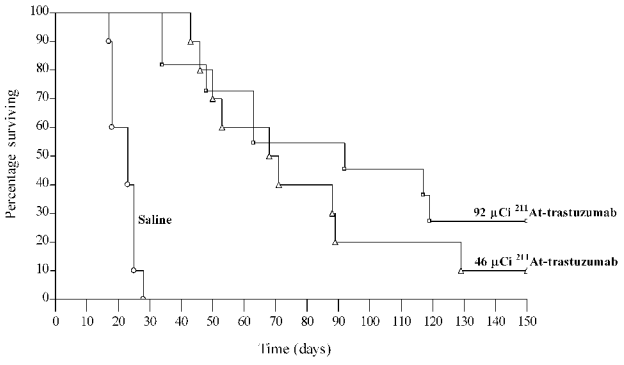
short range (55 μm) and has a 7.2 h half-life, making it an attractive radionuclide for targeted radiotherapy of the disseminated, thin-sheeted CM. We investigated the therapeutic effect of intrathecal administration of ^{211}At-labeled trastuzumab in an animal model of HER2-transfected breast CM.

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.

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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.

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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.

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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).