Abstract #3688

CLN-617 is an IL-2/IL-12 fusion protein with a collagen-anchoring domain that induces potent systemic anti-tumor immunity upon intratumoral administration

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Background

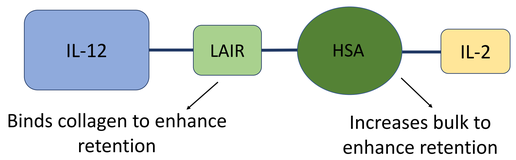
- IL-2 and IL-12 synergistically trigger the stimulation and proliferation of T cells and NK cells to mediate anti-tumor immunity, but have been hindered in the clinic due to significant toxicity[1-3]
- Although aldesleukin, a high-dose IL-2 intravenous (IV) infusion regimen, has been approved for the treatment of melanoma and renal cell carcinoma, adoption in clinical practice has been limited by frequent grade 3 and 4 severe adverse events
- IL-2 and IL-12 separately fused to tumor retention domains demonstrated improved efficacy and safety in multiple murine tumor models when delivered intratumorally[4]. When delivered in combination with radiation, canine versions of these fusion proteins cured spontaneous oral melanoma in pet dogs *(AACR Abstract 4171)*

Cullinan Amber Rationale

- Cullinan Amber is a first-in-class fusion protein that effectively and safely delivers IL-2 and IL-12 as a single agent
- Amber was designed by integrating three primary principles:
  1. Cytokines are autocrine/paracrine in nature, not endocrine
     - ➢ Amber is designed for intratumoral (IT) administration
  2. A protein injected locally will not stay local without retention[4]
     - ➢ Amber is designed with two modes of local retention:
       - (i) a LAIR-2 collagen binding domain
       - (ii) a human serum albumin (HSA) domain to increase MW
  3. Natural immune responses trigger a cytokine milieu, and do not rely on an individual cytokine
     - ➢ Amber combines IL-2 and IL-12 in a single polypeptide

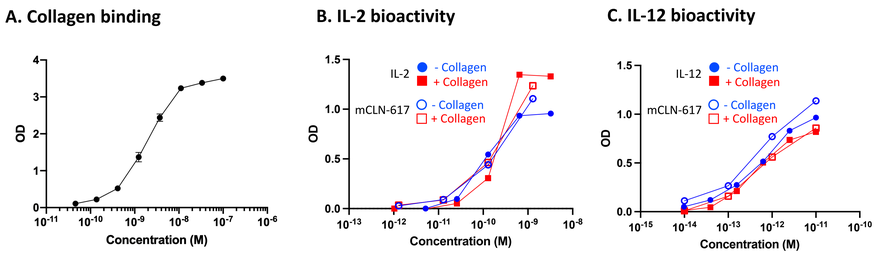
Figure 1: Schematic of Amber design

IL-12 and IL-2 act synergistically to promote Th1 anti-tumor immunity



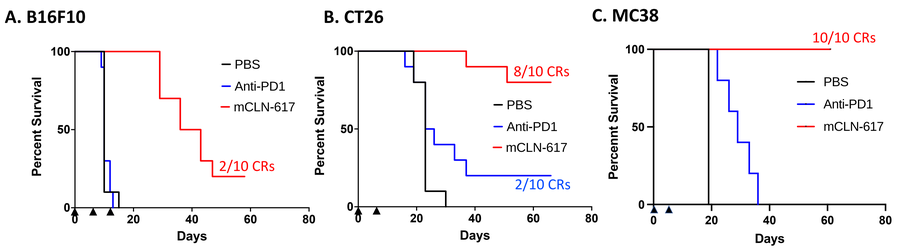
Results

Figure 2: In vitro characterization of Amber bioactivity



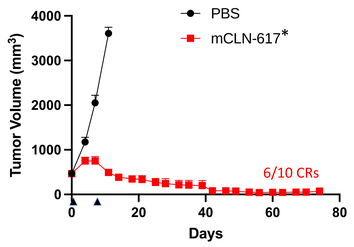
A murine surrogate of Amber (mCLN-617) was evaluated for bioactivity. (A) Collagen binding was evaluated by ELISA. (B) IL-2 bioactivity was evaluated by proliferation of CTLL-2 cells. (C) IL-12 bioactivity was evaluated by proliferation of 2D6 cells. Bioactivity was measured in the presence and absence of collagen

Figure 3: Amber generates robust anti-tumor activity in checkpoint-resistant syngeneic models



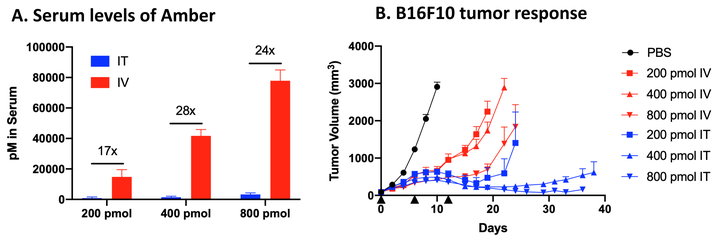
WT mice were inoculated with the indicated tumor cell line (A – B16F10, B – CT26, C – MC38). Established tumors were treated IT with mCLN-617 on day marked with ▲. As controls, mice were treated with PBS IT or with anti-PD1 IP (RPM1-14, 10 mg/kg, BIW).

Figure 4: Large established tumors



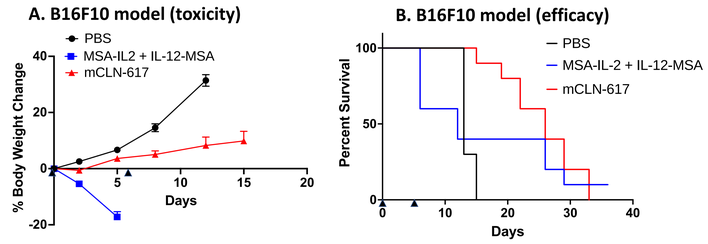
C57BL/6 mice were inoculated with MC38 tumors. 500 mm^3 tumors were treated IT with a *previous generation of mCLN-617 on days marked with ▲.

Figure 5: IT administration reduces systemic exposure and enhances efficacy



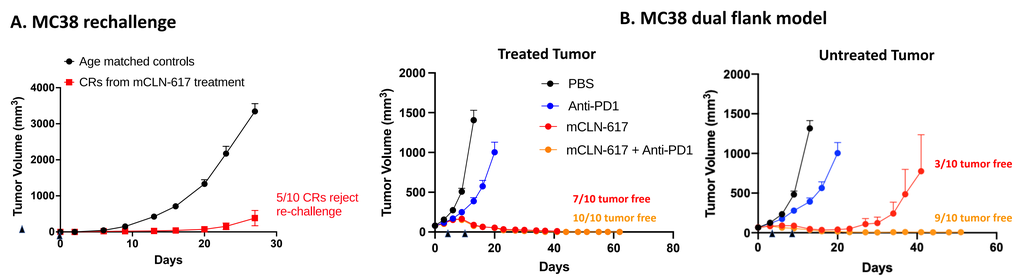
C57BL/6 mice were inoculated with B16F10 cells and established tumors treated with mCLN-617 either IT or IV. (A) Two hours after the first treatment, Amber concentration in serum was measured by ELISA. (B) Tumor growth curves show superior efficacy following IT delivery of Amber.

Figure 6: Retention of IT-administered cytokines decouples efficacy and toxicity



C57BL/6 mice were inoculated with B16F10 tumors. Mice were treated IT with mCLN-617 or cytokine-albumin fusions that lack the collagen-binding retention domain on days marked with ▲. Shown are (A) body weight measurements and (B) survival curves.

Figure 7: Local injection triggers systemic immunity, and shows synergy in combination with anti-PD1 therapy



(A) Surviving mice from Fig. 3C and age-matched controls were re-challenged with MC38 tumors on the opposing flank. (B) C57BL/6 mice were implanted with two MC38 tumors, one tumor treated on days marked with ▲ and the other tumor untreated.

Conclusions

- IT delivery of cytokines mimics the natural cytokine response, but retention strategies are required to prevent toxicity
- Cullinan Amber offers the unique opportunity of combining IL-2 and IL-12 in a single molecule in a safe and effective manner
- Amber's large size and binding to collagen efficiently retains the cytokines in the tumor microenvironment
- Amber can eradicate large, established primary and distal checkpoint-resistant tumors, and a memory response is developed
- Amber synergizes with anti-PD1 therapy
- Preclinical data suggests that Amber may be effective as a single-agent in the clinic for the treatment of solid tumors with minimal toxicities. We have selected a development candidate and IND enabling studies are ongoing

References

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