PET Imaging, Biodistribution and *In Vitro* Stability Assessment of [^{134}Ce]Ce-RPS-088: A Versatile Surrogate for Non-Invasive Dose Calculation of [^{225}Ac]Ac-RPS-088 in Prostate Cancer?

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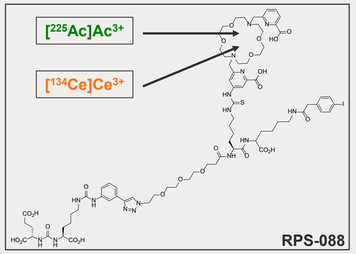
Introduction

Presently, standardized doses are administered to patients receiving targeted alpha therapy with Ac-225 as the low administered activities and the complex decay chain of Ac-225 complicate the calculation of a potentially more effective personalized patient dose. [^{134}Ce]Ce^{3+} has chemical and physical properties that match well with [^{225}Ac]Ac^{3+} and decays by electron capture to positron-emitting La-134 ($t_{1/2}$ = 6.5 min). We hypothesize that ^{134}Ce-labeled compounds could be used to predict Ac-225 dose by PET imaging. The biodistribution profiles of PSMA-binding [^{134}Ce]Ce-RPS-088 and [^{225}Ac]Ac-RPS-088 were therefore compared at four different time points and PET images acquired of the same mice for later dose calculations. Additionally, *in vitro* and ex vivo properties of both compounds were assessed and compared.



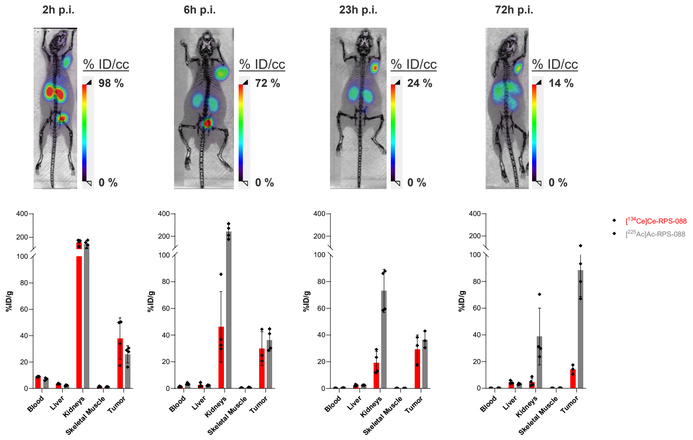


Fig. 1 PET Imaging and biodistribution of [^{134}Ce]Ce-RPS-088 and [^{225}Ac]Ac-RPS-088. 3.1-4.6 MBq [^{134}Ce]Ce-RPS-088 or 45 kBq [^{225}Ac]Ac-RPS-088 were administered to LNCaP-tumor bearing nude mice (n = 4 for every time point). The mice were euthanized at 2h, 6h, 23h and 72, post injection. A 30 min static PET image was acquired of mice, which had received [^{134}Ce]Ce-RPS-088, prior to euthanasia. Ac-225 doses will be calculated based on the Ce-134 PET and biodistribution data and compared with the doses derived from the Ac-225 biodistribution. The %ID/cc values from the PET images were corrected using an internal 2% ID/cc standard.

Fig. 3 [^{134}Ce]Ce-RPS-088 and [^{225}Ac]Ac-RPS-088 digital autoradiography. LNCaP tumor xenograft mice were co-injected with 1.6 MBq [^{134}Ce]Ce-RPS-088 and 45 kBq [^{225}Ac]Ac-RPS-088. The mice were euthanized 6h p.i. and 7 mm sections of the tumor, the kidneys, the spleen and the liver were read on a digital autoradiography system (iQID). The individual signals of β- and α-decays were separated using a method developed by Dr. Brian Miller and colleagues. Differences in the accumulation of [^{134}Ce]Ce-RPS-088 and [^{225}Ac]Ac-RPS-088 could thus be distinguished, although a differentiation between intact tracer, radiometabolites and released radiometal was not possible.

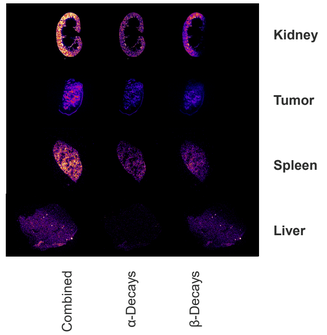
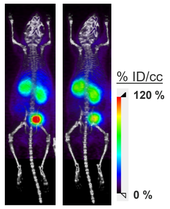
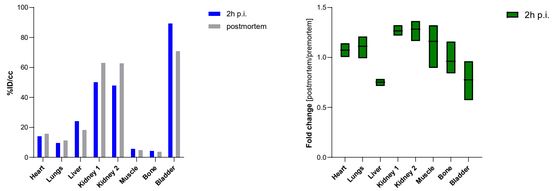


Fig. 2 PET Imaging of [^{134}Ce]Ce-RPS-088 pre- and post-euthanasia. 3.3 MBq [^{134}Ce]Ce-RPS-088 were administered to 4 non-tumor bearing nude mice. A 30 min static PET image was acquired 2h p.i. and the mice subsequently euthanized. Following a waiting period of just under 2h to allow the secular equilibrium to be established between Ce-134 and La-134, the mice were returned to the PET camera. The signal intensity in selected organs was compared. The %ID/cc values were corrected using an internal 2% ID/cc standard.





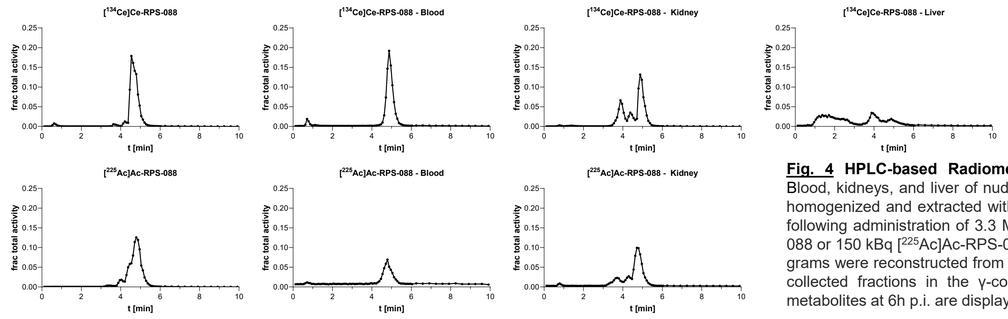


Fig. 4 HPLC-based Radiometabolite Analysis. Blood, kidneys, and liver of nude mice (n = 3) were homogenized and extracted with MeCN/H_2O/MeOH following administration of 3.3 MBq [^{134}Ce]Ce-RPS-088 or 150 kBq [^{225}Ac]Ac-RPS-088. Radiochromatograms were reconstructed from the measurement of collected fractions in the γ-counter. Formation of metabolites at 6h p.i. are displayed.

Fig. 5 Release of Ce-134/Ac-225 from [^{134}Ce]Ce-RPS-088, [^{134}Ce]Ce-RPS-063 and [^{225}Ac]Ac-RPS-088. 0.3-1.1 MBq (5-10 µL) [^{134}Ce]Ce-RPS-088 or the DOTA-conjugated analogue [^{134}Ce]Ce-RPS-063 were incubated at 37 °C in 150 µL human serum or PBS. Aliquots were taken for TLC analysis in 50 mM citrate buffer, pH 5.5 at 2h, 24h, and 72h after the start of incubation to separate released Ce-134 from bound Ce-134. Similarly, 22-50 kBq (2-4 µL) [225Ac]Ac-RPS-088 were analyzed for stability in human serum, using PBS as the control. Finally, both [^{134}Ce]Ce-RPS-088 and [^{134}Ce]Ce-RPS-063 were incubated in mouse urine over 24h and the release of Ce-134 monitored by TLC.

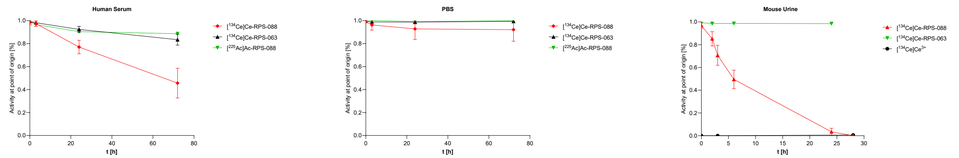
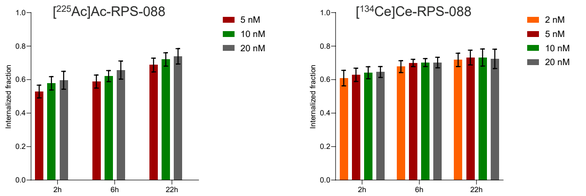


Fig. 6 Internalization of [^{134}Ce]Ce-RPS-088 and [^{225}Ac]Ac-RPS-088. LNCaP cells were plated at 2x10^5 cells/well in poly-L-lysine coated 24-well plates 24h prior to start of the experiment. 2nM, 5nM, 10nM, or 20nM [^{134}Ce]Ce-RPS-088 (0.5-1.9 MBq/nmol) or [^{225}Ac]Ac-RPS-088 (0.1-0.2 MBq/nmol) were added in OPTIMEM + 5% FCS and the cell surface bound fraction separated from the internalized fraction at 2h, 6h and 22h after addition of the compound. 10 µM MIP-1095 was used in blocking studies to demonstrate specificity (not shown here). The counts in the 2nM wells for [^{225}Ac]Ac-RPS-088 were too low and therefore not used in the analysis.



Conclusion

At early time points, i.e. 2h post injection, the biodistribution profile of [^{134}Ce]Ce-RPS-088 appears to be an adequate match for the actual [^{225}Ac]Ac-RPS-088 biodistribution. With later time points, however, [^{134}Ce]Ce-RPS-088 clears faster from both the tumor and critical organs like the kidneys. This was also highlighted by regional differences in the kidney accumulation as shown by digital autoradiography. The reason may be the increased release of Ce-134 when incubated both in human serum and in mouse urine.

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