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.

283 POSTER

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.

284 POSTER

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.

285 POSTER

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.

286 POSTER

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

and weight loss 7C10 was humanized and h7C10 shares the same *in vivo* properties as 7C10. The present results indicate that the humanized anti-IGF-IR antibody h7C10 has a great potential for cancer therapy.

287 POSTER

A phase Ib study of pertuzumab (P), a recombinant humanized antibody to HER2, and capecitabine (C) in patients with advanced solid tumors

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Background: P represents the first in a new class of targeted therapeutics known as HER dimerization inhibitors (HDIs). It blocks ligand-associated heterodimerization of HER2 with other HER-kinase family members (HER1, HER3 and HER4), and thereby inhibits intracellular signaling through MAPkinase and PI3kinase. This phase Ib study of the combination of P and C is being performed to determine the maximum tolerated dose (MTD), to assess the safety profile and dose limiting toxicities (DLTs), to evaluate if there is pharmacokinetic (PK) interaction of the combination and to determine any anti-tumor activity.
Patients and Methods: DLT is assessed in cycle 1 and is defined as: non-hematological toxicity $\geqslant$ grade 3, grade 4 neutropenia of > 7 days, thrombocytopenia grade 4 or any thrombocytopenia requiring platelet transfusion, any subjectively intolerable toxicity felt to be related to either one of the compounds. If DLT is observed in $\geqslant$ 2 pts out of 6 pts at a dose level, the MTD has been exceeded. Selection criteria are: performance status (PS) ECOG 0 or 1, measurable or evaluable disease, baseline LVEF $\geqslant$ 50%, adequate bone marrow-, hepatic- and renal function, no prior therapy with C, other oral fluoropyrimidines or infusional 5-FU > 48 hrs, no history of cardiac failure or poorly controlled cardiovascular disease. Patients are treated with a fixed dose of 1050 mg of P administered as an IV infusion on day 1, and C administered orally bid on days 1–14, q 3 weeks. In combination with P, three dose levels of C are explored: 825, 1000, and 1250 mg/ m* bid.
Results: To date, a total of 7 pts (2 male/5 female) have been included. Mean age 59 years (range 39–68), ECOG PS 0/1: 4/3 pts. Tumor types: breast, ovarian, hepatocellular, colorectal, prostate, fallopian tube and pancreatic cancer. To date, 5 pts have been treated at dose level 1 and 2 pts at dose level 2. A total of 16 cycles have been administered, median 2 (range 1–5). No DLTs have been observed. Most frequent toxicities included: diarrhea, hand-foot syndrome and asthenia, all of grade 1 or 2, in 44%, 31% and 44% of cycles, respectively. Other toxicities were nausea, vomiting, anorexia and mucositis, all of grade 1, in 6% of cycles each. Preliminary data of 2 pts in the first cohort suggest that PK parameters of C are not altered in combination with P. Tumor assessment has been performed in 5/7 pts: 3 pts had stable disease and 2 progressed.
Conclusion: MTD is to be determined and recruitment is ongoing. Results will be updated at the meeting.

288 POSTER

A trivalent bispecific fusion protein produced in myeloma cells for improved pretargeting and therapy of CEA-expressing cancers

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Background: A novel trivalent bispecific agent for improved pretargeted delivery of radionuclide payloads carried by bivalent haptens to tumors expressing carcinoembryonic antigen (CEA) has been produced in myeloma cells. Pretargeting with BS14HP, a trivalent bispecific fusion protein expressed in *Pichia pastoris*, which binds divalently to CEA, resulted in a 3-fold increase in tumor uptake of radio-peptide as compared to constructs with monovalent CEA binding. Here, a construct similar in design to BS14HP was produced in myeloma cells.
Methods and Results: hBS14 is an 80-kDa recombinant fusion protein consisting of two heterologous polypeptide chains associated non-covalently to form two binding sites for CEA from the variable domains of hMN-14 (a humanized anti-CEA antibody; labetuzumab) and one binding site for histamine-succinyl-glycine (HSG) from the variable domains of h679 (a humanized anti-HSG antibody). The V-domains were engineered into a single DNA construct that was stably transfected into SP2/0 cells. Bispecificity was demonstrated on BIAcore using an HSG-coupled sensor chip by measuring the additional increase in response units upon successive injections of hBS14 followed by an anti-id antibody to hMN-14. SE-HPLC analysis of the binding between CEA and hBS14 demonstrated two functional CEA binding groups of hBS14. The efficacy of hBS14 for tumor pretargeting was evaluated in CEA-expressing GW-39 human colonic tumor-bearing nude mice using a bivalent HSG hapten (IMP-245) labeled with ^{99m}Tc. Forty-eight hours after mice were given the hBS14, the ^{99m}Tc-peptide was administered. Animal groups were then imaged at 1, 3, and 24 h, or necropsied at 1, 4, or 24 h. Excellent high contrast images were obtained as early as 1 h after injection of ^{99m}Tc peptide, with tumor uptake at 21% [$\pm$ 2.5] ID/g while other tissues such as liver (1.1%ID/g) and blood (1.6%ID/g) showed very low activity. Although IMP-245 is excreted very rapidly by urinary clearance, tumor/kidney ratios were 2.7 [$\pm$ 0.5] allowing for clear delineation of the tumor compared to the kidney. Image contrast improved over time with only tumor signal detectable after 24 h.
Conclusion: These results indicate that hBS14 is an attractive candidate for use in a variety of pretargeting applications, particularly tumor therapy with radionuclides and drugs. The very early visualization of tumors suggests that this technique could be used with SPECT and PET imaging systems with a suitably radiolabeled peptide. [Supported in part by PHS grant EB002114.]

289 POSTER

Peptide-targeted alpha-radiation for melanoma therapy

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A unique combination of a melanoma targeting peptide and an α-particle-emitting radioisotope were investigated for their melanoma therapy potential. The alpha-emitting radionuclide ^{212}Bi was targeted to melanoma tumors by the DOTA-ReCCMSH peptide, which binds melanocortin-1 receptors expressed on melanoma cells. Radiotherapeutic treatment of relatively radio-resistant melanoma cells with peptide targeted alpha particles is attractive due to the high linear energy transfer properties of alpha radiation: dense ionization with irreparable DNA double strand breaks, lack of oxygen effects and enhanced efficiency by cell internalization of peptide. The DOTA-ReCCMSH peptide was radiolabeled with ^{212}Pb in a 0.05 M NaOAc solution at pH 5.5 at 85ºC for 45 min. The ^{212}Pb[DOTA]-ReCCMSH product was purified by reverse phase high performance chromatography and stabilized in buffered saline with ascorbic acid. Lead-212 ($t_{1/2}$=10.6 h) is the parent of ^{212}Bi ($t_{1/2}$=60.6 min) that decays to stable ^{208}Pb via an alpha beta decay sequence. The radioisotopes were eluted from a ^{224}Ra-^{212}Pb/^{212}Bi radionuclide generator. Biodistribution and therapy studies were performed in a B16/F1 melanoma bearing C57 mouse flank tumor model.
Biodistribution studies demonstrated that the radiolabeled peptide rapidly accumulated in the tumor reaching a maximum level of 13.49% injected dose per gram (ID/g) at 5 min. Tumor activity levels remained constant over 4 hrs then gradually declined to 4.59% ID/g 24 h post injection. Normal organ disappearance was rapid as the peptide is primarily cleared by the kidneys. The radiation dose delivered to the tumor was estimated to be 61 cGy/uCi ^{212}Pb administered.
Therapy studies were performed in tumor bearing mice 4 days post melanoma cell implantation when tumors were palpable. Groups of mice (n=8–10) were given 50, 100 and 200 uCi of ^{212}Pb[DOTA]-ReCCMSH or a saline placebo via the tail vein. The mice tolerated all dose levels with no observable signs of acute toxicity. Survival data were evaluated according to the method of Kaplan and Meier. Placebo treated mice had a 14.6 day mean survival. Treatment with 50 uCi and 100 uCi doses extended mean survival to 22.0 days (p=0.004) and 28.0 days (p=0.002), respectively. The 200 uCi treatment group exhibited the best survival statistics (45.0 days mean survival, P=0.01). Forty-four percent of the mice receiving a 200 uCi dose and twenty percent of the mice from the 100 uCi treatment group were free of tumor and survived the entire 100 day study. These striking results highlight the therapy potential of peptide-targeted α-radiation for malignant melanoma.