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.

References

[1] Pietras, *et al. Cancer Res*. 62, 5476 (2002).
[2] Pietras, *et al. Clin Cancer Res*. 9 (10 Pt 1), 3779 (2003).
[3] Nuti *et al. Int J Cancer*. 29, 539 (1982).
[4] Thor, *et al. Cancer Res*. 46, 3118 (1986).

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*

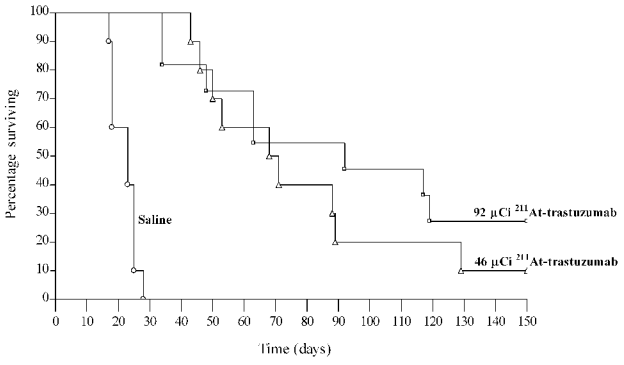
Carcinomatous meningitis (CM) is a devastating disease that results from the dissemination of tumor cells into the subarachnoid space along the brain and spine. Breast carcinoma is one of the two most frequent non-CNS origin of CM. Systemic treatment with the monoclonal antibody (mAB) trastuzumab (Herceptin®) 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

short range (55 μm) and has a 7.2 h half-life, making it an attractive radionuclide for targeted radiotherapy of the disseminated, thin-sheeted CM. We investigated the therapeutic effect of intrathecal administration of ^{211}At-labeled trastuzumab in an animal model of HER2-transfected breast CM.

Material and Methods: Athymic female rats were injected intrathecally with HER2-transfected MCF-7 breast carcinoma cells through a previously surgically-implanted intrathecal catheter. The same catheter was used for intrathecal treatment injection 3 days after tumor inoculation. In experiment 1, animals were treated with 33 or 66 μCi ^{211}At-trastuzumab, cold trastuzumab, or saline. In experiment 2, animals were inoculated with a lower tumor burden and treated with 46 or 92 μCi ^{211}At-trastuzumab, or saline. In experiment 3, animals were treated with 28 μCi ^{211}At-trastuzumab, 30 μCi ^{211}At-TPS3.2 (control mAB), or saline. Animals were neurologically evaluated daily thereafter. At the end of the study, their brain and spine were removed for histopathological analysis.

Results: In experiment 1, median survival was increased from 21 days when treated with saline or cold trastuzumab to 45 and 48 days when treated with 33 and 66 ?μCi ^{211}At-trastuzumab, respectively. In experiment 2 (fig.), median survival was increased from 23 days when injected with saline to 68 and 92 days when treated with 46 and 92 μCi ^{211}At-trastuzumab, respectively. In experiment 3, median survival was increased from 20 days when treated with saline to 29 and 36 days when treated with ^{211}At-TPS3.2 and ^{211}At-trastuzumab, respectively. Long-term survivors were observed exclusively in the ^{211}At-trastuzumab-treated groups.

Conclusion: The therapeutic efficacy of ^{211}At-trastuzumab was better than that obtained previously with cold trastuzumab administered at considerably higher levels in a multi-dose protocol in a similar animal model. Targeted radiotherapy with intrathecal ^{211}At-trastuzumab is a potentially viable treatment for patients with HER2-positive breast CM; further investigations are in progress to define its pharmacokinetics.



Survival of athymic rats with HER-2-positive breast carcinomatous meningitis after treatment with intrathecal injection of ^{211}At-labeled trastuzumab.

297 POSTER

RAV12: a glycotope-specific chimeric antibody that exhibits potent cytotoxic activity against gastrointestinal tumor cell lines in vitro and in vivo

N.K. Pryer, D. Loo, P. Young, R. Winter, K. King, K. Kang, S. Coberly, T. Liang, G. Vehar, J.P. Mather. *Raven Biotechnologies, Inc., South San Francisco, CA, USA*

RAV12 is a chimeric monoclonal antibody directed against a novel carbohydrate antigen highly expressed (defined as intense staining in >75% of tumor cells) by more than half of gastric, colon and pancreatic adenocarcinomas, and smaller proportions of prostate, ovarian, breast and renal cell carcinomas as well as liver adenocarcinoma. RAV12 was constructed based on its murine homolog, KID3, which was generated by immunization of mice with a kidney progenitor cell line. Like KID3, RAV12 exhibits cytotoxic activity in vitro (IC50=5–10ug/mL) against human gastrointestinal tumor-derived cell lines expressing high and uniform levels of the RAV12 antigen, RAAG12. Cytotoxic activity also appears to correlate with internalization of RAV12. The mechanism of action of RAV12 in vitro cytotoxicity is consistent with the induction of necrosis, in that treated cells increase in volume, followed by bursting of the plasma membrane, with no observed expression of classical markers of apoptosis. Biochemical studies demonstrate that RAV12 recognizes a specific N-linked glycotope expressed on one or more proteins present on the cell surface of tumor cell lines. In vivo analysis confirmed that the cytotoxicity observed in vitro correlates with antitumor activity in the rodent subrenal capsule model. RAV12 potently reduces the size of multiple human tumor cell lines grown beneath the renal capsule of mice, quantified by QPCR analysis of human DNA in tumors at the end of the dose period. RAV12 activity against COLO201 subrenal capsule xenografts is seen following six doses as low as 1 mg/kg, with complete tumor eradication in all treated animals at 50mg/kg and higher. The pharmacokinetics of RAV12 in mice are consistent with other chimeric antibodies, with a T 1/2eff of ~5 days. PK/efficacy correlations are in progress. RAAG12 expression on normal human tissue is limited to ductal epithelium (sweat gland, bile, pancreatic) and gastrointestinal epithelium; primarily on the apical surface of these epithelial cells. Pilot tolerance studies of KID3, the murine precursor to RAV12, showed that KID3 was well tolerated in Cynomolgus monkeys, which express cross-reactive RAAG12. RAV12 safety and PK studies are in progress in Cynomolgus monkeys, to support an anticipated IND filing later this year.

298 POSTER

Development of anti-EGFR immunoliposomes for specific delivery and enhanced efficacy in EGFR-overexpressing tumors

C. Mamot[1,3], C. Rochlitz[1], R. Herrmann[1], W. Küng[1], R. Ritschard[1], J. Reuter[1], D.C. Drummond[2], K. Hong[2], D.B. Kirpotin[2], J.W. Park[2,3]. *[1]University Hospital Basel, Division of Oncology, Basel, Switzerland; [2]Hermes Biosciences, South San Francisco, USA; [3]University of California, Division of Hematology/Oncology, San Francisco, USA*

We have developed immunoliposomes (ILs) that bind EGFR or mutant EGFRvIII and internalize in target tumor cells, enabling intracellular delivery of potent anticancer agents.

ILs were constructed modularly with various MAb fragments, including C225 (cetuximab)-, EMD72000-Fab' and novel human scFvs from phage antibody libraries, covalently linked to liposomes containing various drugs or probes. Fluorescence-labeled anti-EGFR ILs efficiently bound to EGFR-overexpressing cells (A-431, MDA-MB-468, U-87) demonstrated extensive internalization in the cytoplasm of target cells consistent with receptor-mediated endocytosis. Non-targeted (no Mab) liposomes and irrelevant (anti-HER2) immunoliposomes did not bind to or accumulate in these EGFR-overexpressing cells. Quantitative studies of uptake and internalization showed binding to MDA-MB-468 cells within 5 minutes, followed by intracellular accumulation detectable at 15 min and increasing to a plateau after 240 min. Total uptake of ILs at 240 min was 1.70 fmol phospholipid/cell, corresponding to approx. 13,000 ILs/cell. Anti-EGFR ILs were used to deliver various drugs (doxorubicin, vinorelbine, methotrexate) against these cell lines in vitro. In each case, anti-EGFR ILs were markedly more cytotoxic than the corresponding liposomal drug in target cells, while equivalent to liposomal drug in control cell lines lacking EGFR. Remarkably, in an EGFR-overexpressing multi-drug-resistant cell line ILs loaded with doxorubicin produced greatly more cytotoxicity in comparison to the corresponding free drug which by it self can penetrate cell membranes easily. PK and biodistribution studies confirmed long circulation half-life and high accumulation in tumors. In vivo efficacy studies in EGFR- or EGFRvIII-overexpressing xenograft models demonstrated the superiority of immunoliposomal delivery in target cells. In each study, anti-EGFR ILs containing various drugs (e.g. doxorubicin, epirubicin and vinorelbine) showed potent antitumor effects, including tumor regressions and cures in many mice, significantly superior to all other treatments, such as free drug, liposomal drug or free MAb + liposomal drug.

In conclusion, ILs provide efficient and targeted drug delivery to EGFR or EGFRvIII-overexpressing tumor cells, and might be helpful in overcoming drug resistance mechanisms. In principle, this targeting approach can be used for the delivery of various probes, drugs and genes.

299 POSTER

Preclinical in vivo evaluation of a doxorubicin-antibody conjugate for treating multiple myeloma

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

Background: We reported recently on the excellent efficacy of the humanized monoclonal antibody conjugate IMMU-110 [(doxorubicin)$_8$-hLL1 {anti-CD74}] in curing SCID mice given a lethal, systemic injection of Raji non-Hodgkin's lymphoma B-cell tumors, using a single 350 μg dose of conjugate given 5 days after tumor challenge [*Clin. Cancer Res.*, 9:6567–6571, 2003]. We tested now the IMMU-110 conjugate against a second B-cell neoplasm expressing the CD74 antigen, multiple myeloma (MM).