Preclinical evaluation of ^{225}Ac-rhPSMA-10.1, a novel radiohybrid PSMA compound for targeted alpha therapy of prostate cancer

Daniel J. Stevens[1], Bradley Waldron[1], Alexander Wurzer[2], Calogero D'Alessandria[2], Rikke Veggerby Grønlund[3], Mathias Wikke Hallund[3], David E. Gauden[1] and **Caroline Foxton[4]**

1. Blue Earth Therapeutics, Oxford, UK.
2. Department of Nuclear Medicine, Technical University of Munich, Germany.
3. Minerva Imaging, Ølstykke, Denmark.
4. Blue Earth Diagnostics, Oxford, UK.

Disclosures

- This study was supported by Blue Earth Therapeutics, Oxford, UK.

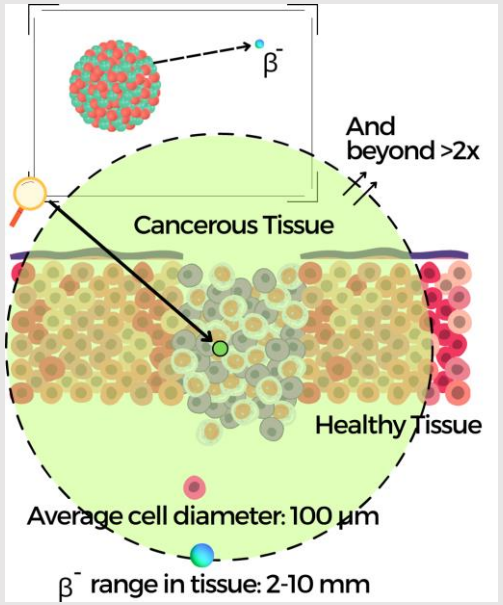
- Caroline Foxton, Bradley Waldron, David E. Gauden and Daniel J. Stevens are employees of Blue Earth Diagnostics / Blue Earth Therapeutics, Oxford, UK.

Introduction

- Radioligand therapy targeting prostate-specific membrane antigen (PSMA) is an effective therapy in men with metastatic castration-resistant prostate cancer[1]
- To date, therapeutic PSMA ligands have typically been labelled with β-emitting ^{177}Lu, although α-emitting ^{225}Ac-labelled ligands are in development

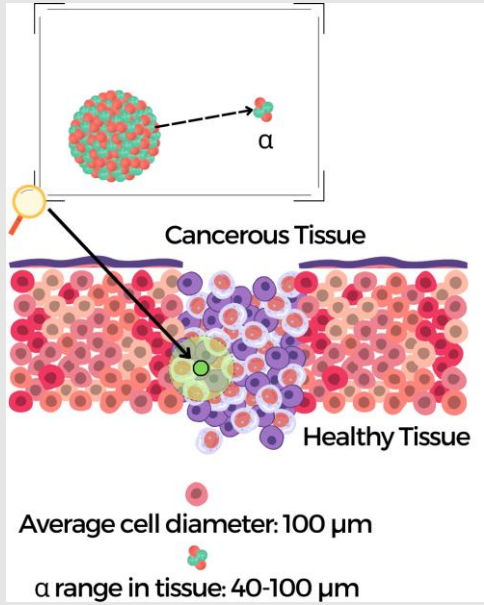
β-radiation

- e.g., ^{177}Lu, ^{67}Cu
- Longer range in human tissue
- Data suggest approximately 1/3 of patients may not respond to ^{177}Lu-based radioligand therapy[2]



α-radiation

- e.g., ^{225}Ac, ^{212}Pb
- Short range in human tissue
- May be appropriate in late-stage disease and/or for patients with micrometastases
- High linear energy transfer may overcome radiation resistance[3]



1. Sartor et al. NEJM. 2021;385:1091–1103; 2. Yadav et al. AJR Am J Roentgenol. 2019;213:275–285; 3. Pouget et al. Front. Med. 2021;8:692436.

Radiohybrid prostate-specific membrane antigen (rhPSMA) ligands

Blue Earth Therapeutics
A Bracco Company

Radiohybrid platform provides two binding sites for radionuclides, enabling theranostic potential[1]

- Radiolabel with ^{18}F for diagnostic imaging (e.g., flotufolastat ^{18}F)[2,3]
- Radiolabel with α- or β-emitting radiometals (**^{225}Ac** or **^{177}Lu**) for therapeutic use:
  - Pre-clinical assessments of ^{177}Lu-rhPSMA-10.1 show it to have:
    - encouraging *in vitro* parameters (e.g., high PSMA affinity and internalization)
    - favourable biodistribution and greater tumour suppression vs ^{177}Lu-PSMA-I&T[4,5]
  - ^{177}Lu-rhPSMA-10.1 is currently being investigated in a Phase 1/2 trial (NCT05413850)

Here, we evaluate ^{225}Ac-rhPSMA-10.1 as a potential alpha-targeted therapy for prostate cancer

1. Wurzer et al. J Nuc Med. 2020;61:735–42; 2. Surasi et al. Eur Urol. 2023;doi.org/10.1016/j.eururo.2023.06.018; 3. Jani et al. J Urol. 2023;210(2):299–311; 4. Vassileva et al. 2023. J Nucl Med. 2023;64(2):P621; 5. Foxton et al. J Nucl Med. 2022;63(2):2567.

Objectives

To characterize the potential of ^{225}Ac-rhPSMA-10.1 as a therapy for prostate cancer by conducting a series of preclinical assessments:

- PSMA binding affinity
- Lipophilicity
- Cellular internalization
- Therapeutic response in a prostate cancer xenograft model (22Rv1)

Preclinical analyses

Blue Earth Therapeutics
A Bracco Company

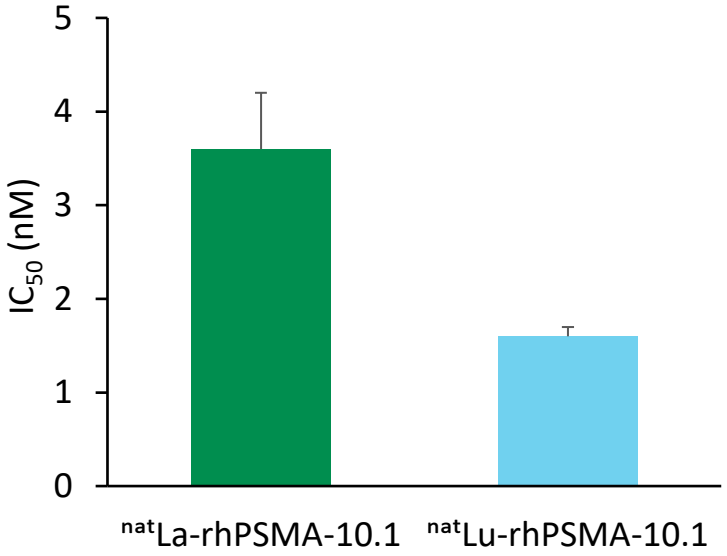
PSMA binding affinity (IC_{50})	• Competitive binding assay in LNCaP cells using rhPSMA-10.1 complexed with natural Lanthanum (^{nat}La, serving as a cold surrogate for ^{225}Ac), or natural Lutetium (^{nat}Lu) • N = 3 times per ligand
Lipophilicity	• The distribution coefficient was measured in n-octanol and phosphate-buffered saline at pH 7.4 (log $D_{7.4}$) using the shake-flask method • N = 8 experiments
Cellular internalization	• % Internalization (normalized vs ^{177}Lu-PSMA-I&T as reference) was assessed by measuring (γ-counting) free, surface-bound, and internalized activity in LNCaP cells after 1-hour incubation • N = 3 experiments
Therapeutic response in 22Rv1 tumour-bearing NMRI nu/nu mice	• Single IV administration of ^{225}Ac-rhPSMA-10.1 (30 kBq) or ^{177}Lu-rhPSMA-10.1 (30 MBq).† Efficacy assessments comprised tumour volume and survival vs untreated controls ≤Day 49. Body weight was monitored for toxicity assessment • N = 8 mice per group

†1000-fold lower dose for ^{225}Ac-rhPSMA-10.1 determined based on data derived with $^{225}Ac/^{177}Lu$-PSMA-617.[1]

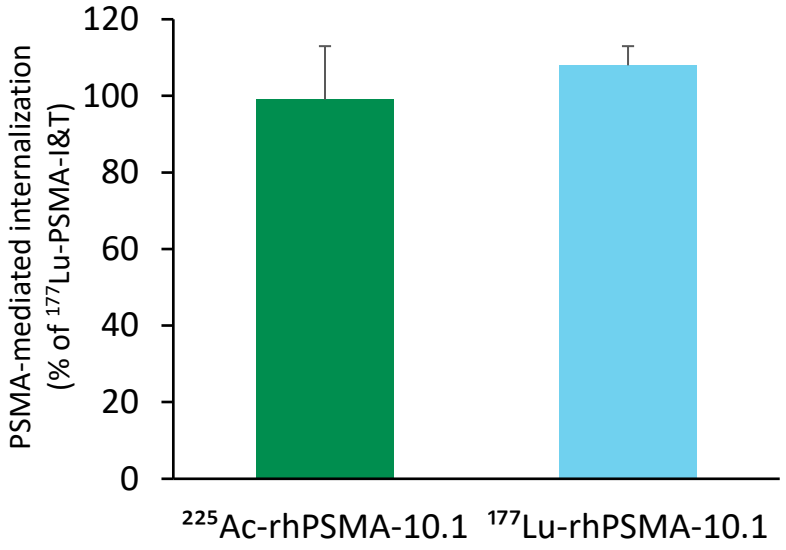
1. Lee. Mol Imaging Radionucl Ther. 2022;31(1):1–6.

In vitro assessments

Substituting ^{225}Ac for ^{177}Lu did not impact key *in vitro* parameters



Ligand	$logD_{7.4}$ ± SD
^{225}Ac-rhPSMA-10.1	-3.4 ± 0.2
^{177}Lu-rhPSMA-10.1	-3.8 ± 0.1



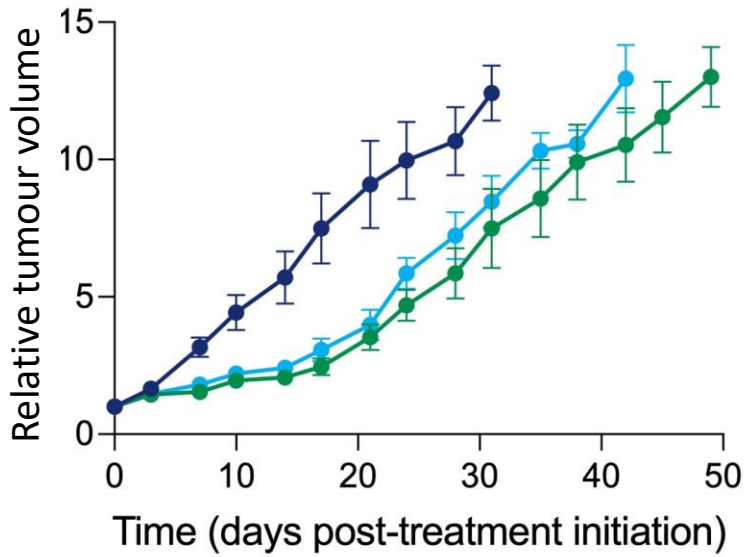
Both ligands demonstrated **PSMA binding affinities** in the low nM range

Both ligands demonstrated similar **lipophilicity**

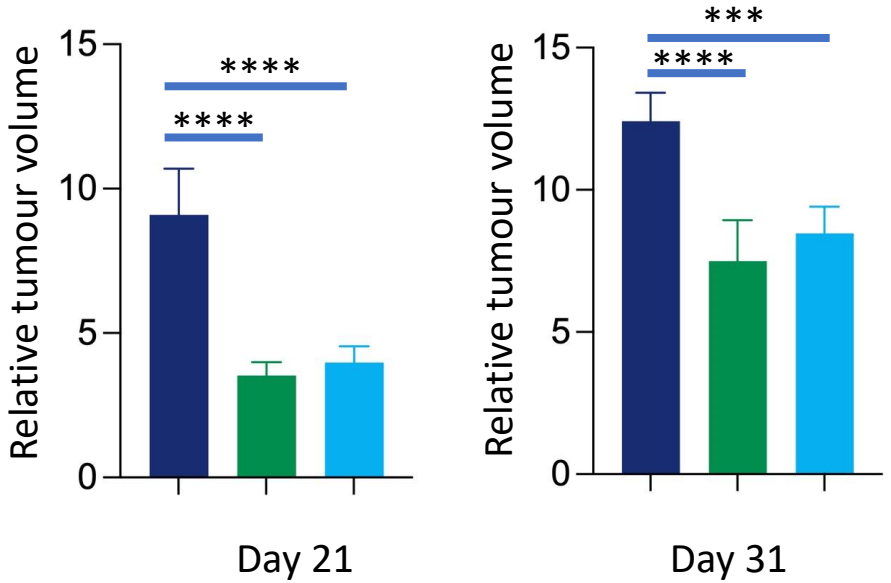
High cellular **internalization** was observed for both ligands

Both plots present data as mean ± SD (n=3).

Therapeutic efficacy – tumour growth in 22Rv1 xenografts



p<0.052 for both ligands from day 14 onwards (two-way ANOVA)



p=0.0006,*p<0.0001.

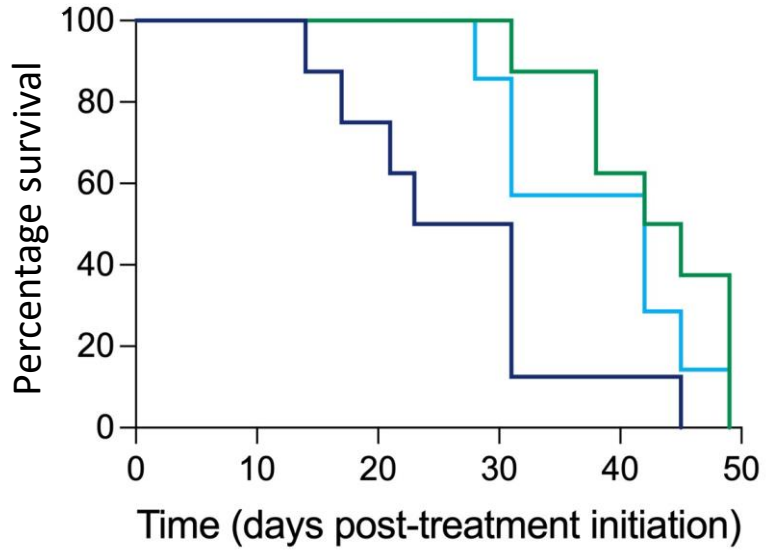
Untreated | ^{225}Ac-rhPSMA-10.1 (30 kBq) | ^{177}Lu-rhPSMA-10.1 (30 MBq)

^{225}Ac-rhPSMA-10.1 significantly reduced tumour growth compared with untreated controls. No significant differences were noted between ^{225}Ac-rhPSMA-10.1 and ^{177}Lu-rhPSMA-10.1 groups.

Foxton et al. EANM Annual Meeting 2023. #OP-145

Data presented as mean ± SEM (n=8).

Therapeutic efficacy – survival in 22Rv1 xenografts



p=0.006 for ^{225}Ac-rhPSMA-10.1 vs untreated (Log-rank test)

Ligand	Median survival (days)
Untreated	27.0
^{225}Ac-rhPSMA-10.1	43.5
^{177}Lu-rhPSMA-10.1	42.0

- Treatments were well tolerated, with no significant effects on body weight observed

Untreated ^{225}Ac-rhPSMA-10.1 (30 kBq) ^{177}Lu-rhPSMA-10.1 (30 MBq)

^{225}Ac-rhPSMA-10.1 significantly prolonged survival compared with untreated controls. No significant differences were noted between ^{225}Ac-rhPSMA-10.1 and ^{177}Lu-rhPSMA-10.1 groups.

Survival curve data (n=8 except ^{177}Lu-rhPSMA-10.1 [n=7]).

Conclusions

- These preclinical analyses demonstrate a promising therapeutic profile for ^{225}Ac-rhPSMA-10.1, using a 1000-fold lower activity than ^{177}Lu-rhPSMA-10.1
- Similar *in vitro* characteristics and *in vivo* therapeutic efficacy results were observed for both ^{225}Ac-rhPSMA-10.1 and ^{177}Lu-rhPSMA-10.1
- ^{225}Ac-rhPSMA-10.1 represents a novel alpha particle-targeted therapy, with clinical trial application submission planned in 2023

Acknowledgements

Blue Earth Therapeutics, Oxford, UK

- Dan Stevens
- Brad Waldron
- David Gauden

Technical University of Munich, Germany

- Alexander Wurzer
- Calogero D'Alessandria

Minerva Imaging, Ølstykke, Denmark

- Rikke Veggerby Grønlund
- Mathias Wikke Hallund

- The authors would like to thank Dr Vessela Vassileva for her contribution to this study and for her work on the abstract associated with this presentation.