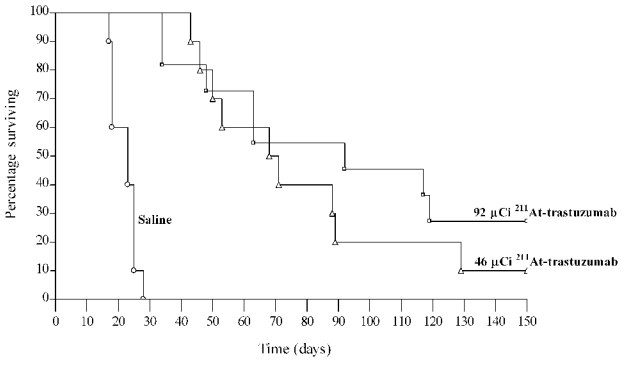
short range (55 μm) and has a 7.2 h half-life, making it an attractive radionuclide for targeted radiotherapy of the disseminated, thin-sheeted CM. We investigated the therapeutic effect of intrathecal administration of $^{211}$At-labeled trastuzumab in an animal model of HER2-transfected breast CM.

**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**

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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**

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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**

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**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).

**Material and Methods:** The IMMU-110 conjugate was prepared as described in the above reference, and tested in a newly developed model of disseminated MM. The MC/CAR human MM cell line was maintained in tissue culture in RPMI 1640 media supplemented with fetal bovine serum, penicillin/streptomycin (1%), and glutamine (2%). Cells were split the day before injection to ensure log-phase growth. C.B-17 FOX CHASE SCID$^{TM}$ mice were pretreated with Fludara (0.4 mg/mouse) and Neosar (2 mg/mouse) 5 days prior to an *i.v.*-injection of $10^7$ MC/CAR cells, and thereafter monitored daily for signs of hind-leg paralysis, at which point they were sacrificed for humane reasons. Survival studies were analyzed by Kaplan-Meier plots (log-rank analysis) using the GraphPad Prism software package.
**Results:** SCID mice, left untreated, succumbed to paralysis, due to disseminated disease, at a median of 32 days post-tumor cell injection. A single injection of 350 μg of IMMU-110 in MC/CAR-bearing SCID mice resulted in 6/10 survivors at > 175 days post-tumor cell challenge. Unconjugated hLL1 alone, at 350 μg, showed considerable efficacy, with an increase in median life extension to ca. 60 days, while an equivalent mixture of hLL1 and free doxorubicin demonstrated an increase in median life extension to 68 days. In a second experiment, delaying treatment to 5, 10 and 14 days post-MC/CAR cell injection resulted as follows: in the day-5 group, 3/10 animals survived to 150 days, and in the day 10 group, 5/10 animals survived at 150 days. Animals with advanced disease treated at 14 days post-tumor cell challenge succumbed, but with a statistically significant improvement in survival time from 28 to 35 days ($p < 0.002$), compared to matched, untreated control animals. In ongoing dose-finding experiments, the maximum tolerated single dose of IMMU-110 has not been reached at 2.5 mg/mouse, while early efficacy is being seen at single doses ranging from 35–2000 μg/mouse.
**Conclusion:** IMMU-110 is a doxorubicin-anti-CD74 conjugate that can cure some mice treated with only a single injection of conjugate, at an essentially non-toxic dose, and therefore exhibits significant potential as a new therapeutic agent for the treatment of MM. Future studies will examine multiple dosing of the IMMU-110.

**300** POSTER

**Cetuximab/irinotecan/HD-FU/LV in first line therapy of metastatic colorectal cancer (CRC)**

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Cetuximab (C225) has be shown to be active in patients with CRC failing irinotecan (Cunningham, ASCO 2003).
We performed a phase I/IIa study to evaluate toxicity, efficacy and PK of C225 combined with CPT-11, infusional 5-FU and in the first line therapy of patients with EGFR+ metastatic colorectal cancer.
Pts. received 250 mg/m2 cetuximab weekly after a loading dose of 400 mg/m2.
Chemotherapy consisting of 80 mg/m2/1h CPT-11, 500 mg/m2/2h LV, and infusional 5-FU (24h) in dose levels of 1.500 mg/m2 and 2.000 mg/m2 was administered weekly × 6, q d50.
Dose limiting toxicities (DLT) were defined as neutropenia or skin toxicity > grade 3, any febrile neutropenia/leukopenia, or thrombopenia, diarrhea, mucositis, hepatic toxicity > grade 2 and other relevant organ toxicity > grade 1, each in the first cycle.
After inclusion of 6 patients at the dose level of 1.500 mg/m2 5-FU without occurrence of DLTs, 15 pts. were enrolled at the dose level of 2.000 mg/m2. At this dose level, 3 DLTs were observed (2 pts. diarrhea grade 3, 1 pt. diarrhea grade 4). Skin toxicity grade 3/4 occurred in 3/19 pts during the first cycle.
Dose modification of chemotherapy during the first cycle was necessary in 2/6 and 7/13 pts. in the dose level of 1.500 and 2.000 mg/m2, respectively. Therefore, we recommend 1.500 mg/m2 5-FU for the phase II trials.
Pharmacokinetics of cetuximab was not influenced by the different 5-FU dose levels.
14 out of 19 evaluable patients achieved objective response (74%, 95% CI 51–88%; 2 pts. CR, 12 pts. PR). Secondary resection of liver metastases was performed in 4/19 patients (21%).
The combination of cetuximab with irinotecan/inf. 5-FU/LV has a promising activity. Final data of this phase I/IIa study will be presented at the meeting.

**301** POSTER

**Genomic discovery, characterization and validation of a transmembrane protein overexpressed in human ovarian and pancreatic cancers: a promising new target for therapeutic monoclonal antibodies**

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We utilized comprehensive data mining and subtractive library evaluation to identify >30,000 sequences differentially regulated in cancer. Genes encoding secreted and transmembrane proteins showing upregulated mRNA expression by microarray and quantitative PCR with 208 human tumor and normal tissues were selected for progression as diagnostic and therapeutic antibody targets, respectively. One gene, dDx115o, encodes a transmembrane serine protease. QPCR showed that dDx115o mRNA is overexpressed in human ovarian and pancreatic cancer tissue with little or no expression in any normal tissues. Recombinant proteins were used to raise a series of monoclonal antibodies that recognize dDx115o. We demonstrated that dDx115o is a glycoprotein which can be specifically identified by western blot analysis using extracts of human tumor cell lines and ovarian tumors but not other normal tissues tested. dDx115o protein was localized to the membrane of dDx115o-expressing tumor cell lines by FACS and immunofluorescence of live cells. Immunohistochemical studies using monoclonal antibodies against dDx115o revealed strong cell surface staining in sections of human ovarian and pancreatic cancers. In functional validation experiments overexpression of dDx115o, but not a dDx115o mutant lacking protease activity, induced growth of test cells in soft agar as well as induced tumor growth in SCID mouse xenograft studies. Furthermore, siRNA-mediated knockdown of dDx115o expression in cultured tumor cells led to apoptosis and increased caspase activity. Monoclonal antibodies able to bind live cells demonstrated an ability to inhibit tumor cell proliferation in culture. The restricted nature of dDx115o over-expression and the demonstrated functional role in promoting phenotypes of malignant transformation makes this cell surface antigen an ideal target for a monoclonal antibody therapeutic strategy. Mouse xenograft efficacy studies are in progress.

**302** POSTER

**Patterns of gene expression can prospectively predict Panitumumab (ABX-EGF) monotherapy responsiveness in xenograft models**

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**Background:** Epidermal growth factor receptor (EGFr) is a transmembrane tyrosine kinase expressed on many different tumor types. There is increasing preclinical and clinical evidence suggesting that blocking the EGFR signaling pathway can provide clinical benefit to patients whose tumors express EGFR. Panitumumab, a fully human antibody, binds to the EGFR with high affinity ($5x10^{-11}$ M) preventing ligand-induced activation resulting in arrest of tumor cell proliferation and apoptosis in some cases [1,2]. The objective of this study was to determine a gene array profile that could predict responsiveness to panitumumab monotherapy.
**Methods:** Responsiveness to panitumumab in ten xenograft models was determined. Animals were treated twice per week with 20, 100, 200, and 500 μg/mouse per dose and response was determined as a 40% reduction of tumor volume (versus control). To determine a set of genes that could potentially help prospectively stratify patients based on responsiveness, untreated xenograft samples, with known responsiveness to panitumumab, were arrayed on the Affymetrix human U133 gene chip. Supervised ANOVA, univariate and multivariate analysis were performed to determine transcripts that predict responsiveness to panitumumab.
**Results:** Panitumumab treatment of mice bearing 300 $mm^3$ established xenografts determined A431, PC-3, MIAPaCa and HT-29 models were responsive and NIH H1299, SK MES PD, MCF-7, U87, ZR75–1 and Colo 205 models were non-responsive. An initial unsupervised cluster analysis demonstrated that the tissue type had greater influence on the clustering of genes than the responsiveness to panitumumab. A two-way analysis of variance that modeled tissue affect and drug responsiveness revealed 2156 genes that were differentially expressed in responders and non-responders (FDR corrected p-value <0.05). Concurrently, a supervised univariate and multivariate classification technique was used to identify 11 genes in a training set of 10 responsive/ non-responsive xenograft models. The gene set was used to prospectively determine the outcome on 9 more xenograft models for which the response to panitumumab was previously unknown.