Blood Collection and Processing

Blood samples (5 mL) for all patients were collected immediately before, and up to 72 h after, LuTate injection and processed within 2 h after collection. The samples were diluted with an equal volume of phosphate-buffered saline (PBS) and layered over a density gradient medium (Ficoll-Paque Plus; Amersham Biosciences). Tubes were centrifuged at 700*g,* 13°C, for 25 min. Lymphocytes were harvested, washed 3 times with PBS, and fixed in 4% paraformaldehyde for 30 min at room temperature before being deposited onto microscopy slides using a Cytospin 4 cytocentrifuge (Thermo Scientific) for 4 min at 800 rpm. The slides were stored at 4°C in 70% ice-cold ethanol for up to a week before immunostaining.

For lymphocyte counts, blood samples were taken before each PRRT cycle and at the 2-wk follow-up.

Blood, Bone Marrow, and Spleen Dosimetry

The administered activity of LuTate prescribed for each patient was adapted to tumor burden and renal function. Renal filtration rate (mL/min) was determined by ^{51}Cr EDTA measurement. Tumor burden was expressed as octreotate-avid tumor volume (Table 2) and measured on ^{68}Ga-octreotate PET/CT as described previously (*1*). Briefly, octreotate-avid tumor volume was delineated by volumes of interest above a threshold SUV of 5.0, ignoring activity in high-uptake healthy organs.

Dose prescription was determined by an empiric formula with 3 dose ranges: 5–6 GBq for patients with a small tumor burden and impaired renal function, 7–8 GBq for small tumor burden and normal renal function or high tumor burden and impaired renal function, and 9–10 GBq for large tumor burden and normal renal function.

Absorbed radiation dose to the blood was calculated on the basis of 9 serial blood sample measurements obtained at the same time points for each patient and up to 72 h after LuTate injection when available. For each patient, trapezoidal integration of area under the blood clearance curve was performed to estimate cumulated activity (Bq*h/mL) up to each sampling time point. Self-dose was calculated with MIRD S-value ($7.30*10^{-5}$ mGy*mL/Bq*h) (*2*).

Absorbed dose values for bone marrow, spleen, and tumor were calculated on the basis of serial quantitative SPECT/CT imaging using previously described methods (*3*, *4*). Images of therapeutic LuTate were recorded at 4, 24, and 72 h after injection. Organs of interest were segmented using Siemens TrueD software, and dose estimates were performed with OLINDA (version 1.1) on the basis of standard male or female models (*2*).

Immunostaining and Image Analysis

Immunostaining of γ-H2AX was performed as described previously (*5*), with minor modifications. After Cytospin preparations had been incubated in PBS for 15 min, they were blocked with 8% bovine serum albumin (BSA) in PBS containing 0.5% Tween-20 and 0.1% Triton X-100 (PBS-TT) for 60 min at room temperature. Samples were incubated for 2 h at room temperature with a mouse monoclonal anti-γ-H2AX antibody (ab18311; Abcam) diluted at 1:500 in PBS-TT containing 1% BSA and then washed 3 times 10 min with PBS and incubated for 1 h with a goat anti-mouse Alexa Fluor 488–conjugated IgG (A11029; Invitrogen) diluted at 1:500 in PBS-TT containing 1% BSA. After 3 washes of 5 min each with PBS, slides were mounted using Vectashield mounting medium containing propidium iodide (Vector Laboratories) and sealed. For each patient, a positive control of DNA DSBs was generated to monitor γ-H2AX foci staining. Blood samples taken at baseline were irradiated with 1 Gy and incubated 30 min at 37°C before being processed as described above.

Laser-scanning confocal microscopy was performed with an Olympus Fluoview FV1000 microscope coupled with FV10-ASW1.7 acquisition software (Olympus). Optical sections (0.6 μm) through the nuclei were captured and images were obtained by projection of the individual sections. Thus, all detectable foci were visible in a single plane.

The number of γ-H2AX foci per nucleus in a population of 50–100 PBLs was quantified with a dedicated computational algorithm using TGIR foci-counting software (*6*). Sixteen samples per time point were analyzed. In some cases, because of immunostaining failure or loss of a blood sample, the number of samples was down to 14.

Statistical Analysis and Foci Kinetics Simulation

Results are expressed as mean ± SEM unless otherwise stated. The Mann–Whitney test was used to compare groups, and a *P* value of less than 0.05 was considered significant. GraphPad Prism6 was used for statistical analysis.

Simulation of γ-H2AX foci kinetics was implemented on the basis of data reported for lymphocytes of ex vivo irradiated human blood obtained from our NIH Blood Bank normal-donors collection (*5*, *6*).

The radiation dose response and the kinetic pattern of accumulation and repair of foci were adopted. An 80-h simulation range was split into 1,000 equal intervals.

For each time interval:

1. The radiation dose was calculated from LuTate activity kinetics in blood, corresponding to administration of the amount of ^{177}Lu to yield an accumulated average radiation dose to blood of 42 mGy,
2. The number of consequent foci generated was recorded for each subsequent time interval,
3. Steps 1 and 2 were repeated and the accumulated foci counts were then numerically integrated over the 80-h range.

A second simulation involved administration of the entire 42-mGy dose at the beginning of the 80-h period.

REFERENCES

1. Beauregard JM, Hofman MS, Kong G, Hicks RJ. The tumour sink effect on the biodistribution of ^{68}Ga-DOTA-octreotate: implications for peptide receptor radionuclide therapy. *Eur J Nucl Med Mol Imaging.* 2012;39:50–56.

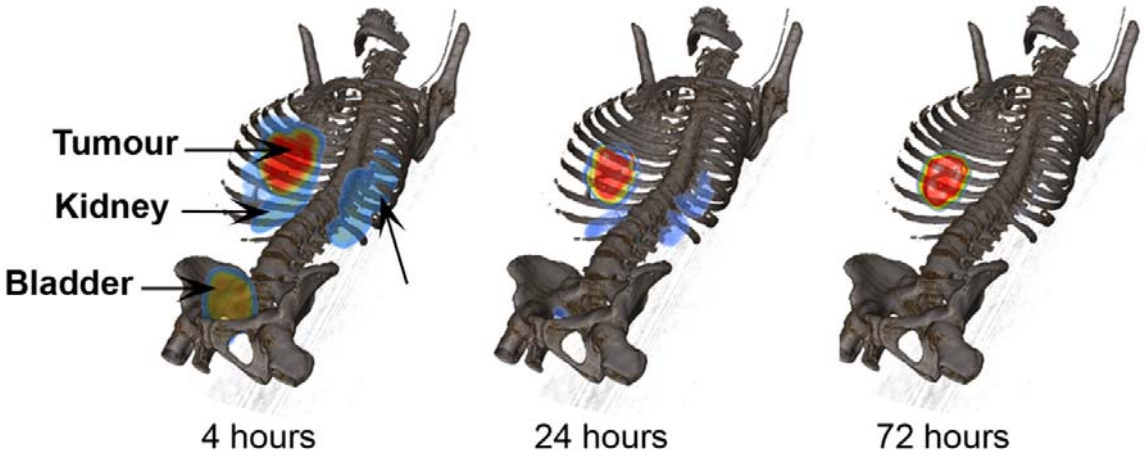
2. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med.* 2005;46:1023–1027.

3. Beauregard JM, Hofman MS, Pereira JM, Eu P, Hicks RJ. Quantitative ^{177}Lu SPECT (QSPECT) imaging using a commercially available SPECT/CT system. *Cancer Imaging.* 2011;11:56–66.

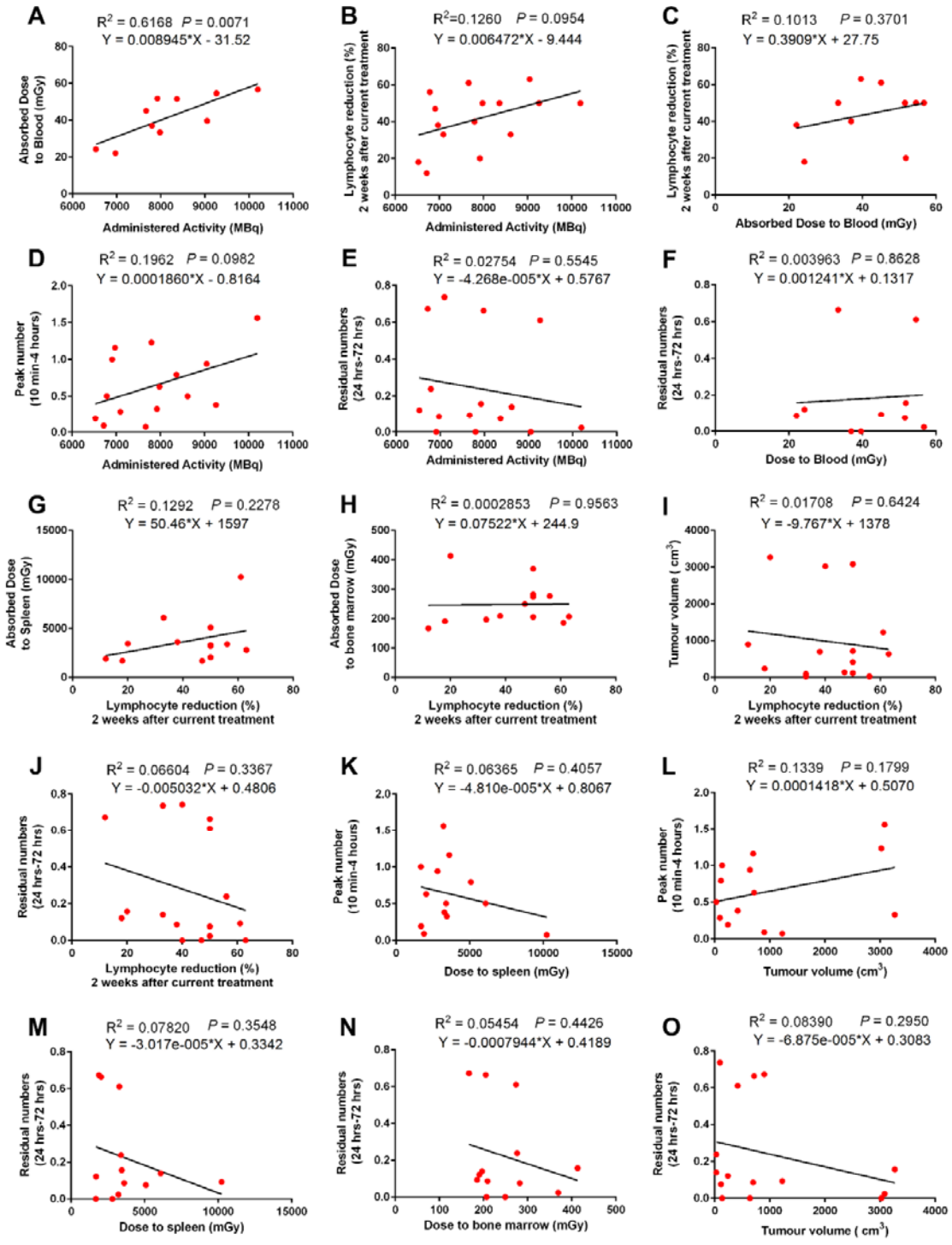
4. Jackson PA, Beauregard JM, Hofman MS, Kron T, Hogg A, Hicks RJ. An automated voxelized dosimetry tool for radionuclide therapy based on serial quantitative SPECT/CT imaging. *Med Phys.* 2013;40:112503.

5. Redon CE, Dickey JS, Bonner WM, Sedelnikova OA. Gamma-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin. *Adv Space Res.* 2009;43:1171–1178.

6. Ivashkevich AN, Martin OA, Smith AJ, et al. GammaH2AX foci as a measure of DNA damage: a computational approach to automatic analysis. *Mutat Res.* 2011;711:49–60.



Supplemental Figure 1. Representative SPECT/CT scans at 4, 24, and 72 h after PRRT. In this patient, there was a large tumor volume in the abdomen that showed long-term LuTate retention.



Supplemental Figure 2. Relationship with dosimetry. (A) Correlation between absorbed dose to blood at 72 h and administered activity. (B) Correlation between percentage of lymphocyte reduction measured before current treatment to 2 wk after current treatment and administered

activity. (C) Correlation between percentage of lymphocyte reduction measured before current treatment to 2 wk after current treatment and absorbed dose to blood at 72 h. (D) Correlation between peak number of excess γ-H2AX foci per cell measured in the 10-min to 4-h interval (peak number) and administered activity. (E) Correlation between peak number of excess γ-H2AX foci per cell measured in the 24-h to 72-h interval (residual number) and administered activity. (F) Correlation between peak number of excess γ-H2AX foci per cell measured in the 24-h to 72-h interval (residual number) and absorbed dose to blood at 72 h. (G) Correlation between absorbed dose to spleen and percentage of lymphocyte reduction measured before current treatment to 2 wk after current treatment. (H) Correlation between absorbed dose to bone marrow and percentage of lymphocyte reduction measured before current treatment to 2 wk after current treatment. (I) Correlation between tumor volume and percentage of lymphocyte reduction measured before current treatment to 2 wk after current treatment. (J) Correlation between peak number of excess γ-H2AX foci per cell measured in the 24-h to 72-h interval (residual number) and percentage of lymphocyte reduction measured before current treatment to 2 wk after current treatment. (K) Correlation between peak number of excess γ-H2AX foci per cell measured in the 10-min to 4-h interval (peak number) and absorbed dose to spleen. (L) Correlation between peak number of excess γ-H2AX foci per cell measured in the 10-min to 4-h interval (peak number) and tumor volume. (M) Correlation between peak number of excess γ-H2AX foci per cell measured in the 24-h to 72-h interval (residual number) and absorbed dose to spleen. (N) Correlation between peak number of excess γ-H2AX foci per cell measured in the 24-h to 72-h interval (residual number) and absorbed dose to bone marrow. (O) Correlation between peak number of excess γ-H2AX foci per cell measured in the 24-h to 72-h interval (residual number) and tumor volume. For each graph, the solid line is the linear fit to all data points. Regression equation, fitting quality (R^2), and *P* value are shown. $P \leq 0.05$ was considered statistically significant.