients with advanced solid tumors not previously exposed to natural mistletoe preparations. Translational research includes the determination of cytokine levels in plasma and monitoring of a possible anti-rViscumin antibody induction.
Methods: Sequential plasma levels of interleukin (IL)-1β, IL-6, IL-10, IL-12, interferon (IFN)gamma and TNFalpha were determined by standard ELISAs (Becton Dickinson) at baseline and on the days of the first and eleventh administration of the drug. Anti-rViscumin antibodies were detected in plasma at baseline, repeated every 3 weeks.
Results: Increased concentrations of IL-1β and IL-6 in plasma of treated patients were observed over the entire dose range administered to date (10-4800 ng/kg). Increased release of IFNgamma as a marker of activated T-cells occurred after higher doses of 3200 and 4000 ng/kg. T-cells might be activated by the cytokine release of the innate immune system. Increase in these cytokine levels was highest 4 and 8 h after the first infusion and declined with further treatment duration. No increases of IL-10, IL-12 or TNFalpha were noted. During the course of treatment, IgG and IgM anti-rViscumin antibodies were mainly detected in plasma of patients treated with rViscumin doses * 800 ng/kg. No clear dose dependency was observed. The titers measured are low (serum dilution of 1:50 and 1:100) and of as yet unclear clinical relevance.
Summary: The i.v. administration of rViscumin stimulates the immune system with a significant release of cytokines such as IL-1β, IL-6 and IFNgamma and is associated with an induction of anti-rViscumin antibodies of IgM and IgG class of unclear clinical significance.

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Pharmacokinetics of the intravenous administration of rViscumin in patients with solid tumours - First results from EORTC Phase I Study 16002

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Background: rViscumin (proposed INN: aviscumine) is a recombinant E.coli-derived type II ribosome-inactivating protein composed of a binding (B-) and an active (A-) chain. The compound has potent antitumor activity *in vitro*, in syngeneic and xenograft models. The drug is currently being investigated in the first intravenous dose-escalating phase I clinical trial performed by the NDDG (Early Clinical Studies Group) in Hannover, Germany, and Nantes, France.
Methods: Pts with progressive solid tumors refractory to conventional treatment are eligible for the trial. rViscumin treatment is given twice weekly by a central i.v. infusion over 1 hour. The number of pts required per dose level is defined by a Continuous Reassessment Method. Assessment of human pharmacokinetics is a key research endpoint of the trial. PK samples were obtained at 0, 1, 1.25, 1.5, 2, 4, 8 and 24 h after start of the 1st and 11th infusion. Sample analysis was performed by immuno-PCR using a polyclonal antibody allowing to detect both the intact holoprotein and each chain of the recombinant molecule.
Results: To date, 26 pts have been entered and doses have been escalated interindividually from 10 to 4800 ng/kg per administration. The maximum tolerated dose has not been reached so far. Pharmacokinetic data of 19 pts are being processed. According to this preliminary analysis, the intact protein has a t1/2 alpha of 30 min. The terminal t1/2 is currently estimated to be in the range of 24 h, but may be shorter because of the polyclonal nature of the antibody which may also detect degradation products. Comparing PK data after the 1st and 11th infusion there is no evidence of drug accumulation with the twice weekly administration. The correlation of AUC and Cmax values with doses indicates a linear relationship for rViscumin dose levels *1600 ng/kg. In pts given 4800 ng/kg per dose, plasma levels of about 20 ng/ml were achieved and maintained briefly. These plasma concentrations were effective in animal models. In human tumor xenograft clonogenic assay, the mean *in vitro* IC70 is 3 ng/ml. Based on our pharmacokinetic findings and the short t1/2, EORTC NDDG is currently considering two additional phase I trials with more frequent or more prolonged administration of rViscumin.