First-in-class antibody radioconjugate ATNM-400 exhibits superior anti-tumor activity and overcomes resistance to enzalutamide and 177Lu-PSMA-617 in prostate cancer models

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BACKGROUND

Targeted radiotherapies are revolutionizing cancer treatment, particularly for patients with advanced disease unresponsive to conventional therapies. FDA-approved beta-emitting radiopharmaceuticals, including 177Lu-PSMA-617 (Pluvicto®) for metastatic castration-resistant prostate cancer (mCRPC) and 177Lu-DOTATATE (Lutathera®) for neuroendocrine tumors, have demonstrated meaningful clinical benefit and validated the potential of this modality. However, these agents predominantly target cell surface markers with limited roles in tumor progression. Here, we report the preclinical development of ATNM-400, a novel antibody radioconjugate using Actinium-225 to target a non-PSMA protein that is overexpressed in advanced prostate cancer and functionally implicated in cell survival signaling and resistance mechanisms. Unlike PSMA which serves primarily as a cell surface marker, the target for ATNM-400 contributes directly to disease progression, with expression correlating with shorter time to castration resistance and poorer survival in CRPC patients. Furthermore, target expression is elevated in patients who develop resistance to the androgen receptor inhibitor enzalutamide (Xtandi®), underscoring its role in therapy resistance. By directly engaging a disease-driving mechanism, ATNM-400 has the potential to overcome current therapeutic limitations and improve outcomes beyond what is achievable with Xtandi® or Pluvicto®. In this study, we evaluated the anti-tumor efficacy of ATNM-400 in preclinical prostate cancer models, benchmarking its activity directly against both Xtandi® and Pluvicto®.

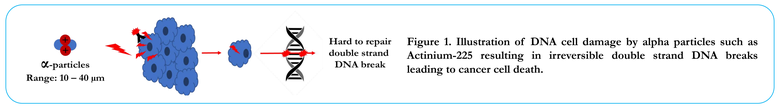
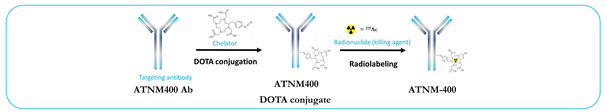


Figure 1. Illustration of DNA cell damage by alpha particles such as Actinium-225 resulting in irreversible double strand DNA breaks leading to cancer cell death.

METHODS

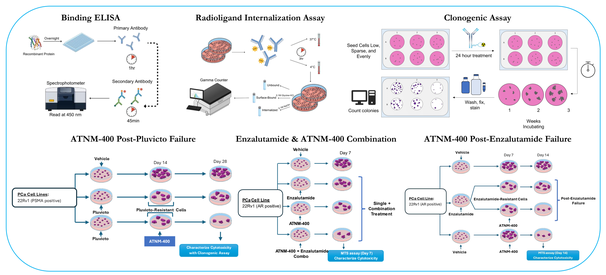
Radiochemistry

- Radiolabeling
- Characterization



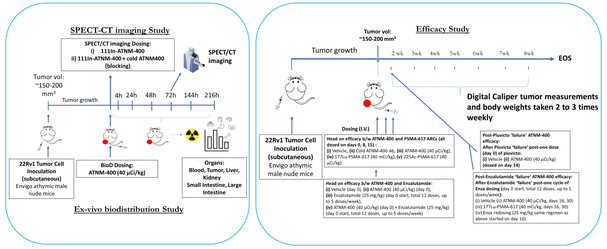
In Vitro

- Target Binding
- Internalization
- Clonogenicity



In Vivo

- Imaging
- Biodistribution
- Dosimetry
- Efficacy



RESULTS

ATNM-400 Binds, Internalizes and Causes Cytotoxicity in Human Prostate Cancer Cells

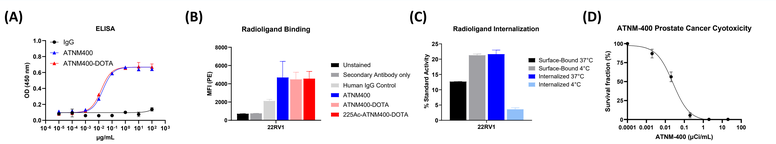


Figure 2. In vitro preclinical evaluation of ATNM-400 in prostate cancer cell line (22Rv1). (A) ATNM-400 specifically bind to the recombinant human target receptor protein by ELISA (EC50 = 0.020 µg/mL and 0.015 µg/mL for ATNM-400 and ATNM-400-DOTA, respectively). (B & C) The ability of [111In]-radiolabeled ATNM-400 to bind to the target receptor protein and to internalize in 22RV1 cells in vitro was quantified in a radioligand internalization assay. (D) ATNM-400 cell killing efficacy shown by clonogenic survival of 22Rv1 cells treated with increasing concentrations of ATNM-400 for 3 hours. All graphs were plotted using GraphPad Prism 10.4.1.

ATNM-400 Exhibits Tumor Uptake and Clearance from Essential Normal Organs

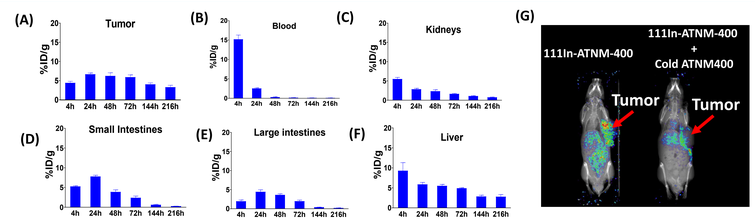
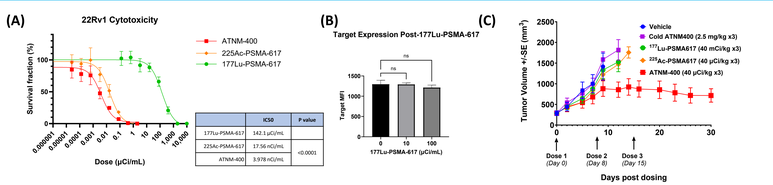


Figure 3: The biodistribution of ATNM-400 was assessed in male athymic nude mice bearing established subcutaneous huPCa 22Rv1 tumor xenografts. Mice were enrolled when tumor size reached ~150-200 mm³ and administered intravenously with ATNM-400 (n = 4), as described in the methods. Tumors and tissues were harvested, weighted and measured for radioactivity at 4, 24, 48, 72, 144 and 216 h after injection, as described. The percentage of injected dose normalized to the mass of the tissue was calculated as (%ID/g) and plotted as %ID/g ± SEM using GraphPad Prism 10.4.1. (A) Consistent uptake of ATNM-400 in the tumor up to 216 h. (B) Rapid clearance of ATNM-400 from the blood by 48 h. From the (C) kidneys, (D) small intestines, and (E) large intestines by 144 h. (F) There was gradual decline of ATNM-400 uptake in the liver till 216 h. (G) Representative SPECT/CT images of 111In-ATNM-400 (left panel) and 111In-ATNM-400 + ATNM-400 (blocking) (right panel) in mice bearing 22Rv1 72 h post injection showing tumor uptake.

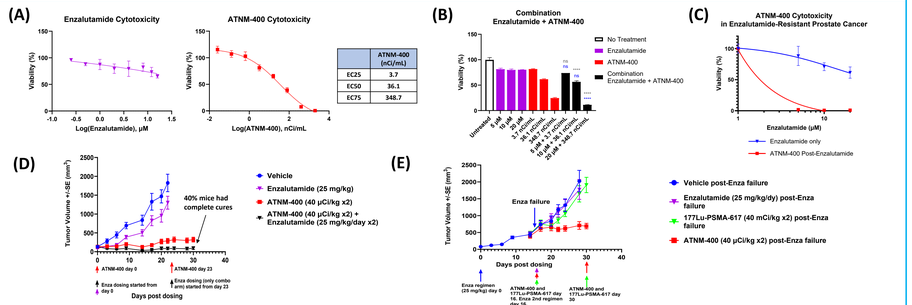
ATNM-400 is More Efficacious than 177Lu-PSMA-617 and 225Ac-PSMA-617



	IC50	P value
177Lu-PSMA-617	142.1 µCi/mL	
225Ac-PSMA-617	17.56 nCi/mL	<0.0001
ATNM-400	3.978 nCi/mL	

Figure 4. ATNM-400 is more efficacious than both 177Lu-labeled and 225Ac-labeled PSMA-617. (A) Survival fraction of 22Rv1 colonies was significantly reduced by ATNM-400, in comparison to 225Ac-PSMA-617 and 177Lu-PSMA-617. (B) Surface expression of the ATNM-400 target was not altered post-177Lu-PSMA-617, ns = not significant. (C) Three doses of ATNM-400 (40 µCi/kg/dose) or 177Lu-PSMA-617 (40 mCi/kg/dose) or 225Ac-PSMA-617 (40 µCi/kg/dose) or Cold ATNM-400 Ab (2.5 mg/kg/dose) or Vehicle (n=6 mice) were administered intravenously as indicated. ATNM-400 was more efficacious than 177Lu-PSMA-617 or 225Ac-PSMA-617 in controlling 22Rv1 PCa tumor growth. All graphs were plotted using GraphPad Prism 10.4.1.

ATNM-400 is Efficacious in Enzalutamide Resistant Prostate Cancer and has Combination Activity



	ATNM-400 (nCi/mL)
EC25	3.7
EC50	36.1
EC75	348.7

Figure 5. ATNM-400 is highly efficacious in enzalutamide (Enza) resistant prostate cancer models. (A) 22Rv1 prostate cancer cells are Enza resistant but ATNM-400 treatment caused potent dose-dependent cytotoxicity. (B) Combination of Enza with ATNM-400 exhibited significantly enhanced cytotoxicity compared to Enza alone treatment. Multiple t-test statistical analysis comparing combination therapy to each monotherapy (asterisk colors match the compared monotherapy, ns = not significant, ****p<0.0001, error bars represent SD). (C) Enza resistant 22Rv1 cells survived (over 50%) enzalutamide treatment for 7 days. ATNM-400 treatment post-enzalutamide killed all cells that survive enzalutamide. (D) ATNM-400 (40 µCi/kg on days 0, 23 (i.v.), n=7) treatment had superior anti-tumor efficacy compared to Enza treatment (25 mg/kg, day 0 regimen start, total 12 doses, up to 5 doses/week, (p.o.), n=7) in 22Rv1 PCa xenograft model. The combination treatment of ATNM-400 (days 0, 23) and Enza (regimen started on days 0 and 23) (n=7) had improved anti-tumor efficacy compared each monotherapy group. (E) Post-Enza treatment (25 mg/kg/day, day 0 start, regimen of total 12 doses, up to 5 doses/week, (p.o.), n=23) and failure, ATNM-400 (40 µCi/kg on days 16, 30 (i.v.), n=5) treatments had markedly better anti-tumor efficacy when compared to Vehicle treatments (days 0, 23 (i.v.), n=6) or Enza redosing (25 mg/kg, regimen start on day 16 (p.o.), n=6) or 177Lu-PSMA-617 treatments (40 mCi/kg on days 16, 30 (i.v.), n=6). All graphs were plotted using GraphPad Prism 10.4.1.

ATNM-400 is Highly Efficacious after 177Lu-PSMA-617 Resistance in Prostate Cancer Models

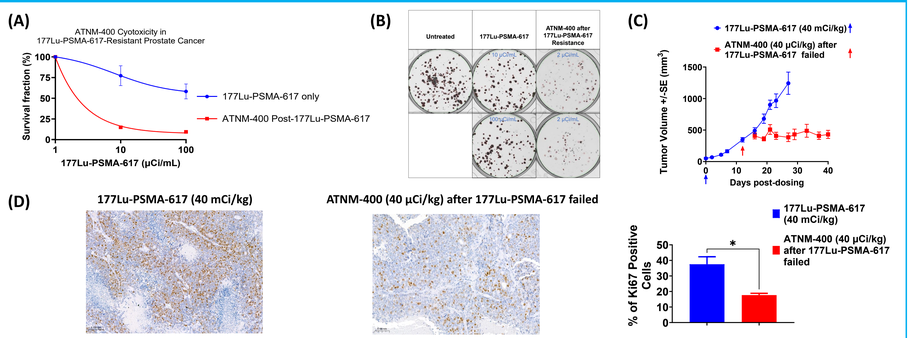


Figure 6. ATNM-400 efficacy after 177Lu-PSMA-617 failure in preclinical models. (A) ATNM-400 inhibits in vitro colony growth of 177Lu-PSMA-617-resistant cells. While 177Lu-PSMA-617 induced some reduction of survival, over 50% of the 22Rv1 cells tolerated 177Lu-PSMA-617 (10 and 100 µCi/mL), retained proliferation functions and grew into healthy colonies. (B) ATNM-400 (2 µCi/mL) induced cell death in the 177Lu-PSMA-617-resistant colonies. (C) Mice bearing post-177Lu-PSMA-617 failed 22Rv1 tumors were administered with single doses of ATNM-400 (40 µCi/kg, i.v.) (n=6) or Vehicle (n=7) on day 14 from 177Lu-PSMA-617 dosing. ATNM-400 was successful in eliciting antitumor activity in 177Lu-PSMA-617 failed tumors. (D) 177Lu-PSMA-617-failed tumors were dosed with ATNM-400 on day 14 post-177Lu-PSMA-617 dosing and were harvested at day 20 post-177Lu-PSMA-617 dosing/day 6 post-ATNM-400 dosing for Ki67 IHC staining. IHC analysis showed significant reduction in the % of Ki67+ proliferating cells from 40 µCi/kg ATNM-400 dosing post-177Lu-PSMA-617 failure (middle panel, right panel) compared to control 177Lu-PSMA-617 failed tumors (left panel, right panel) (*p< 0.05). Statistical analysis was performed using Welch's t test (n=4). IHC images are shown at 10X magnification with a scalebar of 0.1 mm. All graphs were plotted using GraphPad Prism 10.4.1.

CONCLUSIONS

ATNM-400 demonstrated superior and durable anti-tumor activity in preclinical models of prostate cancer, including models resistant to enzalutamide and PSMA-targeted radiotherapies (177Lu-PSMA-617 and 225Ac-PSMA-617). By targeting a disease-driving protein intimately linked to tumor progression and resistance to other targeted therapies, ATNM-400 offers a compelling mechanism-based approach distinct from agents such as Pluvicto® which just targets the cell surface marker PSMA and also agents in development that target tumor microenvironment markers. Our findings support the clinical translation of ATNM-400 as a next-generation Actinium-225 radiotherapy - either as monotherapy or in sequence with existing therapies - to address critical gaps in the prostate cancer treatment landscape.

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