Supplementary data for

In vivo tracking of adoptively transferred natural killer cells in rhesus macaques using ^{89}Zirconium-oxine cell labeling and PET imaging

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SUPPLEMENTARY MATERIALS AND METHODS

Detailed methods for collection and purification of NK cells

For T cell depletion, a biotinylated anti-CD3 antibody (10D12) and anti-biotin magnetic beads were used. For depletion of B cells and monocytic cells, biotinylated anti-CD20^{+} (2H7) and CD14^{+} (ME5E2) antibodies (Biolegend), respectively, followed by anti-biotin magnetic beads were used. For selection of NK cells, a combination of a biotinylated anti-NKp80 antibody (4A4.D10) and anti-biotin magnetic beads for RM NK cells or anti-CD56 (NCAM16.2) magnetic beads for human NK cells were used (Miltenyi). Apheresis was performed using CS3000 Plus Cell Separator (Baxter International). Ficoll (GE Healthcare) was used for density-gradient centrifugation.

NK cell culture

RM or human NK cells were cultured or expanded in X-VIVO 20 medium (Lonza) with 10% heat-inactivated RM serum (healthy NHLBI RM blood donors) or human AB serum (Gemini Bioproducts), supplemented with 500 IU/ml recombinant human IL-2 (Hoffmann-La Roche). *In vitro* studies using ^{89}Zr-oxine labeled or unlabeled RM and human NK cells were performed using the culture medium without IL-2. SMI-EBV-LCL cells were developed by PACT Group for our lab (authenticated by The Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, on 3/4/2009 using HLA typing) and cells thawed from original frozen stocks were used for the study.

Antibodies and reagents used for analyses of phenotype, apoptosis, and proliferation of NK cells

Antibodies against CD16 (3G8), CD56 (NCAM 16.2), and CD107a (H4A3) were purchased from BD Biosciences and anti-NKG2A antibody (REA110) was purchased from Miltenyi. Annexin V (BioLegend) staining was performed using an annexin V binding buffer (R&D Systems) and propidium iodide (PI, Millipore Sigma), and the sum of annexin $V^{+}PI^{-}$ and annexin $V^{+}PI^{+}$ cells, determined by flow cytometry (LSRII, BD Biosciences), was used for analysis. For Ki67 expression analysis, the cells were fixed and permeabilized using -20°C 80% ethanol, washed 3 times using 5% fetal calf serum in PBS, and stained with an anti-Ki67 antibody (BD Pharmingen, B56) or an IgG1 kappa isotype control (BD Pharmingen, MOPC-21) as reference.

Assessing ^{89}Zr transfer from labeled NK cells to neighboring cells

NK cells ($5x10^6$ cells) from 3 healthy donors were labeled with ^{89}Zr-oxine and co-cultured with non-^{89}Zr-labeled Raji or Ramos CD19$^+$ lymphoblastic B cells ($5x10^6$ cells, ATCC, authenticated on 5/3/2017 by ATCC using short-tandem repeat profiling) for 24-36 hours. Co-cultures were then labeled with anti-CD19 (SJ25-CI) magnetic beads, and passed through a separation column (LS columns, Miltenyi). In another set, co-culture of ^{89}Zr-labeled NK cells ($8x10^6$ cells) from 3 HLA-A2 negative healthy donors and PBMCs ($8x10^6$ cells) from healthy HLA-A2 positive donors underwent magnetic separation using a fluorescein isothiocyanate (FITC)-anti-HLA-A2 antibody (REA517) and anti-FITC magnetic beads (Miltenyi). ^{89}Zr-radioactivity of the antibody-labeled cells (flushed from the columns) and non-labeled cells (pass through) was then measured by a γ-counter (γ-*WIZARD*2). Radioactivity of ^{89}Zr-labeled human NK cells incubated alone and passed through the column was measured as a control.

Mobilization and purification of hematopoietic stem and progenitor cells

To mobilize hematopoietic stem and progenitor cells (HSPCs), a RM received subcutaneous injections of granulocyte-colony stimulating factor for 5 days (10 mg/kg/day, Amgen) and a single dose of plerixafor (1 mg/kg, Sigma-Aldrich) 3-4 hours prior to apheresis (CS3000 Plus Cell Separator Baxter International). CD34$^+$ HSPCs were purified using an anti-CD34 antibody (murine clone 12.8, cross reactive with rhesus CD34, obtained from Fred Hutchinson Cancer Center) and anti-murine IgM beads (Miltenyi Biotec) (20,21).

PET/CT imaging and reconstruction of the images

Serial PET/CT images of RMs receiving ^{89}Zr-oxine labeled cells were acquired for up to 7 days using a clinical PET/CT scanner (Gemini TOF, Philips Medical System, or Discovery MI DR

PET/CT, GE Healthcare). The entire torso was imaged in 4 overlapping bed-positions, 5 minutes per bed at 0-4 hour and 6.5, 7, and 9 minutes on days 1, 2 and 7, respectively. Reconstruction of acquired images were performed using the default reconstruction algorithm for Gemini TOF (BLOB-OS-TF, a 3-dimensional ordered subset iterative time-of-flight reconstruction technique using 3 iterations, 33 subsets, Philips Medical System) or Discovery MI DR (VUE Point FX-3D Time of Flight Iterative, 2 iterations, 24 subsets, GE Healthcare).

Detailed methods for microscopy analysis of liver biopsy

Fresh liver biopsy samples were analyzed using an inverted Leica SP5 five channels confocal multiphoton microscope (Leica Microsystems). The samples were placed in 35-mm glass bottom #1.5 chamber for microscopy (Mat Tek corporation), covered with 20 µl PBS and imaged immediately using Leica HC-PL-IRAPO 20x/ 0.75 water immersion objective, employing 561-nm laser for excitation and capturing 570-640nm emission range. Images were collected over large regions using the tile function of the Leica LAS-AF software to automatically generate stitched volumes (xyz) comprising 1 mm x 2 mm x 50-100 µm (depth) tissue. For 3D renderings and picture snapshots, Imaris v 9.2.1 software (Bitplane) was used.

Detailed methods for dosimetry analysis

Contours were drawn delineating the lungs, liver, spleen and bone marrow, where the notable cell concentrations were observed, as well as a contour delineating the whole-body, which was used to estimate the residual activity. Time activity curves were generated and scaled to % ID (without decay correction) for each organ and the whole-body from which the residence time of the ^{89}Zr was measured. The residence times were then extrapolated to human equivalent

residence time using percent organ mass ratios of human to RMs (37,38). The human extrapolated residence times were used in the OLINDA 1.1 (Vanderbilt University) dosimetry software package to generate dosimetry estimates.

References for Supplemental Methods (continued from the main text)

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Legends for Supplementary Figures

Figure S1. **^{89}Zr-oxine labeling does not alter surface phenotype of NK cells.**

A. Expression of CD56 and CD16 on *ex vivo* expanded RM NK cells from 3 unrelated RMs was examined by flow cytometry up to 24 hours after labeling. Surface expression of these markers was not altered by ^{89}Zr-oxine labeling. **B**. The majority of *ex vivo* expanded ^{89}Zr-labeled and non-labeled RM NK cells remained NKG2A positive during the 24 hours post-labeling tested (n=3). **C**. Similarly, CD56 and CD16 expression of e*x vivo* expanded NK cells from 3 unrelated humans was not altered by ^{89}Zr-labeling (n=3).

Figure S2. **Quantitation of PET/CT images of adoptively transferred ^{89}Zr-oxine-labeled NK cell reveals low BM homing.**

A. Kinetics of SUV values with magnified X-axis up to day 1. **B**. % ID curves with magnification of X-axis up to day 1 indicated rapid migration of the cells to the liver while homing to the BM was limited.

Figure S3. ^{89}Zr-oxine-labeled CD34^{+} HSPCs traffick to the BM.

A. Axial PET/CT image of mid-4th lumber spine 1 day after autologous transfer of ^{89}Zr-oxine labeled CD34^{+} HPSCs showed high cell accumulation in the center of the vertebral body which is considered to be BM area. Note higher scale setting of the image compared to that of *ex vivo* expanded NK cells (Fig. 2B) because of very strong ^{89}Zr-signals with CD34^{+} HPSCs. **B**. % ID curves demonstrated rapid migration of ^{89}Zr-oxine-labeled HSPCs to the BM.