Secondary endpoint was the preliminary evaluation of the clinical response. This was an open label, uncontrolled, multi-centric Phase **I/II** clinical trial, in which patients received 6 weekly infusions of h-R3 at the dose of 200 mg in combination with external beam radiotherapy. Twenty-four patients, mean age 44 years, were enrolled in the trial. Primary tumors corresponded to glioblastoma multiforme (15 patients) and anaplastic astrocytoma (9 patients). All patients underwent debulking surgery or biopsy before entering the trial. No evidences of grade 3/4 adverse events were detected. One patient developed a serious adverse event that consisted in grade 2 dysphasia and sensory alteration. No acneiform rash or other dermatological toxicity was detected. In this patient set, 4 subjects (16.7%) have achieved complete response while 5 patients (20.8%) have reached partial response. In total, 20 patients (87.5%) achieved disease stabilization or an objective response. Overall survival from trial inclusion has increased after the combined therapy in comparison with the historical figures for standard radiation regimen and chemoradiation schemes. With a median follow up time from treatment beginning to the closeout date of 10.3 months (range 2.57 to 26.13 months), the mean and median survival for all the patients is 16.76 and 14.77 months.

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Targeting of human glioma xenografts with an anti-EGFRvIII minibody (MR1-1scFv-CH3)

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Background: Low tumor uptake and normal tissue toxicity after delivery limit the efficacy of radioimmunotherapy for the treatment of solid tumors. The glioma-associated variant EGFRv**III** molecule contains a unique antigenic sequence, which functions as a tumor specific epitope. We have genetically engineered a bivalent minibody reactive with the EGFRv**III** extracellular domain (ecd) that should display rapid tumor targeting and blood clearance.
Material and Methods: The high affinity anti-EGFRv**III** single-chain antibody MR1-1scFv was attached to the human IgG1 C_H3 domain via V_H using a modified human IgG_1 hinge peptide linker, LEPKSCDKTHTCP-PCGSGGGSGGGGSS. This minibody MR1-1scFv-C_H3 was expressed in *E. coli* and accumulated in inclusion bodies; recovered minibodies were properly refolded in a redox-shuffling buffer. The purified MR1-1 minibody had assembled into 80 kDa dimers as shown by size exclusion chromatography and MALDI-TOF-MS. The minibody was radioiodinated with *N*-succinimidyl 4-guanidinomethyl-3-[^{131}I]iodobenzoate (SGMIB; *Bioconjugate Chem.* 2001; 12: 428-38), a positively charged template known to enhance tumor retention of radioactivity from internalizing antibodies.
Results: The purified divalent minibodies retained the same specificity but had higher affinity for EGFRv**III** ecd (K_D 4.7×10^{-10} M) than univalent MR1-1scFv. The immunoreactive fraction of the SGMIB-labeled MR1-1 minibody was 73%. Binding affinity remained constant after incubation at 37ºC for 72 h. Tumor targeting properties were evaluated in athymic mice bearing s.c. U87MG ΔEGFR tumor xenografts. [^{125}I]SGMIB-MR1-1 minibodies demonstrated a maximum tumor uptake of 14% ID/g at 6 h following *i.v.* infusion. Radioiodinated minibodies also cleared rapidly from the circulation, yielding high tumor: blood ratios: 20:1 at 12 h and >100:1 at 24 h. In contrast, for intact anti-EGFRv**III** human/mouse chimeric antibody L8A4 (chL8A4), the tumor: blood ratio was 1.6 at 24 h.
Conclusions: The enhanced binding *in vitro* and better performance in biodistribution studies *in vivo* exhibited by the minibodies as contrasted to either scFv or intact chL8A4 is a reflection of the combined attributes of divalency and optimal clearance rates inherent in the 80 kDa minibody. We are currently investigating the specific localization and extent of distribution of these three molecules in intracranial microdiffusion models to choose the optimal construct for clinical trial in malignant glioma patients.

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Effects of erlotinib on HER2/HER3 receptor activation and downstream signaling events in cells lacking EGFR expression

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Erlotinib (Tarceva™), is an orally available, selective, reversible inhibitor of purified epidermal growth factor receptor (EGFR, HER1) tyrosine kinase which has shown inhibitory activity on purified HER2 kinase at much higher concentrations. The inhibition of HER1 kinase prevents receptor phosphorylation and activation of downstream signaling events. In vitro and in vivo studies show that erlotinib has an inhibitory activity against a variety of tumor types. Preclinical and clinical studies demonstrate that erlotinib responsiveness does not always correlate with EGFR expression levels. Additionally, there are studies that show erlotinib inhibits the growth of tumors driven by HER2 activation. To further elucidate the effect of erlotinib on HER2 signaling we generated an EGFR-HER2 chimeric receptor system that can be activated by exogenous TGFalpha. Erlotinib directly inhibited the TGFalpha-induced EGFR-HER2 kinase activity as well as the downstream signaling molecules MAPK and Akt at submicromolar concentrations. We also investigated whether erlotinib had an effect on the ligand dependent HER2/ HER3 activation in cells lacking endogenous HER1 expression. NR6 cells that are devoid of HER1 were stably transfected with HER2 and HER3. Upon erlotinib treatment, inhibition of heregulin induced receptor phosphorylation as well as inhibition of p42/p44 MAPK and Akt were seen in an erlotinib dose dependent manner. More importantly, erlotinib treatment suppressed ligand induced cell proliferation of HER2/HER3 expressing cells. In conclusion, in addition to erlotinib's inhibitory effects on EGFR dependent tumor proliferation, erlotinib may also effectively inhibit the growth of tumors driven by HER2 activation.

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Degradation of the epidermal growth factor receptor occurs upon cetuximab treatment

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The IgG1 anti-Epidermal Growth Factor Receptor (EGFR) monoclonal antibody Erbitux™ (cetuximab) has been shown to induce regression of certain colorectal carcinoma by inhibiting EGFR phosphorylation in both pre-clinical and clinical studies. To further understand the mechanism by which cetuximab inhibits EGFR activation, we studied the effects of cetuximab on EGFR internalization and degradation in the DiFi colorectal cell line. In dose response and time course experiments we detected both EGFR phosphorylation inhibition and receptor degradation in response to 3nM cetuximab treatment at 14hrs. In contrast, a small molecule inhibitor of the EGFR kinase domain only inhibited EGFR phosphorylation with no effect on EGFR degradation. Treatment with non-blocking anti-EGFR monoclonal antibodies induced EGFR degradation but did not prevent EGFR phosphorylation, indicating that degradation of EGFR is a phenomenon seen with antibodies to the receptor, but not small molecules. Treatment of DiFi cells with a proteasomal inhibitor, MG115, had no effect on EGFR degradation by cetuximab. However, data indicates that EGFR is ubiquitinated upon cetuximab treatment The present study suggests that in addition to the ability of cetuximab to block EGFR activation by prevention of ligand binding, it is also inducing degradation of EGFR. Our initial data suggests that the ubiquitin pathway may mediate this degradation.

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Generation of a recombinant humanized anti-insulin-like growth factor receptor type I antibody (h7C10) with an antitumor activity in a variety of human cancer xenograft models

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Interaction of Insulin-like growth factor receptor I (IGF-IR) with its ligands has been reported to induce cell proliferation, transformation and blockade of cell apoptotic functions. IGF-IR is overexpressed on numerous tumor cell types and its blockade could be of importance for anti-cancer therapy. To generate a humanized antibody, a set of murine monoclonal antibodies (MAb) has first been generated by immunizing BALB/c mice subcutaneously (s.c.) with a soluble α 2-β 2 heterotetrameric recombinant human IGF-IR. Resultant hybridomas were initially screened for secretion of anti-IGF-IR MAb by ELISA on the recombinant receptor and by FACS analysis on MCF-7 cells. Positive reactors were cloned and subsequently screened for their non reactivity against insulin receptor (IR), by FACS analysis on Sf9 cells (ATCC) infected with baculovirus constructs encoding either for IR or IGF-IR MAbs with a positive reactivity on IGF-IR cells and a negative one on IR cells were evaluated for their growth inhibiting activity *in vitro* and *in vivo*. We have identified a monoclonal antibody 7C10 that recognizes specifically IGF-I receptor and not insulin receptor. To explore the activity of anti-IGF-IR antibodies on *in vivo* tumor growth, we analyzed their effect *in vivo* on various xenograft tumor models
Treatment of nude mice bearing either human breast cancer cells (MCF-7), prostate cancer cells (DU145), osteosarcoma cells (SKES1) or non small lung cancer cells (A549) with 7C10 inhibited significantly tumor growth. Among all the anti-IGF-IR antibodies generated, 7C10 was the most efficacious to diminish tumor volumeThe anti-IGF-IR antibody administration was non-toxic, as indicated by non-modified animal survival