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.

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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.

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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.

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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,