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

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Background

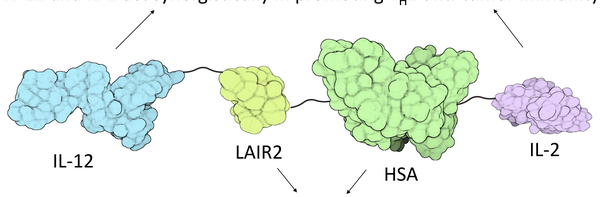
- IL-2 and IL-12 synergistically trigger the stimulation and proliferation of T cells and NK cells to mediate anti-tumor immunity, but have been hindered in the clinic due to significant toxicity[1-3]
- Although aldesleukin, a high-dose IL-2 intravenous (IV) infusion regimen, has been approved for the treatment of melanoma and renal cell carcinoma, adoption in clinical practice has been limited by frequent grade 3 and 4 severe adverse events
- No IL-12 therapy has been approved yet due to toxicity

CLN-617 Rationale

- CLN-617 is designed for intratumoral delivery of both IL-2 and IL-12 and retention in the tumor microenvironment via a LAIR2 collagen-binding domain
- CLN-617 was designed by integrating three primary principles:
  1. Cytokines are autocrine/paracrine in nature, not endocrine
     - ➢ CLN-617 is designed for intratumoral (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

IL-12 and IL-2 act synergistically in promoting T_H1 anti-tumor immunity

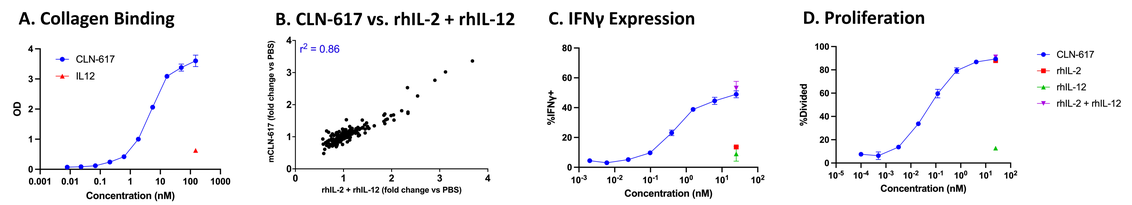


Two mechanisms of retention:
- ➢ By collagen binding
- ➢ By bulky overall size

CLN-617 is a single-chain polypeptide for ease in manufacturing

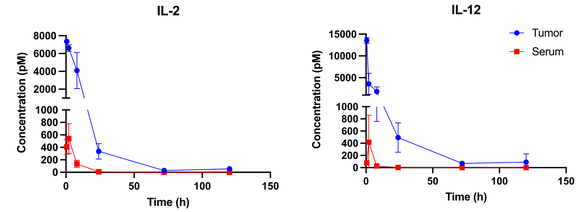
Results

Figure 2: CLN-617 binds to collagen and exhibits full bioactivity of IL-2 and IL-12 *in vitro*



(A) Collagen binding was evaluated by ELISA on collagen I-coated plates using an anti-IL12 detection antibody. (B-D) Following overnight CD3 stimulation, PBMCs were cultured with CLN-617, recombinant IL-2 (rhIL-2), IL-12 (rhIL-12), or a combination of rhIL-2 and rhIL-12 for (B) 2h, (C) 48h, or (D) 72h. (B) PBMC lysate was evaluated using a protein signaling microarray. Each dot is representative of a detected signaling protein. Results demonstrate indistinguishable signaling between CLN-617 and the combination of rhIL-2 and rhIL-12. (C-D) CD8+ T cells were evaluated by flow cytometry. Proliferation was measured by viability dye dilution.

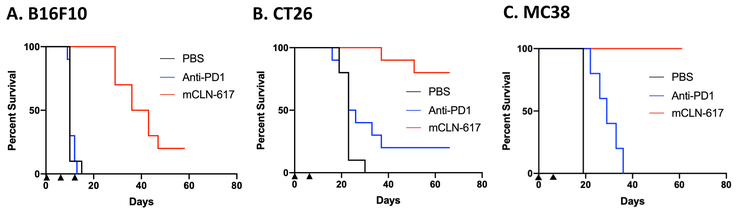
Figure 3: IT administration reduces systemic exposure of mCLN-617



>10x greater C_{max} in tumor than in serum

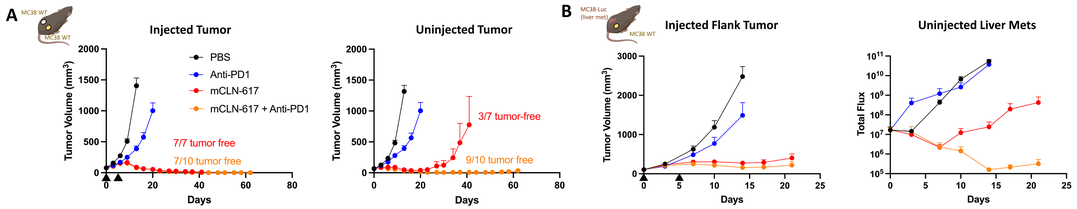
C57BL/6 mice were inoculated with MC38 tumors and injected intratumorally with mCLN-617. IL-2 and IL-12 levels were measured by MesoScale Discovery in serum and in homogenized tumor at the indicated timepoints.

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



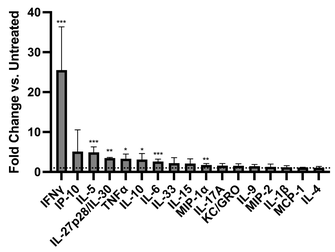
C57BL/6 mice were inoculated with the indicated syngeneic tumor cell line (A – B16F10, B – CT26, C – MC38). Established tumors were treated IT with mCLN-617 on days marked with ▲. As controls, mice were treated with PBS IT or with anti-PD1 IP (RPM1-14, 10 mg/kg, BIW).

Figure 5: Local administration triggers systemic immunity and shows synergy in combination with anti-PD1 therapy



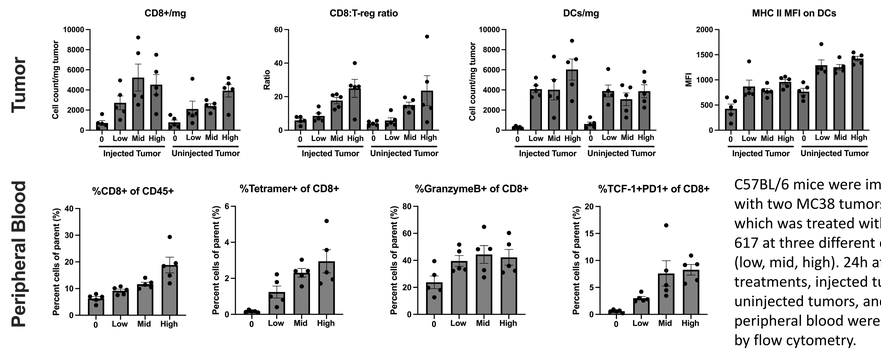
(A) C57BL/6 mice were implanted with two MC38 tumors, only one of which was treated IT with mCLN-617 on days marked with ▲ and the other tumor untreated. (B) C57BL/6 mice were implanted with one MC38 WT tumor in the flank, and MC38-luciferase (luc) tumor cells intrasplenically, leading to luc+ liver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

Figure 6: mCLN-617 mediates local release of interferon-gamma



C57BL/6 mice were inoculated with MC38 tumors and treated intratumorally with mCLN-617. 24h after treatment, tumors were homogenized and intratumoral cytokines evaluated by MesoScale Discovery.

Figure 7: Local administration mobilizes a systemic, tumor-specific cellular immune response, remodeling both the injected and uninjected tumors



C57BL/6 mice were implanted with two MC38 tumors, one of which was treated with mCLN-617 at three different dose levels (low, mid, high). 24h after two treatments, injected tumors, uninjected tumors, and peripheral blood were evaluated by flow cytometry.

Conclusions

- CLN-617 combines IL-2 and IL-12 in a single molecule in a safe and effective manner via retention domains
- CLN-617 activates T cells in a manner indistinguishable from recombinant 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 drives robust, functional, and systemically mobilized tumor-specific cellular immunity
- Preclinical data suggests that CLN-617 may be effective for the treatment of solid tumors with minimal toxicities in the clinic
- IND filing has cleared, and clinical trial initiation is expected in 2023

References

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