ATNM-400 is a novel non-PSMA targeting Actinium-225 Antibody Radioconjugate with Potent Efficacy in Preclinical Models of Prostate Cancer

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Abstract # 578

BACKGROUND

In 2024, an estimated 299,010 new prostate cancer (PCa) cases will be diagnosed in the US, with 35,250 deaths. While PCa initially responds to androgen deprivation therapy (ADT), it often progresses to metastatic castration-resistant prostate cancer (mCRPC), a challenging condition with limited treatment options. Targeted radiotherapies like Pluvicto, a beta-emitting treatment targeting prostate-specific membrane antigen (PSMA), have shown promise but face limitations due to radiation properties of lutetium-177 and variability in PSMA expression. mCRPC is driven by persistent androgen receptor signaling and other pathways, often mediated by overexpressed cell surface receptors. We designed ATNM-400 against a target highly expressed in mCRPC that has been previously targeted with naked antibodies or antibody drug conjugates but have been ineffective clinically. Here, we tested ATNM-400, a first-in-class non-PSMA targeting actinium-225 (Ac-225) antibody radioconjugate (ARC) in preclinical models of huPCa (human prostate cancer) with the hypothesis that targeted delivery of an alpha-emitter would offer stronger therapeutic potential than existing therapies to address a critical unmet need in mCRPC patients.

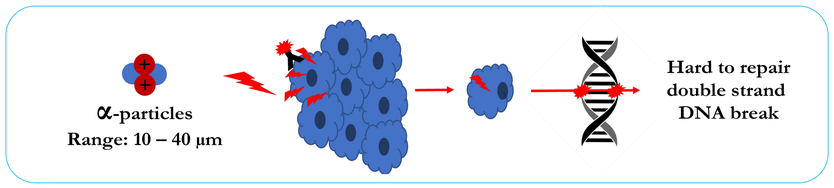
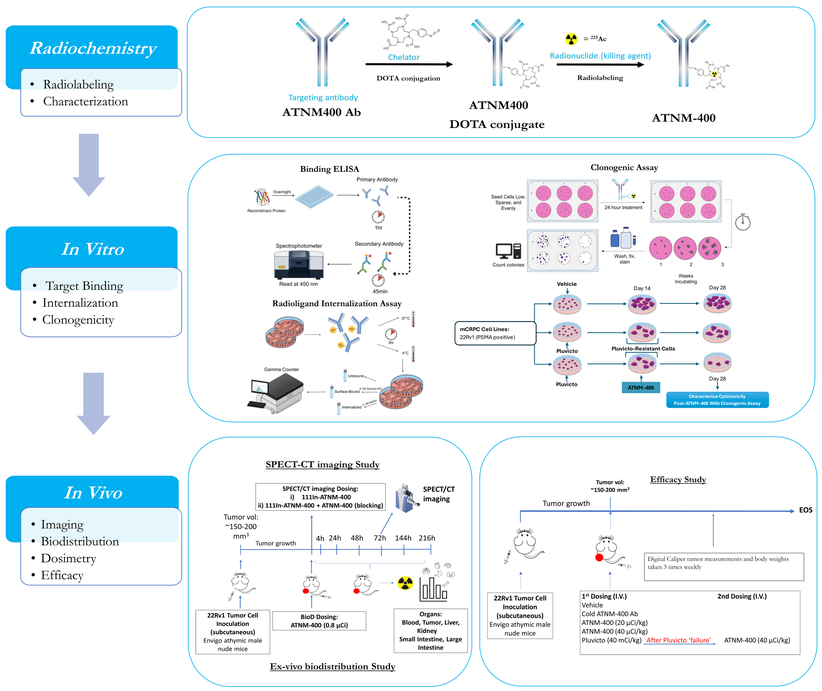


Figure 1. Illustration of DNA cell damage by alpha to cells resulting in irreversible double strand DNA breaks leading to cell death.

METHODS



RESULTS

ATNM-400 Targets and Kills huPCa Cells via Double Strand DNA Breaks

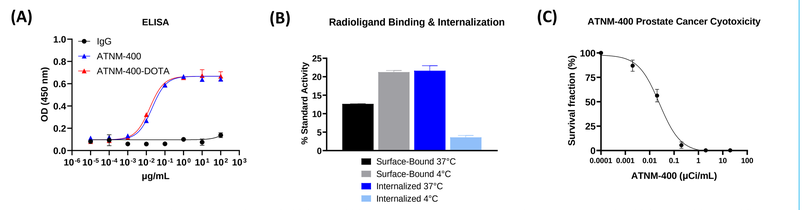


Figure 2. In vitro preclinical evaluation of ATNM-400 in prostate cancer cell line (22Rv1). (A) ATNM-400 specifically bind to the recombinant human target receptor protein by ELISA (EC50 = 0.020 µg/mL and 0.015 µg/mL for ATNM-400 and ATNM-400-DOTA, respectively). (B) The ability of [111In]-radiolabeled ATNM-400 to bind to the target receptor protein and to internalize in 22RV1 cells *in vitro* was quantified in a radioligand internalization assay. (C) ATNM-400 cell killing efficacy shown by clonogenic survival of 22Rv1 cells treated with increasing concentrations of ATNM-400 for 3 hours and allowing for colony growth of 14 days.

ATNM-400 Exhibited Uptake in Tumor and Clearance from Essential Organs

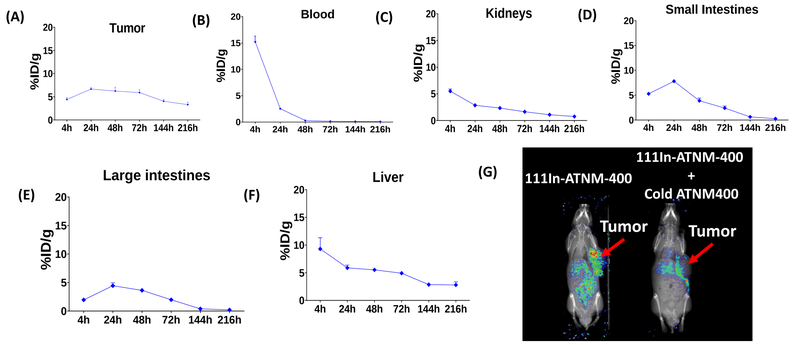


Fig 3: The biodistribution of ATNM400 was assessed in male athymic nude mice bearing established subcutaneous huPCa 22Rv1 tumor xenografts. Mice were enrolled when tumor size reached ~150-200 mm^3 and administered intravenously with ATNM-400 (n = 4 per group), as described in the methods. Tumors and tissues were harvested, weighted and measured for radioactivity at 4, 24, 48, 72, 144 and 216 hours 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 216h. (B) Rapid clearance of ATNM-400 from the blood by 48h. 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 72h post injection showing tumor uptake.

ATNM-400 Inhibits Tumor Growth in a Preclinical Model of Prostate Cancer

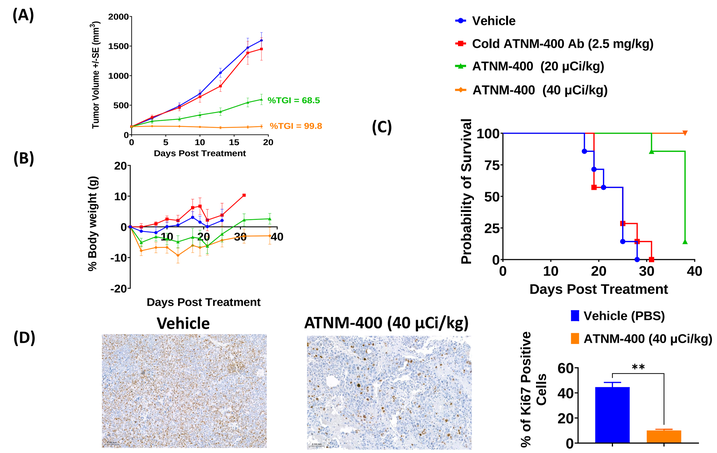


Figure 4. In vivo efficacy study of ATNM-400 in male athymic nude mice bearing huPCa 22Rv1 xenograft tumors. See the methods section for study design. (A) Single doses of ATNM-400 (20 or 40 µCi/kg) or cold ATNM-400 Ab (2.5 mg/kg) or Vehicle (PBS) were administered intravenously on day 0 (n = 7 per group). ATNM-400 at 40 and 20 µCi/kg demonstrated 99.8% and 68.5% tumor growth inhibition (TGI) respectively, compared to Vehicle control, suggesting dose dependent efficacy. (B) % body weight (BW) analysis showed BW recovery, tolerance and no apparent toxicity at both doses. (C) Survival analysis showed extended survival in the 40 µCi/kg-dosed cohorts compared to the 20 µCi/kg group and control groups. (D) Ki67 IHC staining analysis of day 13 post-dosed 22Rv1 tumors showed significant reduction in the % of Ki67+ proliferating cells from 40 µCi/kg ATNM-400 dosing (middle panel, right panel) compared to Vehicle-treated tumors (left panel, right panel) (**p< 0.001). Statistical analysis was performed using Welch's t test (n=4/group). IHC images are shown at 10x magnification with a scalebar of 0.1 mm. All graphs were plotted using GraphPad Prism 10.4.1.

ATNM-400 is More Efficacious than Pluvicto and is Highly Efficacious after Pluvicto Resistance in Preclinical Models of Prostate Cancer

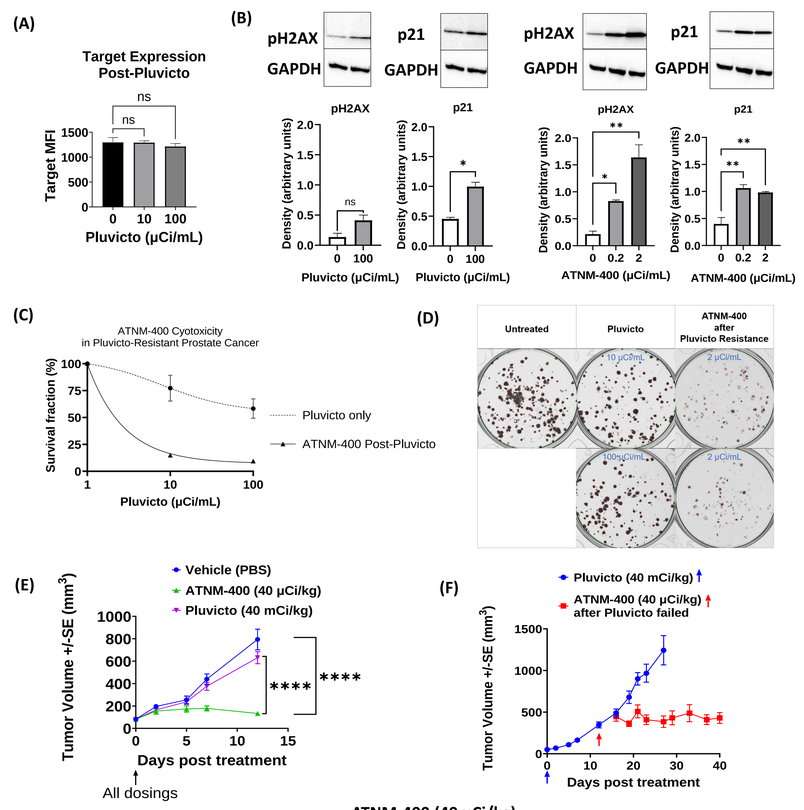


Figure 5. (A) 22Rv1 cells were treated with Pluvicto and the surviving cells were grown for 18 days. Flow cytometry confirmed that the expression of the target receptor on the cells was not altered by the Pluvicto treatment. (B) Mechanism of action for Pluvicto and ATNM-400 were studied with Western Blot. The significant increase in phosphorylation of H2AX is a result of ATNM-400 induced double stranded DNA damage and p21 correlates with the induction of cell growth arrest. (C) ATNM-400 inhibits in vitro tumor growth of Pluvicto-resistant cells. (D) While Pluvicto induced some reduction of survival, over 50% of the 22Rv1 cells tolerated Pluvicto, retained proliferation functions and grew into healthy colonies. ATNM-400 induced cell death in the Pluvicto-resistant colonies. (E) Single doses of ATNM-400 (40 µCi/kg, n=6 mice) or Pluvicto (40 mCi/kg, n=38 mice) or Vehicle (n=6 mice) were administered intravenously on day 0. ATNM-400 was significantly more efficacious than Pluvicto or Vehicle in controlling 22Rv1 PCa tumor growth ((****p < 0.0001) for ATNM-400 vs. Pluvicto or Vehicle). (F) Mice bearing post-Pluvicto failed 22Rv1 tumors were administered intravenously with single doses of ATNM-400 (40 µCi/kg) or Vehicle on day 14 from Pluvicto dosing (n=7 mice for both groups). ATNM-400 was successful in eliciting antitumor activity in Pluvicto failed tumors. Tumor volume statistical analysis was performed using two-way ANOVA. (G) Pluvicto failed tumors were dosed with ATNM-400 on day 14 post-Pluvicto dosing and were harvested at day 20 post-Pluvicto 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-Pluvicto failure (middle panel, right panel) compared to control Pluvicto failed tumors (left panel, right panel) (*p< 0.05). Statistical analysis was performed using Welch's t test (n=4/group). 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

In this study, ATNM-400 demonstrated significant antitumor activity in a dose-dependent manner and showed a favorable safety profile in a PCa tumor xenograft model. Additionally, ATNM-400 was superior to Pluvicto in anti-PCa activity and was efficacious after Pluvicto failure. These results highlight ATNM-400's potential as a transformative therapeutic option for PCa patients with unmet clinical needs.

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