Tissue-Targeted Lipid Nanoparticle Delivery for mRNA Encoding Bispecific T-cell Engager Demonstrated Potent Antitumor Effects on Both Hematological Malignancies and Solid Tumors

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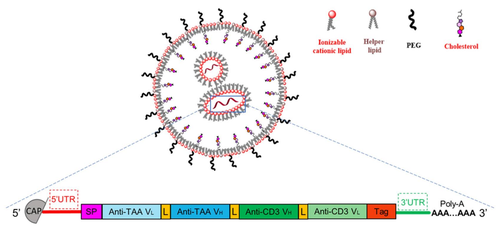
Abstract

The recent success of LNP-mRNA COVID vaccines has accelerated the development of lipid nanoparticles (LNP) as an attractive delivery approach for next wave of genomic medicines. Bispecific T cell engager (BiTE) therapy is a clinically approved immunotherapy to treat hematological malignancies, however, challenges in production, manufacturing, short serum half-life, and reported CRS and neurotoxicity of recombinant BiTEs have limited their applications. At Hopewell therapeutic, we formulated mRNA encoding BiTE with tissue-targeted Lipid Nanoparticle (ttLNP) to address current challenges of recombinant BiTEs and enable a new generation T-cell redirect therapy.

Hopewell's proprietary ionizable lipid HTX-L01 formulated LNP-mRNA primarily targets liver, spleen, and less extent BM via intravenous injection, with high reporter protein expression in hepatocytes, Kupffer cells and immune cells. HTX-L01-003 and HTX-L01-008 encapsulated with mRNA encoding optimized single chain sequence of CD19-CD3 and GPC3-CD3 produce extended therapeutic levels of BiTE protein at the targeted tumor sites. The results from multiple preclinical studies have demonstrated systemic administration of ttLNP-BiTE mRNA induce the activation of T cells to eliminate tumor cells in both hematological malignancy and solid tumor mouse models as well as deplete circulating B-cell in a NHP model.

ttLNP-BiTE mRNA Design



- Compartmentalized protein expression provides highly enriched T-cell recruitment in targeted tissue and minimizes systematic toxicity and adverse immune reaction
- High and flexible payload capacity for simple multiplexing and combination
- Simple and cost-effective manufacturing process for rapid novel target R&D

Figure 1. Tissue Targeted LNP-BiTE mRNA has Multiple Advantages over Conventional Antibody Therapy.

Desired Attributes of Ionizable Lipid HTX-L01 for LNP-mRNA Therapy

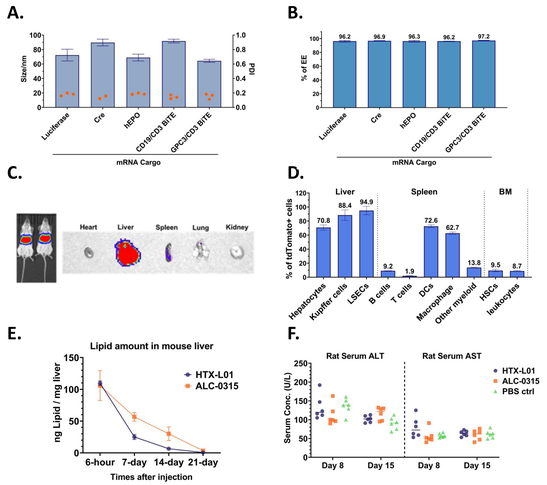


Figure 2. Ionizable lipid HTX-L01 has desired attributes for LNP-mRNA. HTX-L01 formulated with different mRNA cargoes shows: (A) particle size<100nM, PDI<0.2; (b) encapsulation efficiency >90% (C) IVIS images showed HTX-L01 targeting primarily liver and less spleen. (D) High level transfection efficiency of Cre mRNA in hepatocytes, Kupffer cells and immune cells isolated from liver, spleen and bone marrow. 7 days post HTX-L01-Cre mRNA injection in Ai9 mouse (E) Rapid clearance of HTX-L01 in liver by LC-MS after IV injection of 0.5mg/kg LNP-hEPO mRNA in CD1 mice. (F) Normal serum concentration of liver enzyme ALT and AST in Sprague Dawley Rats following 5 doses of 0.1 mg/kg HTX-L01-hEPO mRNA twice per week, IV injection.

HTX-L01-003 Induces High CD19/CD3 BiTE Expression in Mice

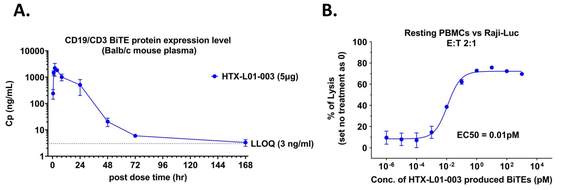


Figure 3. HTX-L01-003 shows high protein expression with robust bioactivities against CD19+ hematological malignancies. (A) *In vivo* production of CD19/CD3 BiTEs was achieved via single dose of 5μg HTX-L01-003 IV injection into Balb/c mice (N=5). (B) HTX-L01-003 produced BiTEs show high potent TDCC activity against CD19+ Raji cells.

Anti-tumor Efficacy of HTX-L01-003 (CD19-CD3 BiTE) in Lymphoma Bearing Mice

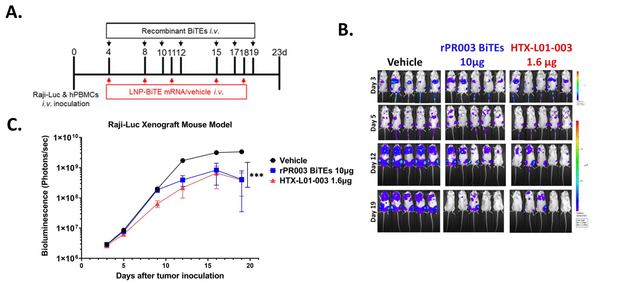


Figure 4. HTX-L01-003 Induces Robust Antitumor Activity in a Raji-luc xenograft mouse model. (A) Raji-Luc lymphoma cells and hPBMCs were IV inoculated into B-NDG mice (N=5) (B) 3/5 animal treated with HTX-L01-003 showed complete remission of Raji tumor flux monitored D19. (C) Lower and less frequent dosing of HTX-L01-003 achieved similar tumor regression or elimination efficacy as recombinant BiTEs.

Deep and Long-last B-cell Depletion Achieved in NHP Treated by HTX-L01-003

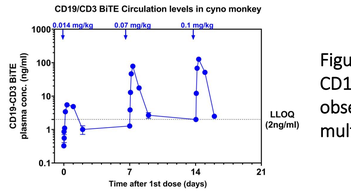


Figure 5. Robust and dose-dependent CD19/CD3 protein expression observed in the NHP treated with multiple-dose of HTX-L01-003

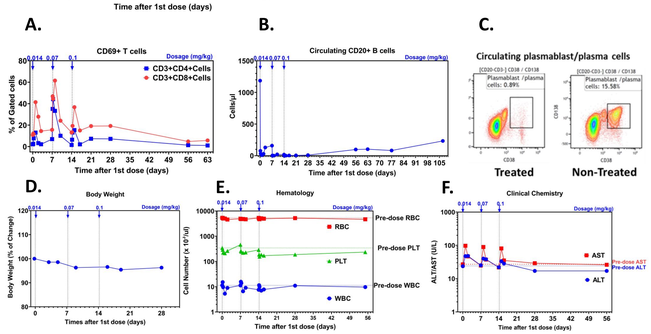


Figure 6. HTX-L01-003 treatment in NHP induced transient T cell activation and sustained B Cell Depletion and were well tolerated (A) Transient activation of CD4+ and CD8+ T-cell; (B) Circulating B-cell in circulation completely depleted with 80% diminished up to 105 days post treatment; (C) Circulating CD19+ late-stage ab-secreting plasmablast/plasma cells depleted as measured on D105; (D) No body weight loss (E) normal hematology (F) and clinical chemistry finding.

HTX-L01-008 Induces High GPC3/CD3 BiTE Expression in Mice

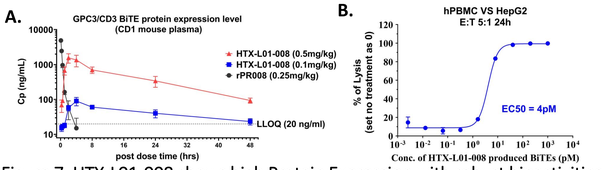


Figure 7. HTX-L01-008 shows high Protein Expression with robust bioactivities against GPC3+ hepatocellular carcinoma. (A) Dose dependent plasma BiTE protein expression following single dose IV injection of HTX-L01-008 in CD1 miice (N=5), comparing with recombinant GPC3/CD3 protein; (B) HTX-L01-008 produced BiTEs show high potent TDCC activity against GPC3+ HepG2 cells.

HTX-L01-008 Eliminates Orthotopic Hepatocellular Carcinomas

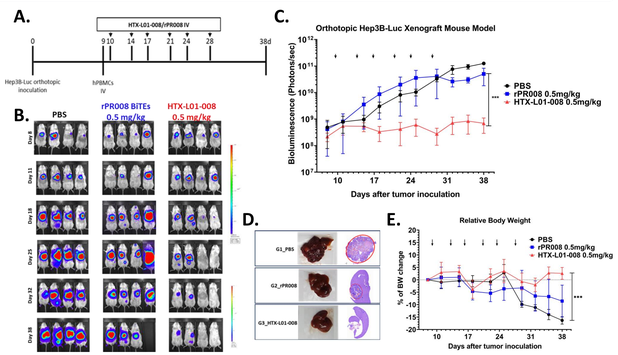


Figure 8. Systemic administration of HTX-L01-008 induced effective in situ HCC cell killing in an orthotopic Hep3B-Luc xenograft mouse model. (A) Hep3B-Luc HCCs cells were orthotopic inoculated into B-NDG B2M KO mice at D0 following by IV injection of hPBMCs at D9. 6 doses, twice a week, IV administration of 0.5 mg/kg HTX-L01-008, or recombinant GPC3/CD3 protein rPR008 started at D10. (B) Animal images at indicated timepoints show HepG2 tumor flux monitored twice weekly. (C) Results of Hep3B HCC burden based on bioluminescence value indicate strong anti-tumor efficacy of HTX-L01-008 in vivo. (D) HCC elimination in situ were demonstrated through liver histopathology at end timepoint (E) No body weight changes in treated group.

Conclusions

- Ionizable lipid HTX-L01 shows great potential for LNP-mRNA therapy.
- Specific tissue targeting profile of HTX-L01-003 resulted in highly potent and long-lasting depletion of target cells, including late-stage ab-secreting plasma cells, in both mouse and NHP studies.
- The HTX-L01-008 study with an orthotopic HCC mouse model demonstrated the targeted production of GPC3/CD3 protein which effectively eliminated solid tumors in situ via IV injection.
- The HTX-L01-mRNAs were well-tolerated in all pre-clinical studies.