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

M.F. Mayo[1], A.P. Leung[2], L. Wang[2], P. Wunderli[3], G. Payne[4], H. Xie[1], R.J. Lutz[5]. *[1]ImmunoGen Inc., Pharmacology, Waltham, USA; [2]ImmunoGen Inc., Analytical, Waltham, USA; [3]ImmunoGen Inc., Biological Evaluation, Waltham, USA; [4]ImmunoGen Inc., Translational Research, Waltham, USA; [5]ImmunoGen Inc., Preclinical Development, Waltham, USA*

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

H. Chang[1], L. Obenauer-Kutner[1], A.Q. He[1], G. Xing[1], A. Truong[1], P. Kayne[1], A. Flesher[1], N. Siemers[1], M. Jure-Kunkel[1], M. Grace[1]. *[1]Bristol-Myers Squibb Co., Research & Development, Princeton, USA*

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

N. Laing[1], B. McDermott[1], S. Wen[1], M. Pandya[1], A.M. Mazzola[1], D. Lawson[1], P. Hall[1], A. Drake[2], S. Klakamp[2], Z. Cao[1]. *[1]AstraZeneca Pharmaceuticals, Cancer Biosciences, Waltham, MA, USA; [2]MedImmune, Translational Sciences, Hayward, USA*

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

D. Surguladze[1], M.J. Plym[1], I. Duignan[1], M. Prewett[1], D.J. Hicklin[2], Y. Wu[3], L. Witte[4], J.R. Tonra[1]. *[1]ImClone Systems Incorporated, Preclinical Pharmacology, NYC, USA; [2]ImClone Systems Incorporated, Experimental Therapeutics, NYC, USA; [3]ImClone Systems Incorporated, Antibody Technology, NYC, USA; [4]ImClone Systems Incorporated, Research, NYC, USA*

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,

cetuximab (60 mg/kg, twice a week) significantly inhibited the growth of OVCAR-5 tumors with a T/C% of 48 ($p = 0.0001$). When both antibodies were combined no additional antitumor benefits were observed (T/C% = 44; $p = 0.22$ versus ME1, $p = 0.23$ versus cetuximab). No partial tumor regressions were observed with any treatment. Tumor histological analysis utilizing MECA 32 staining revealed that ME1 treatment had no effect on blood vessel density in this model ($p = 0.72$), while cetuximab significantly ($p = 0.015$) decreased blood vessel density compared to the control group. The combination of both ME1 and cetuximab showed a trend towards greater antivascular effects compared to cetuximab monotherapy, but the difference did not reach statistical significance ($p = 0.20$). These data suggest that the antivascular effects following EGFR inhibition in the OVCAR-5 model result from targeting tumor cell EGFR.
In the GEO colorectal cancer model, ME1 and cetuximab significantly inhibited tumor growth with T/C% values of 61 ($p = 0.039$) and 31 ($p < 0.0001$), respectively. The combination of ME1 and cetuximab resulted in significantly increased efficacy compared to monotherapies (T/C% = 19; $p < 0.0001$). Moreover, the combination was associated with a partial tumor regression frequency of 83%, compared to 17% with cetuximab and 25% with ME1. Histological analysis in the GEO model is pending. Nevertheless, results support the conclusion that while targeting EGFR expressed outside the tumor cell, presumably on the endothelial cells, contributes towards the antitumor effects of an EGFR targeting antibody strategy, antitumor effects are mostly related to targeting tumor cell EGFR.

537 POSTER

Detection of EGFR mutation in the sample of pleural effusion is contributive as a determinant of EGFR-TKI-therapy for the patients with lung cancer

A. Uchida[1], T. Shibayama[1], S. Takahashi[1], N. Hamada[1], N. Kawata[1], A. Tada[1], R. Soda[2], K. Takahashi[2]. *[1]NHO Minami-Okayama Medical Center, Respiratory Medicine, Tsukubo-gun, Japan; [2]NHO Minami-Okayama Medical Center, Internal Medicine, Tsukubo-gun, Japan*

Background: Activating mutations in the epidermal growth factor receptor (EGFR) underlying responsiveness of non-small cell lung cancer (NSCLC) to EGFR tyrosine kinase inhibitor (TKI). Recently, EGFR mutation can be easily examined using cytological specimens.
Methods: We examined the EGFR mutation status in eight pleural effusion samples from the patients with NSCLC using peptide nucleic acid-locked nucleic acid (PNA-LNA) PCR clamp assay in our institution.
Results: Eight patients (Median age; 69 y.o. [ranging from 50−82 y.o]; man/female: 6/2; smoker/non-smoker 2/6; adeno/adenosquamous 7/1; ECOG performance status 2/3/4: 3/4/1), were enrolled. PNA-LNA PCR clamp assay detected three EGFR-mutative cases, all of which represented exon21 L858R in EGFR and the rest five showed wild type EGFR. Two of the three patients carrying L858R mutation and one patient with were treated with gefitinib. One patient with the wild type EGFR was treated with erlotinib. Consequently, all of the patients treated with EGFR-TKI revealed clinical response, and side effects were tolerable.
Conclusion: Pleural effusion is a common complication of advanced lung cancer, which is easily obtained from the patients. The results suggest that detection of the EGFR mutations by PCR from these samples is considered to be useful as a choice of application of EGFR-TKI for the advanced therapy.

538 POSTER

ARH460-16-2, targeting the CD44 cancer stem cell marker, uses multiple mechanisms to achieve its therapeutic anti-cancer effects

L.A.G. da Cruz[1], K. O'Reilly[2], F. McConkey[2], X.W. Wang[3], T. Antoshchenko[4], N. Aziz[1], A.K. Gupta[5], S.E. Hahn[6], D.S. Pereira[6], D.S. Young[6]. *[1]ARIUS Research Inc., Antibody Characterization, Toronto, Canada; [2]ARIUS Research Inc., Assay Support, Toronto, Canada; [3]ARIUS Research Inc., In Vivo, Toronto, Canada; [4]ARIUS Research Inc., Antibody Production, Toronto, Canada; [5]ARIUS Research Inc., Antibody Engineering, Toronto, Canada; [6]ARIUS Research Inc., Vice-President Product Development, Toronto, Canada*

CD44 is expressed on the cancer stem cells of breast, colon, prostate, head and neck, pancreatic cancer and AML where it is thought to play key roles in tumor maintenance, cell survival, adhesion, migration and invasion. The murine, chimeric, and humanized versions of a highly effective anti-CD44 anti-cancer antibody (ARH460-16-2) have similar binding, apogenic and anti-tumor properties. A murine monoclonal antibody was generated using the ARIUS' FunctionFIRST™ platform and demonstrated potent anti-tumor efficacy and a significant increase in survival time in models of human breast, liver, prostate and pancreatic cancer and AML. A dose-ranging toxicology study was carried out in cynomolgus monkeys with the chimeric form of the antibody and revealed no demonstrable dose limiting toxicity. Based on these results a humanized version of the antibody was generated for clinical testing. The mechanism by which ARH460-16-2 exhibits anti-tumor activity was studied. ARH460-16-2 directly inhibited tumor cell adhesion to hyaluronic acid (HA)-coated surfaces. Blocking of the binding of MB-231 breast cancer cells to HA-coated surfaces is consistent with the role of CD44 in promoting adhesion and may have important physiological effects and include blocking of motility and/or migration of tumor cells, and induction of apoptosis. ARH460-16-2 induced apoptosis in several cancer cell lines and suppressed phosphorylation of the Tie-1 receptor tyrosine kinase in breast cancer cells. CD44 is involved in cell survival in different cell types through distinct mechanisms, and induction of early apoptosis events by ARH460-16-2, as assessed by Annexin-V staining, in MB-231, MB-468 and in HUVEC cells, indicate that the antibody has an apogenic effect. The apogenic effect of ARH460-16-2 in HUVEC cells and decreased Tie-1 phosphorylation in MB-231 cells is consistent with the anti-neoangiogenic effect of CD44 deficiency in the tumors of tumor-bearing CD44 knockout animals. ARH460-16-2 may also recruit effector cells to enhance tumor ADCC. In vitro assays showed that the antibody failed to induce complement activation; however, in ADCC assays there was evidence of increased lysis of target cells. These results show that ARH460-16-2 can affect tumor growth by multiple mechanisms, and further studies to characterize the impact of the antibody on cell signaling pathways are ongoing. Humanized ARH460-16-2 is currently in development for treatment of solid and hematological cancers.

539 POSTER

Translational pharmacokinetic (PK), pharmacodynamic (PD) modeling and simulation analysis of MetMAb

B. Bender[1], H. Xiang[2], A.E. Reyes II[2], L.A. Damico[2], M. Merchant[3], A. Peterson[4], W. Forrest[5], N.L. Jumbe[1]. *[1]Genentech Inc, Clinical PK/PD, SSF, USA; [2]Genentech Inc, Early Dev. PK/PD, SSF, USA; [3]Genentech Inc, Translational BioOncology, SSF, USA; [4]Genentech Inc, Clinical BioOncology, SSF, USA; [5]Genentech Inc, NonClinical Biostatistics, SSF, USA*

Background: MetMAb is a recombinant humanized, monoclonal, monovalent (one armed) anti Met antibody with potential as a novel targeted therapy for cancer. The goal of this analysis was to predict a minimally effective MetMAb dose regimen for objective response using cynomolgus monkey PK and KP4 xenograft mice anti tumor efficacy data.
Materials and Methods: Human MetMAb serum concentrations were predicted using species invariant time transformations of cynomolgus monkey data (0.5, 3, 10, and 30 mg/kg MetMAb), and nonlinear mixed effects modeling of composite individual animal data. A separate mixed effects PK/PD model describing anti-tumor efficacy in KP4 xenograft mice was also developed from single and time dose-fractionated multiple dose regimens of MetMAb (0.825–120 mg/kg). The predicted human PK model was subsequently imposed on the established MetMAb exposure/anti-tumor activity relationship for clinical simulations of expected tumor responses at various treatment dose regimens. The exposure/target predictor of clinical treatment success, i.e. 'progression-free objective response defined as = 20% increase in tumor mass, was identified by classification and regression tree analysis. Additionally, simulated tumor responses were analyzed by Cox proportional hazards tests.
Results: A population PK/PD model, comprised of a two-compartment nonlinear PK model with direct KP4 tumor growth inhibition, was developed for MetMAb. The probability of attainment of 'progression-free objective response' was achieved at an AUC/tumoristatic concentration ratio = 16 in an individual patient as determined from analysis of projected clinical MetMAb exposure/anti-tumor activity simulations. Progression-free time to event analyses of simulated MetMAb tumor responses, compared with an identified standard of care, showed that the dose regimens of = 12.5 mg/kg once weekly (Q1W) and = 20.0 mg/kg once every 3 weeks (Q3W) increased treatment success (hazard ratio = 0.75) and achievement of the MetMAb exposure/target.
Conclusions: MetMAb dose regimens demonstrating a target stable disease treatment outcome of = 20% increase in tumor mass were determined via modeling and simulation methodologies which utilized efficacy data from KP4 xenograft mice and PK data from monkeys. Dose regimens of = 12.5 mg/kg Q1W and = 20.0 mg/kg Q3W are projected to result in a significant improvement in progression free disease (hazard ratio = 0.75) over an identified standard of care.