in CTC was detected in 11/19 p. CTC IGF-1R was undetectable following treatment with C at doses above 3 mg/kg.
Conclusions: This combination is safe and feasible with no significant toxicity attributed to C and encouraging antitumor activity in CRPC.

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Denosumab is a selective inhibitor of human receptor activator of NF-kB ligand that blocks osteoclast formation in vitro and in vivo

R. Elliott[1], P. Kostenuik[1], C. Chen[1], M. Kelley[1], N. Hawkins[1], J. Housman[1], S. McCabe[1], V. Mukku[1], J. Sullivan[1], W. Dougall[2]. *[1]Amgen Inc., Thousand Oaks, USA; [2]Amgen Inc., Seattle, USA*

Introduction: Receptor activator of NF-κB ligand (RANKL), a member of the TNF superfamily, is an essential mediator of osteoclast formation, function, and survival. Increased osteoclast activity is critical in the pathogenesis of diseases that result from excessive bone resorption, including cancer-related bone metastasis and multiple myeloma. Denosumab is a fully human monoclonal antibody to RANKL that is in clinical trials for the treatment of bone disorders associated with pathologically increased bone resorption. Here we describe the results of studies that characterized the RANKL-binding properties of denosumab and evaluated its effects on osteoclast function in vitro and in vivo.
Methods: Denosumab binding to human RANKL (huRANKL) was determined by flow cytometry and ELISA, and the binding affinity was measured using BIAcore and a kinetic exclusion assay. The effects of denosumab on osteoclast formation in vitro were assessed using the mouse RAW 264.7 cell line. To evaluate the effect of denosumab on osteoclast function in vivo, mice were administered soluble huRANKL (twice daily at 1.0 mg/kg/day for 5 days), which produced hypercalcemia due to increased bone resorption. Concurrent with the first huRANKL dose, mice were treated with vehicle, another RANKL inhibitor, OPG-Fc (3 mg/kg), or various single doses of denosumab (1, 3, or 10 mg/kg).
Results: Binding assays showed that denosumab bound both soluble and membrane-bound forms of huRANKL. Moreover, denosumab binding to either form of huRANKL was inhibited by excess huRANKL, but not by TNF-α, TNF-β, TRAIL, or CD40 Ligand. Using BIAcore methods and a kinetic exclusion assay, the dissociation constants of denosumab were calculated to be 9.5×10^{-11} M and 3×10^{-12} M, respectively. Denosumab neutralized the ability of soluble huRANKL to stimulate the differentiation of RAW 264.7 cells into osteoclasts in vitro (IC_{50} of 1.64 ng/ml vs OPG-Fc IC_{50} of 1.15 ng/ml). Administration of either denosumab or OPG-Fc delayed the development of hypercalcemia in huRANKL-treated mice, indicating that denosumab neutralized the activity of soluble huRANKL in vivo. Denosumab caused dose-dependent suppression of hypercalcemia in this model.
Conclusion: These data demonstrate that denosumab binds human RANKL with high affinity and does not bind TNF-α, TNF-β, TRAIL, or CD40 ligand, thereby inhibiting osteoclast function in vitro and in vivo.

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Multi-targeted inhibition of the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) pathways: a phase I study of cetuximab (C), erlotinib (E), and bevacizumab (B) in patients with solid tumors

C. Lin[1], G. Preston[2], E. Calvo[1], K. Papadopoulos[1], A. Patnaik[1], J. Sarantopoulos[1], P. O'Rourke[1], C. Takimoto[1], A. Tolcher[1]. *[1]Cancer Research and Research Center, Institute for Drug Development, San Antonio, TX, USA; [2]Brook Army Medical Center, Department of Hematology/Oncology, San Antonio, TX, USA*

Background: Complex interrelationships exist between the EGFR and VEGFR pathways. EGFR activation elicits cell proliferation, and downstream effects increase expression of VEGF. In renal cell carcinoma, mutations increase hypoxia inducible factor-1alpha, stimulating VEGF and transforming growth factor expression. Moreover, there is additive tumor inhibition from combined EGFR targeting with C, and a tyrosine kinase inhibitor. To maximally inhibit EGFR, and then inhibit downstream VEGFR activity, this phase I study was initiated to determine the maximum tolerated dose (MTD) of E with a fixed dose of C, and then the MTD of B with combined E and C in patients with advanced malignancies.
Methods: Patients with advanced malignancies likely to express EGFR were entered in part 1 to daily oral E (starting at 100 mg, planned initially to increase to 150 mg), with fixed dose C (400 mg/m^2 loading and 250 mg/m^2 IV weekly). Once the MTD was determined for E in combination C, part 2 incorporated the addition of escalating doses of B (5 mg/kg IV every 2 weeks, to increase to 10 mg/kg) to the combination of E and C.
Results: 30 patients were entered and received 113 courses over 4 dose levels. In part 1 grade 3 rash occurred in 2 patients at E at 100 mg daily, and the MTD of E for this combination was 50 mg daily with standard dose C (11 patients treated). Other adverse events included rash, diarrhea, fatigue, and hypomagnesemia. Part 2: B at 5 mg/kg IV q14 days can be added to the MTD of E with C, with additional non-dose limiting toxicities of headache, proteinuria, and hypertension. There is one partial response in a patient with renal cell carcinoma. Durable stable disease has been observed in 4 patients for 7 (head and neck sqaumous cell); 10+, 12, and 12+ (renal cell) months.
Conclusions: The MTD for E combined with standard C is 50 mg daily. B at 5 mg/kg can be combined safely with this combination and dose escalation is ongoing.

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Efficacy evaluation of the humanized anti-EGFR MAb h-R3 (nimotuzumab) in combination with radiotherapy in the treatment of patients with unresectable squamous cell carcinomas of the head and neck

T. Crombet[1], M. Osorio[2], T. Cruz[3], J. Alert[4], J. Marinello[4], J. González[5], E. Neninger[6], E. De Armas[7], M. Cedeño[8], M. Frómeta[8]. *[1]Center of Molecular Immunology, Clinical Immunology Department, Havana, Cuba; [2]National Institute of Oncology, Experimental Chemotherapy, Havana, Cuba; [3]National Institute of Oncology, Head and Neck, Havana, Cuba; [4]National Institute of Oncology, Radiotherapy, Havana, Cuba; [5]National Institute of Oncology, Experimental Chemotherapy, Havana, Cuba; [6]Hermanos Ameijeiras Hospital, Oncology, Havana, Cuba; [7]Celestino Hernández Hospital, Oncology, Havana, Cuba; [8]Center of Molecular Immunology, Histology, Havana, Cuba*

Background: The incidence of head and neck tumors is worldwide increasing. High levels of Epidermal Growth Factor Receptor (EGFR) are associated with malignant transformation of squamous cells and are observed in head and neck squamous cell carcinomas (SCCHN). There is evidence of a relationship between EGFR expression and tumour cell proliferation, metastases development and radiation resistance.
Material and Methods: h-R3 (nimotuzumab) is a humanized monoclonal antibody (mAb), with high affinity and specificity to the EGFR. In *vitro* as well as *in vivo*, h-R3 demonstrated a remarkable anti-proliferative, pro-apoptotic and anti-angiogenic effect.
In order to assess the efficacy of h-R3 in combination with radiotherapy in the treatment of advanced SCCHN patients, a controlled, double blinded, Phase II clinical trial was conducted. Patients received 6 weekly infusions of a placebo or h-R3 at the dose of 200 mg. Immunohistochemical evaluation of EGFR expression in tumours was done before trial inclusion. A second biopsy was taken after the 4th dose of the mAb or placebo.
Results: Thus far, 72 evaluable patients, median age 66, with documented unresectable SCCHN have been randomly assigned to groups A or B. Ionizing radiation was delivered in doses of 2 Gy to a total dose of 66−70 Gy. Fifty-seven patients (79%) had either T3 or T4 at presentation. The most common toxicities were fatigue, anemia, fever, hypotension and cephalea. These events were classified as mild or moderate, according to the NCI-CTC scale. None of the patients had skin rash or allergic reactions. Seven patient developed grade 3 adverse events consisting in fatigue, anemia and peripheral arterial ischemia. The most frequent radiation associated toxicities were mucositis, radiodermitis and dysphagia. Objective response (complete or partial response) was achieved in 70 % of the patients, in spite of the treatment group. With a median follow up time of 23 months, the median survival is 16.50 months for all patients treated with mAb or placebo. Pre-treatment tumor biopsies as well as second biopsies were taken to compare h-R3 and placebo impact on the EGFR signal transduction cascade, proliferative activity and angiogenesis. Trial blinding will be open once 84 patients had been recruited.
Conclusions: Nimotuzumab is well tolerated. Preliminary efficacy, safety and pharmacodynamic results per treatment group are intended to be presented at the meeting.

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Strictly target cell-dependent activation of T cells by bispecific single-chain antibody constructs of the BiTE class

K. Brischwein, L. Parr, S. Pflanz, J. Volkland, J. Lumsden, M. Klinger, B. Schlereth, M. Locher, P. Kufer, P.A. Baeuerle. *Micromet AG, Munich, Germany*

Background: Bispecific antibodies have been extensively studied in vitro and in vivo for their use in redirected tumor cell lysis. A particular challenge of bispecific antibody constructs recognizing the CD3 signaling complex is a controlled polyclonal activation of T cells that, ideally, is entirely dependent on the presence of target cells. If this is not the case, systemic production of inflammatory cytokines and secondary endothelial reactions may occur as side effects, as are observed with the murine anti-human

CD3ε antibody OKT-3 (muromab, Orthoclone®). Here we present evidence that two bispecific single chain antibodies of the BiTE class, targeting CD19 or EpCAM in addition to CD3, induce T-cell activation exclusively in the presence of target cells.
Material and Methods: Peripheral blood mononuclear cells from healthy donors were prepared by Ficoll density centrifugation. In a typical experiment, PBMC were incubated for 24 hours with bispecific single chain antibody in presence or absence of specific target cells. Target cell lysis was determined by measurement of adenylate kinase activity released from dead cells. De novo expression of activation markers CD69 and CD25 on T cells was assessed by flow cytometry using directly conjugated monoclonal antibodies, concentration of cytokines in the supernatant was determined by a commercial FACS based bead array.
Results: Two distinct bispecific single-chain antibody constructs of the BiTE class, called MT110 and MT103 (or MEDI-538), were analyzed for conditional T cell activation. In the presence of target-expressing cell lines, low picomolar concentrations of MT110 and MT103 were sufficient to stimulate a high percentage of peripheral human T cells to express cytokines and surface activation markers, enter into cell cycle and to induce redirected lysis of target cells. However, in the absence of target cells, the two BiTE molecules did no longer detectably activate human T cells even at concentrations exceeding the ED50 for redirected lysis and conditional T cell activation by more than five orders of magnitude. In the case of MT110, T-cell activation was no longer observed with a cell line harbouring a single amino acid substitution in human EpCAM inactivating the binding epitope for MT110.
Conclusion: Our data show that T cell activation by monomeric forms of MT110 and MT103 is highly conditional in that it is strictly dependent on the presence of cells expressing the proper target antigen. BiTE molecules therefore qualify for a highly controlled polyclonal T cell therapy of cancer.

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Denosumab safety, pharmacokinetics (PK), and pharmacodynamics (PD) in a phase 1 study of Japanese women with breast cancer-related bone metastasis

H. Minami[1], K. Kitagawa[1], K. Yonemori[2], Y. Fujiwara[2], H. Fujii[3], T. Arai[3], M. Ohkura[4], G. Jang[5], T. Ohtsu[4]. *[1]National Cancer Center Hospital East, Kashiwa, Japan; [2]National Cancer Center Hosptial, Tokyo, Japan; [3]Tochigi Cancer Center, Utsunomiya, Japan; [4]Amgen KK, Tokyo, Japan; [5]Amgen Inc., Thousand Oaks, CA, USA*

Background: Receptor activator of NF-kB ligand (RANKL) is an essential mediator of osteoclast formation, function, and survival. Denosumab is an investigational fully human monoclonal antibody that inhibits RANKL. This open label, dose escalation study evaluated the safety, PK, and PD of denosumab in Japanese women with breast cancer metastatic to bone.
Methods: Patients (n = 18; 6/cohort) received either a single 60- or 180-mg SC dose of denosumab, or denosumab 180 mg SC every 4 weeks (Q4W) for 3 doses. Patients could receive concurrent chemotherapy or hormonal therapy if the regimen was stable. Adverse events (AEs) were monitored for up to 20 weeks. Serum concentrations of denosumab and levels of the bone resorption markers serum C-telopeptide (sCTx) and urine N-telopeptide/creatinine (uNTX) were measured.
Results: The AE profile in these breast cancer patients receiving denosumab was similar to that of advanced breast cancer patients receiving systemic treatment. The most common AEs were fatigue, anorexia, headache, malaise, and nausea. One serious AE of myositis occurred in the 180-mg dose group and, per the investigator, was attributed to a paraneoplastic syndrome, although a possible relationship to study drug could not be excluded. No deaths occurred during the study, and no patients developed antibodies to denosumab. The increase in exposure to denosumab observed between the 60-mg and 180-mg doses was approximately proportional. In the 180-mg Q4W group, approximately 2-fold accumulation was observed by the third dose. Suppression of sCTX and uNTX was rapid (within 48 hours), extensive (to levels observed in healthy adults), and sustained (for at least 12 weeks) after dosing. Reductions were similar across the dose groups with overall median (interquartile range) reductions in sCTX of 84.5% (73.1%, 91.7%) and in uNTX of 71.2% (41.2%, 88.5%) 12 weeks after dosing.
Conclusions: In this group of patients, the safety profile of denosumab given as a single dose or as multiple doses (Q4W for 3 doses) was similar to that expected in treated advanced breast cancer patients. Suppression of bone resorption markers was rapid, extensive, and sustained in all cohorts tested. Both the PK and PD results were comparable to those observed in non-Japanese women treated with similar doses of denosumab (Body, Clin Can Res 2006). Further investigation of denosumab treatment in women with advanced cancer and bone metastases will be conducted in a phase 3 trial.

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Mapatumumab, a fully human agonistic monoclonal antibody that targets TRAIL-R1, in combination with gemcitabine and cisplatin: a phase 1 study in patients with advanced solid malignancies

C.H. Mom[1], S. Sleijfer[2], J.A. Gietema[1], N.L. Fox[3], C. Piganeau[4], L. Lo[3], D.R.A. Uges[5], W. Loos[6], E.G.E. de Vries[1], J. Verweij[6]. *[1]University Medical Center Groningen, Medical Oncology, Groningen, The Netherlands; [2]Erasmus University Medical Center, Medical Oncology, Rotterdam, The Netherlands; [3]Human Genome Sciences, Rockville, USA; [4]Human Genome Sciences, Düsseldorf, Germany; [5]University Medical Center Groningen, Hospital Pharmacy, Groningen, The Netherlands; [6]Erasmus University Medical Center, Medical Oncology, Rotterdam, The Netherlands*

Background: Mapatumumab (HGS-ETR1, TRM-1) is a fully human monoclonal antibody that targets and activates the tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1). Adding mapatumumab to gemcitabine and cisplatin results in increased cytotoxicity in human tumor cell lines and mouse xenograft models. Prior phase 1 and 2 studies have shown that mapatumumab can be safely administered as a single agent. In this phase 1 study, the safety and tolerability of escalating doses of mapatumumab in combination with gemcitabine and cisplatin are being evaluated.
Methods: Patients with advanced solid malignancies received gemcitabine 1250 mg/m^2 IV on days 1 and 8, and cisplatin 80 mg/m^2 IV on day 1 every 21 days, for a maximum of 6 cycles. In case of clinical benefit, patients were allowed to continue on mapatumumab monotherapy. Following the first gemcitabine and cisplatin infusion, and concomitant in other cycles, mapatumumab was administered IV every 21 days. Planned dose escalation levels were 1, 3, 10 and 20 mg/kg (with extension of the 10 and 20 mg/kg cohorts to a maximum of 12 patients depending on toxicity). Toxicity was evaluated according to CTCv3.0. Tumor measurements were performed as appropriate. Pharmacokinetic analysis of plasma mapatumumab, gemcitabine, dFdU, unbound and total platinum was performed.
Results: To date, 26 patients have been enrolled at 1 mg/kg (n = 4), 3 mg/kg (n = 7), 10 mg/kg (n = 12) and 20 mg/kg (n = 3) mapatumumab. A median of 6 cycles (range 1–6 cycles) was administered. The adverse events most commonly observed reflect the toxicity profile of gemcitabine and cisplatin, with hematologic toxicity, nausea and vomiting, ototoxicity and liver function disturbances. To date, 4 patients have experienced transient grade 3 elevations of ALT (one in combination with AST elevation grade 3 and one with GGT elevation grade 3) and one additional patient has experienced a grade 3 elevation of AST. Gemcitabine was considered to be the most likely cause. In one patient with pancreatic cancer, bile duct obstruction resulted in ALT and GGT elevations. The 20 mg/kg cohort will be expanded to 12 patients. Eight patients have experienced a partial response thus far. Stable disease was seen in 12 patients. Pharmacokinetic analyses showed no signs of drug interactions.
Conclusions: The combination of mapatumumab with gemcitabine and cisplatin is safe in doses up to 20 mg/kg. Further phase 2 studies of this combination are warranted.

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Antitumor efficacy of DX-2400, a potent and selective human antibody MMP-14 inhibitor discovered using phage display technology

L. Devy[1], T. Arulanandam[2], D. Buckler[2], R. Finnern[2], L. Naa[1], D. Rank[2], R.C. Ladner[2], S. Rabbani[3], D.T. Dransfield[2], P. Henderikx[2]. *[1]Dyax s.a., Liege 1, Belgium; [2]Dyax corp., Cambridge, MA, USA; [3]Basic Cancer research, McGill University, Oncology, Montreal, Canada*

Selective inhibition of the activity of matrix metalloproteinases (MMPs) could provide an attractive non-cytotoxic approach towards improving the therapy of aggressive, metastatic cancers. To date, no MMP inhibitors have been approved probably for reasons of insufficient specificity and/or dose-limiting side effects. The challenge in the MMP field is to design highly potent and selective inhibitors with optimal pharmacokinetics and minimal toxicity. This provides a unique opportunity for protein-based inhibitors. Utilizing our human Fab-displaying phage library and using a recombinant active catalytic domain of human MMP-14 as a target, we have discovered DX-2400. DX-2400 binds and specifically inhibits MMP-14 activity (Ki = 0.9±0.3 nM) and does not inhibit activity of a panel of other MMPs tested. DX-2400 blocks pro-MMP2 activation on MMP-14/MMP-2 expressing cancer cells and therefore has the potential to inhibit extracellular matrix proteolysis. In addition, DX-2400 blocks in vitro invasion of select cancer cell lines through Matrigel. We evaluated the anti-cancer activity of DX-2400 in the orthotopic MDA-MB-231 breast cancer model. DX-2400 reduced tumor progression by 70% compared to an isotype control, with activity comparable to doxorubicin. Importantly, DX-2400 resulted in no body weight loss through the treatment. This specific