Allometric scaling of preclinical dosimetry for the Nectin-4 miniprotein binders AKY-807 and AKY-1189 accurately predicts human absorbed dose to major organs

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INTRODUCTION

- Nectin-4 is a clinically validated target in urothelial cancer and has shown promise in other Nectin-4-expressing tumors but has been underserved by radiopharmaceuticals.
- AKY-807 and AKY-1189 are synthetic miniproteins targeting Nectin-4, with sequence differences in 4 locations and similar pharmacokinetic profiles.
- After administering a radiopharmaceutical, serial imaging in animals or humans shows retention and excretion patterns over time, which are then used to calculate the cumulative absorbed dose of radiation to an organ or tumor (dosimetry).
- A major challenge facing therapeutic radiopharmaceutical development is accurate prediction of cumulative organ doses in patients based on serial images in preclinical models (allometric scaling).
- Specific issues include:
  - inherent differences in animal and human physiology,
  - use of imaging isotopes as surrogates (in animals and/or humans) to estimate cumulative absorbed doses of the therapeutic, and
  - multiple-step dosimetry processes, with each step contributing to overall uncertainty in estimates of cumulative dose.
- Direct intrapatient comparisons of agents with distinct preclinical dosimetry profiles are rarely performed but reduce the potential sources of variability and therefore may provide additional insight into optimal scaling methods.

AIM

To evaluate the utility of an allometric scaling method appropriate for predicting the therapeutic index for miniprotein binders to Nectin-4.

AN ALLOMETRIC SCALING METHOD TO ESTIMATE CLINICAL DOSE

- Allometric scaling methods take into account differences in surface area, organ mass, and physiology between species.
- The selected method used in this study is based on the molecular characteristics of Aktis miniproteins (**Figure 1**).

A.

$$\text{Human organ exposure} = \text{Mouse organ exposure} * \frac{\left(\frac{\text{organ mass}}{\text{whole body mass}}\right)_{\text{human}}}{\left(\frac{\text{organ mass}}{\text{whole body mass}}\right)_{\text{mouse}}}$$

B.

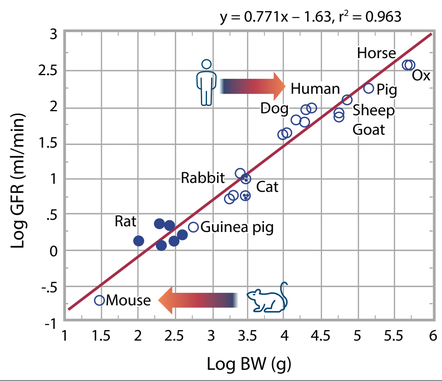


Figure 1. A. Selected allometric scaling equation. **B.** Log-log relationship between glomerular filtration rate and body size across species.[1]

CLINICAL ADMINISTRATION OF OPTIMIZED NECTIN-4-SPECIFIC MINIPROTEINS

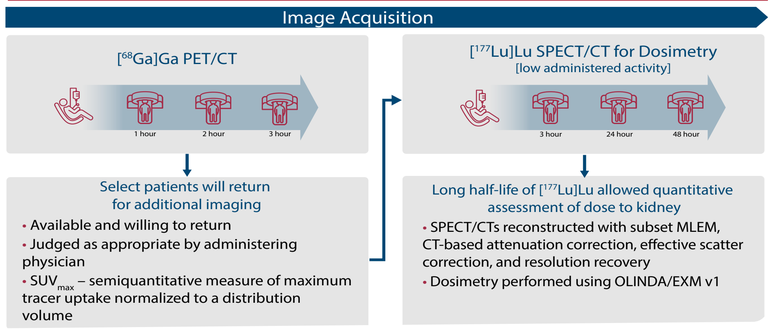


Figure 2. Injection and imaging schema. Assessments were made under the South African Health Products Regulatory Authority Section 21 regulatory path. Manufacturing was performed on-site at the Nuclear Medicine Research Infrastructure (NuMeRI, Pretoria, South Africa).

AKY-807 AND AKY-1189 HAVE SIMILAR STRUCTURE, SELECTIVITY, AND EFFICACY

- AKY-807 and AKY-1189 were optimized for the delivery of therapeutic radiopharmaceuticals targeting Nectin-4-positive disease.
- AKY-807 and AKY-1189 are structurally similar, rapidly cleared, and efficacious with a single administration in a urothelial cancer preclinical model (**Table 1**).
- AKY-1189 has a higher affinity to Nectin-4 and is predicted to exhibit less kidney retention, resulting in a lower absorbed dose to kidneys in humans (**Tables 1 & 2**).

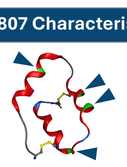
Category	AKY-807 Characteristics	AKY-1189 Characteristics
Structure		
Affinity	K_i = 16.3nM K_D = 3.0nM	K_i = 0.82nM K_D = 0.22nM
On-Cell Binding	Selectivity for HT-1376 No binding to the isogenic HT-1376 Nectin-4 KO cell line	Selectivity for HT-1376 No binding to the isogenic HT-1376 Nectin-4 KO cell line
Selectivity	No off-target binding via Retrogenix screen	No off-target binding via Retrogenix screen
PK Profile	Clearance at GFR	Clearance at GFR
Efficacy	Target and dose-dependent efficacy	Target and dose-dependent efficacy

Table 1. Comparison of AKY-807 and AKY-1189. Differences appear in red font. Arrow heads indicate sequence difference in 4 locations. **Please see preclinical abstract #118, presented on Wednesday, October 23, 2024, for miniprotein development details and immunohistochemical characterization of cell lines.**

SEQUENCE CHANGES TO AKY-1189 RESULTED IN INCREASED TUMOR UPTAKE AND REDUCED KIDNEY UPTAKE COMPARED TO AKY-807

- Tumor uptake and retention were observed for both AKY-807 and AKY-1189; however, the intensity of uptake and retention was higher for AKY-1189 (**Figure 4A, B**).
- Uptake in the kidney was greatly reduced for AKY-1189 relative to AKY-807 (**Figure 4A, B**).
- Both molecules had potential clinical utility and were further assessed.

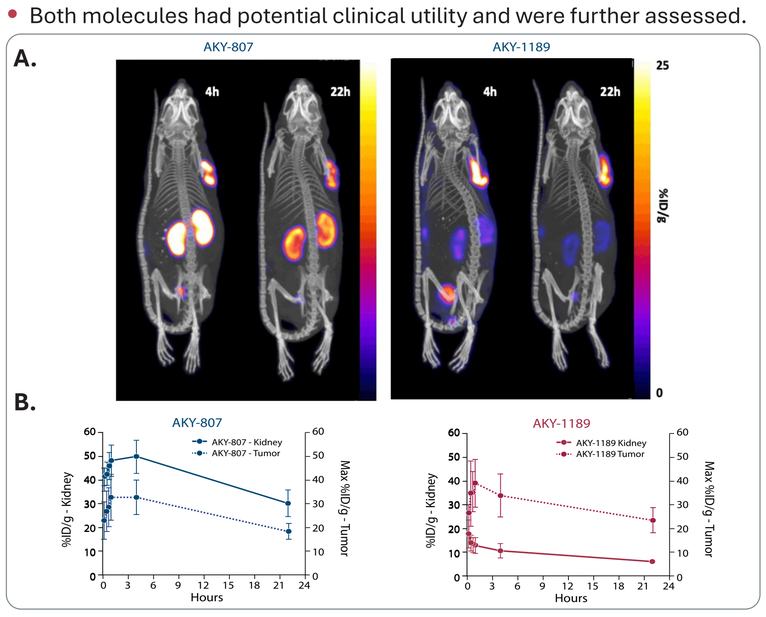


Figure 4. A. Isogenic xenograft models were generated using HT-1376 (human urinary bladder carcinoma) cells exogenously expressing Nectin-4. Single intravenous injection of [^{111}In]In-AKY-807 or [^{111}In]In-AKY-1189 was followed by SPECT/CT imaging at 4 and 22 hours. Absorbed dose estimates were generated utilizing OLINDA 2.2.3. **B.** Injected dose (ID)/gram (g) (kidney) and maximum ID/g (tumor) over time in each molecule.

ALLOMETRIC SCALING PREDICTED FAVORABLE KIDNEY ABSORBED DOSE IN HUMANS FOR AKY-1189

- Estimated kidney absorbed dose was lower for AKY-1189.
- Identical absorbed doses were predicted in bone marrow for both molecules.

	AKY-807 Characteristics		AKY-1189 Characteristics	
Location	Absorbed Dose per GBq (Gy/GBq)	Estimated Absorbed Dose After 6 Administrations of 7.4 GBq (Gy)	Absorbed Dose per GBq (Gy/GBq)	Estimated Absorbed Dose After 6 Administrations of 7.4 GBq (Gy)
Kidney	0.86	38.18	0.22	9.77

Table 2. Comparison of estimated absorbed doses in kidney for AKY-807 and AKY-1189.

IMAGING AND DOSIMETRY IN PATIENTS RECEIVING AKY-807 AND AKY-1189

Compound	Patient (#)
[^{68}Ga]Ga-AKY-807	11
[^{177}Lu]Lu-AKY-807	3
[^{68}Ga]Ga-AKY-1189	20
[^{177}Lu]Lu-AKY-1189	8

Table 3. Number of patients undergoing [^{68}Ga]Ga imaging and [^{177}Lu]Lu dosimetry for each molecule.

- One patient received [^{68}Ga]Ga-AKY-807 and [^{177}Lu]Lu-AKY-807 followed by [^{68}Ga]Ga-AKY-1189 and [^{177}Lu]Lu-AKY-1189 approximately 2 months later.
- The patient received no treatment between images and experienced significant disease progression.

INTRAPATIENT COMPARISON CONFIRMS TUMOR UPTAKE OF BOTH [^{68}Ga]Ga-AKY-807 AND [^{68}Ga]Ga-AKY-1189

- Tumor uptake was observed with both AKY-807 and AKY-1189 (**Figure 5**).
- Uptake with AKY-1189 appears increased in representative lesions as assessed by SUV_{max} (**Table 4**).

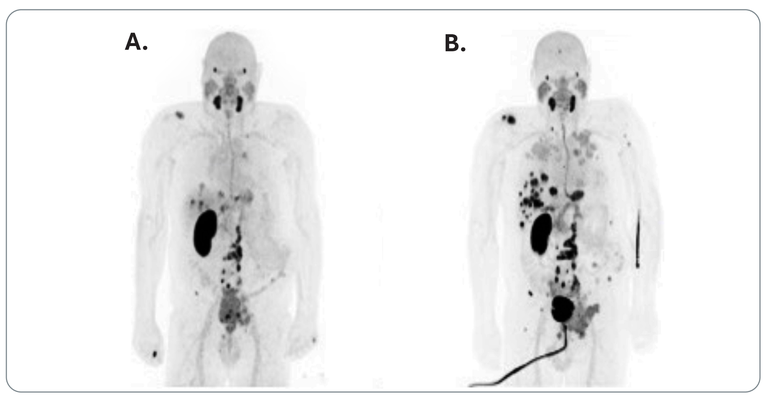


Figure 5. [^{68}Ga]Ga-AKY-807 (**A**) and [^{68}Ga]Ga-AKY-1189 (**B**) PET/CT images (1-hour timepoint) in the same patient approximately 2 months later. Single kidney due to prior nephrectomy. Scaling is the same in both images. **Please see abstract #10, presented on Friday, October 25, 2024, for additional clinical details.**

Location	AKY-807 SUV_{max}		AKY-1189 SUV_{max}	
	1 hour	3 hours	1 hour	3 hours
Bone (right scapula)	10.83	11.57	27.74	27.75
Lung lesion	7.78	12.61	10.34	16.22
Liver lesion	15.06	21.41	38.47	49.32
Lymph node (left para-aortic)	24.73	31.49	28.39	29.44

Table 4. Representative lesions that could be identified on both AKY-807 and AKY-1189 images were selected for evaluation of SUV_{max} at the 1- and 3-hour timepoints.

RAPID WASHOUT OBSERVED IN NON-KIDNEY NORMAL TISSUES WITH [^{177}Lu]Lu-AKY-1189

- Physiologic uptake observed in the salivary and lacrimal glands (**Figure 5**), as well as the GI tract, is not expected to be dose-limiting due to washout at 48 hours on SPECT/CT imaging (**Figure 6**).
- The kidney is the potentially dose-limiting normal tissue.

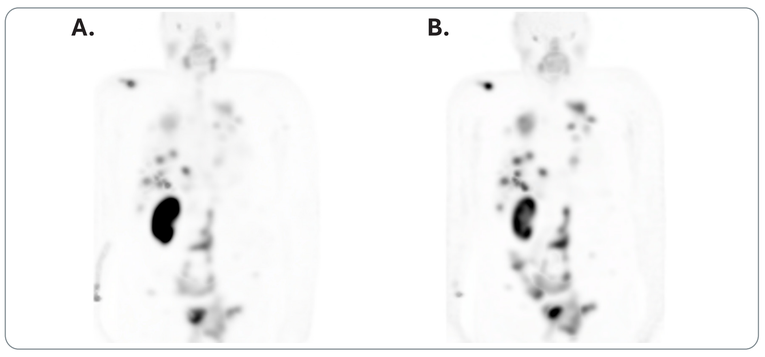


Figure 6. [^{177}Lu]Lu-AKY-1189 images in the same patient as in **Figure 5** at the 3-hour (**A**) and 48-hour (**B**) timepoints. Scaling is the same in both images. **Please see abstract #10, presented on Friday, October 25, 2024.**

INTRAPATIENT COMPARISON CONFIRMS REDUCED KIDNEY DOSES FOR AKY-1189 AS PREDICTED IN PRECLINICAL MODELS

- Clear differentiation in predicted absorbed dose to the kidneys can be seen between AKY-807 and AKY-1189 in mice and in the intrapatient comparison (**Table 5**).
- Although there is expected variation in absolute predicted doses in the mouse and human, the relative reduction is consistent.

Compound	Absorbed Dose per GBq of ^{177}Lu (Gy/GBq)	Relative reduction
Mouse		
AKY-807	0.86	74%
AKY-1189	0.22	
Human		
AKY-807	1.41	80%
AKY-1189	0.28	

Table 5. Comparison of absorbed dose to the kidneys with AKY-807 and AKY-1189 based on preclinical models and human dosimetry.

CLINICALLY MEANINGFUL REDUCTION IN ABSORBED DOSE TO KIDNEYS IS PREDICTED OVER A FULL TREATMENT COURSE

- Cumulative dosimetry estimated preclinically confirmed with clinical dosimetry (**Figure 7A, B**).

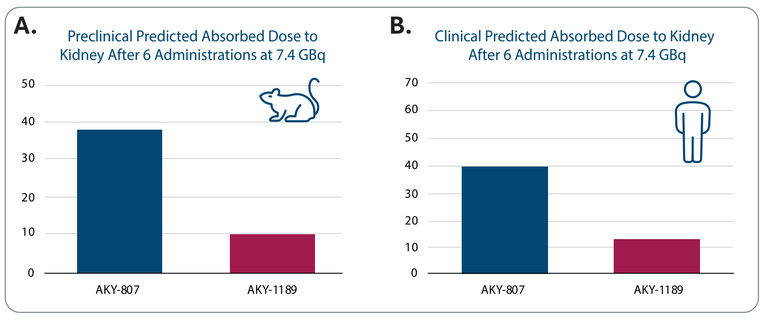


Figure 7. Cumulative estimated preclinical (**A**) and clinical (**B**) absorbed dose to kidney for AKY-807 and AKY-1189.

SIMILAR TRENDS OBSERVED IN THE LARGER GROUP OF PATIENTS UNDERGOING DOSIMETRIC EVALUATION

- Patients receiving AKY-807 demonstrate higher initial activity and greater activity retention in kidneys compared to those receiving AKY-1189 (**Figure 8**).

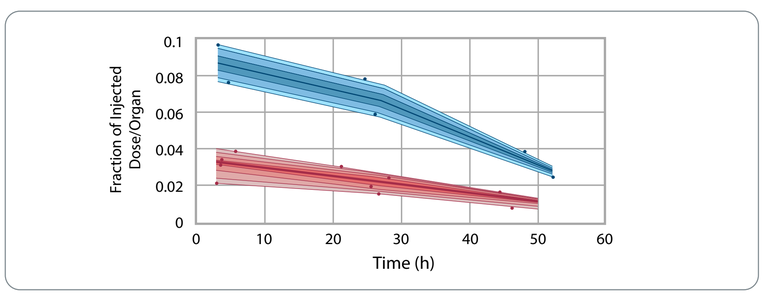


Figure 8. Comparison of time activity curves for patients receiving AKY-807 (n=2, blue) compared to patients receiving AKY-1189 (n=4, red). Thick lines, median; spread, 5% - 95%. Patients with R^2 values >0.9 for curve fit included in analysis.

CONCLUSIONS

- AKY-1189 shows robust tumor deposition and has favorable kidney dosimetry.
- Based on this translational evaluation, differences in preclinical scaled dosimetry for radiolabeled miniproteins appear useful for comparing compounds and predicting therapeutic indices in patients.
- Further studies with larger patient cohorts are essential to validate dosimetry findings and establish best practices for dosimetry and treatment protocols that delicately balance safety and efficacy in patients.
- [^{225}Ac]Ac-AKY-1189 will be evaluated as a therapeutic option for patients with metastatic urothelial cancer, as well as other Nectin-4-expressing tumors, in studies in South Africa and the US.

REFERENCE

1. Singer MA, Morton AR. (2000) Mouse to elephant: Biological scaling and Kt/v. *AJKD*. 35(2):P306-309.