222-I A SINGLE-DOMAIN ANTIBODY-BASED PET TRACER TO VISUALIZE HUMAN CD4-POSITIVE CELLS IN VIVO

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Background $CD4^+$ T cells play essential roles in the development and regulation of autoimmune diseases and cancer. Recently, we have developed single-domain antibodies that bind with high affinity to human CD4 receptor without affecting T cell function. The lead candidate was radiolabeled with NODAGA and Cu-64 (^{64}Cu-αCD4-sdAb) for PET imaging. The aim of this study was to evaluate the applicability of our ^{64}Cu-αCD4-sdAb to visualize and differentiate minimal changes in the $CD4^+$ immune infiltrate during cancer immunotherapy and in inflamed tissue.

Methods ^{64}Cu-αCD4-sdAb was administered intravenously in two orthotopic cancer models and a cutaneous Delayed-Type-Hypersensitivity-Reaction (cDTHR) model. In the first approach, PyMT breast cancer and B16 melanoma cells were implanted in C57BL/6J human-CD4 knock-in ($hCD4^{KI}$) or wildtype (WT) mice. A subgroup of animals was additionally treated with therapeutic αPD1/α41BB antibodies. To induce cDTHR, $hCD4^{KI}$ mice were sensitized at the abdomen and repetitively challenged at one ear with trinitrochlorobenzene (TNCB). ^{64}Cu-αCD4-sdAb PET/MRI was conducted 24 h after the 5th TNCB-ear challenge and organs of interest were harvested for *ex vivo* biodistribution, immunohistochemistry or mass cytometry (CyTOF) analyses immediately after the last imaging time-point.

Results Dynamic ^{64}Cu-αCD4-sdAb PET/MRI of PyMT tumors over 6 h revealed the best differentiation between $hCD4^{KI}$ and WT mice at 1.5 h and 3 h imaging time-point post tracer injection. In line with *ex vivo* analyses of intratumoral $CD4^+$ cell densities, αPD1/α41BB-treated PyMT tumors of $hCD4^{KI}$ mice showed significantly higher ^{64}Cu-αCD4-sdAb uptake (0.46±0.01%ID/ml) compared to untreated littermates (0.30 ±0.03%ID/ml) and PyMT tumors of WT mice (0.21±0.02% ID/ml). In contrast, B16 tumors with histologically almost absent $CD4^+$ cell infiltrates yielded an uptake of 0.23±0.02% ID/ml similar to WT mice. In the cDTHR model, the ^{64}Cu-α CD4-sdAb uptake in the inflamed ears was 3-fold higher compared to control ears. Importantly, a control sdAb tracer did not reveal such differences in the inflamed and non-inflamed ears, excluding relevant non-specific perfusion effects. Immunohistochemistry revealed a rare number of CD4+ cells in the inflamed tissues being detectable by out extremely sensitive PET/MRI approach.

Conclusions Due to the important role of $CD4^+$ cells in health and disease, precise non-invasive imaging approaches to visualize and monitor $CD4^+$ cells *in vivo* are urgently needed. Our newly developed ^{64}Cu-αCD4-sdAb PET tracer is able to detect small amounts of $CD4^+$-cell infiltrates in both cancer and cDTHR models, pointing out its potential to serve as a versatile probe for a broad range of T cell-associated diseases.

Ethics Approval All animal experiments were carried out in accordance with the German Animal Welfare Act and with consent of regulatory authorities (Regierungspräsidium Tübingen).

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