PET imaging of CD8+ T-cells using 89Zr-Crefmirlimab berdoxam in healthy Non-Human Primates

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Introduction

A $CD8^+$ PET imaging probe using a radiolabeled human CD8 minibody (^{89}Zr-crefmirlimab berdoxam, ImaginAb) can help to select effective single or combination immunotherapies in oncology clinical trials [1,2]. $CD8^+$ T-cells are not only a major biomarker in cancer immunotherapy, [3] but they are also a critical biomarker in vaccine development [4]. The COVID-19 pandemic generated significant interest for new vaccine development and the ideal species to understand the immune response to vaccines is the non-human primate (NHP) [5]. This study is the first CD8 PET imaging study in NHPs to provide a non-invasive method of visualizing the biodistribution of $CD8^+$ leukocytes in the lymphatic organs.

Radiochemistry

Crefmirlimab berdoxam was obtained from ImaginAb, Inc. (Inglewood, CA). 89Zr-oxalate was purchased from 3D imaging (Little Rock, AK). PD-10 desalting columns were purchased from Cytiva Life Science (Marlborough, MA). Crefmirlimab berdoxam was radiolabeled at the Radiochemistry and Molecular Imaging Probe Facility at Memorial Sloan Kettering Cancer Center (New York, NY). Briefly, approximately 2.5mg of Crefmirlimab berdoxam was labelled with 89Zr and purified by a PD-10 column. The final radiolabeled product was tested for appearance, pH, radiochemical identity, and purity by size-exclusion high-performance liquid chromatography (radio SEC) and instant thin-layer-chromatography (iTLC), and for immunoreactivity by the bead method. The radiolabeling efficiency was above 99%, radiochemical purity was above 95% (as determined by radio SEC), and minibody binding was above 95%.

Ethical approval:

The study was conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and was reviewed by the Institutional Animal Care and Use Committee at GSK

Study design

Two female Cynomolgus NHPs with body weights of 4.25 kg and 5.45 kg were included in this study. Each NHP received a bolus IV injection of a GMP grade ^{89}Zr-Crefmirlimab berdoxam (^{89}Zr-Df-IAB22M2C, ~1mCi) in the concentration of 0.18mg/ml for a total dose 0.25mg/kg. The dose calculation was based on the estimated affinity of Crefmirlimab in Cynomologus NHP. ADA (anti-drug antibody) assay was performed prior to the imaging study to confirm immunogenicity. Full body PET/CT imaging was performed on a Mediso LFER scanner at 17 hours, and on days 2, 5, and 7 post injection. SUVmean uptake of ^{89}Zr-Crefmirlimab berdoxam was measured in the heart, liver, spleen, kidneys, and lymph nodes.

Results

The PET images (Figure 1) showed high uptake of ^{89}Zr-Crefmirlimab berdoxam in the liver, spleen, kidneys, and lymph nodes and a low background uptake in CD8 poor tissues (e.g. muscle and lung). The quantitative SUVmean (Figure 2) showed a decreasing uptake over time in the heart matching the ex-vivo blood uptake. A steady state uptake was observed in the liver and spleen after the end of the distribution phase (SUVmean: 13.87, and 5.21 on day 5, respectively. Figure 3 upper). Interestingly, the uptake in the kidney was very high compared to any other organ with a distinguished uptake in the cortex compared to the medulla (SUVmean: 18.44, and 9.95 on day 5, respectively). The SUVmean uptake gradually increased over time in lymph nodes (LNs) with the highest uptake in the cervical LN compared to the axillary and inguinal LNs (SUVmean: 15.4, 9.4, and 9.18 on day 5, respectively. Figure 3 bottom). The tissue:blood ratios in different organs: brain, heart, liver, spleen, kidney, lymph nodes, bone marrow, vertebrae are presented in Figure 4. The highest tissue:blood ratio in lymph nodes are 12.44 in cervical LN at day 5 and 12.12 at day 7.

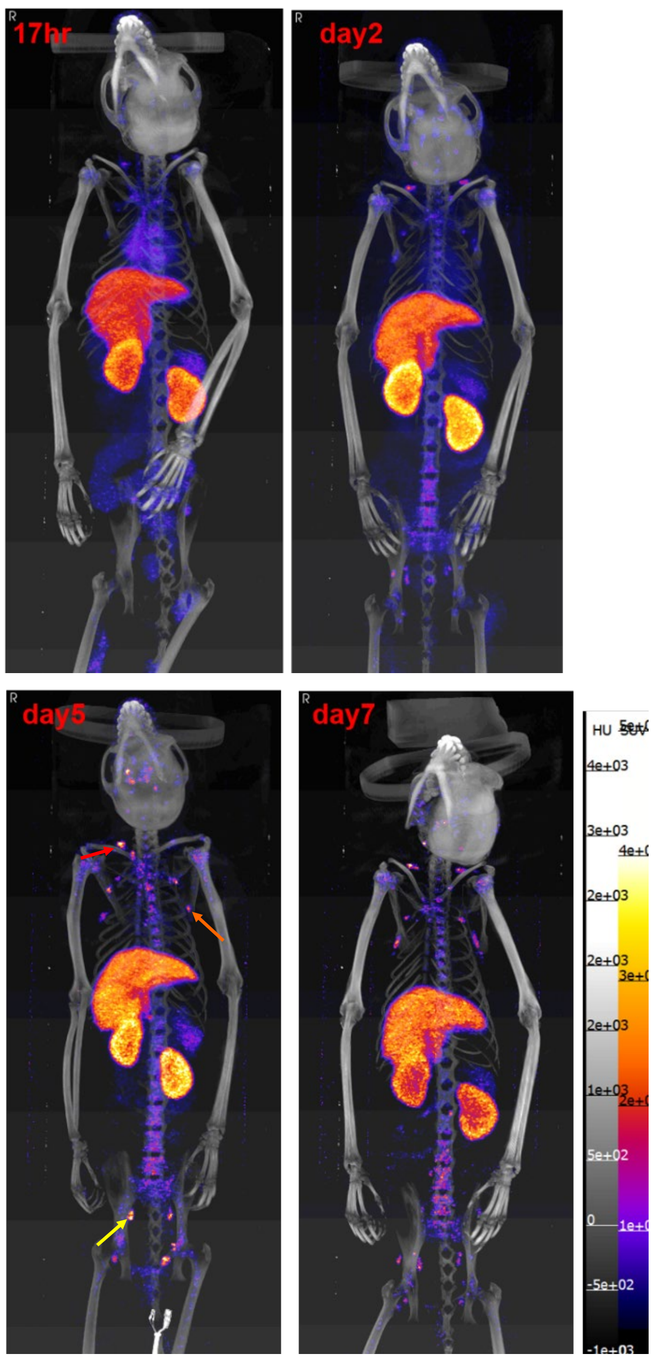

Figure 1: Full body PET/CT images of an NHP post injection of ^{89}Zr- Crefmirlimab berdoxam showing high uptake in the liver and kidneys with distinguished higher uptake in the renal cortex. A high uptake can be observed in the cervical, axillary, inguinal lymph nodes (red, orange, yellow arrows, respectively).

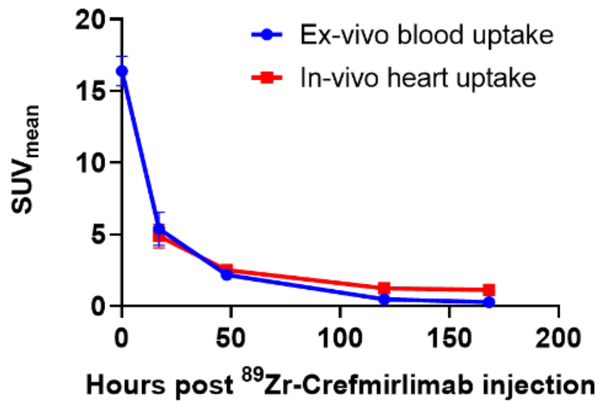

Figure 2: The quantitative SUVmean showed a decreasing uptake over time in the heart matching the ex-vivo blood uptake.

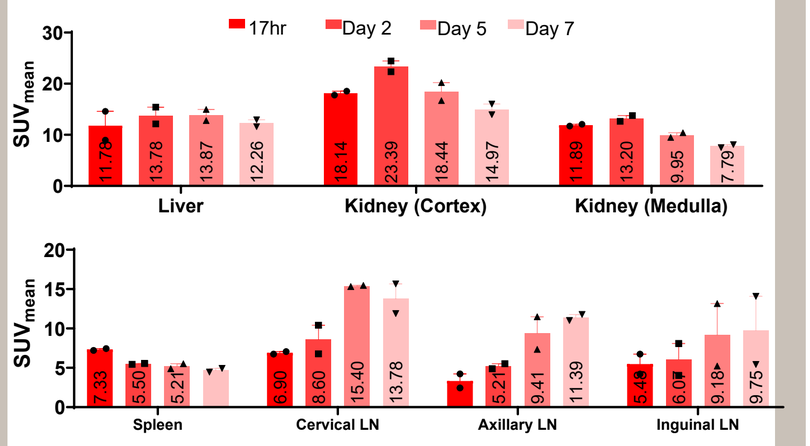

Figure 3: The in-vivo uptake of ^{89}Zr-Crefmirlimab berdoxam in different organs at 17 hrs, 2 days, 5 days, and 7 days post injection.. The quantitative uptake (SUVmean) was analyzed in liver, kidney, spleen and lymph node. The liver and kidneys (cortex, and medulla) out to 7 days post injection. The spleen, and lymph nodes (cervical, axillary, inguinal LNs) showed a higher accumulation in the cervical LN as opposed to axillary or inguinal LNs.

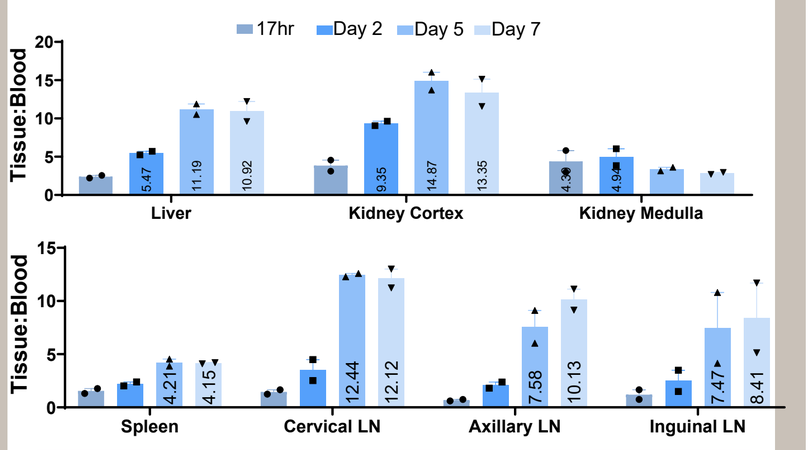

Figure 3: The tissue blood ratio was generated by PET uptake of each tissue (organs) normalized by PET uptake of heart. The highest tissue:blood ratio in lymph nodes are 12.44 in cervical LN at day 5 and 12.12 at day 7.

Conclusions

In this study, we assessed the baseline distribution of ^{89}Zr-Crefmirlimab berdoxam in healthy NHPs. The data confirmed Crefmirlimab has a high uptake in the lymphatic system with a low background which makes it an ideal PET probe to non-invasively monitor whole body biodistribution of CD8+ T-cells for vaccine and infection disease drug development.

References

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Conflict of Interest

Shih-Hsun Cheng, Steve Lenhard, Heather Haag, Shelly Marshall, and Hasan Alsaid are employees of GSK.
Fang Jia, Alessandro Mascioni, Ian Wilson are employees of ImaginAb, Inc.