responses and one partial response in the 7 animals treated at 10 mg/kg. IMGN388 also demonstrated efficacy against established human tumors of colon (HT-29), large cell lung (H460), pancreatic (AsPC-1), ovarian (A2780, SKOV-3), and breast (MDA-MB-231.OT.F2) carcinomas in nude rat models. Additionally, IMGN388 has been found to inhibit angiogenesis using an in vivo model of basic fibroblast growth factor-induced angiogenesis in nude rats. Thus, the anti-tumor effects of IMGN388 can be attributed to two distinct mechanisms of action: direct tumor-cell killing and anti angiogenic activity.
Conclusion: The broad expression of the target integrin among solid tumors and the observed anti-tumor efficacy of IMGN388 in xenograft models of pancreatic, colon, lung, breast, and ovarian carcinomas in rats support the clinical evaluation of IMGN388 for the treatment of solid tumors.

530 POSTER

Use the humanized anti-EGFR MAb (nimotuzumab) and radiotherapy for the treatment of high grade glioma patients

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Background: The incidence of brain tumors is worldwide increasing and despite advances in neurosurgery and radiotherapy, limited progress has been made in the treatment of patients with high-grade gliomas. Amplification and rearrangement of the Epidermal Growth Factor Receptor (EGFR) have been found in primary high-grade astrocytomas. For primary brain tumors, over-expression of EGFR has been associated with poor survival due to growth advantages. Nimotuzumab is a humanized monoclonal antibody that recognizes EGFR with high affinity, inhibiting tyrosine kinase activation.
Material and Methods: A Phase II/III clinical trial was conducted to evaluate the efficacy and safety of nimotuzumab in combination with radiotherapy in newly diagnosed high-grade glioma patients. It was a multicentric, controlled, double blinded trial where 80 patients bearing high grade glioma were randomized to receive radiotherapy and nimotuzumab or irradiation plus a placebo. Patients received 6 weekly infusions of the placebo or nimotuzumab (200 mg) while they were receiving radiotherapy. After irradiation, patients received a maintenance dose of the investigational drug, every 21 days until completing a year of treatment.
Results: So far, 65 patients have been enrolled, 30 patients bearing glioblastoma and 35 bearing anaplastic astrocytomas. Fifteen additional anaplastic astrocytoma patients should be enrolled to finish the trial. All patients had surgery (biopsy, partial or total resection) before the inclusion in the trial. Both groups were very well balanced in relation to the factors that predict the outcome of the disease: Karnofsky index, previous surgery and age. Since the enrollment of the glioblastoma stratum is finished, a preliminary evaluation of safety and survival was done. The antibody was very well tolerated. Adverse events were more frequent in the placebo arm as compared to the nimotuzumab arm. The antibody did not provoke skin rash or allergic reactions. A preliminary survival analysis was done for all subjects bearing glioblastoma that received curative intent radiotherapy. The mean and median survival time for the patients treated with nimotuzumab plus radiotherapy was 14.31 and 16.43 months, respectively, while the mean and median survival time for the placebo arm was 8.67 and 10.49 months. The median survival time is similar to the one reached in the previous single arm study in patients bearing glioblastoma treated with nimotuzumab and radiotherapy (17.43 months) and compares favorably with the overall survival after irradiation and temozolomide (14.6 months). The evaluation of human anti-humanized antibody response (HAHA) is ongoing.
Conclusions: For the subgroup of glioblastoma multiforme patients, nimotuzumab combined with radiotherapy was safe and showed a trend toward a survival benefit as compared to the placebo arm.

531 POSTER

Junctional complexes as a factor limiting the extravascular penetration of trastuzumab

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The tumor microenvironment presents many barriers to drug penetration, including abnormal microvessel structure and function, deficient or absent lymphatics and variable extracellular matrix composition. Previously, using immunohistochemical mapping of MDA-435/LCC6^{HER2} xenografts we found the extravascular distribution of trastuzumab (generic, Herceptin®), to be incomplete and highly heterogeneous. To characterize properties of the tumor microenvironment that govern trastuzumab penetration, we extended these studies using HER2 over-expressing MCF-7 breast cancer cells (MCF-7^{HER2}) and a tight junction marker, ZO-1. Additionally, we used multilayered cell cultures (MCCs) in conjunction with transmission electron microscopy (TEM) to assess trastuzumab penetration through MCF-7^{HER2} tumor cells in vitro.
In mapping studies, mice bearing MCF-7^{HER2} tumors were given single doses of 4 mg/kg trastuzumab with tumor harvest at various time points thereafter; bound trastuzumab was imaged in tumor cryosections using fluorescent anti-human secondary antibodies. Combinations of additional markers, including HER2, 5-bromo-2-deoxyuridine, CD31, DioC7, and ZO-1 were also mapped on the same tumor sections. MCF-7^{HER2} MCCs were exposed from both sides to 60ug/mL trastuzumab for 1−24 h before drug removal, washing, and freezing. MCCs were cryosectioned and immunostained for trastuzumab, HER2, and ZO-1. For TEM studies untreated MCCs were fixed in gluteraldehyde, treated with osmium tetroxide and embedded in epoxy resin; ultra-thin 60 nm sections were imaged.
Similar to the MDA-435/LCC6^{HER2} model, 4 mg/kg trastuzumab did not saturate MCF-7^{HER2} tumors even after 72 h following administration. Trastuzumab exposure to both sides of MCF-7^{HER2} MCCs revealed an interesting phenomena wherein trastuzmab penetrated from only one surface of the discoid culture, despite ubiquitous HER2 expression. Staining for ZO-1 revealed the presence of continuous tight junctions along the surface of the culture disallowing trastuzumab penetration. TEM images confirmed the existence of tight junctions along the surface of MCF-7 MCCs. This suggests paracellular transport is required for trastuzumab penetration and implicates the need for structurally aberrant vasculature within tumors to facilitate the extravascular distribution of trastuzumab. This observation warrants further investigation into the junctional complexes of tumor tissue and endothelium as a factor limiting the penetration of anti-cancer agents.

532 POSTER

A chimerized anti-CD4 monoclonal antibody for the treatment of T cell lymphomas acts through activation of membrane acid sphingomyelinase leading to increased ceramide release and CD4/ZAP-70 protein redistribution in membrane rafts

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Background: recombinant IgG1 antibody 13B8.2 (rIgG1 13B8.2) binds to the CDR3-like loop on the D1 domain of CD4, and both inhibits proliferation and induces complement- and antibody-dependent cell cytotoxicity of T lymphoma cells. The biological effects of rIgG1 13B8.2 are partly due to signals that prevent NF-κB nuclear translocation, but precise mechanisms of action, particularly at the level of membrane proximal-signalling, remains obscure.
Materials and Methods: upon crosslinking of Jurkat T lymphoma cells with rIgG1 13B8.2, membrane rafts were extracted using Brij98 as detergent at 37ºC and subsequently separated by sucrose gradient centrifugation. Protein analysis was performed by western blot using appropriate antibodies. Lipid composition was measured by using Amplex red kits for cholesterol and acid sphingomyelinase (Invitrogen), and thin-layer chromatography.
Results: rIgG1 13B8.2 was found to induce an accumulation/retention of the CD4 molecule inside Brij 98 detergent-resistant membrane rafts, together with recruitment of TCR, CD3, p56 Lck, Lyn and Syk p70 kinases, LAT and Cbp/PAG adaptor proteins, and PKCtheta, but excluded ZAP-70 and its downstream targets SLP-76, PLCgamma1, and Vav-1. Analysis of key upstream events such as ZAP-70 phosphorylation showed that modulation of Tyr292 and Tyr319 phosphorylation occurred concomitantly with 13B8.2-induced ZAP-70 exclusion from the membrane rafts. rIgG1 13B8.2 did not affect membrane cholesterol but increased ceramide synthesis in membrane raft, in correlation with enhanced acid

sphingomyelinase activity. Treatment of T lymphoma cells with bacterial sphingomyelinase altered raft distribution of CD4 and ZAP-70, similarly to those observed following CD4 antibody cross-linking. Membrane rafts, mainly comprising C16:0 and C18:0 ceramides, also demonstrated reduced phosphatidylserine level following CD4-specific antibody treatment.
Conclusion: to induce its therapeutic effects, baculovirus-expressed CD4 monoclonal antibody 13B8.2 could activate membrane acid sphingomyelinase leading to increased ceramide release, and subsequent protein redistribution in membrane rafts. The analysis of the lipid-protein rheostat in membrane rafts upon treatment with biotechnological drugs could open new strategies for raft-based therapeutics in oncology.

533 POSTER

In vivo stability in mice of SAR566658 (huDS6-DM4), an immunoconjugate targeting solid tumours

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SAR566658 (huDS6-DM4) is a conjugate consisting of the cytotoxic maytansinoid, DM4 linked to the humanized antibody DS6, which targets a Muc1 sialoglycotope on solid tumors such as breast, ovarian, and pancreatic. The plasma clearance of SAR566658 in mice was evaluated using ELISA-based assays for total antibody and conjugate at various times after administration of a single bolus injection of 20 mg of SAR566658. The clearance is characterized by biphasic pharmacokinetics, with an initial distribution phase of 8 to 24 hours followed by a terminal elimination phase with the half-life for SAR566658 being about 5 days. The volume of distribution (90 mL/kg) suggests that the conjugate is essentially confined to the plasma compartment. The slow clearance and small volume of distribution of the conjugate is in sharp contrast to that observed for the unconjugated DM4, which has a terminal half-life of 2 hours and a volume of distribution of 3−10 L/kg. The clearance of the antibody moiety of the conjugate was about 1.7-fold slower than that measured for the conjugate indicating that the number of DM4 molecules per antibody molecule slowly declines during circulation. Therefore, the conjugate clearance rate is influenced by both the clearance of antibody as well as the slow loss of DM4.
Plasma samples were also analyzed for changes in the DM4 distribution profile by mass spectrometry (MS) after protein A-HPLC purification of antibody and conjugate. The DM4 distribution profile showed that SAR566658 is a mixture of conjugate species having various numbers of DM4 molecules per antibody. Over time in circulation, the profile shifts to species with lower DM4 molecules per antibody, consistent with the clearance results as measured by ELISA. UV spectroscopic determination of the DM4/antibody ratio also confirmed the ELISA and MS results, suggesting that 20−30% of the conjugated DM4 is lost during 2 days in circulation. The integrity of the antibody-linked DM4 during circulation was demonstrated by HPLC analysis following release of the DM4 by reduction with TECP.
These results demonstrate that SAR56658 is stable in circulation with pharmacokinetic properties similar to those of humanized antibodies. These favorable pharmacokinetics likely contribute to the profound difference in anti-tumor activity comparing SAR566658 and unconjugated DM4 in tumor xenograft models in mice and provide support for the clinical development of SAR566658.

534 POSTER

Expression profiling demonstrates co-stimulatory activity of BMS-663513, an anti-CD137 antibody

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Background: CD137 (4−1BB) is a major co-stimulatory receptor that promotes the survival and expansion of activated T cells. Anti-tumor activities have been reported in murine models using agonistic anti-CD137 antibodies to enhance anti-tumor immune responses. BMS-663513 is an agonistic monoclonal antibody specific to human CD137 currently under clinical development by BMS for use as an anti-tumor therapeutic agent. To further understand role of the anti-CD137 antibody in T-cell activation, we used gene expression profiling to study the effects of BMS-663513 in a human T-cell line.
Material and Methods: The CEM T-cell line was used as a model for evaluating T-cell activation from BMS-663513 treatment. CEM cells were treated on a time course with BMS-663513 at two concentrations with or without an anti-CD3 antibody. A matching IgG_4 isotype control was analyzed as specificity control in parallel. Gene expression data were measured by Affymetrix HT_HG-U133A chips and analyzed with bioinformatics and statistics methods.
Results: Robust transcriptional responses were observed in BMS-663513 treated CEM cells in a time and dose-dependent manner. The observed changes were specific, requiring both anti-CD137 and anti-CD3 signals. BMS-663513 induced transcriptional responses that increase with time, starting with low-level gene expression changes at 16 hours and becoming robust by 72 hours. Many of the regulated transcripts were for genes linked to pathways known to be critical for T-cell activation and differentiation, for example the MAPK and NFkB pathways. BMS-663513 also induced the expression of many anti-apoptosis genes, for example Bcl2l1. Other genes up-regulated by BMS-663513 included cytokines and secreted cytotoxic proteins reported to have anti-tumor activities (IL23A and TRAIL). Interestingly the expression of OX40 and OX40L transcripts were also up-regulated by BMS-663513, suggesting cross-regulation with other T-cell co-stimulatory signals.
Conclusions: The described gene expression data in the CEM cell line support the hypothesis that BMS-663513 provides specific co-stimulatory signals for T-cell activation, differentiation and control of apoptosis, and is consistent with the proposed therapeutic mechanism of action. As well, the expression array data provide new insight into signaling pathways resulting from direct engagement of CD137.

535 POSTER

Characterization of a fully human PDGFRa antibody that reduces tumor growth and stromal infiltration in a xenograft model of non-small cell lung cancer

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PDGFRa is a receptor tyrosine kinase that promotes cell survival and growth, and is expressed in both the tumor and the stromal components of multiple human cancers. We have developed a fully human monoclonal antibody (MEDI-575) that binds to human PDGFRa with high affinity and selectivity, with no significant affinity for either murine PDGFRa or human PDGFRb. In order to more fully characterize the role of PDGFRa in the regulation of tumor stroma, we characterized the in vivo antitumor effects of MEDI-575 in tumor-bearing SCID mice and in genetically altered SCID mice expressing human PDGFRa in place of murine PDGFRa (h-PDGFRa/SCID). We utilized the Calu-6 non-small cell lung cancer line for these studies, since it lacks an in vitro proliferative response to PDGFRa activation. Antitumor efficacy was observed when the study was performed in h-PDGFRa/SCID, but not in the regular SCID mice expressing the murine receptor. Immunohistological analysis of tumors from h-PDGFRa/SCID showed a highly significant reduction in stromal fibroblast content and only minor changes in tumor proliferative index in tumors exposed to MEDI-575 compared to vehicle-treated tumors. Additional in vitro studies with primary cancer-associated human fibroblasts indicated that MEDI-575 can directly impact key signaling pathways in these stromal cells. These results highlight the potential for observing antitumor activity with MEDI-575 through modulation of the stromal component of tumors and confirm that the PDGFRa pathway plays significant role in maintaining a tumor microenvironment conducive to tumor growth.

536 POSTER

Comparison of the tumor growth inhibitory effects of tumor cell and non-tumor cell EGFR targeted antibodies in cancer models

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In tumors EGFR can be expressed by tumor cells as well as endothelial cells lining blood vessels. The extent to which targeting tumor blood vessel EGFR or tumor cell EGFR contributes to the overall antitumor effects of EGFR antibody therapy is a matter of debate. To address this issue, we have developed a rat monoclonal antibody, ME1, that specifically targets mouse EGFR. We have compared the antitumor effects of ME1 to that of a chimeric antibody specific to human EGFR, cetuximab, in subcutaneous xenograft cancer models. To evaluate treatment effects, tumor growth and blood vessel density were evaluated.
In the OVCAR-5 ovarian cancer model, ME1 (60 mg/kg twice weekly) did not significantly inhibit tumor growth (T/C% = 71, $p = 0.07$). In contrast,