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

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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.

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

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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.

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

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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.

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

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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,