ATNM-400, a first-in-class Actinium-225 antibody radioconjugate, has superior anti-tumor activity compared to approved drugs (Osimertinib, Dato-DXd, and Amivantamab) in EGFR-mutant lung cancer preclinical models

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BACKGROUND

Non-small cell lung cancer (NSCLC) is the most prevalent type of lung cancer, comprising of about 85% of all lung cancer cases. EGFR (Epidermal Growth Factor Receptor) mutant NSCLC is detected in about 10-15% of NSCLC cases in Western countries and up to 50% in some Asian populations. EGFR tyrosine kinase inhibitors (TKIs) standard-of-care, such as Osimertinib (Osi) and other drugs such as Dato-DXd (TROP2-ADC) and Amivantamab (EGFR-cMET bispecific) have revolutionized treatment for advanced EGFR-mutated NSCLC, but acquired resistance eventually develops, requiring innovative strategies to overcome resistance. Here, we report on the development of ATNM-400, a novel antibody radioconjugate using Actinium-225 (Ac-225). ATNM-400 targets an antigen that is associated with poor prognosis in NSCLC. The target is overexpressed in lung cancer and is linked to Osi resistance. Furthermore, clinical studies have previously shown improved progression-free survival by combining Osi with external beam radiotherapy. Thus, we evaluated the anti-tumor efficacy of the targeted radiotherapy ATNM-400 in EGFR-mutant NSCLC preclinical models versus Osi, Dato-DxD and Amivantamab to overcome current therapeutic limitations.

METHODS

Figure 1. Schematic representation of the methodology used to prepare the antibody radioconjugates and to evaluate the preclinical properties of ATNM-400 *in vitro* and *in vivo*.

RESULTS

ATNM-400 Binds, Internalizes, and Exhibits Potent Cytotoxicity in Human Lung Cancer Cells

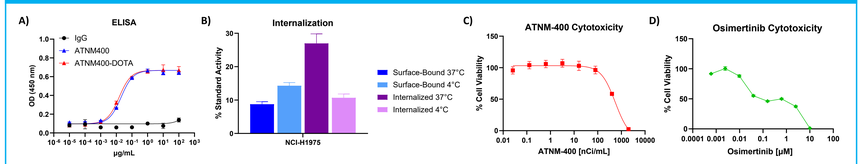

Figure 2. *In vitro* preclinical evaluation of ATNM-400 in lung cancer cell line, NCI-H1975 that harbors EGFR mutations in L858R and T790M. (A) ATNM-400 specifically binds to the recombinant human target receptor protein quantified by ELISA (EC50 = 0.020 µg/mL and 0.015 µg/mL for ATNM-400 and ATNM-400-DOTA, respectively). (B) The ability of [111In]-radiolabeled ATNM-400 to bind to the target receptor protein and to internalize in NCI-H1975 cells *in vitro* was quantified in a radioligand internalization assay. (C) Dose-dependent cytotoxicity in NCI-H1975 cells with ATNM-400 treatment was demonstrated using an MTS Cytotoxicity Assay. ATNM-400 exhibits dose-dependent cytotoxicity in NCI-H1975 cells. (D) The Osimertinib dose response curve was generated using an MTS Cytotoxicity Assay.

EGFR Inhibitor Osimertinib Triggers Increased Target Expression in EGFR-Mutant NCI-H1975 Lung Cancer Cells which Enhances the Cytotoxic Potency of ATNM-400

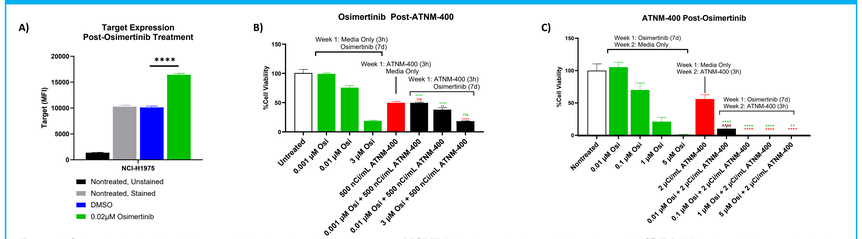

Figure 3. Osimertinib significantly increased the target protein expression in NCI-H1975 cells and enhanced the potency of ATNM-400 treatment when applied after Osimertinib, but not when the regimen order is reversed. (A) Osimertinib treatment for 7 days significantly increased target expression in NCI-H1975, as quantified in a flow cytometry binding assay. (B) Sequential treatment of ATNM-400 followed by Osimertinib does not significantly alter ATNM-400 potency. (C) Increased target expression post-Osimertinib treatment significantly augmented susceptibility to ATNM-400 cytotoxicity. Multiple t-test statistical analysis comparing combination therapy to each monotherapy (asterisk colors match the compared monotherapy, ns = not significant, **p<0.01, ****p<0.0001, error bars represent SD).

ATNM-400 Has Sustained Tumor Uptake and Rapid Clearance from Normal Organs in Lung Cancer Model

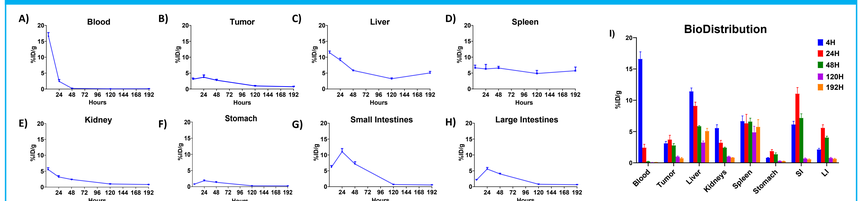

Figure 4. Biodistribution of ATNM-400 was assessed in BALB/c nude female mice bearing established subcutaneous NCI-H1975 tumor xenografts. Mice were enrolled when tumor size reached ~200 mm^3 and administered intravenously with ATNM-400 (n = 4). Tumors and tissues were harvested, weighed and measured for radioactivity at 4, 24, 48, 120 and 192 h after injection. (A) Rapid clearance from blood by 48 h (B) Consistent uptake of ATNM-400 in the tumor up to 48 h. There was gradual decline of ATNM-400 uptake in the (C) Liver and (D) Spleen. There was rapid clearance of ATNM-400 in the (E) Kidneys, (F) Stomach, (G) Small intestines, and (H) Large intestines by 120 h. (I) The percentage of injected dose normalized to mass of tissue was calculated as (%ID/g) and plotted as %ID/g ± SEM.

ATNM-400 Exhibits Potent Efficacy as Monotherapy and has Superior Synergism in Combination with Osimertinib in EGFR-mutant Lung Cancer NCI-H1975 Model

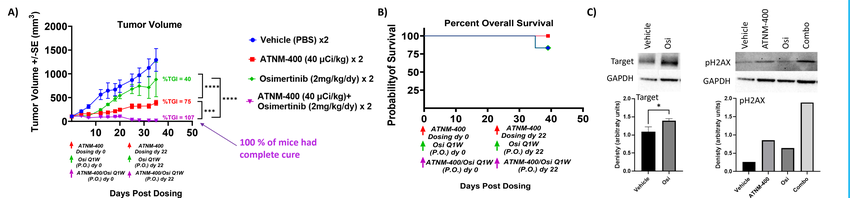

Figure 5. *In vivo* efficacy study of ATNM-400 in BALB/c nude female mice bearing human lung cancer NCI-H1975 (harbors L858R and T790M EGFR mutations) xenograft tumors. (A) Two doses of ATNM-400 (40 µCi/kg) or two cycles of osimertinib (Osi) or ATNM-400 (x2)+Osi (x2) combination or Vehicle (PBS) were administered on day 0 and 22 (n = 6-7 per group). At day 35 from first dosing, ATNM-400 and Osi monotherapy demonstrated tumor growth inhibition (TGI) of 75% and 40%, respectively. ATNM-400+Osi combination showed a TGI of 107% compared to Vehicle and showed complete tumor regression, suggesting strong synergism. (B) Survival analysis showed the treatments were well tolerated with no apparent toxicity. Error bars represent ± SEM. (****p < 0.0001, ***p < 0.001). (C) Western blot analysis of tumor lysates demonstrated Osimertinib treatment significantly increase in the target protein expression (*p < 0.05, n=4). Increase in phosphorylation of H2AX (pH2AX) indicates double strand DNA damage by ATNM-400, and this effect was enhanced in the combination of Osimertinib and ATNM-400.

ATNM-400 Exhibits Superior Efficacy as Monotherapy in Comparison to approved drugs Osimertinib (1L), Dato-DXd (2L), and Amivantamab (3L) in EGFR-mutant Lung Cancer NCI-H1975 Model

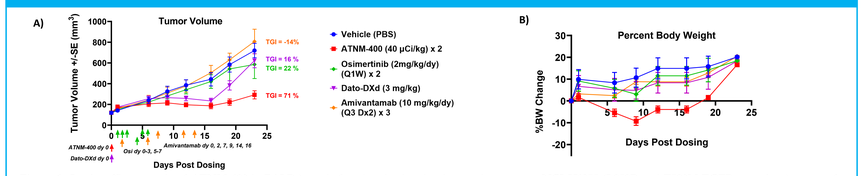

Figure 6. *In vivo* efficacy study of ATNM-400 in BALB/c nude female mice bearing human lung cancer NCI-H1975 (L858R and T790M EGFR mutations) xenograft tumors. (A) One dose of ATNM-400 (40 µCi/kg) or Osimertinib (2 mg/kg/day), or Amivantamab (10 mg/kg/day) or Vehicle (PBS) were administered on day 0 (n = 7 per group). At day 23 from first dosing, ATNM-400 monotherapy demonstrated tumor growth inhibition (TGI) of 71% and Osi next best with 22%. (B) Percent body weight (%BW) analysis showed BW recovery and treatments were well tolerated with no apparent toxicity. Survival was monitored and remained 100% at this timepoint, consistent with ongoing study timeline. Error bars represent ± SEM.

ATNM-400 also Exhibits Robust Efficacy in EGFR-wt, K-Ras-mutant Lung Cancer Calu-3 Model

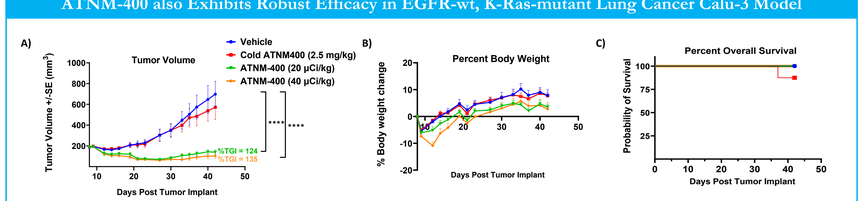

Figure 7. *In vivo* efficacy study of ATNM-400 in female athymic nude mice bearing human lung cancer Calu-3 (amplified HER2, wild-type EGFR, mutant K-Ras (G13D), and TP53 and CDKN2A mutations) xenograft tumors. (A) Single doses of ATNM-400 (20 or 40 µCi/kg), cold ATNM400 Ab (2.5 mg/kg), or Vehicle (PBS) were administered intravenously on day 0 (n = 8 per group). ATNM-400 at 20 and 40 µCi/kg demonstrated 124% and 135% tumor growth inhibition (TGI), respectively, compared to Vehicle. (B) Percent body weight (BW) analysis showed BW recovery, tolerance and no apparent toxicity with both doses. (C) Survival analysis. Error bars represent ± SEM. (****p < 0.0001).

CONCLUSIONS

ATNM-400 shows strong anti-tumor efficacy and favorable tolerability across multiple lung cancer subtypes, including Osimertinib resistant models, and is also superior to both Dato-DXd (Trop2-ADC) and Amivantamab (EGFR-cMET bispecific). The synergy of Osimertinib with targeted radiotherapy ATNM-400 is in accordance with previous clinical data showing improved progression-free-survival when Osimertinib was combined with external beam radiation. Furthermore, the findings here support the continued development of ATNM-400 as a novel therapeutic approach for lung cancer patients with high unmet needs following TKI therapy failure, both as monotherapy, in rational combination regimens or post-resistance to TKIs.

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