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

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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

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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

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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

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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