^{225}Ac-FL-020 is a novel PSMA-targeting radionuclide drug conjugate (RDC) with superior *in vivo* anti-tumor activity

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Introduction

Despite significant developments over the last few decades, metastatic castration-resistant prostate cancer (mCRPC) remains incurable. Prostate specific membrane antigen (PSMA), directly correlates with androgen independence, metastasis, and disease progression. PSMA has been well-established as a radioligand target for the diagnosis and treatment of mCRPC[1]. Lutetium (177Lu) vipivotide tetraxetan (Pluvicto®) was approved in 2022 for the treatment of progressive PSMA-positive mCRPC[2]. However, only 30% of patients showed a radiological response in the registrational trial together with a grade1/2 xerostomia in around 39% of patients[3]. These data request for further improvement in the clinical benefit and toxicity profile of PSMA-targeted radiotherapy. Actinium-225 (^{225}Ac), an alpha emitter, demonstrated potent cancer cell killing and a shorter range in tissue penetration when compared to the beta emitter ^{177}Lu[4, 5]. This profile supports the development of ^{225}Ac based radiotherapies. Using our proprietary Clear-X™ technology platform, we developed ^{225}Ac-FL-020, a novel ^{225}Ac-based PSMA radioligand therapy candidate.

Methods and Materials

- Binding affinity of FL-020 was determined by competing with Cy5-labeled PSMA ligand on LNCaP cells.
- Selectivity of FL-020 was evaluated by Safety Panel screening.
- Internalization in LNCaP cells was measured by ^{177}Lu-labeled FL-020 on LNCaP cells at various time points.
- *In vivo* biodistribution was assessed by SPECT/CT of ^{111}In-labeled FL-020 and *ex vivo* cut-and-count of ^{177}Lu-labeled FL-020 on LNCaP xenograft model at different time points.
- *In vivo* efficacy was determined with ^{225}Ac-FL-020 in comparison with ^{225}Ac-PSMA-617 in LNCaP tumor-bearing nude mice.
- Safety profile of ^{225}Ac-FL-020 was evaluated by the measurements of body weight and hematology parameters in LNCaP tumor-bearing nude mice.
- Mechanism of action was determined by quantitative image analysis of γH2AX and cCASP3 in tumor tissues following drug treatments.

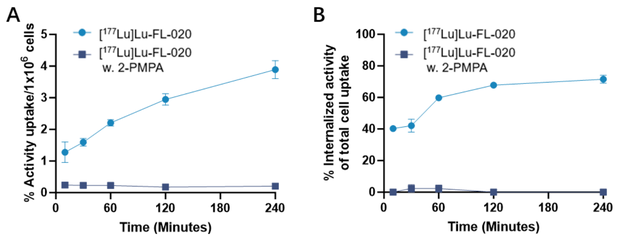
Results

Table 1. Binding affinity of FL-020 in LNCaP cells

Compound	IC_{50} nM
FL-020	51.6*

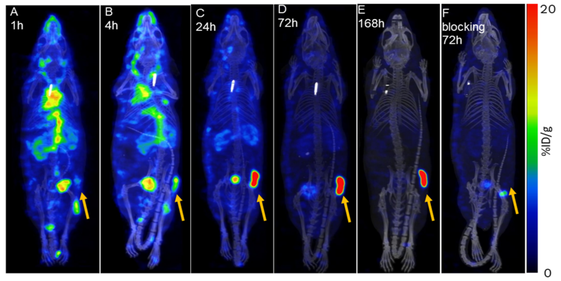
- *Mean of two independent experiments.
- Less than 50% inhibition of binding or activity was observed by FL-020 at 10 µM against 85 targets including receptors, ion channels, enzymes, and transporters.

Figure 1. Cell uptake and internalization in LNCaP cells



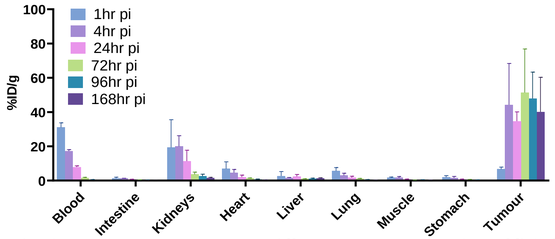
- Percent of total cell uptake (A) and internalized activity of total cell uptake (B) in LNCaP cells treated with [^{177}Lu]Lu-FL-020 alone or in combination with the PSMA inhibitor 2-PMPA. Data are presented as mean of three independent experiments ± SEM

Figure 2. *In vivo* SPECT/CT Imaging with [^{111}In]In-FL-020



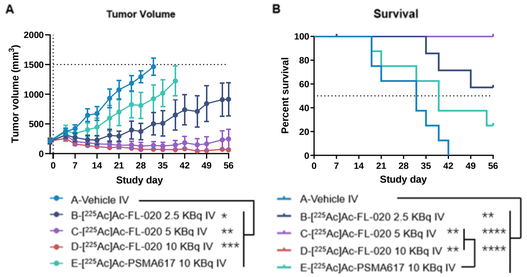
- Representative SPECT/CT image (maximum intensity projection) after [^{111}In]In-FL-020 administration in LNCaP xenograft model at 1h (A), 4h (B), 24h (C), 72h (D), 168h (E), and 72h (H) with blocking post dosing. The tumor is indicated with an arrow.

Figure 3. Biodistribution of [^{177}Lu]Lu-FL-020 in LNCaP model



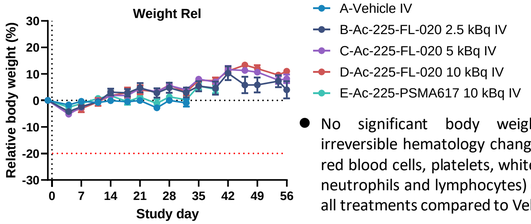
- Biodistribution data in major organs at different time points post dose of [^{177}Lu]Lu-FL-020 in LNCaP. Data are represented as mean ± SD (N=3~4).

Figure 4. *In vivo* efficacy of [^{225}Ac]Ac-FL-020 in LNCaP xenograft model



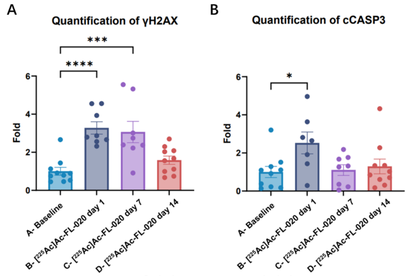
- (A) Mean tumor growth after therapy initiation. Statistical analysis on tumor volume at day 18 was conducted by one-way ANOVA with Tukey's multiple comparison test. *p<0.05, **p<0.01 and ***p<0.001. (B) Survival curves for each efficacy group. Statistical analysis on median survivals was conducted by Log-rank, Mantel-Cox analysis without correction for multiple comparison on the pair-wise comparisons. N=8. **p<0.01 and ****p<0.0001.

Figure 5. *In vivo* safety of [^{225}Ac]Ac-FL-020 in LNCaP xenograft model



- No significant body weight loss or irreversible hematology changes (including red blood cells, platelets, white blood cells, neutrophils and lymphocytes) observed for all treatments compared to Vehicle group.

Figure 6. DNA damage and cell apoptosis induced by [^{225}Ac]Ac-FL-020



- Quantitative image analysis of (A) γH2AX and (B) cCASP3. Immunoreactivity in comparison with Baseline (one-way ANOVA and Dunnett). *p<0.05, ***p<0.001, ****p<0.0001.

Conclusion

- FL-020 selectively bound to PSMA with an IC_{50} value of 51.6 nM.
- FL-020 could be internalized into LNCaP cells in time- and PSMA-dependent manners.
- ^{111}In-FL-020 and ^{177}Lu-FL-020 displayed a very promising *in vivo* distribution profile with high and sustained tumor uptake and fast systemic clearance.
- ^{225}Ac-FL-020 exhibited superior anti-tumor activity compared to ^{225}Ac-PSMA-617 at the same dose level (10 KBq/mouse) in the LNCaP xenograft model with a favorable safety profile as indicated by body weight and hematological parameters.
- ^{225}Ac-FL-020-treated LNCaP tumor samples where DNA double-strand breaks and tumor cell apoptosis were observed, confirming the MOA of alpha emitters.
- Taken together, these results collectively demonstrate that ^{225}Ac-FL-020 is a potent and selective PSMA-targeting radioligand therapy candidate with superior anti-tumor activity and a favorable safety profile that will be investigated in a Phase I clinical trial in 2024.

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