CXCR4-targeted radio-theragnostics based on the endogenous ligand EPI-X4 for oncological applications

Raghuvir H. Gaonkar[1], Jacopo Millul[1], Rosalba Mansi[1], Mirja Harms[2], Jan Münch[2,3], Melpomeni Fani[1]

[1]*Division of Radiopharmaceutical Chemistry, University Hospital Basel, Basel, Switzerland*

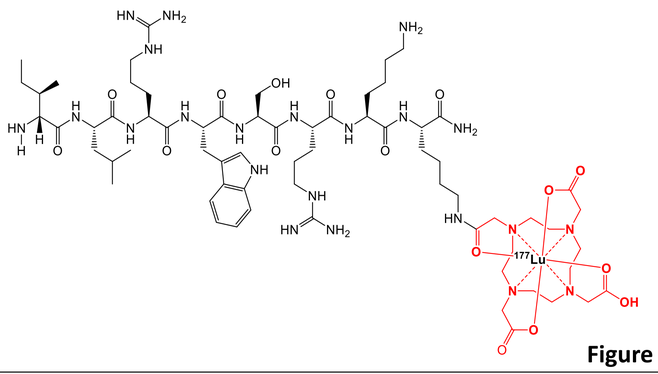
[2]*Institute of Molecular Virology, Ulm University Medical Center, Ulm, Germany*

[3]*Core Facility Functional Peptidomics, Ulm University Medical Center, Ulm, Germany*

CXCR4-targeted imaging and therapy has been an area of intensive research due to the overexpression of CXCR4 in several cancer types. We utilized the **E**ndogenous **P**eptide **I**nhibitor of C**X**CR**4** (EPI-X4), a human serum albumin fragment (1), as scaffold for developing novel CXCR4-targeting radio-theragnostics. Out of a small library of EPI-X4 derivatives, we identified ^{68}Ga-/^{177}Lu-JMF-04 (DILRWSRKK(^{68}Ga-/^{177}Lu-DOTA)-NH_2) as the derivative able to visualize CXCR4-expressing tumors. However, ^{68}Ga-/^{177}Lu-JMF-04 had undesirably high kidney uptake. In a follow-up structure optimization study, we developed two optimized derivatives, JMF-10 (lacking one lysine) and JMF-11 (in addition having 6-aminohexanoic acid as a spacer. Both the new derivatives were evaluated *in vitro* and *in vivo* and compared with JMF-04.

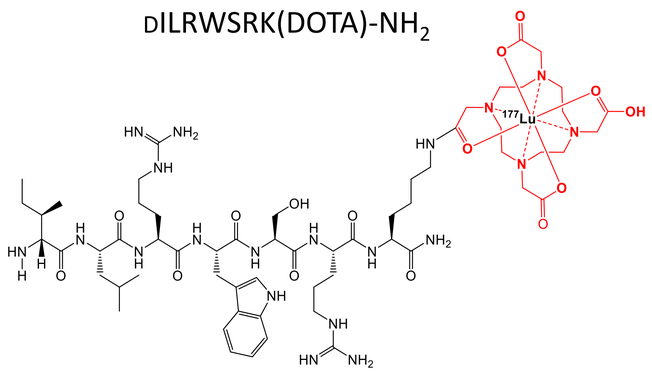
JMF-04

DILRWSRKK(DOTA)-NH_2



JMF-10

DILRWSRK(DOTA)-NH_2



JMF-11

DILRWSRK(Ahx-DOTA)-NH_2

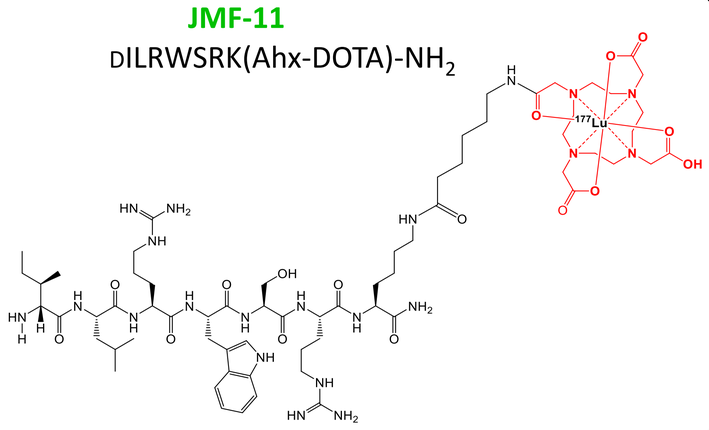


Figure 1. Structures of the ^{177}Lu-labeled DOTA-conjugated EPI-X4 derivatives.

EPI-X4-based radiotracers: The DOTA-conjugated EPI-X4 derivatives were incubated with $^{177}LuCl_3$ (40-150 MBq) in ammonium acetate buffer (0.4 M, pH 5.0) for 20-30 min at 95°C. Quality control was performed by radio-HPLC. Radiolabeling yield was ≥97% and radiochemical purity ≥95% at apparent molar activities of 30-80 MBq/nmol.

Table 1. Physicochemical properties of the ^{177}Lu-labeled radiotracers.

Compound code	Molecular weight	Retention time t_R (min)	Radiochemical Purity (RCP)
^{177}Lu-JMF-04	1471.7	8.4	≥95 %
^{177}Lu-JMF-10	1343.1	8.6	≥95 %
^{177}Lu-JMF-11	1456.3	8.7	≥95 %

*Column: Phenomenex Jupiter Proteo C12 (90 Å, 250 × 4.6 mm) column
Gradient: Gradient: 5-50% solvent B in 15 min (*A* = H_2O [0.1%TFA], *B* = ACN [0.1% TFA])

Log *D*: The octanol-water distribution coefficient at pH 7.4 (log *D*) was determined by shake-flask method. The two new radiotracers, ^{177}Lu-JMF-10 and ^{177}Lu-JMF11, had very similar log *D* (-2.99±0.31 and -2.88±0.31, respectively), being more lipophilic than the reference ^{177}Lu-JMF-04 (log *D* = -3.45±0.06).

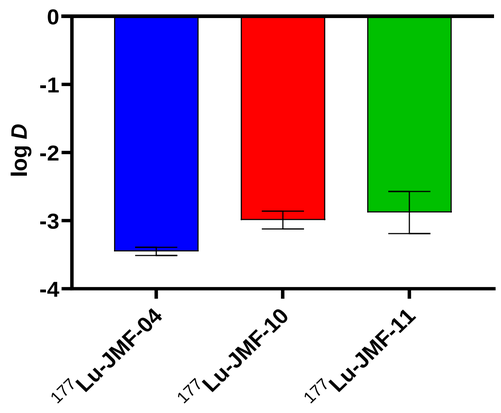


Figure 2. Lipophilicities of the ^{177}Lu-labeled radiotracers.

Shelf-life: The shelf-life of the radiotracers was studied up to 24h. ^{177}Lu-JMF-10 and ^{177}Lu-JMF11 showed improved stability, being >90% intact after 4h at RT. ^{177}Lu-JMF-10 showed the highest stability (80% at 24h), among all.

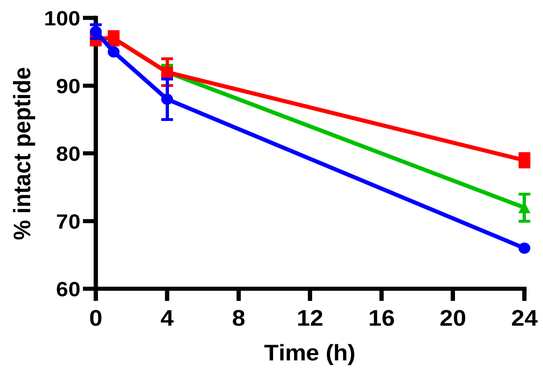


Figure 3. Shelf-life of the ^{177}Lu-labeled radiotracers.

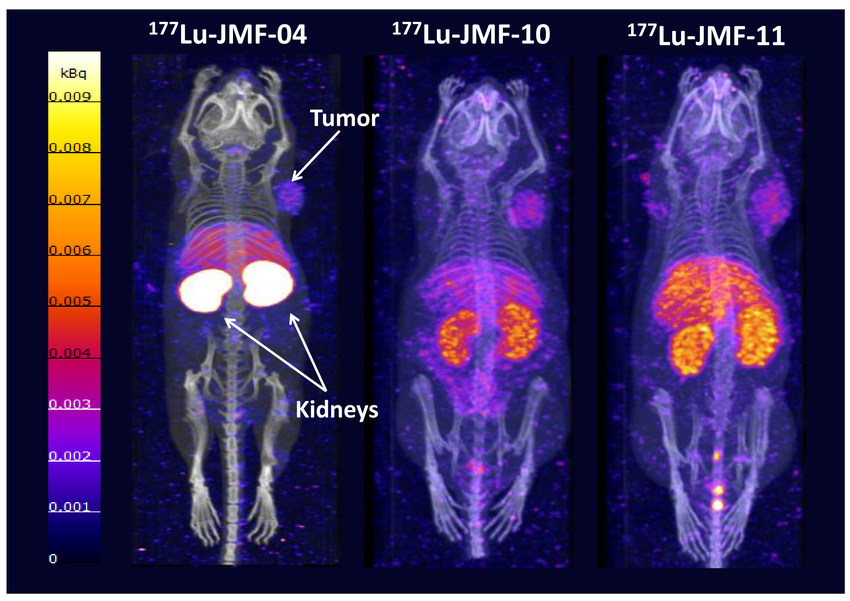


Figure 3. SPECT/CT images of ^{177}Lu-labeled radiotracers at 1h p.i.

SPECT/CT imaging: Nude mice bearing Jurkat xenografts were euthanized 1 hour post injection (p.i.) of 200 pmol/~15 MBq of the ^{177}Lu-labeled radiotracers (Figure 3). ^{177}Lu-JMF-10 and ^{177}Lu-JMF-11 showed massive reduction in kidney uptake in comparison to ^{177}Lu-JMF-04, while the accumulation in the tumor remained at the same level.

Biodistribtuion: Quantitative biodistribution was performed for all three ^{177}Lu-labeled tracers (200 pmol/0.8-1 MBq) in Jurkat xenograft tumor model at 1h p.i. While, there was no significant difference in tumor uptake between ^{177}Lu-JMF-04 and ^{177}Lu-JMF10 (2.25±0.43 vs 1.71±0.17 % injected activity per gram of tissue (% I.A./g), ^{177}Lu-JMF-11 had a lower tumor uptake (1.24±0.21 %I.A./g) and hence was omitted from further evaluation. As also seen from the SPECT/CT imaging, a 15- and 19-fold reduction in the kidney uptake was observed for ^{177}Lu-JMF-10 and ^{177}Lu-JMF-11, compared to ^{177}Lu-JMF-04.

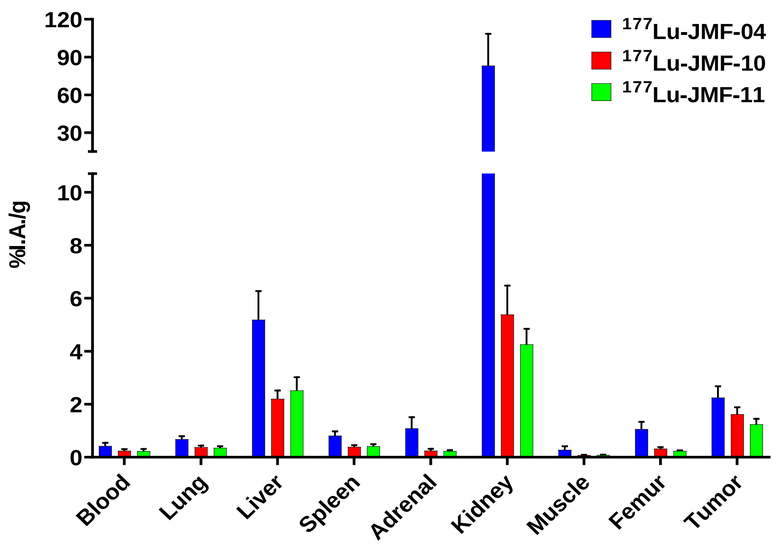


Figure 4. Biodistribtuion of ^{177}Lu-labeled radiotracers at 1h p.i. The results are expressed as % I.A/G.

PET/CT imaging and biodistribution: The ^{68}Ga-counterparts behaved very similar to the ^{177}Lu-labeled ones. They displayed similar tumor uptake and massive reduction in kidney uptake for the ^{68}Ga-JMF-10 in comparison to ^{68}Ga-JMF-04. When quantified the tumor uptake was found to be 2.24±0.57 %I.A/g and 2.45±0.46 %I.A./g for ^{68}Ga-JMF-04 and ^{68}Ga-JMF-10 respectively.

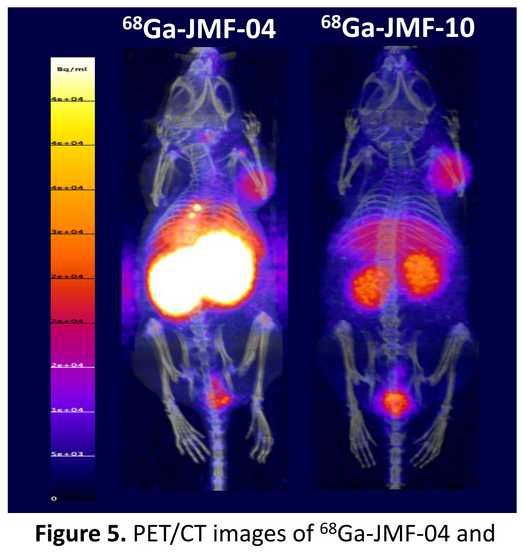


Figure 5. PET/CT images of ^{68}Ga-JMF-04 and ^{68}Ga-JMF-10 at 1h p.i.

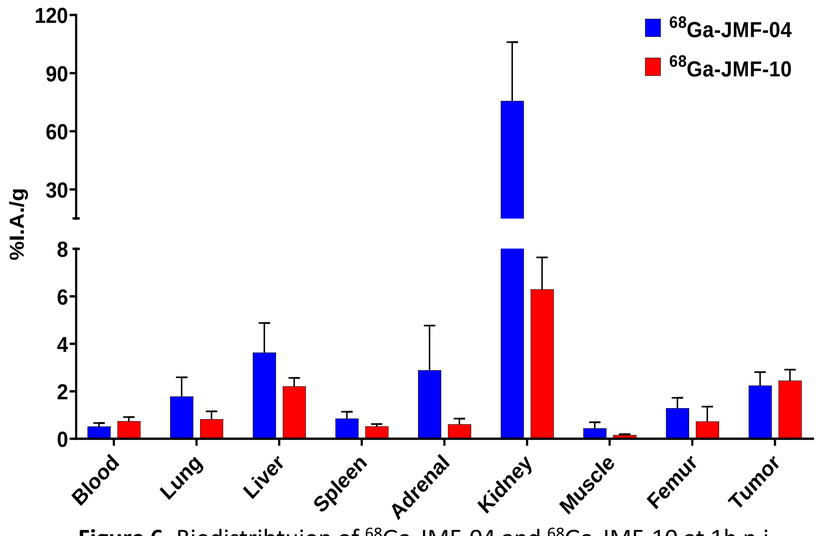


Figure 6. Biodistribtuion of ^{68}Ga-JMF-04 and ^{68}Ga-JMF-10 at 1h p.i.. The results are expressed as % I.A./g.

***In vivo* metabolic stability: :** Mice were injected with 500 pmol/~18 MBq of ^{177}Lu-JMF-04 and ^{177}Lu-JMF-10 and at 5, 15, and 30 min blood was collected, centrifuged and the plasma was separated and analysed. Both radiotracers displayed very similar stability at all investigated time points, remaining ~50 % intact in plasma after 30 min.

Table 2. *In vivo* metabolic stability of ^{177}Lu-JMF-04 and ^{177}Lu-JMF-10.

Compound code	% intact peptide		
	5 min	15 min	30 min
^{177}Lu-JMF-04	71 ± 5	58 ± 7	53 ± 6
^{177}Lu-JMF-10	73 ± 1	58 ± 3	49 ± 7

Conclusion

- The kidney uptake was significantly reduced in the two optimized derivatives while maintaining similar tumor uptake for JMF-10.
- This study designates JMF-10 as the lead derivative and further optimization in the direction of improving affinity is in progress.

References

1. Ziraffi, O., et al. *Cell Rep* **2015**;11(5):737-47.