**Supplementary Information**

# A Positron Emission Tomography Strategy for Visualizing Activated T Cells in Acute Graft-Versus-Host Disease

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## Supplementary Materials and Methods

### CHO Cell Transfection

CHO-K1 ($3x10^5$) cells were plated in 6 well-plates and transfected the next day with 6 μg of empty, human dCK-expressing, or human dGK-expressing (transcript variant 1) vectors (pCMV promoter; Origene Technologies, Inc., Rockville, MD). Vectors were complexed with a linear polyethylenimine transfection agent (12 μl of agent; jetPEI, Polyplus-transfection, Inc., Illkirch, France). Tracer uptake experiments were performed 48 hours after transfection.

### Tracer Uptake and Efflux Assays for Adherent Cells

For adherent cells (HeLa, MeWo, MDA-MB-231, and PyMT), $1x10^5$ cells per well (24-well plate) were seeded and 24 hours later tracer ([$^{18}$F]F-AraG (5 μCi) or [$^3$H]F-AraC (1 μCi)) was added in 0.5 ml HBSS for 1 hour to measure uptake. For CHO-K1 cells, $1x10^5$ transfected cells per well (24-well plate) were seeded 24 hours after transfection and tracer assays were performed the next day. For the efflux experiments, cells were washed twice with HBSS and incubated for an additional hour in HBSS without tracer. To measure radioactivity, cells were washed with cold PBS, lysed with 250 μl of 0.1N NaOH, and radioactivity in lysates was measured with either an automated gamma counter ([$^{18}$F]F-AraG; Cobra II; Packard) or an LS 6500 Multi-Purpose scintillation counter ([$^3$H]F-AraC; Beckman Coulter). Adjacent wells were used for measuring total cell number by washing in cold PBS, followed by trypsinization (100 μl), neutralization with media (300 μl), and cell counting using an automated cell counter (Nexcelom Bioscience, Lawrence, MA). Data was expressed as percentage uptake per $10^5$ cells.

### Western Blot

We performed dCK Western blot analysis on whole cell lysates using a rabbit polyclonal anti-dCK antibody (OriGene) and an HRP-conjugated anti-rabbit secondary antibody (Cell Signaling Technologies). Recombinant dCK with a C-terminal DDK tag was used as a positive control (OriGene). These antibodies were removed from blots using the Re-Blot Plus Western Blot Recycling kit (EMD Millipore) and re-probed with a rabbit anti-Actin antibody (Cell Signaling Technologies) and the same secondary as above. For detection, we utilized a Pierce ECL Western Blotting kit (ThermoFisher).

**Immunofluorescence Staining of Lymphoid Organs**

Spleen and CLNs were harvested from the $GFP^+$ T cell-transplanted mice on day 3 and day 10. Thin slice cryosections (5 μm) were prepared using the Kawamoto method (1). The sections were fixed with 4% paraformaldehyde and stained with an anti-mouse CD4 antibody (Biolegend). An Alexa-594 conjugated-goat anti-rat IgG secondary antibody (Life Technologies) was used and slides were mounted with Vector Shield containing DAPI (Vector). Fluorescence images were obtained with a TCS SP2 confocal microscope (Leica Microsystems).

***Ex Vivo* Donor T Cell Proliferation Analysis**

From aGVHD mice, cervical lymph nodes were collected at 1, 3, 5, 7, 9, 11, and 14 days post-transplantation. Cell suspensions from 3 mice at each time point were combined due to scarcity of cells. Pooled suspensions were immunostained with CD45.1 (for donor cells), CD45.2 (for recipient cells), Ki67, CD25, CD4, CD8, NK1.1, CD11b and B220 primary antibodies and analyzed on an LSRII cytometer (BD Biosciences). The percentage of donor T cells that were Ki-67-positive and CD4-positive were determined.

**Automated preparation of [$^{18}$F]FAraG for human imaging using the Neptis® perform PET synthesizer.**

Briefly, no carrier-added [$^{18}$F]fluoride ion was prepared on the UCSF GE PET trace cyclotron, transferred to the Neptis® perform PET synthesizer and captured on a QMA cartridge. The [$^{18}$F]fluoride ion was eluted with a solution of $K_2CO_3$ (1.5 mg) and kryptofix 2.2.2 (6 mg) in 500 µl $CH_3CN/H_2O$ (1:1). The fluoride complex was azeotropically dried twice with $CH_3CN$ at 95 °C. The residue is taken up in a solution of 5:1 t-amyl alcohol/$CH_3CN$ (1.3mL) containing 7-9 mg of the precursor, 2-N-Acetyl-6-O-((4-nitrophenyl)ethyl)-9-(3,5-di-O-trityl-2-trifyl- β-D-ribofuranosyl)guanine. The solution was heated to 115°C for 30 minutes followed by the first deprotection for 10 minutes at 110°C with 1.2 mL of 0.5 M NaOMe in methanol. Excess acid (1.7 mL of 1.0M HCl) was added and heated to 110°C for 10 minutes to afford the second deprotection followed by partial neutralization with 1.0 mL of $NaHCO_3$. The resulting mixture was purified via HPLC (Phenomenex Luna 10 µm C18, 10x250 mm, 97% 50 mM ammonium acetate/3% ethanol at 6 mL/min, UV was monitored at 254 nm and radioactivity detected with a built-in synthesizer detector). The product was collected between 12 and 14 minutes and passed through a 0.22 µm sterilizing filter into a multidose vial.

A sample was collected for quality testing. [$^{18}$F]F-AraG met all quality specifications required for human intravenous injection. The radiochemical yield was 2-4% (decay corrected, $n$ = 10). The chemical and radiochemical purities of [$^{18}$F]F-AraG were greater than 95%. The radiosynthesis time was 90 minutes and specific activity was 5-8 Ci/µmol.

**Imaging Studies in Humans**

Four 30-minute whole body PET scans (5 minutes per bed for 6 beds) per volunteer were acquired over 3 hours after a bolus intravenous injection of 244-329 MBq of [$^{18}$F]F-AraG. PET data were acquired

using a time-of-flight (TOF) PET combined with 3-tesla MRI (GE SIGNA PET/MR). The PET raw data were then reconstructed using a 3-dimensional ordered subsets expectation maximization (3D-OSEM) algorithm enabled with time-of-flight information with the following parameters: 60-cm transverse field of view, 2 iterations, 28 subsets, 5 mm post reconstruction Gaussian filter, and MRI-based attenuation correction provided by the scanner manufacturer. The transverse voxel size was 3.125 mm × 3.125 mm with a slice thickness of 2.78 mm. The reconstructed images were analyzed using the AMIDE software.

Regions of interest were drawn carefully over key sites on static PET images, obtained 47-77 minutes post tracer injection, for all six subjects. These were used to generate standard uptake values, normalized to body weight (SUV-BW) for the purpose of quantitative analysis of tracer distribution. Body weight ranged from 45-95 kg (mean =64.0± 18.9 kg). Dosimetric calculations were performed using the OLINDA/EXM 1.1 software using methods previously employed with other tracers (2).

**Statistics**

All statistical analysis was performed using the software Prism 6.0 (GraphPad). Comparison of 2 groups was performed using unpaired two-way T tests. Comparison of 3 or more groups was done using an analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparisons test. A nominal p-value less than 0.05 was considered significant.

**References:**


1. Kawamoto T, Shimizu M. A method for preparing 2- to 50-micron-thick fresh-frozen sections of large samples and undecalcified hard tissues. Histochem Cell Biol **2000**;113:331-9
2. Yaghoubi S, Barrio JR, Dahlbom M, Iyer M, Namavari M, Satyamurthy N, *et al.* Human pharmacokinetic and dosimetry studies of [(18)F]FHBG: a reporter probe for imaging herpes simplex virus type-1 thymidine kinase reporter gene expression. J Nucl Med **2001**;42:1225-34