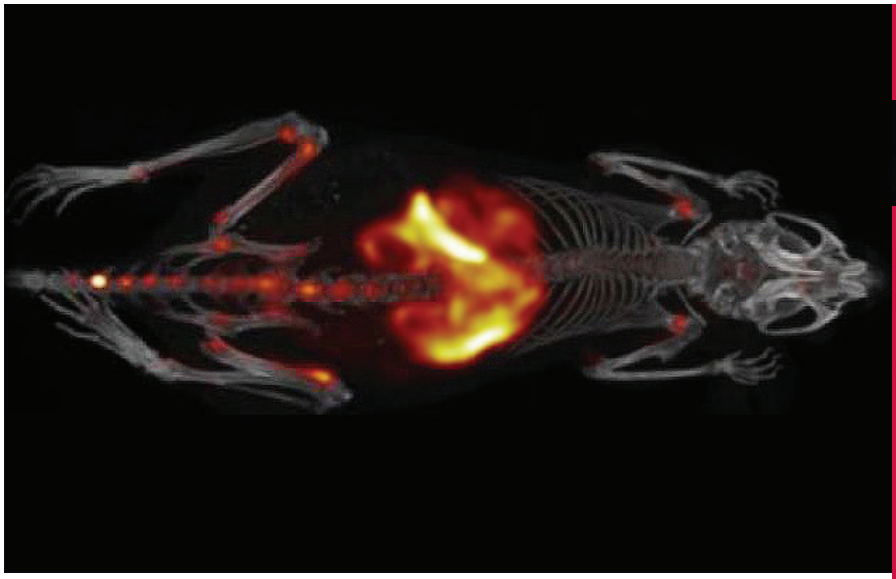

APPLICATION NOTE

Preclinical *In Vivo* Imaging

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Immuno-PET: PET Imaging using Radiolabeled Antibodies

Positron Emission Tomography (PET) is a highly sensitive functional imaging modality that provides 3-dimensional and quantifiable visualization of biological processes. To obtain anatomical information, PET is often combined with a CT scan. The effectiveness of PET imaging, is dependent on the specificity of the biomolecule portion of the injectable radiotracer, to the desired target. PET imaging quality can be compromised by high background signal due to unbound isotopes or nonspecifically targeted agents.

Monoclonal Antibodies (mAbs) are high affinity molecules that can be used for specific binding and delivery to cell surface molecules, thanks to their strict targeting abilities. In 1975, the antibody revolution began thanks to the introduction of the hybridoma technology by Kohler and Milstein. This technology made it possible for an unlimited range of mAbs to be obtained against any type of cellular antigen. As a result, the widespread availability of these antibodies with the unmatched ability to identify highly specific protein targets, has been extensively exploited for both *in vitro* diagnostics and *in vivo* therapeutics. The first generation of mAbs were of murine origin making them immunogenic thus limiting their clinical use. Consequently, chimeric mAbs, humanized mAbs, and complete human mAbs were produced to solve this issue. However, the use of full-sized antibodies can be limited in the context of PET as their considerable size (~150 kDa) results in a long circulatory half-life and reduced tissue penetration. To improve the imaging performance, alternative ligands have been studies and developed including antibody fragments and engineered variants $F(ab')^2$ and F(ab'), single-chain variable fragments (scFv), diabodies, and minibodies (~25–100 kDa) and other types of therapeutic proteins. e.g. affibodies and nanobodies. Facilitated by these developments, therapeutic antibodies have been designed to bind specifically to cancer cells, induce cell death and engage host immune effector responses[1].

Antibody targets can be identified and generated for a variety of applications including cancer detection and staging, tumor and metastasis phenotyping, stratification of patients into treatment groups and the evaluation of tumor targeting and therapy response. Once the optimal antibody has selected, it can be labelled with a radionuclide, a combination used for Immuno-PET imaging.

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Radioisotopes for Immuno-PET

Positron emitters for Immuno-PET are typically classified by their physical half-life (t½) and divided into three subgroups – short-lived, intermediate-lived and long-lived. The t½ should match the biological t½ of the antibody fragment. Short-lived isotopes, Fluorine-18 (^{18}F) and Gallium-68 (^{68}Ga), are suitable for rapidly clearing fragments such as scFv and diabodies. Copper-64 (^{64}Cu) is an intermediate-living radioisotope and typically bound to antibody fragments with intermediate clearance like minibodies and modified scFv-Fc fragments. Both Zirconium-89 (^{89}Zr) and Iodine-124 (^{124}I) are long-lived positron emitters, more suitable for full-sized intact antibodies and cell tracking studies.

Table 1. Commonly used radioisotopes for Immuno-PET and their half-lives. ^{68}Ga, Gallium-68; ^{18}F, fluorine-18; ^{64}Cu, Copper-64; ^{124}I, iodine-124; ^{89}Zr, Zirconium-89.

Radionuclide	Half-Life (Hours)
^{69}Ga	1.1
^{18}F	1.8
^{64}Cu	12.7
^{89}Zr	78.4
^{124}I	100.3

Imaging Immune Responses and Disease

The immune system plays a vital role in many diseases. Clinical outcome is often tied to the activation or suppression of the immune system. Immuno-PET is a non-invasive way to investigate the kinetics of immune responses to further understand their role in cancers, infectious diseases and inflammation as well as image the disease itself. Van Elssen and Rashidian *et al.*[2] developed an Immuno-PET method to image human immune responses in a humanized mouse model.

BLT mice (mice reconstituted with human fetal thymus, liver and liver-derived hematopoietic stem cells) were injected with VHH4 was radiolabeled with ^{64}Cu. Figure 1 shows PET/CT images (G8 PET/CT, PerkinElmer) indicating high SUV uptake in spleen, marrow and liver in stage 3 graft versus host disease (GvHD) mice exhibiting alopecia and blepharitis. The graph shows this SUV uptake is higher in the liver and bone marrow in stage 3 versus stage 0 (no symptoms) GvHD mice. This is indicative of the infiltration of activated Class II MHC$^+$ T cells in mice who developed a GvHD-like condition. Results suggest Immuno-PET could be used to diagnosis and evaluate the treatment of graft-versus-host disease and other diseases characterized by inflammation.

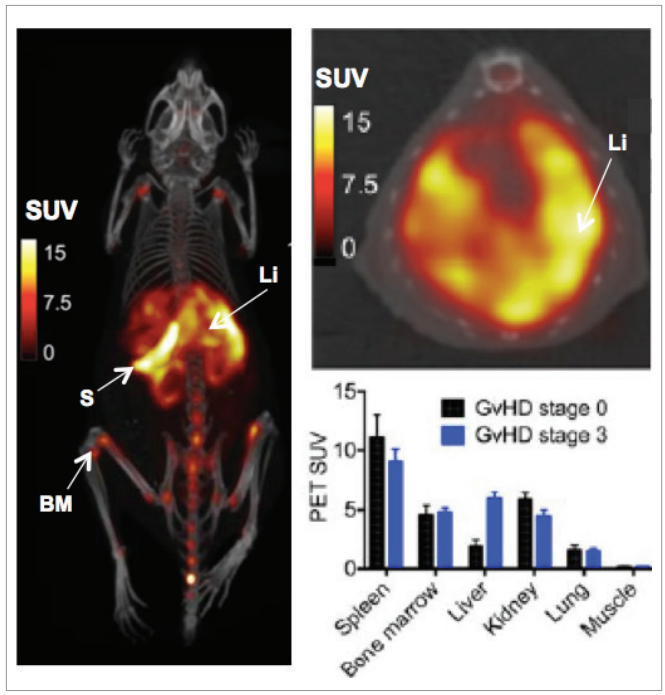

Figure 1. PET/CT images with ^{64}Cu-labeled VHH4 GvHD stage 3 BLT mouse showing uptake in spleen (S), bone marrow (BM) and liver (Li). Graph shows higher PET SUV uptake in liver and bone marrow in GvHD stage 3 versus stage 0 mice[2].

Immuno-PET on the G8 PET/CT: Customer Testimonial

Professor Hidde Ploegh, Massachusetts Institute of Technology

"The G8 PET/CT, installed at the Koch Institute at MIT in 2015, has proven to be an excellent tool for PET probe development and use. Using site-specifically modified antibody fragments as probes, the G8 has allowed us and others to image immune responses and uncover new aspects of tumor biology in ways not previously possible. We just moved to a new lab facility to continue our imaging research at Boston Children's Hospital and were delighted we did not need to request infrastructure changes or claim large amounts of space for our new bench-top G8 PET/CT instrument."

Summary

Immuno-PET can be used to detect virtually any cell surface tumor biomarker, and precise target selection can yield valuable information about the tumor e.g. location, phenotype, susceptibility to therapy, and treatment response. Currently the US Food and Drug Administration has approved at least twelve antibody therapeutics for the treatment of both solid and hematologic malignancies, with many more being evaluated in phase I to III trials. Such clinical success makes it possible to combine the delivery of tumor-targeted antibodies to their target antigens *in vivo* with PET imaging agents and encourages the rapid advancement of this field using preclinical Immuno-PET[1]. With the highest sensitivity on the market, the preclinical G8 PET/CT can detect very low levels of radioactivity. This is ideal for Immuno-PET probe development, where yields are often low, and studies investigating immune responses, where large doses of carrier or radioactivity could elicit unexpected therapeutic effects. This, in combination with its small footprint, makes it the ideal tool for the advancement of Immuno-PET research.

References

1. Knowles SM and Wu AM. Advances in Immuno–Positron Emission Tomography: Antibodies for Molecular Imaging in Oncology, *J Clin Oncol.* 2012 Nov 1;30(31):3884-92.
2. Van Elssen CH, Rashidian M, Ploegh H et al, Noninvasive imaging of human immune responses in a human xenograft model of graft-versus-host-disease. *J Nucl Med.* 2017 Feb 16. PET/CT Images used with permission from JNM. This research was originally published in JNM. © by the Society of Nuclear Medicine and Molecular Imaging, Inc.

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