**Supplementary Material**

**Supplementary Material and Methods**

**Isolation and genetic modification of CD8+ central memory T cells (TCM) by retroviral gene transfer**

For isolation of human CD8+ TCM, peripheral blood mononuclear cells (PBMC) were depleted by CD4+ and CD45RA+ cells and enriched for CD62L+ cells using microbead technology (Miltenyi Biotec). A bi-cistronic construct separated by a P2A element consisting of TCR2.5D6 beta and alpha chains containing murinized constant domains and an additional disulfide bridge was generated in silico and codon optimized (Life technologies) as described(23). For in vivo depletion analyses, the TCR2.5D6 was linked downstream to near-infrared red protein (iRFP) by a T2A element. The constructs were cloned into the retroviral vector pMP71, supernatants were generated and CD8+ TCM were transduced.

**aTCRmu-IgG and aTCRmu-F(ab’) preparation and affinity determination**

The aTCRmu-IgG was affinity purified from supernatant of H57-597 hybridoma cells using Protein A-Sepharose (GE Healthcare). F(ab’)2 fragments of aTCRmu and isotype-IgG (Ha4/8, BD Bioscience) were generated by Pepsin digestion followed by Protein A purification using F(ab’)2 Preparation Kit (Thermo Scientific Pierce™). For Kd determination, TCR2.5D6-transduced Jurkat76-CD8α cells were incubated with different concentrations of aTCRmu-IgG or F(ab’)2 followed by staining with aTCRmu-FITC/7-AAD and subsequent analysis by flow cytometry (LSRII, BD Bioscience). The mean fluorescence intensity (MFI) of aTCRmu-IgG/F(ab’)2 was subtracted from the MFI of cells stained exclusively with aTCRmu-FITC and the Kd was calculated by nonlinear regression analysis.

**Analysis of Interferon γ (IFNγ) secretion and apoptosis of TCM**

TCR2.5D6-transduced or non-transduced TCM were incubated with different concentrations of aTCRmu-IgG, Isotype-IgG, aTCRmu-F(ab’)2 or Isotype-F(ab’)2 for 12h. To determine IFNγ secretion of TCM in response to tumor cells, TCM were co-incubated with ML2-B7 using a 1:1 effector to target ratio. Supernatants were analyzed by IFNγ-ELISA according to the manufacturer´s instructions (BD Bioscience). Apoptosis was determined by Annexin-5 (BD Bioscience) and 7-AAD (Sigma-Aldrich) staining according to manufacturer’s instructions and analyzed by flow cytometry (LSRII).

**Zirconium-89(89Zr) labeling of aTCRmu-F(ab’)2**

The labeling of aTCRmu-F(ab’)2 was performed based on the labeling protocol of Perk et al. (28) with slight modifications. Briefly, 37 MBq of 89Zr in 1M oxalic acid (BV Cyclotron VU) was adjusted with 2M sodium carbonate/0.5M HEPES after addition of 100µg DFO-aTCRmu- F(ab’)2. The 89Zr-Df-immunocomplex was purified by size exclusion chromatography and radiochemical purity was assessed by radio-ITLC and by SE-HPLC (BioSep™ SEC-s3000 LC Column, Phenomenex).

**Autoradiography**

Sections (20µm) of tumors and organs were exposed to a FujiFilm Imaging Plates (BAS-IP MS2025) for 48h followed by detection by a Phosphor-imager (CR35BIO, Dürr-Biomedical) and analyzed using AIDA Image analyzer software 4.21.

**Determination of 89Zr-aTCRmu-F(ab`)2 stability in vivo**

48 hours post intravenous injection of 89Zr-aTCRmu-F(ab`)2, organs of mice were frozen by liquid nitrogen and homogenized using Mikro Dismembrator II ball mill (B. Braun, Melsungen, Germany) followed by depletion of high density cellular components by centrifugation. Directly after withdrawal, blood was centrifuged and serum was collected. SDS-PAGE of protein solutions was performed under non-reducing conditions and separation gels were exposed to a FujiFilm Imaging Plates (BAS-IP MS2025) for 12h followed by detection by a Phosphor-imager (CR35BIO). Freshly labelled 89Zr-aTCRmu-F(ab`)2 was used as a control. Semiquantitative analysis was performed using AIDA Image analyzer 4.21 software by drawing region of interest (ROI) on bands representing stable and fragmented 89Zr-aTCRmu-F(ab`)2. Signal intensity in defined ROIs expressed as quantum level unit was background subtracted and shown for stable and fragmented 89Zr-aTCRmu-F(ab`)2 within different organs and blood.

**qPCR**

Genomic DNA (gDNA) was isolated using QiAamp DNA mini kit (Qiagen). Part of the murinized TCR beta region (TCRb) was amplified by quantitative RT-PCR in a StepOnePlus (Applied Biosystems) using the Primer 5’-GACCACGTGGAACTGTCTTG-3’ and 5’-CTCTCAGTCTGCTGGACAGG-3’ including the probe 5’FAM-ACACGCCGCTGTGCACCTCT-3’TAMRA. Amplification of the human constant TCR-beta region was analyzed using the primer 5’CGAGTCTTACCAGCAAGGG-3‘and 5’-ATACAAGGTGGCCTTCCCTA-3’ combined with the probe 5’FAM-TCCTGTCTGCCACCATCCTCTATGA-3’TAMRA. All reactions were performed using the universal PCR Master mix (KAPA™ PROBE FAST Universal) according to the manufacturer´s recommendations. Dilutions of pMP71 vector containing TCR with human or murinized constant beta gene sequences were used as a standard for number of molecule calculation and number of murinized TCR beta molecules were normalized to the amount of total human DNA (human TCR as reference molecule).

**Supplementary Figure legends**

**Figure S1: Principle of targeting of TCR2.5D6-transduced T cells by aTCRmu-IgG and aTCRmu-F(ab’)2**

Principle of the targeting strategy of human TCR-transgenic T cells: Transgenic TCR contain a murinized constant domain included to improve TCR-expression and pairing. The TCR are retrovirally transduced into human central memory T cells (TCM) and are specifically detected by the 89Zr-labeled aTCRmu-IgG or aTCRmu-F(ab`)2 using PET imaging.

**Figure S2. Investigation of different imaging time points to track TCR-transduced TCM after injection of 89Zr-aTCRmu-F(ab’)2**

(A) Overview of the experimental setting: 1x107 TCR-transduced TCM were injected directlyinto subcutaneously inoculated ML2-B7 tumors and 1x107 non-transduced TCM were injected into ML2-WT tumors. In control mice, PBS was injected into both tumors. 89Zr-aTCRmu-F(ab’)2 was subsequently injected intravenously and PET/CT imaging was performed 12h (B), 24h (C) or 48h (D) post injection (p.i.). Representative 3D-PET (MIP) images 12h (B), 24h (C) or 48h (D) p.i. of 89Zr-aTCRmu-F(ab’)2 with marked tumors are shown on the left graph. Scale bar: 0-15%ID/g. Quantitative ROI analyses of ML2-B7 and ML2-WT tumors of PET images 12h (B), 24h (C) or 48h (D) p.i. of 89Zr-aTCRmu-F(ab’)2 are depicted on the middle graphs. Mean ± s.d. of %ID/g in defined ROI is depicted. Graphs on the right side show tumor to blood ratio of %ID/g following ex vivo biodistribution analysis 12 (B), 24h (C) or 48h (D) post injection of 89Zr-aTCRmu-F(ab’)2. Mean ± s.d. of %ID/g ratio is depicted. Analysis has been performed with (B) TCM (n=5) and PBS (n=2), (C) TCM (n=6) and PBS (n=2), (D) TCM (n=3) and PBS (n=2). R=right, L=left, non-transd. = non-transduced.

**Figure S3: Investigation of dynamics of tumor rejection mediated by TCR2.5D6-transduced TCM to determine biologically critical imaging time points in an ML2-based myeloid sarcoma model**

(A) Overview of the experimental setting. NSG mice were inoculated subcutaneously with 1x107 ML2-B7 cells into the right and ML2-WT cells in to the left flank followed by 1Gy total body irradiation (TBI) and intravenous transfer of TCR-transduced TCM, non-transduced TCM or PBS. Irradiated (80Gy) human interleukin-15 producing NSO-cells were injected intraperitoneally three times per week. (B) Tumor size of ML2-B7 and ML2-WT at indicated days post T-cell injection is shown. Mean ± s.d. of tumor size is indicated in mm2 of n=6 animals. Flow cytometry analyses of single cell suspensions from tumors and organs were performed at day 10 post T-cell injection. (C) The percentage of viable human T cells and (D) percent TCR2.5D6-transduced T cells of viable T cells within the ML2-B7 or ML2-WT tumors, spleen and lung is shown. Mean ± s.d. of viable T cells (%CD45+ CD3+CD5+) or TCR-transduced T cells (%CD5+TCRmu+) of viable T cells is depicted for n=6 animals; Mann-Whitney Test: \*\*p<0.01.

**Figure S4. Investigation of uptake by biodistribution and autoradiography as well as stability of 89Zr-aTCRmu-F(ab’)2 within a clinically relevant ML2-based myeloid sarcoma model of adoptive T-cell transfer**

(A) Ex vivo biodistribution analysis of 89Zr-aTCRmu-F(ab’)2 in indicated organs 48h post injection. %ID/g ± s.d. is depicted for n=5 animals in each group treated with TCM and n=2 treated with PBS. Representative data of one out of three experiments is shown. Mann-Whitney test: \*\*p<0.01. (B) Representative data of autoradiography of indicated organs are shown. (C) In vivo stability of 89Zr-aTCRmu-F(ab`)2 48h post injection into NSG mice. Intensity in quantum level units of stable versus fragmented 89Zr-aTCRmu-F(ab`)2 in blood as well as the excretory organs kidney and liver are depicted.

**Figure S5: Differential distribution of 89Zr-aTCRmu-F(ab`)2 signals within ML2-B7 tumors of mice injected with 2.5D6TCR-transgenic TCM**

(A) Representative 3D-PET (MIP) pictures of ML2-B7 tumors 48h post intravenous injection of 89Zr-aTCRmu-F(ab’)2 are shown (experimental design is shown in Figure 2A). Classifications of tumors to group I (n=4), group II (n=4) and group III (n=3) depending on PET signal distribution and tumor size are shown. Scale bar = 0-15%ID/g; T=Tumor; K=Kidney. (B) ML2-B7 tumor development in mice injected with TCR2.5D6-transduced TCM or non-transduced TCM according to the classification within group I, group II and group III. Tumor size in mm2 at indicated days post TCM injection is depicted. Arrows indicate the days of TCM injection and PET/CT imaging. (C) Quantitative analysis of PET-signal of ML2-B7 tumors according to group classification. Mean ± s.d. of %ID of defined ROI is depicted. (D) Quantitative analysis of PET/CT images of ML2-B7 tumors according to group classification. Mean ± s.d. of %ID/g of defined ROI is depicted. (A-D) Data of 3 experiments are combined and include animals treated with TCR2.5D6 TCM (n=11).

**Figure S6: Determination of different tumor rejection kinetics according to tumor size**

(A) Overview of experimental design. NSG mice were inoculated subcutaneously with 1x107 ML2-B7 cells into the right and ML2-WT cells in to the left flank at different time points as indicated followed by 1Gy total body irradiation (TBI). Subsequently, TCR2.5D6-transduced TCM, non-transduced TCM or PBS were adoptively transferred. Three days after TCM injection, 18F-FDG imaging was performed followed by intravenous injection of 89Zr-aTCRmu-F(ab’)2 and PET/CT imaging after 48h. (B) Tumor size of subcutaneous ML2-B7 tumors at indicated days post intravenous injection of TCR2.5D6-TCM (n=4 for group I, n=7 for group II, n=5 for group III) or non-transduced TCM (n=3 for group I, II and III) is shown. Arrows indicate the day of subcutaneous tumor cell injection and intravenous TCM injection. Mean ± s.d. of tumor size is indicated in mm2. Mann-Whitney test: \*p<0.05; n.s.=not significant.