ATNM-400, a first-in-class Actinium-225 antibody radioconjugate, demonstrates potent anti-tumor activity and overcomes resistance to enzalutamide and 177Lu-PSMA-617 in prostate cancer models

Amanda S. Chin, Sumit Mukherjee, Jason Li, Karina Peregrina, Debbie Lewis, Le-Cun Xu, Dhiren Patel, Monideepa Roy, Adeela Kamal

Actinium Pharmaceuticals, Inc. New York, NY USA

BACKGROUND

Prostate cancer (PCa) initially responds to androgen deprivation therapy, but most cases progress to metastatic castration-resistant prostate cancer (mCRPC), a lethal stage with limited options. Standard treatments with androgen receptor (AR) inhibitors such as enzalutamide (Enza) often lead to treatment resistance. Targeted radiotherapies have emerged as promising alternatives. The PSMA-targeted radiotherapy Pluvicto® (Novartis, active agent 177Lu-PSMA-617) is approved for treatment of mCRPC, yet many patients eventually develop resistance or fail to respond. To address these unmet needs, we developed ATNM-400, a first-in-class antibody radioconjugate armed with the alpha-emitter Actinium-225 (225Ac). ATNM-400 targets a non-PSMA antigen overexpressed in PCa that drives tumor survival and resistance pathways. The expression of this target correlates with rapid disease progression, shorter time to castration resistance, and poor survival in mCRPC. We hypothesized that ATNM-400 would provide superior efficacy compared to current therapeutic options and overcome resistance to AR inhibitors (Enza) and PSMA-directed radioligands (177Lu-PSMA-617 or 225Ac-PSMA-617).

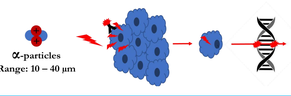
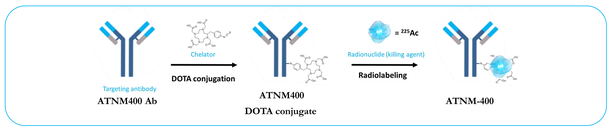


Figure 1. Illustration of DNA cell damage by alpha particles such as Actinium-225 resulting in irreversible double strand DNA breaks leading to cancer cell death.

METHODS

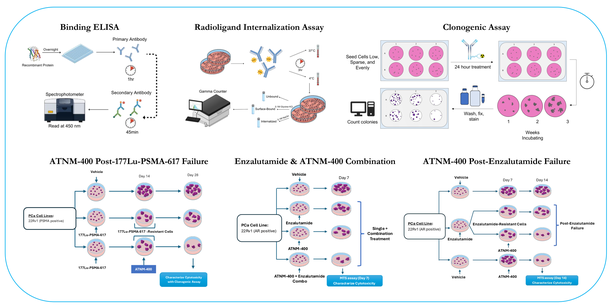
Radiochemistry

- Radiolabeling
- Characterization



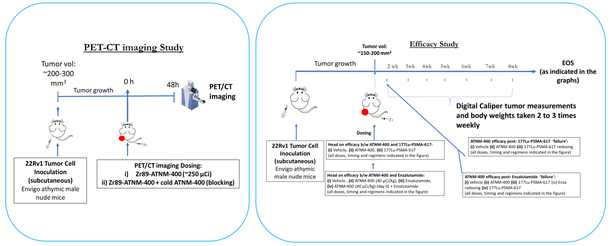
In Vitro

- Target Binding
- Internalization
- Clonogenicity



In Vivo

- Imaging
- Dosimetry
- Efficacy



RESULTS

ATNM-400 Targets and Internalizes in Human Prostate Cancer Cells and Exhibits Tumor Specific Uptake in Prostate Cancer Bearing Animals

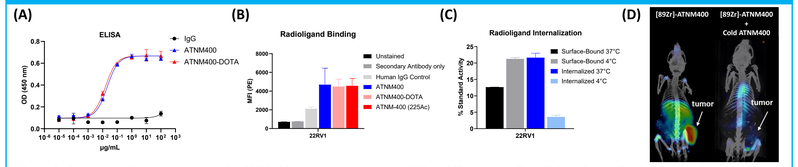


Figure 2. ***In vitro*** **preclinical evaluation of ATNM-400 in a prostate cancer cell line (22Rv1). (A & B) ATNM-400 specifically binds to the recombinant human target receptor protein measured by ELISA (EC50 = 0.020 µg/mL and 0.015 µg/mL for ATNM400 and ATNM400-DOTA, respectively) and in 22Rv1 cells measured by flow cytometry. (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) Representative PET/CT images of [89Zr]-radiolabeled ATNM-400 (left panel) and [89Zr]-radiolabeled ATNM-400 + ATNM-400 (blocking) (right panel) in mice bearing 22Rv1 48 h post injection showing tumor uptake.**

ATNM-400 is Highly Efficacious, Increases Overall Survival in Enzalutamide Resistant Prostate Cancer, and has Synergistic Activity with Enzalutamide

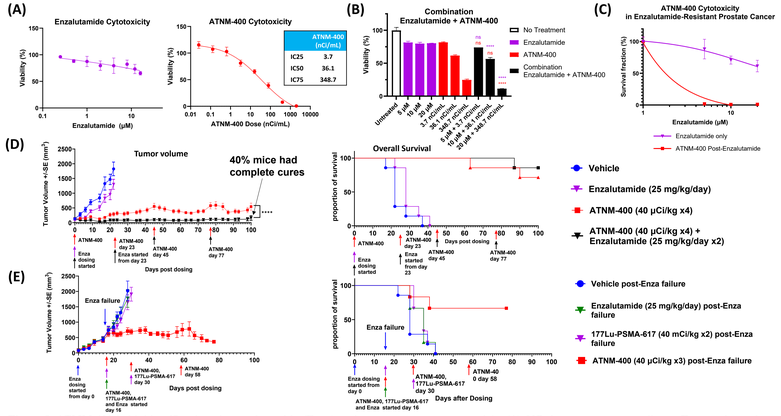


Figure 5. ATNM-400 is highly efficacious in enzalutamide (Enza) resistant prostate cancer models. (A) 22Rv1 prostate cancer cells are Enza resistant but ATNM-400 treatment caused potent dose-dependent cytotoxicity. (B) Combination of Enza with ATNM-400 exhibited significantly enhanced cytotoxicity compared 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) Four doses of ATNM-400 (40 µCi/kg/dose IV) 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 PO) in 22Rv1 PCa xenograft model. The combination treatment of ATNM-400 (4 doses total, 40 µCi/kg/dose) and Enza (two regimens) had synergistic anti-tumor efficacy compared to each monotherapy group (n=7, ****p < 0.0001) (left panel). Long-term durable response was seen in both ATNM-400 alone and combination group with overall survival for up to 100 days (right panel). (E) Post-Enza treatment (previously described dose, n=23) and failure, three doses of ATNM-400 (40 µCi/kg/dose IV, n=5) treatments had markedly better anti-tumor efficacy when compared to Vehicle treatments (n=6) or Enza redosing (previously described dose, n=6) or two doses of 177Lu-PSMA-617 (40 mCi/kg/dose IV, n=6, left panel). Long-term durable response with improved overall survival for up to 77 days was seen in the ATNM-400 dosed arm (right panel).

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

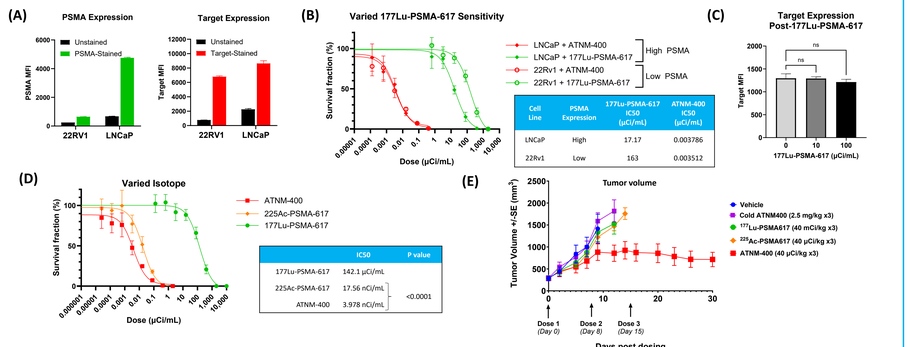


Figure 3. ATNM-400 is more efficacious than 177Lu-PSMA-617 in both sensitive and resistant cell lines, as well as both 177Lu-labeled and 225Ac-labeled PSMA-617. (A) The levels of PSMA and the target of ATNM-400 were characterized in Pca cell lines, LNCaP and 22Rv1. LNCaP cells are PSMA^{high} and 22Rv1 are PSMA^{low}, making them 177Lu-PSMA-617 sensitive and resistant, respectively. (B) PSMA^{high} LNCaP were more responsive to 177Lu-PSMA-617 than 22Rv1 (PSMA^{low}), which was anticipated. Both models responded equally to ATNM-400. (C) Surface expression of the ATNM-400 target was not altered post-177Lu-PSMA-617, ns = not significant. (D) ATNM-400 is more efficacious than 177Lu-PSMA-617 and 225Ac-PSMA-617 suggesting it is not due to the difference in isotopes. (E) 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) were administered intravenously as indicated. ATNM-400 was more efficacious than 177Lu-PSMA-617 or 225Ac-PSMA-617 in controlling 22Rv1 PCa tumor growth.

ATNM-400 is Highly Efficacious and Increases Overall Survival in 177Lu-PSMA-617-Resistant Prostate Cancer

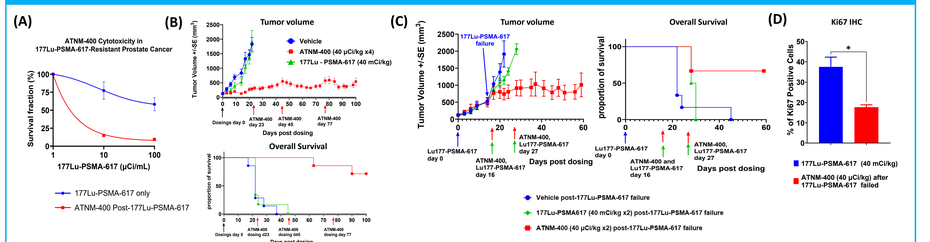


Figure 4. ATNM-400 efficacy after 177Lu-PSMA-617 failure in preclinical models. (A) ATNM-400 inhibits ***in vitro*** **colony growth of 177Lu-PSMA-617-resistant cells. While 177Lu-PSMA-617 induced some reduction of survival, over 50% of the 22Rv1 cells tolerated 177Lu-PSMA-617 (10 and 100 µCi/mL), retained proliferation functions and grew into healthy colonies. ATNM-400 (2 µCi/mL) induced cell death in the 177Lu-PSMA-617-resistant colonies. (B) Four doses of ATNM-400 (40 µCi/kg/dose, n=7) treatment had superior anti-tumor efficacy compared to 177Lu-PSMA-617 (40 mCi/kg, n=7) in 22Rv1 PCa xenograft model (top panel). Long-term durable response with improved overall survival for up to 100 days was also observed in the ATNM-400-dosed arm (bottom panel). (C) Mice bearing post-177Lu-PSMA-617-failed 22Rv1 tumors (n=4-6) were administered with two doses of ATNM-400 (40 µCi/kg/dose) or 177Lu-PSMA-617 (40 mCi/kg) or Vehicle on day 14 from 177Lu-PSMA-617 dosing. ATNM-400 was successful in eliciting antitumor activity in 177Lu-PSMA-617 failed tumors (left panel). Long-term durable response with improved overall survival for up to 60 days was also seen in the ATNM-400 dosed cohort (right panel). (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 for Ki67 IHC staining. IHC analysis showed significant reduction in the % of Ki67 positive proliferating cells from 40 µCi/kg ATNM-400 dosing post-177Lu-PSMA-617 failure compared to control 177Lu-PSMA-617 failed tumors (*p< 0.05). Statistical analysis was performed using Welch's t test (n=4).**

CONCLUSIONS

ATNM-400 showed robust and durable anti-tumor efficacy and a favorable safety profile in preclinical PCa models. These findings support ATNM-400 as a next-generation Actinium-225 therapy - with potential as 1) Monotherapy in CRPC (pre-177Lu-PSMA-617), 2) Combination therapy with AR pathway inhibitors, or 3) Sequential therapy after ARPI or 177Lu-PSMA-617 failure. ATNM-400 overcomes resistance to enzalutamide and PSMA-directed therapies and holds the promise to address critical gaps in mCRPC treatment landscape.

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