Supplementary Data to

[^{177}Lu]pentixather: comprehensive preclinical characterization of a first CXCR4-directed endoradiotherapeutic agent

by Margret Schottelius et al.

Mass spectrometric and HPLC characterization of pentixather and its metal complexes

pentixather ($C_{60}H_{79}IN_{14}O_{14}$): calculated molecular weight = 1346.5
found: *m/z* = 1347.8 $[M+H]^+$, 1370.6 $[M+Na]^+$, 674.6 $[M+2H]^{2+}$

The ^{nat}Lu-, ^{nat}Y- and ^{nat}Bi-complexes of pentixather were prepared by dissolving 200-500 µg of peptide either in 20 mM $LuCl_3$ or YCl_3 in 0.01 M HCl or in 5mM $Bi(OAc)_3$ in 0.5 M NaOAc (pH = 4.5), respectively, to yield a final peptide concentration of 1 mM. After heating to 95°C for 30 minutes in a sealed tube, RP-HPLC analysis (Shimadzu Prominence HPLC system; UV detection at 214 nm; Nucleosil 100 C-18 (5µm, 125 x 4.0 mm) column (CS GmbH, Langerwehe, Germany); linear gradient of 20-35% acetonitrile (0.1% TFA) in 0.1% TFA within 15 min; flow: 1 mL/min) revealed quantitative complex formation. The product solutions were used as such for the preparation of dilution series for binding studies.

^{nat}Lu-pentixather ($C_{60}H_{76}IN_{14}O_{14}Lu$): calculated molecular weight = 1519.2
found: *m/z* = 1521.1 $[M+H]^+$, 760.8 $[M+2H]^{2+}$
HPLC (20-35% B in 15 min): t_R = 11.68 min; K' = 7.55

^{nat}Y-pentixather ($C_{60}H_{76}IN_{14}O_{14}Y$): calculated molecular weight = 1432.5
found: *m/z* = 1433.6 $[M+H]^+$, 1455.8 $[M+Na]^+$, 717.4 $[M+2H]^{2+}$
HPLC (20-35% B in 15 min): t_R = 11.66 min; K' = 7.52

^{nat}Bi-pentixather ($C_{60}H_{76}IN_{14}O_{14}Bi$): calculated molecular weight = 1553.2
found: *m/z* = 1554.3 $[M+H]^+$, 777.8 $[M+2H]^{2+}$
HPLC (20-35% B in 15 min): t_R = 11.30 min; K' = 7.29

Cell culture conditions

Jurkat (human T cell lymphoma), CHO-K1 (Chinese hamster ovary) and the human multiple myeloma cell lines OPM-2 (DSMZ no. ACC50), RPMI 8226 (DSMZ no. ACC402) and MM1.S (ATCC no. CRL-2974) cells were grown in RPMI-1640 medium supplemented with 10% FCS, 2 mM L-glutamine and 100 units/mL of penicillin/streptomycin (P/S). The human acute myeloid leukemia cell lines Molm-13 (DSMZ no. ACC554), MV4-11 (DSMZ no. ACC102) and THP-1 (DSMZ no. ACC16) were maintained in RPMI Medium 1640 supplemented with 20% FCS, 2 mM L-glutamine, 100 units/mL P/S. Daudi (human Burkitt lymphoma) cells were cultured in RPMI-1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 1% non-essential amino acids, 50 µM β-mercaptoethanol and 100 units/mL of P/S. All cell lines were maintained at 37°C in a humidified 5% CO_2 atmosphere.
Media and supplements were obtained from Biochrom (Berlin, Germany) or Invitrogen (life technologies, Darmstadt, Germany). In the assay medium used for uptake and internalization studies, FCS was replaced by 5% bovine serum albumin (BSA; Sigma, St.Louis, USA). For cell counting, cells were stained with trypan blue and counted manually using a Neubauer chamber.

Radiolabeling

Material and Methods
[^{177}Lu]Pentixather for human dosimetry studies (activity ~200 MBq) was formulated in 5-10 mL saline, [^{177}Lu]Pentixather for therapy application (activities ranging from 7.64 to 8.20 GBq) was formulated in 10-20 mL saline. Every single batch of [^{177}Lu]Pentixather was tested for radiochemical purity by RP-radio-HPLC (Knauer HPLC system; UV detection at 250 nm; activity detection via NaI(TI) scintillation detector; Nucleosil 100 C-18 (5µm, 125 x 4.0 mm) column (CS GmbH, Langerwehe, Germany); linear gradient of 0-100% acetonitrile (0.1% TFA) in 0.1% TFA within 10 min; flow: 0.7 mL/min) and thin layer chromatography before release. Additionally, the pH of the radiopharmaceutical preparation was determined and a bubble point test was performed. Acceptance criteria and results from quality control are summarized in Supplementary Table 1.

Results

[^{177}Lu]pentixather: RP-HPLC (0-100 % B in 10 min): t_R = 7.3 min; K' = 2.47
Identity of the product was confirmed by coinjection of the ^{nat}Lu-reference compound.

Supplementary Table 1: *Release specifications and results from quality control of [^{177}Lu]Pentixather for patient application.*

Test	Acceptance Criteria	Results
Appearance	colorless, clear, particle-free solution	complies
Radionuclide purity	> 99 %	> 99.98 %
Radiochemical purity (HPLC)	≥ 98.0 %	98.83 - 100 %
Radiochemical purity (TLC)	≥ 98.0 %	98.14 - 99.64 %
pH	4.0 - 8.0	4.5 - 5.0
Sterile filter integrity test	> 3.45 bar	3.6 - 4.0 bar

Dosimetry - Extrapolation to Humans

Material and Methods

Measurements of the biokinetics in mice were used to determine time-integrated activity coefficients (TIACs) and to estimate dose data for humans. The extrapolation of TIACs in mice to humans was performed by two commonly used methods which have been reported to yield results of comparable reliability [1]:

Method 1 is based on the assumption that the time-integrated activity coefficients (TIACs) for the same organ are identical in mice and humans. Method 2 considered a relative mass scaling where the TIAC in the human organ is assumed to be equal to the TIAC in the animal multiplied by the organ to total body mass ratio in the human divided by the organ to total body mass ratio in the animal. The mouse TIACs were calculated with the software NUKFIT choosing the optimal fit functions as described by Kletting et al. [2]. The dose calculation was performed for a selected group of organs using OLINDA/EXM V1.1 [3].

The following adaptations were made to deduce the TIACs:

1) The masses of the human organs were extracted from OLINDA/EXM or, if not available, from ICRP 89 [4].
2) The mice used for the experiment were female CB-17 SCID mice with 18.5 g nominal total body mass at the age of 9-11 weeks (Charles River, Sulzfeld, Germany). Organ mass data as listed in Supplementary Table 1 were deduced as follows:
    - the organs heart, lungs, spleen, pancreas, stomach (empty), small intestine with content, large intestine with content, uterus with ovaries, adrenals, and brain were prepared and measured completely and exclusively and the mean values of all animals were calculated.
    - One kidney per animal was prepared and the measurement results were doubled, assuming that the other kidney was identical.
    - As it was difficult to completely prepare the livers in each animal, only five livers were prepared completely, resulting in a mean mass of 0.877 ± 0.058 g. The liver masses of all other animals were scaled to this value.
    - Information on blood volumes in mice varies but is consistent with the general requirement of linear scaling of the blood volume with body mass in mammals [5]. The blood to total body mass ratio was assumed to be equal (7.6%) in mice and humans resulting in identical $TIAC_{blood}$ values for both methods.
    - One femur was prepared for each animal. Measured mass and TIAC data were scaled to the total skeleton mass in the mouse of 10.7% of the total body mass [6].
    - Pieces of muscle were taken from the thighs. Data were scaled assuming that the muscle mass in the mouse is 38.4% of the total body mass [6].
    - Pieces of skin were scaled to 16.5% of the total body mass [6].
3) The TIAC extrapolated to the whole skeleton was completely attributed to the red marrow and adapted for the human red marrow for both methods.
4) The TIAC measured for uterus and ovaries was distributed to both organs according to the mass ratio in the human.
5) The TIAC measured for the colon was distributed to upper and lower large intestine according to the mass ratio in the human. Adequate scaling of the intestine data from mouse to human in Method 2 is debatable. As measured masses strongly depend on the feeding status, literature mass data from [6] and [3] were used to scale the TIACS.
6) The activity in the remainder of the body (adipose tissue, blood in large vessels, etc.) was not measured. The TIAC for the remainder was approximated by the TIAC of total blood. This is expected to introduce a minor uncertainty because the contribution by photon radiation from the remainder is small.
7) The TIAC measured for the heart was distributed to heart content (90%) and heart wall (10%) according to the ratio of the blood contents [4].

Results

Supplementary Table 1 lists the time-integrated activity coefficients measured in mice and scaled to humans as estimated by the two scaling methods for the organs relevant for dosimetry. The mouse organ masses used for the determinations were deduced as explained above. The human TIACs were used to estimate absorbed dose coefficients with OLINDA/EXM [3]. The TIACs of organs not listed in Supplementary Table 2 were set to zero. The resulting dose coefficients are listed in Supplementary Table 3.

Supplementary Table 2: *Time integrated activity coefficients (TIACs) measured in mice and scaled to humans using the mouse organ masses listed in column 2.*

Organ	**Mouse**		**Human TIACs (h)**	
	Mass (mg)	**TIACs (h)**	**Method 1**	**Method 2**
Total Body (TB)*	18500			
Adrenals	10.9	0.014	0.014	0.0053
Blood	1406	0.156		
Brain	397	0.009	0.009	0.0081
Colon	202	0.332		
Lower Large Intestine			0.143	0.069
Small Intestine	468	0.382	0.382	0.139
Stomach	119	0.041	0.041	0.01436
Upper Large Intestine			0.189	0.091
Heart	91	0.029		
Heart Content			0.026	0.023
Heart Wall			0.003	0.0025
Kidneys	277	0.688	0.688	0.186
Liver	877	20.441	20.441	11.175
Lungs	124	0.077	0.077	0.156
Muscle	7104	0.688	0.688	0.681
Ovaries			0.006	0.0014
Pancreas	116	0.023	0.023	0.0047
Red Marrow			2.267	2.267
Skeleton	1985	2.267		
Skin	3058	0.651	0.651	0.161
Spleen	192	0.059	0.059	0.141
Uterus			0.056	0.0124
Uterus + Ovaries	99	0.062		
Remainder			0.156	0.156

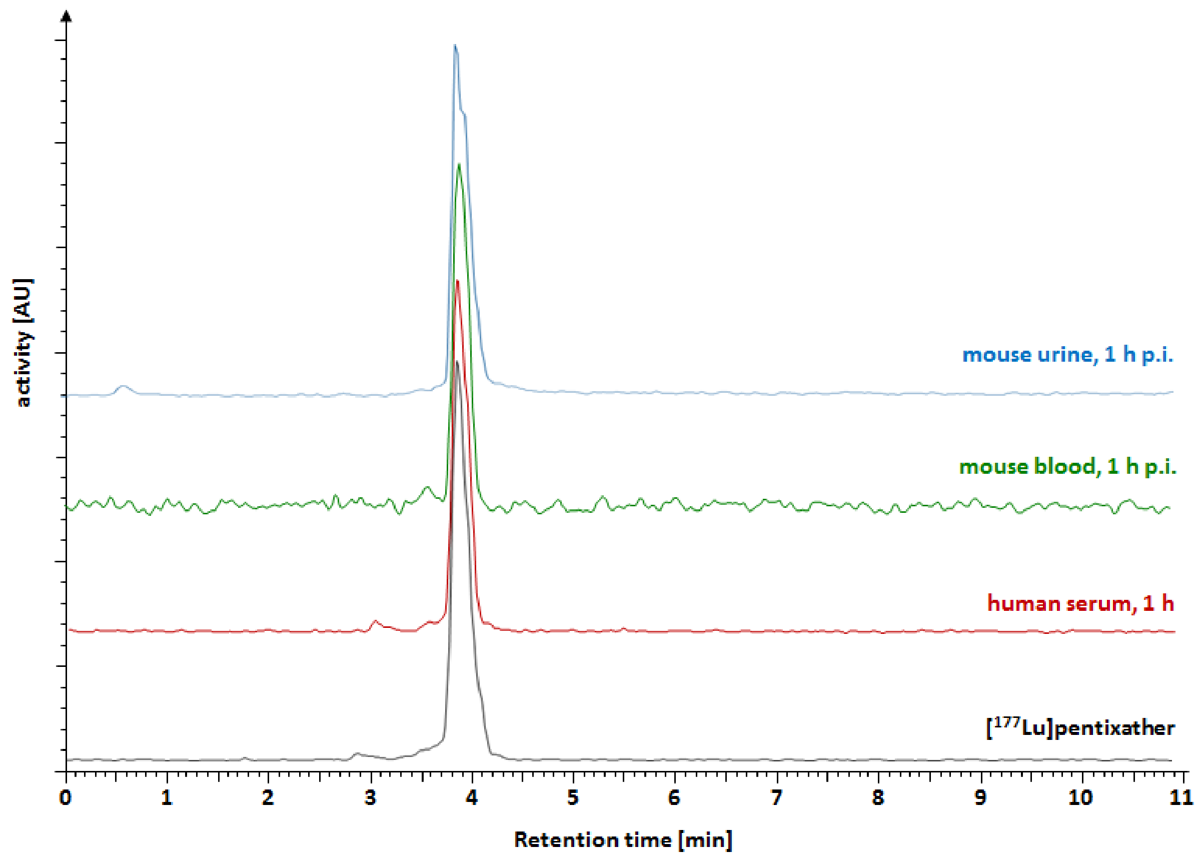
Supplementary Table 3: *Estimates of the absorbed dose coefficients in humans deduced from biokinetics data measured in mice. The contributions by beta and photon radiation as well as the sum of both (Total) are shown in mGy per MBq [^{177}Lu]pentixather.*

Target Organ	**Method 1**			**Method 2**		
	Beta	Photon	Total	Beta	Photon	Total
Adrenals	7.3E-02	1.1E-02	8.4E-02	2.6E-02	6.0E-03	3.2E-02
Brain	5.4E-04	2.6E-04	7.9E-04	4.8E-04	2.4E-04	7.2E-04
Breasts	1.8E-04	1.6E-03	1.8E-03	1.8E-04	1.0E-03	1.2E-03
Gallbladder Wall	1.8E-04	1.9E-02	1.9E-02	1.8E-04	1.1E-02	1.1E-02
Lower Large Intestine Wall	4.3E-02	1.8E-03	4.4E-02	2.1E-02	1.3E-03	2.2E-02
Small Intestine	3.9E-02	3.9E-03	4.2E-02	1.4E-02	3.2E-03	1.7E-02
Stomach Wall	6.9E-03	3.8E-03	1.1E-02	2.5E-03	2.4E-03	4.9E-03
Upper Large Intestine Wall	3.5E-02	5.5E-03	4.0E-02	1.7E-01	5.6E-03	1.7E-01
Heart Wall	3.2E-03	5.3E-03	8.5E-03	3.0E-03	3.1E-03	6.1E-03
Kidneys	2.0E-01	1.0E-02	2.1E-01	5.3E-02	5.0E-03	5.8E-02
Liver	9.1E-01	3.9E-02	9.5E-01	5.0E-01	2.2E-02	5.2E-01
Lungs	6.5E-03	4.6E-03	1.1E-02	1.3E-02	2.8E-03	1.6E-02
Muscle	2.1E-03	2.0E-03	4.1E-03	2.1E-03	1.3E-03	3.4E-03
Ovaries	5.9E-02	2.4E-03	6.1E-02	9.8E-03	2.0E-03	1.2E-02
Pancreas	2.1E-02	9.1E-03	3.0E-02	4.5E-03	5.2E-03	9.7E-03
Red Marrow	9.6E-02	3.1E-03	9.9E-02	9.6E-02	2.3E-03	9.9E-02
Osteogenic Cells	4.5E-02	3.5E-03	4.9E-02	4.5E-02	2.5E-03	4.8E-02
Skin	1.8E-04	9.6E-04	1.1E-03	1.8E-04	6.1E-04	7.9E-04
Spleen	2.7E-02	2.9E-03	3.0E-02	6.5E-02	2.6E-03	6.8E-02
Testes	1.8E-04	2.3E-04	4.1E-04	1.8E-04	2.1E-04	3.9E-04
Thymus	1.8E-04	1.5E-03	1.7E-03	1.8E-04	1.0E-03	1.2E-03
Thyroid	1.8E-04	4.7E-04	6.5E-04	1.8E-04	3.9E-04	5.7E-04
Urinary Bladder Wall	1.8E-04	8.5E-04	1.0E-03	1.8E-04	6.8E-04	8.6E-04
Uterus	6.0E-02	2.5E-03	6.3E-02	1.3E-02	1.5E-03	1.4E-02

Metabolite analysis

Results

Supplementary Figure 1



Supplementary Figure 1 *Exemplary reversed-phase Radio-HPLC analysis of the metabolic stability of [^{177}Lu]pentixather in human serum (1h incubation at 37°C) and in blood and urine of a CB-17 SCID mouse (1 h p.i.). For HPLC conditions, see Materials and Methods section of the main manuscript.*

References

1. Sparks RB AB, Comparison of the Effectiveness of Some Common Animal Data Scaling Techniques in Estimating Human Radiation Dose. Sixth International Radiopharmaceutical Dosimetry Symposium. 1999: 705-716.
2. Kletting P, Schimmel S, Kestler HA, et al., Molecular radiotherapy: the NUKFIT software for calculating the time-integrated activity coefficient. Medical physics. 2013; 40(10): 102504.
3. 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(6): 1023-7.
4. ICRP89, Publication 89: Basic anatomical and physiological data for use in radiological protection: reference values. Annals of the ICRP. 2002; 32(3-4): 1-278.
5. West GB, Brown JH, Enquist BJ, A general model for the origin of allometric scaling laws in biology. Science. 1997; 276(5309): 122-6.
6. Brown RP, Delp MD, Lindstedt SL, et al., Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health. 1997; 13(4): 407-484.