Immuno-PET Imaging of Engineered Human T Cells in Tumors

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Abstract

Sensitive \textit{in vivo} imaging technologies applicable to the clinical setting are still lacking for adoptive T-cell–based immunotherapies, an important gap to fill if mechanisms of tumor rejection or escape are to be understood. Here, we propose a highly sensitive imaging technology to track human TCR-transgenic T cells \textit{in vivo} by directly targeting the murinized constant TCR beta domain (TCRmu) with a zirconium-89 \textsuperscript{89}Zr-labeled anti–TCRmu-F(ab’\textsubscript{2}) fragment. Binding of the labeled or unlabeled F(ab’\textsubscript{2}) fragment did not impair functionality of transgenic T cells \textit{in vitro} and \textit{in vivo}. Using a murine xenograft model of human myeloid sarcoma, we monitored by Immuno-PET imaging human central memory T cells (T\textsubscript{CM}), which were transgenic for a myeloid peroxidase (MPO)–specific TCR. Diverse T-cell distribution patterns were detected by PET/CT imaging, depending on the tumor size and rejection phase. Results were confirmed by IHC and semiquantitative evaluation of T-cell infiltration within the tumor corresponding to the PET/CT images. Overall, these findings offer a preclinical proof of concept for an imaging approach that is readily tractable for clinical translation.

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Introduction

Adoptive transfer of T cells genetically modified by T-cell receptors (TCR) specifically recognizing malignant cells is an evolving therapeutic option for cancer patients. Clinical efficacy of TCR-transgenic T cells with specificity for cancer-associated antigens has been demonstrated (1–3), although long-term benefits are currently still limited (4). Novel target structures and TCR recognizing such targets are under intensive investigation. Understanding the \textit{in vivo} behavior of such genetically modified T cells in preclinical models has a high value for optimization of this approach before clinical application. More importantly, clinically applicable imaging technologies for TCR-modified T cells are considered to be critical for an improved comprehension of pharmacodynamics and pharmacokinetics in the clinical context of immunotherapy.

Positron emission tomography (PET) imaging is a noninvasive imaging technology providing advantages due to high spatial and temporal information on cellular distribution with the potential for clinical translation (5). However, most T-cell imaging approaches using PET are far from clinical application and none has been yet clinically approved. This is mainly caused by species-specific tool development in non-humans, the often applied clinically non-significant \textit{ex vivo} labeling procedures providing only short-term information after adoptive T-cell transfer and the negative impact of the labeling on T-cell function (6–10). Promising novel approaches include the usage of reporter genes or antibody derivatives for imaging. Usage of reporter genes such as HSV1-tk has been closest to clinical translation with few patients treated, although technical and regulatory hurdles so far impede broader application (5, 11–15). \textit{In vivo} imaging by Immuno-PET using antibody derivatives for targeting has been already clinically applied, although mainly for delineation of tumors (16). Recently, this technology has been also investigated to track murine T cells by targeting either general T-cell markers as CD4 and CD8 (17–19) or murine monoclonal TCR (20). However, these tools are mouse-specific and cannot be applied in humans. Moreover, the impact of these targeting strategies on T-cell function has not been analyzed so far. Thus, clinically translatable T-cell imaging strategies with high sensitivity and lack of functional impairment are currently missing although urgently needed.

We here take advantage of the introduction of murine sequences within the constant domain of TCR constructs as general optimization strategy for transgenic human TCR (21). This opens the possibility to use an anti-murine TCR monoclonal antibody (aTCRmu; ref. 22) for detection of TCR-transduced human T cells independent of the specificity of the transgenic TCR (23–25). We developed an F(ab’\textsubscript{2})\textsubscript{-}fragment-based imaging approach in a xenogenic human myeloid sarcoma model for
sensitive tracking of TCR-transgenic T cells by PET/CT imaging in vivo.

**Materials and Methods**

**Primary material and cell lines**

Blood from healthy donors was collected for isolation of peripheral blood mononuclear cells (PBMC) after informed consent following requirements of the local ethical board and principles of the Helsinki Declaration. The following cell lines were used: ML2 (The CABRI consortium, obtained in 2004), NSO-IL15 cells (kindly provided by S.R. Riddell in 2011; ref. 26), the TCRγδ-deficient T-cell line Jurkat76 (27), transduced with the CD8 alpha chain (Jurkat76-CD8t; kindly provided by W. Uckert, Molecular Cell Biology and Gene Therapy, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, in 2007) and the hybridoma H57-597 (HB-218, ATCC, acquired in 2012). All cell lines were routinely analyzed microscopically and tested for Mycoplasma infection (Venan GeM Mycoplasma Detection Kit, Minerva Bios), expression of cell line specifying surface markers, transgene expression, and HLA-typing.

**Genetic modification of CD8<sup>+</sup> central memory T cells and tumor cell lines by retroviral gene transfer**

Human CD8<sup>+</sup> central memory T cells (T<sub>CM</sub>) were isolated from PBMCs and retrovirally transduced with the optimized construct of TCR2.5D6 as described previously (23) and outlined in Supplementary Material. Jurkat76-CD8t cells were transduced with TCR2.5D6 and a cell line with stable transgene expression was generated by single-cell cloning. For in vivo depletion analyses, we used a TCR2.5D6 construct linked to near-infrared red protein (iRFP) by a T2A element. ML2 cells were transduced by the HLA-B<sup>07:02</sup> gene linked to enhanced GFP (eGFP). A stable ML2-B7GFP (ML2-B7) cell line was generated by single-cell cloning.

**Antibodies, flow cytometry-based assays, and analysis of cytokine secretion**

The following antibodies, antibody derivatives, and cell-labeling agents were used: The αTCRmu-IgG and F(ab<sub>2</sub>) were prepared from supernatant of H57-597 hybridoma cells using the Protein A-Sepharose (GE Healthcare) and F(ab<sub>2</sub>)<sup>2</sup> Preparation Kit (Thermo Scientific Pierce; Supplementary Material). For flow cytometry (LSRII; BD Biosciences), anti-human CD3 (UCHT1), anti-murine-TCRγδ-FITC (H57-597; all BD Biosciences), anti-human CD45 (12-2; Beckman Coulter), the MPO<sub>5</sub>-specific multimer (23), Annexin-5 (BD Biosciences), and 7-Aminoactinomycin (7-AAD, Sigma-Aldrich) were used. K<sub>d</sub> determination and apoptosis assays were performed by flow cytometry (LSRII) as described in the Supplementary Material. Cytokine secretion was analyzed as described previously (23) and outlined in the Supplementary Material.

**Tumor model and adaptive T-cell transfer**

C57BL/6 Rag<sup>−/−</sup>Il2rg<sup>−/−</sup>Tcrγδ<sup>−/−</sup>/HicTac (BRG; Taconic) and NOD.Cg-Pkdcd<sup>−/−</sup>Il2rg<sup>−/−</sup>/Slz (NSG; The Jackson Laboratory) were maintained according to the institutional guidelines and approval by local authorities. For depletion analysis, mice were challenged 1 day after intravenous injection of 1.5 × 10<sup>7</sup> TCR2.5D6-iRFP-transduced T<sub>CM</sub> (70% iRFP positivity) with 150 μg of αTCRmu-IgG or isotype-IgG and 110 μg of αTCRmu-F(ab<sub>2</sub>)<sub>2</sub> or isotype-F(ab<sub>2</sub>)<sub>2</sub> (both corresponding to 500 nmol/L in assumption of a 2-ml blood volume). To analyze T cells at the tumor site, 1 × 10<sup>7</sup> ML2-WT and ML2-B7 tumor cells were inoculated subcutaneously into the right and left flank, respectively, of NSG or BRG mice. Tumor size was assessed by caliper measurement. Before intravenous injection of T<sub>CM</sub>, mice were total body irradiated (TBI) with one Gray (Gy; Glimray Irradiator). Human IL15–producing NSO cells, irradiated with 80Gy, were injected three times weekly intraperitoneally. TCR2.5D6-transduced T<sub>CM</sub> nontransduced T<sub>CM</sub> or PBS were injected in the tumor (only Supplementary Fig. S2) or intravenously (all other experiments) at indicated time points.

**Conjugation and zirconium-89-labeling of αTCRmu-F(ab<sub>2</sub>)<sub>2</sub>**

The p-isothiocyanatobenzyl-derivative of desferrioxamine (DFO-Bz-NCS, Macrocycles, Inc.) was used as a bifunctional chelator for zirconium-89<sup>89</sup>Zr) and was added in a 3-fold molar excess to the αTCRmu-F(ab<sub>2</sub>)<sub>2</sub>, followed by purification using size exclusion chromatography (Sephadex G-25 M, PD10 column, GE Healthcare). The labeling of αTCRmu-F(ab<sub>2</sub>)<sub>2</sub>, with <sup>89</sup>Zr was performed on the basis of the protocol of Perk and colleagues (28) with slight modifications (Supplementary Material).

**PET/CT imaging**

Mice were anesthetized and imaged with the Inveon small-animal positron emission tomography/computer tomography (PET/CT) scanner (Siemens) after indicated time points of intravenous <sup>89</sup>Zr-αTCRmu-F(ab<sub>2</sub>)<sub>2</sub>, injection or 10–20 μg injection or 15 minutes after intravenous injection of (18-Fluor)1-Fluor-2-deoxy-D-glucose (18F-FDG, 15MBq). Static PET emission images were acquired for 20 minutes (<sup>89</sup>Zr-αTCRmu-F(ab<sub>2</sub>)<sub>2</sub>) or 20 minutes (18F-FDG) and reconstructed using OSEM-3D algorithm. CT images were reconstructed using a modified Feldcamp algorithm and PET and CT images were fused and analyzed using the Inveon Research Workspace (Siemens). The data were corrected for attenuation and radioactive decay. Tracer uptake was calculated as the percentage of injected dose per gram of tissue (%ID/g, 1 cc = 1 g). For quantification, a region of interest (ROI) encompassing the tumor was drawn based on CT images, transferred to the PET data, and mean values (%ID/g) were determined using a threshold of 50% of maximal uptake inside the tumor region. For quantification of differential distribution patterns, uptake in ROI of respective tumors was analyzed without threshold and expressed as %ID/g or as total activity (%ID) by multiplying the uptake by the region volume.

**Biodistribution analysis**

Mice were sacrificed 48 hours after <sup>89</sup>Zr-αTCRmu-F(ab<sub>2</sub>)<sub>2</sub> administration. Blood and organs were collected, weighted, and radioactivity was counted against a standard of known activity in a gamma-counter (2480Wizard2, PerkinElmer). Tumor and organ uptake was calculated as %ID/g or percent injected dose (%ID) and expressed as ratio to blood uptake.

**qPCR**

Genomic DNA (gDNA) was isolated using the QiAamp DNA Mini Kit (Qiagen). Part of the murinized TCR beta region (TCRb) was amplified by quantitative RT-PCR in a StepOnePlus (Applied Biosystems) as described in Supplementary Material.
PET Imaging of T-cell Receptor-Engineered T Cells

Histology and IHC
During necropsy of mice, ventral and dorsal orientation of tumors was flagged by tissue ink followed by cutting the tumors in axial plane. Tumors were fixed in 10% formalin and embedded in paraffin with downward orientation of the axial cutting site. Two-micron thick sections on different levels of the tumors were stained with hematoxylin and eosin (H&E) and consecutive slides were used to detect human T cells. Anti-CD5 (4C7, Novacastra) and anti-CD3 (MRQ-39, Cell Marque) staining was performed on an automated immunostainer with an iVIEW DAB Detection Kit (Ventana Medical Systems, Roche) according to the company’s protocols with slight modifications. Slides were evaluated with an Olympus BX53 microscope by semiquantitative evaluation of T-cell infiltration in predefined regions depicted by colored codes as indicated. Colocalization of imaging and histologic slices was performed by calculating the sum of total sections, including a variation factor of tissue modification due to the technical procedure.

Statistical analysis
Data are presented as mean ± SD. Statistical analysis of results was performed using GraphPad Prism software version 5.01 using the Mann–Whitney test as indicated in the figure legends.

Results
aTCRmu-F(ab')2 binds specifically and with high affinity to TCR-transduced cells and binding of nonlabeled and labeled aTCRmu-F(ab')2 has no impact on functionality of TCR-transgenic TCM
The hamster aTCRmu antibody (H57-597) specifically recognizes the murine TCR beta domain present in murine T cells as well as engineered TCR constructs (22, 23, 29). We used this antibody (aTCRmu-IgG) as well as its F(ab’)2 fragment (aTCRmu-F(ab’)2) to track specifically human T cells engineered with a tumor-specific TCR (Supplementary Fig. S1). For establishment of an imaging model of TCR-transgenic T cells harboring such modification, we took advantage of the previously described human-derived leukemia-reactive TCR2.5D6 transduced into human CD8-enriched TCM, resulting in specific recognition of a myeloperoxidase-derived peptide (MPO22) in the context of HLA-B7 (23). Flow cytometry–based binding analyses using TCR2.5D6-transducedJurkat76 cells revealed a comparable dissociation constant (Kd) for the full antibody and its F(ab’)2 derivative (Fig. 1A). We then analyzed the influence of aTCRmu-IgG or aTCRmu-F(ab’)2 on transduced TCM. Incubation of TCR-transduced TCM with aTCRmu-F(ab’)2 for 12 hours did not influence the T-cell apoptosis rate, whereas aTCRmu-IgG induced a dose-dependent increase of apoptotic cells (Fig. 1B, top). Similarly, we detected a dose-dependent secretion of IFNγ by TCR2.5D6-transduced TCM after incubation with aTCRmu-IgG but not with aTCRmu-F(ab’)2 (Fig. 1B, bottom). To analyze the functional impact of aTCRmu-F(ab’)2 versus aTCRmu-IgG in vivo, TCM cells were transduced with TCR2.5D6 linked to the near-infrared red fluorescent protein (iRFP) and intravenously injected into NSG-mice. We found reduced percentages of engrafted TCR-transgenic T cells in different organs after intravenous application of aTCRmu-IgG, which was not observed after application of aTCRmu F(ab’)2 (Fig. 1C). After confirmation of a lack of functional influence of TCR2.5D6-transgenic TCM by incubation with aTCRmu-F(ab’)2 in vitro and in vivo, we conjugated the aTCRmu-F(ab’)2 with DFO-Bz-NCS followed by labeling with 89Zr (89Zr-aTCRmu-F(ab’)2) to exclude a potential functional impairment by exposure to the radioactive tracer. After in vitro labeling by 89Zr-aTCRmu-F(ab’)2, we observed similar IFNγ secretion of labeled TCM in response to ML2 cells transduced with HLA-B7 (ML2-B7) as compared with incubation with the nonradioactive aTCRmu-F(ab’)2 (Fig. 1D).

Investigation of suitable time points for PET/CT imaging of TCR-transduced TCM using 89Zr-aTCRmu-F(ab’)2
To investigate the feasibility to visualize TCR-transduced TCM by 89Zr-aTCRmu-F(ab’)2, as well as determination of the optimal imaging timepoint after 89Zr-aTCRmu-F(ab’)2 application, we developed an in vivo model of myeloid sarcoma by subcutaneous inoculation of ML2-B7 on one flank and ML2-WT cells on the other flank of BRC-mice. Transgenic TCM were initially directly injected in ML2-B7 tumors followed by intravenous injection of 89Zr-aTCRmu-F(ab’)2 (Supplementary Fig. S2A). Nontransduced TCM were injected in ML2-WT tumors as control. In comparison with controls, we observed the strongest specific signal in the ML2-B7 tumors 48 hours after injection further confirmed by quantification of the PET signal and ex vivo biodistribution (Supplementary Fig. S2B–S2D). This time point has been therefore selected as most suitable for imaging TCR-transgenic TCM by 89Zr-aTCRmu-F(ab’)2.

Investigation of dynamics of TCM-mediated tumor rejection to determine biologically critical imaging time points in a clinically relevant ML2-based myeloid sarcoma model
To model a clinical scenario and determine biologically critical time points for T-cell imaging, we injected TCM intravenously into NSG mice harboring ML2 tumors as described above (Supplementary Fig. S3A). Significant rejection of ML2-B7 tumors was observed in mice injected with TCR2.5D6 TCM in contrast with mice treated with non-transduced TCM or PBS (Supplementary Fig. S3B). ML2-WT tumors showed no modification in growing kinetics following adoptive transfer of TCR-transduced TCM or controls (Supplementary Fig. S3B). Thus, the established model proved high efficacy of TCR2.5D6-transduced TCM in a clinically relevant scenario and suggested day 4 to 5 after T-cell transfer as turning point during rejection, and therefore highly interesting for imaging. Ex vivo flow cytometry analyses confirmed a significant increase in total human T cells within the ML2-B7 tumors but not in the ML2-WT tumors (Supplementary Fig. S3C). T cells were not enriched in tumors of mice injected with nontransduced TCM, whereas T-cell infiltration was similar in spleen and lung, indicating comparable engraftment ratios (Supplementary Fig. S3C). Within the group injected with TCR2.5D6-transduced TCM around 30% of all T cells present in the ML2-B7 tumor was positive for aTCRmu (Supplementary Fig. S3D).

Tracking of TCR2.5D6-transduced TCM in tumor-bearing NSG mice by 89Zr-aTCRmu-F(ab’)2 PET imaging in the course of tumor rejection
For monitoring of TCR2.5D6-transduced TCM in the established in vivo model, we applied 89Zr-aTCRmu-F(ab’)2 3 days after adoptive T-cell transfer of TCM and analyzed mice by PET/
We detected a highly distinct signal at the ML2-B7 tumor site in animals treated with TCR2.5D6-transduced TCM in comparison with controls (Fig. 2B). Image-based quantification confirmed these findings (Fig. 2C). Ex vivo biodistribution analyses showed a 13-fold increased uptake in relation to blood in ML2-B7 tumors of mice injected with TCR2.5D6-transduced TCM (Fig. 2D). Furthermore, we detected a significant and 4-fold increased tumor-to-blood ratio in ML2-B7 tumors compared with ML2-WT tumors. Mice infused with nontransduced TCM or PBS showed no differences of tumor to blood ratio between both tumors (Fig. 2D). Of note, we detected ex vivo a higher activity in the spleen and lung of mice.
treated with TCR2.5D6-transduced TCM compared with non-transduced TCM (Fig. 2D), whereas distribution of $^{89}$Zr-aTCRmu-F(ab')$_2$ in all other organs was similar independent of the treatment modality (Supplementary Fig. S4A). Autoradiography of tumors and tissue sections confirmed these findings (Supplementary Fig. S4B). In vivo stability of aTCRmu-F(ab')$_2$ was reassured by ex vivo detection of stable $^{89}$Zr-aTCRmu-F(ab')$_2$ in the blood of mice 48 hours after injection (Supplementary Fig. S4C).

We further validated our data by ex vivo detection of T cells after imaging. We observed the increased percentage of total human T cells in ML2-B7 tumors by flow cytometry in mice injected with TCR2.5D6-transduced TCM (Fig. 3A). In contrast, T-cell percentages were comparable in spleen and lung irrespective of the treatment (Fig. 3A). Moreover, we detected high percentages of TCR2.5D6-transduced T cells within the ML2-B7 tumors but not in the ML2-WT tumors (Fig. 3B) confirming the specificity of the PET-signal. Slightly enhanced levels of TCR2.5D6-transduced T cells were detected in the lung. Analysis of gDNA by qPCR revealed elevated copies of TCR2.5D6 in the ML2-B7 tumor, spleen, and lung (Fig. 3C) corresponding to biodistribution data. IHC of tumor sections showed tumor cell necrosis and presence of high numbers of CD5$^+$ T cells within ML2-B7 tumors. In contrast, we observed only few T cells in ML2-WT tumors and no signs of tumor necrosis (Fig. 3D).
Qualitative and quantitative evaluation of $^{89}$Zr-aTCRmu-F(ab')$_2$ signals in ML2-B7 tumors

In-depth analysis of mice injected with TCR2.5D6 TCM revealed differences in distribution of $^{89}$Zr-aTCRmu-F(ab')$_2$ signals in vivo within ML2-B7 tumors depending on the tumor size (Supplementary Fig. S5A). Tumors derived from different experiments ($n=11$) were therefore classified according to their size and signal distribution pattern (Supplementary Fig. S5A–S5B). Interestingly, within the region of interest, the size correlated with the total injected activity but not with the injected activity per gram (Supplementary Fig. S5C–S5D). To confirm these observations, mice were injected subcutaneously with ML2-B7 at different time points before intravenous application of TCR2.5D6-transduced or nontransduced TCM application to provide different tumor kinetics (Supplementary Fig. S6A and S6B). We primarily performed $^{18}$F-FDG-PET/CT imaging and observed a homogenous uptake of $^{18}$F-FDG within ML2-B7 tumors 2 days after TCM transfer (Fig. 4A). In contrast, $^{89}$Zr-aTCRmu-F(ab')$_2$ imaging revealed a different signal distribution with hotspot signal enhancement areas dependent on the tumor size (Fig. 4A).

We confirmed correlation of signal distribution patterns to the different groups, with group I tumors having more intense signals at the tumor border, whereas group II and III demonstrating signals in the center of the tumors (Fig. 4A). The image-based total %ID quantification in the tumor was enhanced in tumors of group I compared with group II and III (Fig. 4B) corresponding to tumor volume (Fig. 4C), whereas %ID/g of
tumors did not show significant differences between the groups (Fig. 4D).

Mapping of adoptively transferred T cells within the tumor by 89Zr-aTCRmu-F(ab')2-based PET/CT

We validated the PET/CT imaging data by IHC of tumor sections on different axial levels (Fig. 5). Intra- and intersectional heterogeneity of CD3+ T-cell infiltrations within tumors of group I was obvious, whereas tumors of group II showed a uniform T-cell distribution. We colocalized tumor sections to respective axial imaging levels for deeper correlation analysis (Fig. 5A–D). The intrasectional T-cell heterogeneity was mapped by semiquantitative analysis of T-cell infiltration within predefined areas correlating to respective PET-imaging levels (Fig. 5B–D). Thus, using 89Zr-aTCRmu-F(ab')2 PET/CT, we were able to map the differential distribution of T-cell infiltrations within the tumor, and thereby classified different tumor rejection phases reflecting the dynamics of T-cell response of TCR-transgenic T cells in this model.

**Discussion**

We here present a novel and highly sensitive imaging approach for mapping of TCR-engineered T cells within the tumor by PET/CT. We directly target the transgene with an antibody construct recognizing the murinized constant TCR beta domain applicable for any TCR independent of the defined specificity.

Our approach provides many advantages of previous published studies essential for clinical translation. It is, to our knowledge, the first approach using a F(ab')2-based technology for specific in vivo imaging of human TCR-transgenic T cells by directly targeting the TCR without any impact on the function of targeted T cells. This is of particular interest as direct targeting of the TCR complex has been previously demonstrated to have an impact on T-cell function as well as viability potentially jeopardizing therapeutic efficacy (20, 30). As previously reported (22, 31–33), we also observed alterations of T-cell function and viability by the full IgG anti-TCRmu but not its...
Figure 5.
T-cell imaging by PET/CT using $^{89}$Zr-aTCRmu-F(ab')$_2$ facilitates mapping of T cells within ML2-B7 tumors of mice injected with TCR2.5D6-transgenic TCM. A, coronal PET image of a representative ML2-B7 tumor of mice injected with TCR2.5D6 transgenic TCM classified into group I (top) and group II (bottom). Dotted lines, a., b., and c. show the position of corresponding axial PET images depicted in B; scale bar, 0–15%ID/g. C, heterogeneous CD3$^+$ T-cell infiltration within ML2-B7 tumor sections corresponding to the respective axial PET images. IHC of CD3$^+$ T cells within ML2-B7 tumor sections is depicted. Differential CD3$^+$ T-cell distribution within respective tissue section was mapped by plotting the percentage of T-cell infiltration in pre-defined areas using a color code as shown in the figure; bar, 2 or 0.9 mm as shown in the figure. D, magnification of ML2-B7 tumor areas showing differential intrasectional heterogeneity of T-cell infiltration. Localization of magnification areas is shown by indicated numbers corresponding to marked areas of tumor sections shown in C; bar, 100 μm.
PET Imaging of T-cell Receptor-Engineered T Cells

F(ab')2. In contrast with direct ex vivo labeling strategies, our in vivo labeling approach has the major advantage to provide the capability to image at any time point of interest. This is important for long-term monitoring of the therapeutic efficacy. Moreover, by choosing the primary transgene as target for labeling usage of reporter genes can be omitted. This is of practical importance as reporter gene expression may be influenced by the metabolic stage of transgenic cells (34) and defined probes have been reported to increase background in replicating organs such as tumors, resulting in reduced sensitivity (35–37). In fact, the high sensitivity and excellent signal-to-noise ratio within the tumor observed in our experiments has not been previously reported for other T-cell imaging studies. In vivo, we detected an absolute uptake of 3.2% to 6.0% ID/g compared with 2.04% ID/g within the control, whereas ex vivo the difference was even more accentuated with 10.7% ID/g compared with 2.2% ID/g background. Imaging-based quantification has been reported to be inferior to ex vivo analyses due to diverse influences as detector performance, dead time, and partial volume effects as described (15, 39–42). Moreover, the number of effector cells infused in our experiments was within the range of those previously applied in clinical trials (1–3, 43). As TCM engraftment can be expected to be superior in humans as compared with NGS mice, this preclinical model may even underestimate the potential of this approach. Our technique has, therefore, the potential to be used as clinically relevant surrogate marker for efficacy of T-cell–based immunotherapies using TCR-transgenic T cells.

Specific signals detected by PET/CT were not only intensively validated by ex vivo biodistribution and autoradiography analyses but also by the direct detection of TCR-engineered TCM by flow cytometry and qPCR confirming data provided by PET/CT. Of particular interest, we were not only able to visualize T cells but concretely map them by PET/CT within the tumor as validated by corresponding IHC. The comprehensive qualitative analysis of imaging data combined with signal quantification with respect to the total injected dose therefore provided information about the stage of tumor rejection.

Background 89Zr-uptake in kidney and liver was expected and results from fragment reabsorption and retention of radioactivity in these organs as described previously (44) and shown in our in vivo stability assays. This may limit the application for metastases affecting liver and kidneys. However, 89Zr-labeling of an anti-HER2-Fab showed much better in vivo stability and tumor uptake compared with 124I-labeling (45). There are a number of options for potential improvement as the usage of smaller constructs with rapid blood clearance (46), amino acid preloading (47), infusion of cold renal–blocking agents (48), introduction of metabolizable linkages facilitating excretion of radiometabolites (44), or infusion of DFO to capture free 89Zr (49), which need to be evaluated in the future.

Potential immunogenicity of partially murinized TCR might be critical for clinical translation. Antibody development against the variable domains of a murine TCR has been previously observed in treated patients. However, this response was neither associated to the persistence of transgenic cells nor response to therapy (50). Reduction of murine gene segments sparing the relevant epitope as well as humanization of the aTCRmu-F(ab’)2, itself may decrease the risk of immunogenicity.

Taken together, our data indicate that this noninvasive 89Zr-aTCRmu-F(ab’)2–based immuno-imaging technology reaches a sensitivity to provide deeper insights in pharmacodynamics of transgenic T cells with the potential for clinical translation. The technology may be useful for the timely identification of T-cell infiltration versus exclusion from the tumor and therefore might be implemented as potential surrogate marker for the identification of responders versus nonresponders.

Disclosure of Potential Conflicts of Interest

A.M. Krackhardt and R. Klar are involved in a patent application currently ongoing for the defined MPO peptide and sequences of TCR2.5D6. M. Schwaiger received a commercial research grant from Siemens Medical Research, received speakers’ bureau honoraria from Siemens Lungh Symposium, and has ownership interest (including patents) in Siemens. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: S. Mall, A.M. Krackhardt Development of methodology: S. Mall, N. Yusufi, R. Wagner, R. Klar, H. Bianchi, M. Straub, M. Schweiger, C. D’Alessandria, A.M. Krackhardt Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Mall, N. Yusufi, R. Wagner, H. Bianchi, M. Straub, S. Ausdehm, I. Laitinen, M. Aichler, S. Ziegler, A.M. Krackhardt Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Mall, N. Yusufi, R. Wagner, H. Bianchi, K. Steiger, M. Straub, I. Laitinen, M. Aichler, S. Ziegler, M. Mustafa, M. Schweiger, C. D’Alessandria, A.M. Krackhardt Writing, review, and/or revision of the manuscript: S. Mall, N. Yusufi, I. Laitinen, M. Aichler, C. Peschel, S. Ziegler, M. Mustafa, M. Schweiger, C. D’Alessandria, A.M. Krackhardt Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Mall, K. Steiger, I. Laitinen, M. Schweiger, A.M. Krackhardt Study supervision: M. Schweiger, A.M. Krackhardt

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