POSTER #1093

CLN-617 is a first-in-class fusion protein that retains IL-2 and IL-12 in injected tumors and potently triggers systemic anti-tumor immunity

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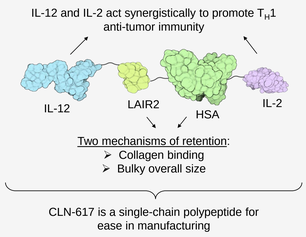
Background

- IL-2 and IL-12 are potent cytokines that can mediate anti-tumor immunity, but their clinical utility has been hindered due to significant toxicity.[1-3]
- IL-2 and IL-12 work synergistically to trigger activation of T cells and NK cells via complementary modes of action.[1-3]
- Anchoring IL-2 or IL-12 to a collagen-binding domain promotes cytokine retention in the tumor and reduces systemic cytokine exposure, thus minimizing toxicity.[4]
- CLN-617 is a fusion protein comprising IL-2, IL-12 and retention domains (Figure 1).
- We have previously shown that intratumoral (IT) delivery of CLN-617 is well tolerated in mice, elicits potent anti-tumor efficacy and extensively remodels the tumor immune microenvironment[5]. Here we further elucidate its mechanism of action.

CLN-617 Rationale

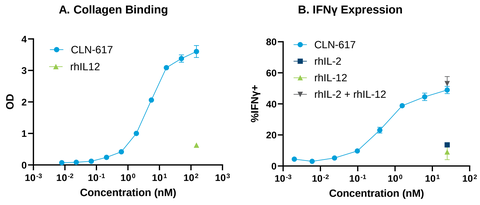
- CLN-617 is designed for intratumoral (IT) delivery and retention of both IL-2 and IL-12 in the tumor microenvironment
- CLN-617 was designed integrating three primary principles:
  1. Cytokines are autocrine/paracrine in nature, not endocrine
     - CLN-617 is designed for IT administration
  2. A protein injected locally will not stay local without retention[4]
     - CLN-617 is designed with two modes of local retention
  3. Natural immune responses trigger a cytokine milieu, and do not rely on an individual cytokine
     - CLN-617 combines IL-2 and IL-12 in a single polypeptide

Figure 1: Schematic of CLN-617 design



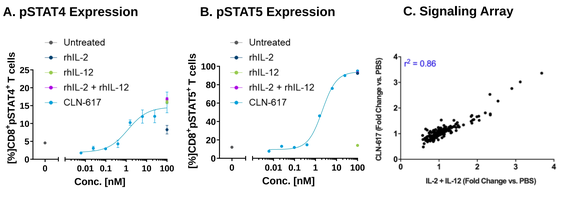
Results

Figure 2: CLN-617 binds to collagen and stimulates IFNγ expression



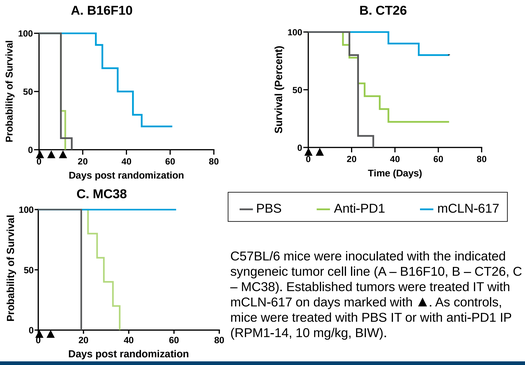
(A) Collagen binding was evaluated by ELISA on collagen I-coated plates using an anti-IL-12 detection antibody. (B) PBMCs were CD3 stimulated and cultured with CLN-617, recombinant IL-2 (rhIL-2), IL-12 (rhIL-12), or a combination of rhIL-2 and rhIL-12 for 48h. IFNγ expression was detected by flow cytometry.

Figure 3: IL-2 and IL-12 activity is maintained on CLN-617



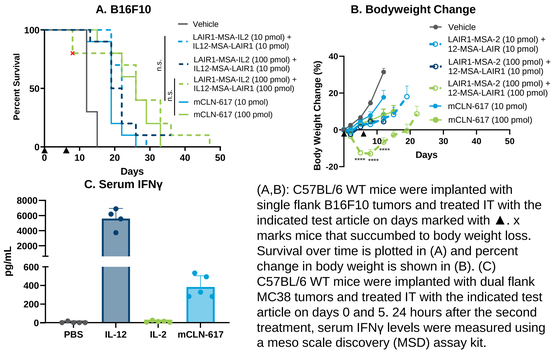
(A) pSTAT4 and (B) pSTAT5 levels assessed in CD8⁺ T-cells by FACS. (C) High correlation between signaling mediated by CLN-617 vs. a combination of recombinant IL-2 and IL-12 as measured in a protein signaling microarray. Human PBMCs were CD3-stimulated overnight and then treated with test article for 10 min (A,B) or 2 hours (C).

Figure 4: mCLN-617 is effective in checkpoint refractory models



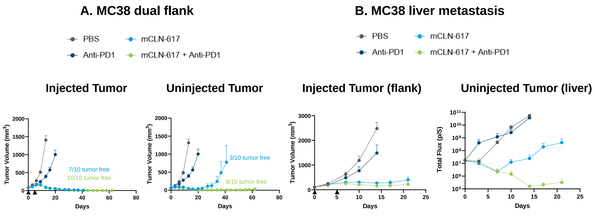
C57BL/6 mice were inoculated with the indicated syngeneic tumor cell line (A – B16F10, B – CT26, C – MC38). Established tumors were treated IT with mCLN-617 on days marked with ▲. As controls, mice were treated with PBS IT or with anti-PD1 IP (RPM1-14, 10 mg/kg, BIW).

Figure 5: mCLN-617 exhibits de-tuned IL-12 activity in vivo resulting in further improved safety



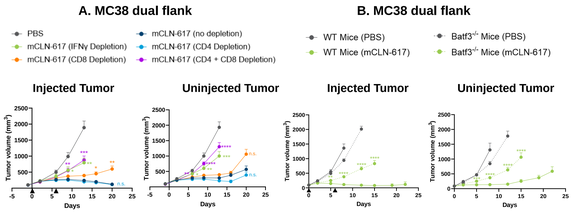
(A,B): C57BL/6 WT mice were implanted with single flank B16F10 tumors and treated IT with the indicated test article on days marked with ▲. x marks mice that succumbed to body weight loss. Survival over time is plotted in (A) and percent change in body weight is shown in (B). (C) C57BL/6 WT mice were implanted with dual flank MC38 tumors and treated IT with the indicated test article on days 0 and 5. 24 hours after the second treatment, serum IFNγ levels were measured using a meso scale discovery (MSD) assay kit.

Figure 6: Local administration triggers systemic immunity and shows synergy in combination with anti-PD1



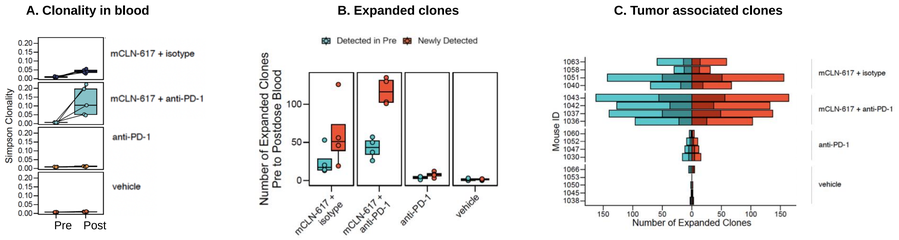
(A) C57BL/6 mice were implanted with two MC38 tumors, only one of which was treated IT with mCLN-617 on days marked with ▲ and the other tumor left untreated. (B) C57BL/6 mice were implanted with one MC38 WT tumor in the flank, and MC38-luciferase (luc) tumor cells intrasplenically, leading to luc+ liver metastases. The flank tumor was treated on days marked with ▲ and liver metastases left untreated. In both studies, anti-PD1 was administered IP (RPM1-14, 10 mg/kg, BIW).

Figure 7: Efficacy of mCLN-617 is dependent on IFNγ, T cells and Batf3⁺ dendritic cells



(A) C57BL/6 WT mice were implanted with two MC38 tumors, only one of which was treated IT with mCLN-617 on days marked with ▲ and the other tumor left untreated. Mice were treated with depletion antibodies BIW, starting 24 hours before the first mCLN-617 treatment and continued for 5 weeks (B) WT or Batf3-/- C57BL/6 mice bearing dual flank MC38 tumors were IT-treated in the right flank with mCLN-617 on days marked with ▲

Figure 8: mCLN-617 mediates clonal expansion of tumor associated T-cells



(A-C) C57BL/6 WT mice were implanted with two MC38 tumors and split into 4 groups as indicated. mCLN-617/vehicle was administered IT while anti-PD1/Isotype was delivered IP. Peripheral blood was collected pre-treatment. Tumors and peripheral blood were collected 24 hours after two test-article treatments. TCRB sequencing was performed on the samples.

Conclusions

- CLN-617 combines IL-2 and IL-12 in a single molecule in a safe and effective manner via tumor retention domains
- CLN-617 induces IFNγ expression in a manner dependent on both IL-2 and IL-12
- mCLN-617 can eradicate large, established primary and distal checkpoint-resistant tumors
- mCLN-617 demonstrates single agent activity and synergizes with anti-PD1 therapy
- mCLN-617 efficacy is partially dependent on T cells, IFNγ and Batf3⁺ dendritic cells
- mCLN-617 drives T cell clonal expansion and clones are shared between mCLN-617 injected and uninjected tumors
- Collectively, the data suggest CLN-617 may be effective for the treatment of solid tumors with minimal toxicities
- CLN-617 is currently being evaluated in a Phase I clinical trial (Trial ID: NCT0603574406; See Poster # 771)

References: **1.** Gollob, JA, et al. *J Immunol*, 1999 **2.** Hanagiri, T, et al. *Cancer Immunol Immunother*, 1996. **3.** Wigginton, JM, et al. *JCI*, 2001. **4.** Momin, N, et al. *STM*, 2019. **5.** Mehta, N, et al. *AACR* 2023.

Acknowledgments: **1.** SBH Biosciences **2.** Charles River Laboratories **3.** Adaptive Biotechnology **4.** Crown Biosciences