

# Eradication of Established Pancreatic Tumors in Mice by Engagement of Extra-tumoral Human T Cells with BiTE Antibody MT110

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## Background

Bispecific T-cell Engager (BiTE) antibodies are single-chain bispecific antibody constructs with specificity for CD3 on T cells and a surface antigen on target cancer cells. By transiently bridging T cells and cancer cells, BiTE antibodies are capable to mount a polyclonal T cell response that is no longer limited by T cell receptor specificity, presence of MHC class I, generation and presentation of peptide antigen, or the need for T cell costimulation.

MT110 is a representative of the BiTE class targeting the epithelial cell adhesion molecule (EpCAM), which is among the best characterized targets for immunotherapeutic approaches and is frequently overexpressed on a wide range of human carcinomas. MT110 is in a phase 1 clinical trial and has previously been shown to have high anti-tumor activity in SCID mouse models against human colorectal cancer xenografts.

In previously established xenograft models, both human tumor and human effector T cells are subcutaneously engrafted into recipient mice and animals daily treated intravenously with MT110 starting with the day of tumor cell implantation. The inhibition of tumor outgrowth is used as a measure of anti-tumor activity. The short half-life of subcutaneously injected effector T cells limits the described model to study the efficacy of BiTE antibodies on early tumor formation or eradication of only small (~50 mm<sup>3</sup>) established tumors.

The aim of the present study was to develop a xenograft mouse model for evaluation of the potency of MT110 against larger established tumors (>300 mm<sup>3</sup>) derived from the AsPC-1-derived pancreas adenocarcinoma line. In this model, T effector cells are no longer mixed with tumor cells but injected i.p. after a solid tumor has established. This approach will also address the question whether circulating T cells are effective in tumor eradication by the EpCAM/CD3-bispecific BiTE antibody MT110.

## Inhibition of s.c. pancreatic tumor outgrowth by MT110

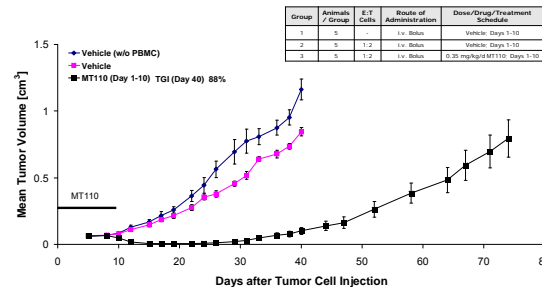


Figure 2: MT110-mediated delay of tumor outgrowth from human AsPC-1 pancreatic cancer line

AsPC-1, human pancreas carcinoma cell line, was subcutaneously injected with or without unstimulated human PBMC derived from healthy human donors at, as applicable, an E:T cell ratio of 1:2 into the right dorsal flank of female NOD/SCID mice (n=5 per group). Mice were daily treated with MT110 from the day of tumor/PBMC inoculation with 0.35 mg/kg/day for ten consecutive days by bolus injection into the lateral tail vein. Controls included two vehicle-treated groups (tumor cells injected with or w/o human PBMC). Progress of tumors was determined by external caliper measurements, and tumor volumes were calculated using a standard hemispherical formula: (length (mm) x width (mm))<sup>2</sup>/2. Values represent mean tumor size (cm<sup>3</sup>) +/- SEM (n=5 per group).

## Reconstitution of tumor-bearing NOD/SCID mice with human T cells

Group	Animals / Group	CD3 <sup>+</sup> T Cells	Route of Administration	Dose/Drug/Treatment Schedule
1	5	-	i.v. Bolus	Vehicle: Days 9-30
2	5	-	i.v. Bolus	Vehicle: Days 9-30
3	5	+	i.p.	0.35 mg/kg/d MT110: Days 9-30
4	5	+	i.p.	Vehicle: Days 9-30
5	5	+	i.p.	0.35 mg/kg/d MT110: Days 9-30

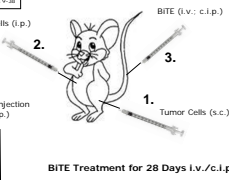


Figure 1: Generation and Mode of Action of EpCAM/CD3-bi-specific BiTE Antibody MT110

Generation of a BiTE antibody from the variable domains of two distinct monoclonal antibodies is depicted on the left. The anti-CD3 specific single-chain antibody (red) is shared by all BiTE antibodies. The target antigen-specific single-chain antibody (green) recognizes EpCAM expressed on cancer cells. As shown on the right, the BiTE-mediated, transient connection between a T cell and an EpCAM-expressing tumor cell triggers T-cell activation and eventually programmed cell death of the cancer cell.

Figure 3: Study design of a novel xenograft NOD/SCID mouse model using engraftment of human T cells in tumor-bearing mice

## Circulating human T cells in tumor-bearing NOD/SCID mice

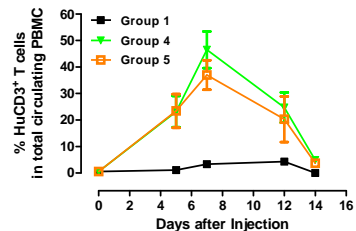


Figure 4: Schematic demonstration of a tumor model in human T cell reconstituted NOD/SCID mice

Human T cells were expanded in vitro by standard T cell conditioning and injected into the peritoneal cavity of  $\gamma$ -irradiated NOD/SCID mice. The percentage of human T cells in total circulating PBMC was determined using fluorescently labeled anti-human CD4 and CD8 antibodies by FACS analysis.

## Pharmacokinetics of continuously i.p. delivered MT110 in NOD/SCID mice

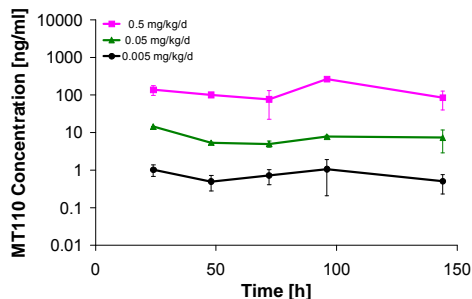


Figure 5: Plasma concentrations versus time profile of MT110 in NOD/SCID mice after continuous intraperitoneal delivery

The serum concentration versus time profiles observed after intraperitoneal continuous infusion by means of Alzet® osmotic pump of 0.5, 0.05 and 0.005 mg/kg/d MT110 are shown in a semi-logarithmic plot. NOD/SCID mice were bled 24, 48, 72, 96 and 144 hours after intraperitoneal Alzet® osmotic pump implantation, respectively.

## Eradication of established pancreatic tumors by MT110 with i.p.-transferred human T cells

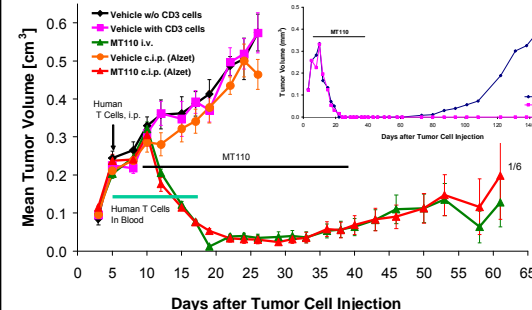


Figure 6: MT110-mediated eradication of human pancreatic AsPC-1 tumors in NOD/SCID mice by i.p.-transferred human T cells

AsPC-1 human pancreas carcinoma cells were subcutaneously injected into the right dorsal flank of female NOD/SCID mice (n=5 per group). After tumors had reached a volume of 250 mm<sup>3</sup>, human T cells were injected into the peritoneal cavity. MT110 (0.35 mg/kg) was administered either by intravenous bolus injections into the lateral tail vein or continuous intraperitoneal release from osmotic pumps (ALZET®) starting on Day 9 and ending on day 28, with one replacement of the pump on Day 23. Controls included three vehicle-treated groups (tumor cells injected with or w/o human PBMC) whereby the vehicle was delivered either by intravenous bolus injection or continuous intraperitoneal release. Growth of tumors was determined by external caliper measurements, and tumor volumes were calculated using a standard hemispherical formula: (length (mm) x width (mm))<sup>2</sup>/2. Values shown represent mean tumor size (cm<sup>3</sup>) +/- SEM (n=5 per group). Numbers indicate the proportion of animals that stayed free of tumor for the entire observation period of 150 days (based on absence of measurable/observable masses during in-life phase/at necropsy). The inset shows the duration of tumor-free survival and time to relapse for two animals treated with MT110 by c.i.p. delivery.

## Conclusions

- Subcutaneous tumors grown subcutaneously in the absence of human T cells in NOD/SCID mice to sizes >300 mm<sup>3</sup> can be eradicated by BiTE antibody MT110 engaging initially extra-tumoral human T cells.
- MT110 and other BiTE antibodies in development may not solely rely on tumor-resident T cells but are also capable of engaging effector cells from the circulation and/or lymphatic organs.
- MT110 may have activity against pancreatic cancer.
- The new xenograft model offers to:
  - test combinations of BiTE antibodies with other therapies be it by sequential, parallel or alternating administration
  - test alternative treatment schedules and activity against even larger tumors
  - use alternative routes of administration such as osmotic pumps for continuous i.p. and s.c. administration
  - determine time to progression during or after cessation of BiTE treatment
  - evaluate anti-tumor activity in orthotopically-implanted tumors
  - analyse the effect of BiTE antibody treatment on invasion and metastasis of orthotopically-implanted tumors