Methodological Details

PET Image Acquisition and Reconstruction Protocol

PET scans were acquired in 2D mode using a PET only scanner (HR+, Siemens) at one of the sites, and a PET/CT scanner (GE Discovery LS4 PET/CT, GE Healthcare) at the two other sites. Dynamic PET images were obtained over the heart for 10 min, followed by either eight or six sequential whole body images over approximately 5 hours. For scanning performed on the PET only scanner, eight whole body images were acquired from head to foot in three imaging sessions, with separate transmission scans acquired for each session. For the scanning on the hybrid PET/CT scanners, six head-to-mid thigh images and two mid-thigh-to-feet images were acquired in three sessions, with CT attenuation scans acquired after each session.

Images were reconstructed using software provided by the PET scanner manufacturers, which used all available corrections for attenuation, dead-time, scatter, and efficiency (normalization). All PET scanners underwent initial qualification, including an assessment of calibration with the dose calibrator to be used in the clinical trial.

Estimation of Dosimetry

Estimates of organ absorbed doses were calculated based on multiple whole-body PET images acquired at scheduled time points after administration of LMI1195 to evaluate organ, blood, and whole-body distribution. For 6 of the subjects (from 2 sites)

whose data were acquired on systems not capable of full whole-body imaging, images of the legs were used to estimate activity not in the field of view in the head to mid-thigh images.

Image data acquisition and dosimetry calculations were performed in accordance with the recommendations of the Medical Internal Radiation Dose (MIRD) Committee. (17) Organ Level Internal Dose Assessment (OLINDA/EXM) software(18) was used to estimate organ absorbed doses in accordance with US FDA guidelines pertaining to dosimetry for experimental radiopharmaceuticals. (19) Estimates of the effective dose equivalent (EDE) and the effective dose (ED) were based on International Commission on Radiological Protection (ICRP) publications ICRP 26 (20) and ICRP 60 (21) , respectively.

Regions of interest were constructed using the coronal slices to determine whole-organ activity. Where reasonable, multiple adjacent coronal slices spanning one or more organs in sagittal extent were summed prior to region drawing to reduce the number of ROIs required to complete the process. Image quantification was performed in general concordance with the methodology described in MIRD Pamphlet No.16. (17) Organ activity values were normalized where necessary, assuming conservation of activity and to ensure that estimates of absorbed dose were conservative so that any errors resulted in overestimation rather than underestimation.

For each patient and organ, voxel sum data in the resulting volumes of interest (VOI) were subjected to nonlinear least squares regression analysis using sums of exponentials of the form shown in Supplemental Equation 1. Between one and four exponential terms were employed, as appropriate.

$$F(t) = \sum_k f_k e^{-\lambda_k t}$$ Supplemental Equation 1

where:

f_k = Fractional dose model parameter (determined in the fitting process and allowed to take only positive values),
λ = Clearance rate model parameter (determined in the fitting process),
$F(t)$ = Fraction of the total injected activity,
t = Time post injection, and
k = k^{th} exponential term.

Urinary excretion for dosimetry purposes was estimated based on the total body radioactivity, quantified from the images, using Supplemental Equation 2.

$$\%AA_{Urine} = 100 - \%AA_{WB}$$ Supplemental Equation 2.

where:

$\%AA_{Urine}$ = Estimated %AA in the cumulative urine, and
$\%AA_{WB}$ = Estimated %AA in the whole body based on the image data.

Residence times (τ) were determined by integration of Supplemental Equation 1 from time of injection to infinity, taking into account physical decay. The remainder of the body residence times was determined by subtraction of the organ residence times from the whole body residence times. Urinary bladder residence times were determined using the parameters determined by fitting the whole body activity data with a urinary bladder model as implemented in the OLINDA/EXM software (version 1.1), (18) with a 3.5 hour bladder voiding interval. Red marrow residence time was determined based on a VOI created for a portion of the lumbar spine. The total lumbar spine was assumed to contain 16.1% (22) of the total red marrow. Absorbed dose estimates for all target

organs were determined with OLINDA/EXM using the hermaphroditic adult “male” phantom. Salivary gland self dose was determined by using a conservative estimate of the S-value for salivary glands based on the reference man total mass of the parotid and submaxillary salivary glands (23), and assuming a spherical shape. S-values for spheres were determined using the OLINDA/EXM software. These S-values are then multiplied by the residence times using the standard MIRD methodology to produce final salivary gland dose estimates as described in the MIRD Primer. (24)