290 POSTER

Pharmacokinetics and tissue biodistribution of a doxorubicin-antibody conjugate in mice

P. Sapra[1], G. Griffiths[1], S. Govindan[1], C. Damoci[2], J. Pickett[1], A. Sheerin[1], T. Cardillo[1], H. Hansen[1], I. Horak[1], D. Goldenberg[2]. [1]*Immunomedics, Inc., Morris Plains, NJ, USA;* [2]*Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville, NJ, USA*

Background: IMMU-110 is a drug immunoconjugate comprised of doxorubicin (DOX) conjugated to the humanized form of the anti-CD74 monoclonal antibody (mAb), hLL1, at a DOX: mAb (mol/mol) ratio of 8:1. Previously, we have demonstrated excellent therapeutic efficacy of IMMU-110 in preclinical xenograft models of human B-cell lymphoma. Here, we examined the pharmacokinetics (PK) and tissue biodistribution (BD) of IMMU-110 in naïve BALB/c mice and compared the results with those obtained for naked hLL1 mAb.
Methods: Benzyl-DTPA (Bz-DTPA) conjugate of hLL1 was prepared as described previously for similar humanized mAbs (J. Nucl. Med., 44:77–84, 2003), and the DOX conjugate of hLL1-Bz-DTPA was made in an identical manner to the DOX conjugate of hLL1 (Clin. Cancer Res., 9:6567–6571, 2003). hLL1-DTPA was radiolabeled with 88Y and IMMU-110-DTPA was radiolabeled with 111In. Naïve BALB/c mice were coinjected i.v. with 88Y-DTPA-hLL1 and 111In-DTPA-IMMU-110, so that each animal was injected with a total of 20 mg of protein (hLL1 + IMMU-110, at a ratio of 1:1). At selected times after dosing, groups of mice were anesthetized and a blood sample and major animal tissues were weighed and counted for 111In and 88Y activity.
Results: IMMU-110 displayed a PK and BD profile almost identical to that of hLL1 mAb. Both hLL1 mAb and IMMU-110 had biphasic clearance from the circulation, characterized by an initial rapid redistribution (a) and a later slower clearance (b) phase. The a and b half-life (t1/2) of IMMU-110 was 4.6 h and 157.9 h respectively, and that of hLL1 was 5.4 h and 151.5 h respectively. IMMU-110 had a mean residence time (MRT) similar to that of hLL1 (222 h for IMMU-110 vs 210 h for hLL1). The clearance (Cl) of both IMMU-110 and hLL1 was 0.015 ml/h. In BD studies, no significant difference was observed between IMMU-110 and hLL1 with regards to normal tissue uptake. Neither IMMU-110 nor hLL1 mAb had a significant association with any normal body tissue.
Conclusions: Coupling DOX to hLL1 mAb does not alter the PK or BD profile of the antibody component in the conjugate, IMMU-110. However, these studies only represent the PK and BD profile of the antibody component of IMMU-110. We are currently performing experiments to determine the PK and BD parameters of both the individual drug and antibody components of IMMU-110.

291 POSTER

Preclinical evaluation of 177Lu-AMBA, a radiolabelled peptide for systemic radiotherapy and imaging of prostate cancer by targeting gastrin releasing peptide receptors

A.D. Nunn, L.E. Lantry, R. Thomas, M.E. Maddalena, J.S. Fox, T. Arunachalam, W. Feng, R.E. Swenson, M.F. Tweedle. *Ernst Felder Laboratories, Bracco Research USA Ltd, Princeton, USA*

We report preclinical data on a Lu-177 octapeptide that is an agonist for the Gastrin Releasing Peptide (GRP) receptor family and which allows targeted radiotherapy and imaging of prostate, breast and other cancers. ^{177}Lu-DOTA-G-[4-aminobenzoyl]-QWAVGHLM-NH_2 [^{177}Lu-AMBA] is a DOTA-containing bombesin derivative that binds to GRP and Neuromedin B [NMB] receptor subtypes but not the BB_3 subtype which is found in the normal human pancreas. The use of Lu-177, which is a gamma and beta emitter allows both imaging and radiotherapy with the same drug.
Competition binding studies with AMBA and ^{177}Lu-AMBA were performed with PC3 human prostate tumor cells at 4ºC. Internalization was studied over 40 min/37ºC and then% efflux from washed cells was followed for 2h/37ºC. Biodistribution of radioactivity was measured at 1, 4, and 24 h in the PC3 tumor-bearing male nude mouse using 5 μCi of ^{177}Lu-AMBA, administered i.v. Single dose radiotherapy studies were performed in the PC3 model; mice were administered a bolus of 30 mCi/kg of ^{177}Lu-AMBA s.c. (n=46), or vehicle control s.c. (n=40), and followed for up to 120 days. ^{177} Lu-AMBA has an IC_{50} of 3 nM, relative to ^{125}I-Tyr^4-Bombesin. 70% of cell-associated counts are internalized with little (12%) washout. After injection into PC3 tumour bearing nude mice clearance is rapid with half the radioactivity excreted into the urine in 1h. Kidney levels are 3–6% ID/g at 1 h and 1.5–3.5% ID/g at 24 h. PC3 tumor uptake was 3–6% ID/g at 1 h, and 2–5% ID/g at 24 h. PC-3 tumor-bearing mice [n=46] treated with a single 750 μCi dose of ^{177}Lu-AMBA showed 39% survival at 30 days vs 5.5% survival for control [n=36]. Treated animals showed 26%, 22% and 17% survival at 60, 90, and 120 days.
Although two radiolabelled antibodies were recently approved for the treatment of lymphoma, systemic radiotherapy using antibodies has been less successful for solid tumours, where the excellent specificity is confounded by long circulation times (irradiating the bone marrow) and suboptimal penetration of the tumour. Encouraging results for neuroendocrine tumours have recently been obtained with radiolabeled peptide based somatatostatin analogues but radiation mediated renal toxicity has required amino acid infusion to achieve a better therapeutic index. In animal models ^{177} Lu-AMBA has lower renal retention than the somatostatin analogues. ^{177}Lu-AMBA has been selected as a clinical candidate for the radiotherapeutic treatment of GRP receptor-positive prostate cancer.

292 POSTER

A preclinical pharmacokinetic/pharmacodynamic study for anti-PDGF receptor alpha antibody 3G3 in a human glioblastoma xenograft model

N. Loizos, Y. Xu, E. Corcoran, C. Joynes, N. Covino, P. Balderas, P. Steiner, J. Tonra, D. Hicklin, P. Kussie. *ImClone Systems Incorporated, Protein Science Department, New York, USA*

The Platelet-derived growth factor (PDGF) Receptor alpha is a type III receptor tyrosine kinase that is normally present on fibroblasts and smooth muscle cells as well as on a variety of tumor types. Neutralizing human monoclonal antibody 3G3 was previously shown to inhibit the growth of glioblastoma (U118 cell line) xenografts in nude mice. In the present study, the pharmacokinetic (PK) parameters associated with efficacious doses of the antibody were determined in this human glioblastoma xenograft model. Prior to in vivo growth, U118 tumor cells were evaluated by Scatchard analysis to identify the receptor number per cell and affinity of 3G3 for cell-surface receptor. An equilibrium constant of 3.8×10^{-11} M was obtained which agrees with the Kd obtained on a BIAcore instrument for the 3G3: human PDGFR alpha interaction. The number of PDGFR alpha molecules per cell was estimated to be between 1,740 and 3,580. The growth of U118 tumors in nude mice was significantly inhibited by 3G3 treatment administered IP twice a week at 6 (p=0.06), 20 (p=0.03) and 60 (p=0.0004) mg/kg. There were 4 of 12, 5 of 11 and 10 of 12 regressions in the 6, 20, and 60 mg/kg treatment groups, respectively, and no regressions in a human IgG control group (p<0.0001). Thus, a 60 mg/kg maintenance dose was especially effective, causing tumor regression in 83% of the mice. The average steady state plasma 3G3 concentration for the 6, 20, and 60 mg/kg dose was 132, 339, and 561 microgram/ml, respectively. The anti-tumor activity of 3G3 was directed at the tumor cells and not stroma as no cross reactivity of 3G3 was detected against the mouse PDGFR alpha. The above studies further support the potential use of the anti-PDGFR alpha antibody 3G3 as a cancer therapeutic.

293 POSTER

Identification of a novel prostate tumor target, RG-1, for antibody based therapy of prostate cancer

R. Parry[1], D. Schneider[1], D. Hudson[2], D. Parkes[1], J. Xuan[1], R. Harkins[1], R. Lin[1], B. Alicke[3], H. Dinter[1], G. Parry[1]. [1]*Berlex Biosciences, Cancer Research Department, Richmond, USA;* [2]*Medarex, Hybridoma, Milpitas, USA;* [3]*Berlex Biosciences, Animal Pharmacology, Richmond, USA*

Mining of a gene expression EST database for genes that were over expressed in prostate tumors identified a novel sequence, termed RG-1, that was abundantly expressed in prostate tumors and normal prostate tissues. RG-1 sequences were expressed at significantly lower levels in other normal tissues. This finding was confirmed by Taqman based analysis and Northern blot analysis of RG-1 mRNA levels in clinical tissue samples. Full-length sequence analysis of RG-1 revealed that RG-1 was a member of the spondin family of extracellular matrix-like proteins and probably represented the human homologue of mindin. The protein has a molecular weight of 40kDa, and is secreted from LNCaP cells and from BHK cells transfected with the RG-1 gene, but is also detected in ELISA assays as a peripheral protein on the surface of LNCap cells. Anti-peptide RG-1 antibodies were generated in rabbits and used in immunohistochemistry studies. RG-1 protein was detected in prostate tumor samples and, at a lower level, in normal prostate epithelium. RG-1 protein could not be detected at significant levels in other tissues in the human male. To determine whether antibodies targeting RG-1 could be used for diagnostic and therapeutic purposes, monoclonal antibodies recognizing RG-1 were then generated in HuMab-mice ®, (Medarex). Two high affinity antibodies, 19G9 and 34E1, were identified that recognized the native RG-1 protein, and could be conjugated with the metal binding chelator p-SCN-benzyl DTPA and labeled with ^{111}In, without compromising their binding affinity. Biodistribution studies performed with these conjugated antibodies in nude mice bearing RG-1 expressing LNCaP cells grown as xenografts,

demonstrated significant accumulation of both antibodies in the tumors. Approximately 40% of the injected dose/g tissue accumulated in the tumors at 90–180 hours after injection, and tumor/blood ratios of 6 to 8 were seen with these antibodies. This accumulation was better than that seen with Prostascint ™ in the same model. Control antibodies did not accumulate in the LNCaP xenografts. Based on this specific accumulation of these antibodies and the selective expression of RG-1 protein, we suggest that antibodies 19G9 and 34E1 may be suitable for in vivo diagnosis and therapy of prostate cancer.

294 POSTER

1D09C3, a human, HLA-DR-specific monoclonal antibody efficiently induces programmed cell death in lymphoid tumors

J.-B. Holz[1], Z. Nagy[1], B. Rattel[1], K. Lobenwein[1], S. Leyer[1], A. Krenn[1], S. Messemer[2], F. Schwaiger[2]. [1]*GPC Biotech AG, Drug Development, Munich, Germany;* [2]*Aurigon GmbH, Tutzing, Germany*

Background: Major histocompatibility complex class **II** molecules (MHC-**II**) are transmemebrane glycoproteins and are only expressed on the surface of immune system cells: B cells, macrophages and mature dendritic cells. In addition to their role of presenting antigen to T-lymphocytes they can serve as receptors triggering programmed cell death. It has been demonstrated, that MHC-**II** induced apoptosis affects activated/tumor transformed cells selectively and proceeds without the involvement of caspases. 1D09C3 is an IgG4 antibody derived from a human antibody phage display library, binding to human leukocyte antigen-DR (HLA-DR) with a sub-nM affinity. The selection of 1D09C3 from a panel of mAbs was based on its ability to kill a selected panel of human HLA-DR$^+$ lymphoma/leukemia cells in vitro while normal, resting HLA-DR$^+$ cells were not affected, thus resulting in a selectivity for the apoptotic effect.
Material and Methods: The in vivo activity of 1D09C3 has been investigated in xenotransplant models of Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell leukemia, and multiple myeloma. 1D09C3 was given at different doses ranging from 1mg/day to 0.04 μg/day in divided doses (iv) in a series of experiments. The dosing was carried out on days 5,7, and 9. To eliminate NK cells the SCID mice were pretreated with anti-asialo GM1 for three days, starting one day prior to the intravenous tumor cell inoculation. In the late stage disease experiments, 1D09C3 was administered for 4 or 5 days at 1 mg/day (iv) once visible symptoms of disseminated lymphoma were present. Rituximab was administered concurrently with 1D09C3 (iv) in a combination study to explore potentially synergistic effects in a non-Hodgkin's lymphoma model. The disease endpoint was paraplegia or death.
Results: The antibody showed very consistent activity across all four tumor models: within a dose range of 2,5 μg to 1 mg/day/mouse, the time to disease progression was delayed in all treated animals, compared to vehicle treated controls. High dose (1 mg/day × 4) treatment at late stages of disseminated lymphoma (~7 days before moribund) could still rescue 3/9 treated animals. The effect of 1D09C3 was compared to that of rituximab in a model of CD20$^+$HLA-DR$^+$ non-Hodgkin's lymphoma. The single agent efficacy of 1D09C3 was comparable to rituximab, however, when administered concurrently, the efficacy of the combination regimen exceeded the efficacy of either drug alone. In addition to malignant lymphoid cells, 1D09C3 has shown to induce death of HLA-DR$^+$ melanoma cells, in vivo studies are underway.
Conclusion: 1D09C3 has consistently demonstrated efficacy in various lymphoid tumors as well as in HLA-DR$^+$ melanoma cell lines. In a terminal-stage disseminated lymphoma model, high-dose treatment with 1D09C3 slowed down disease progression and resulted in 3 long term survivors (2 disease free and one with a single localized tumor) out of 9 treated animals. The combination of 1D09C3 with rituximab showed greater efficacy than either antibody alone in a non-Hodgkin's lymphoma model. The most likely basis for the observed increased efficacy is that the antibodies recognize different target receptors and may have different effector mechanisms.

295 POSTER

Enhancing radioimmunotherapy with the PDGFr-beta inhibitor: imaging and tumor response studies

J. Baranowska-Kortylewicz[1], M. Abe[1], T. Kurizaki[2], K. Pietras[3], Z.P. Kortylewicz[1], J. Nearman[1], R.L. Mosley[4], C.A. Enke[1], A. Östman[5]. [1]*University of Nebraska Medical Center, Radiation Oncology, Omaha, USA;* [2]*National Hospital Organization, Kumamoto Medical Center, Kumamoto City, Japan;* [3]*University of California, Diabetes Center, San Francisco, USA;* [4]*University of Nebraska Medical Center, Pathology and Microbiology, Omaha, USA;* [5]*Karolinska Institute, Pathology-Oncology, Stockholm, Sweden*

Background: The success of radioimmunotherapy (RIT) depends on the tumor-specific delivery of radioisotopes in quantities sufficient to deposit therapeutic radiation doses. In radiosensitive tumors such as lymphoma, this aim is accomplished with the total administered doses that spare normal tissues. Solid tumors are less sensitive to radiation than lymphomas, and as a result they are not responsive to RIT at tolerable doses. Recent studies indicate that the inhibition of PDGFr-β in the tumor stroma with STI571 attenuates tumor hypertension (P_{IF}) and improves influx of chemotherapy to tumors (1, 2). We proposed that a combination regimen of STI571 + RIT may well allow accumulation of therapeutically sufficient radiation doses in solid tumors.
Materials and Methods: All studies were conducted in a mouse model of the human colorectal adenocarcinoma LS 174T grown as subcutaneous (SQ) tumors in athymic mice. Radioimmunotherapy and radioimmunodiagnosis studies were conducted using a monoclonal antibody B72.3 that recognizes TAG-72 antigen common to nearly 90% of human adenocarcinomas (3,4). Imaging studies were done using the LumaGEM™ scintillation camera.
Results: STI571-induced attenuation of P_{IF} had a positive effect on the total uptake as well as the homogeneity of ^{125}I-B72.3 distribution within the tumor. This effect was dose-dependent and under optimized dosing conditions allowed for a 160% enhancement in the absolute tumor uptake of radiolabeled B72.3 as measured in the biodistribution studies. SPECT imaging studies substantiated these results and indicated that the homogeneity of radioisotope distribution was also significantly improved when compared with the control tumors. The increased uptake of RIT into the tumor resulted in >400% increase in the tumor absorbed radiation doses in STI571+RIT-treated mice compared to PBS-treated mice. Two additional causes related to the STI571-induced attenuation of P_{IF}, were identified: improved homogeneity of MAb distribution in tumor; and increased tumor radiosensitivity in response to improved tumor oxygenation.
Conclusions: The attenuation of tumor P_{IF} was identified as the primary reason for the enhanced radioimmunoconjugate uptake and improved RIT of the STI571-treated tumors.

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296 POSTER

Trastuzumab monoclonal antibody labeled with alpha-particle emitter astatine: targeted radiotherapeutic experiments on a HER2-positive breast carcinomatous meningitis animal model after intrathecal administration

A. Boskovitz[1], H. Ochiai[2], T. Okamura[1], X.-G. Zhao[3], D. Bigner[1], M. Zalutsky[3]. [1]*Duke University Medical Center, Dept. of Pathology – Neuro-Oncology Program, Durham, NC, USA;* [2]*Duke University Medical Center, Dept. of Surgery – Neuro-Oncology Program, Durham, NC, USA;* [3]*Duke University Medical Center, Dept. of Radiology – Neuro-Oncology Program, Durham, NC, USA*

Carcinomatous meningitis (CM) is a devastating disease that results from the dissemination of tumor cells into the subarachnoid space along the brain and spine. Breast carcinoma is one of the two most frequent non-CNS origin of CM. Systemic treatment with the monoclonal antibody (mAB) trastuzumab (Herceptin®) is efficient against HER2-positive breast carcinoma and systemic metastasis but does not affect the course of the leptomeningeal disease as the CSF concentration remains 300-fold lower than the systemic one. Intrathecal administration of radiolabeled trastuzumab could result in the delivery of a high radiation dose specifically to the disseminated tumor foci, while reducing systemic exposure. Astatine ^{211}At emits α-particles with a high linear energy transfer (97 keV/μm), a