Abstract # 2077

Preclinical characterization of a next-generation CD19/CD3-bispecific T cell engaging antibody construct CLN-978 with long serum half-life and superior potency against target cells expressing very low levels of CD19

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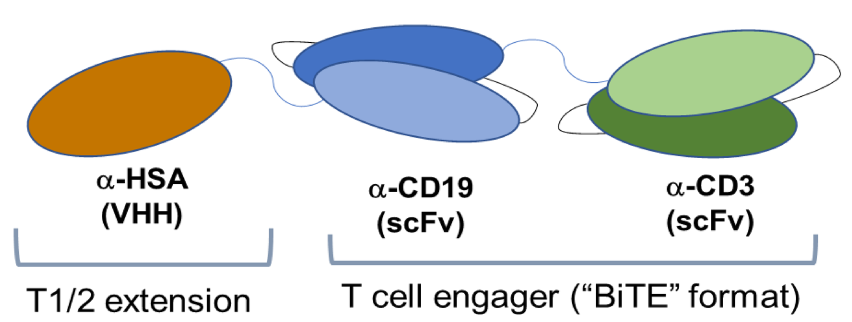
Background

- CD19 is a clinically and commercially validated target for the treatment of various B cell malignancies via T cell engaging antibodies (TCE), CAR-T cells, and monoclonal antibodies
- Blinatumomab (Blincyto®) is a CD19/CD3-bispecific TCE approved for the therapy of relapsed/refractory (r/r) and minimal residual ALL expressing CD19
- Often low expression levels of CD19 or apparent loss of CD19 from malignant cells under therapy severely limit the therapeutic benefit of TCE and CAR-T cell therapies
- CLN-978 is a novel TCE designed with features to improve upon several shortcomings of approved therapies to provide a therapeutic option for refractory or relapsing NHL or ALL patients with cancer cells expressing very low levels of CD19
- CLN-978 is currently in IND-enabling studies

Structure of CLN-978



Features of CLN-978

- A very high-affinity human CD19-binding scFv to recognize and kill NHL and ALL cells with very low levels of CD19 target expression (blue)
- A humanized serum albumin-binding single-domain antibody (VHH) for half-life extension (brown)
- A medium-affinity human scFv to bind to the invariant CD3 epsilon chain of the T cell receptor (TCR) (green)
- CLN-978 is fully cross-reactive with nonhuman primate orthologs
- CLN-978 is produced at high titers in CHO cells, omits a His tag, and is purified via standard protein A binding methods

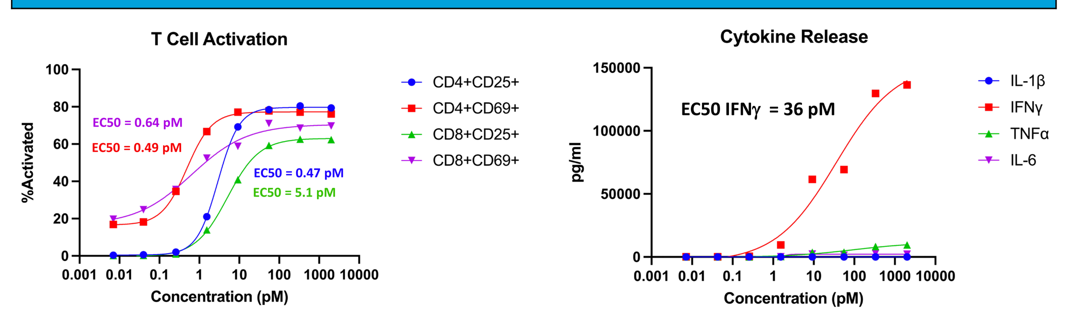
Results

Figure 1: Binding affinities of CLN-978

Characteristic	CLN-978
HSA Affinity (K_D)	0.60 nM
CD19 Affinity (K_D)	0.23 nM
CD3 affinity (K_D)	7.9 nM
Affinity Difference (CD19 vs CD3)	~34-fold

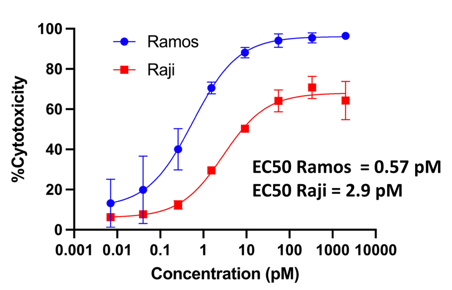
Binding affinities were determined by SPR

Figure 2: Activation of T cells by CLN-978 co-cultured with Ramos B lymphoma cells



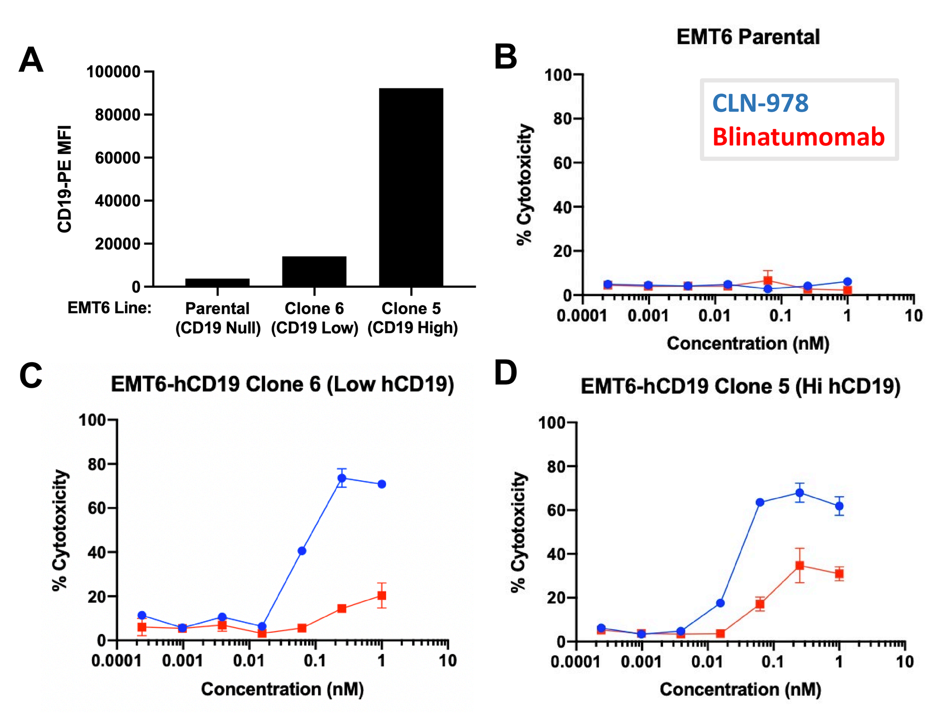
Unstimulated PBMC were co-cultured with Ramos cells at an E:T ratio of 10:1 for 48 hours. Surface expression of CD69 and CD25 was determined by FACS, and cytokine levels by Luminex.

Figure 3: Redirected lysis by CLN-978 of Ramos and Raji B lymphoma cells with T cells *in vitro*



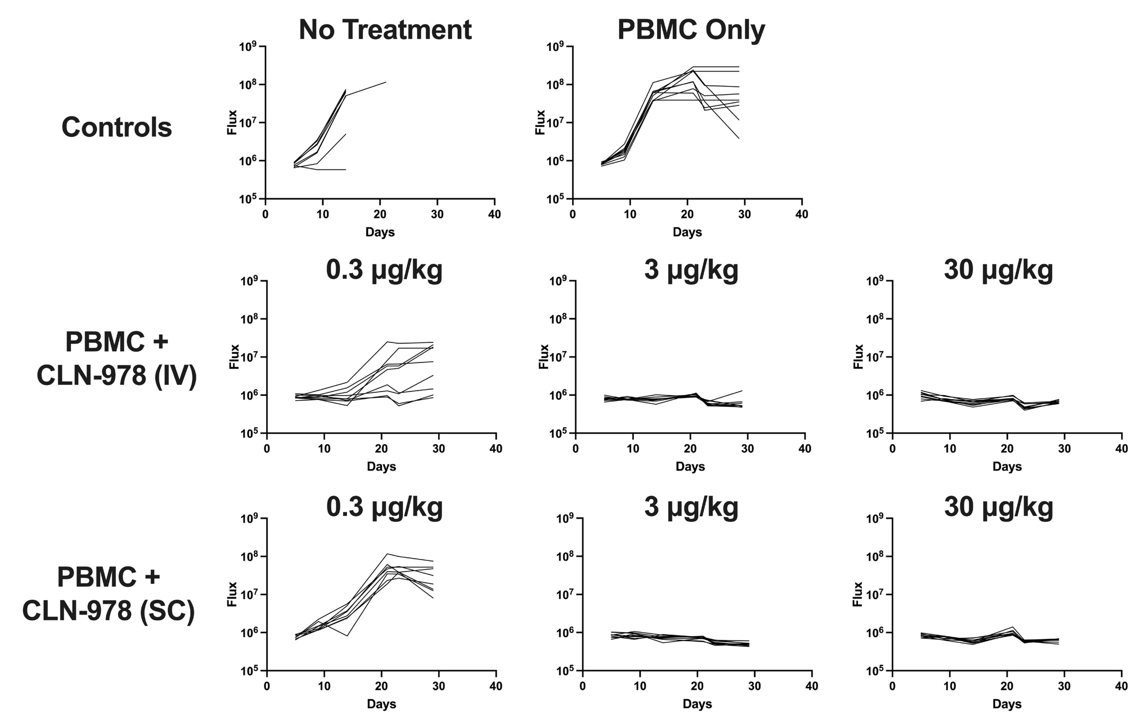
Unstimulated PBMC were co-cultured with Ramos or Raji cells at an E:T ratio of 10:1 for 48 h. Cytotoxicity was determined by flow cytometry.

Figure 4: Comparison of CLN-978 with blinatumomab for their potency of redirected lysis *in vitro*

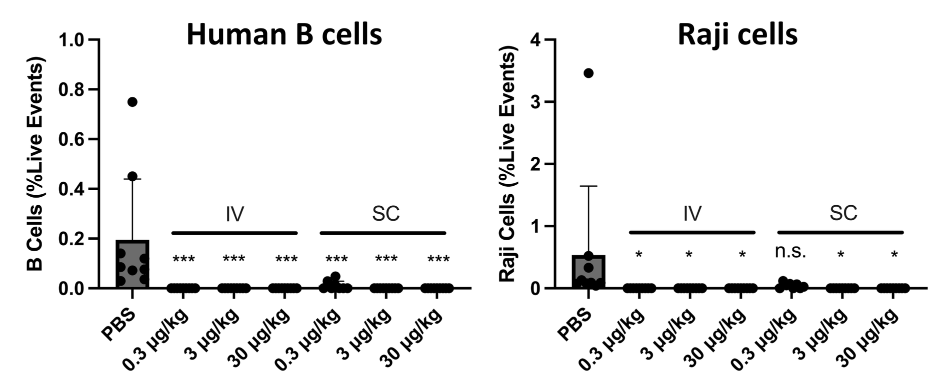


A) EMT6 cells were stably transfected to express hCD19. The parental line, a CD19 low line (clone 6), and a CD19 high line (clone 5) were characterized by flow cytometry. B-D) Unstimulated T cells were co-cultured with the indicated EMT6 cell line at a 3:1 E:T ratio for 48 h, and cytotoxicity was evaluated by flow cytometry.

Figure 5: *In vivo* efficacy of IV- and SC-administered CLN-978 in a Raji-Luciferase NCG mouse model

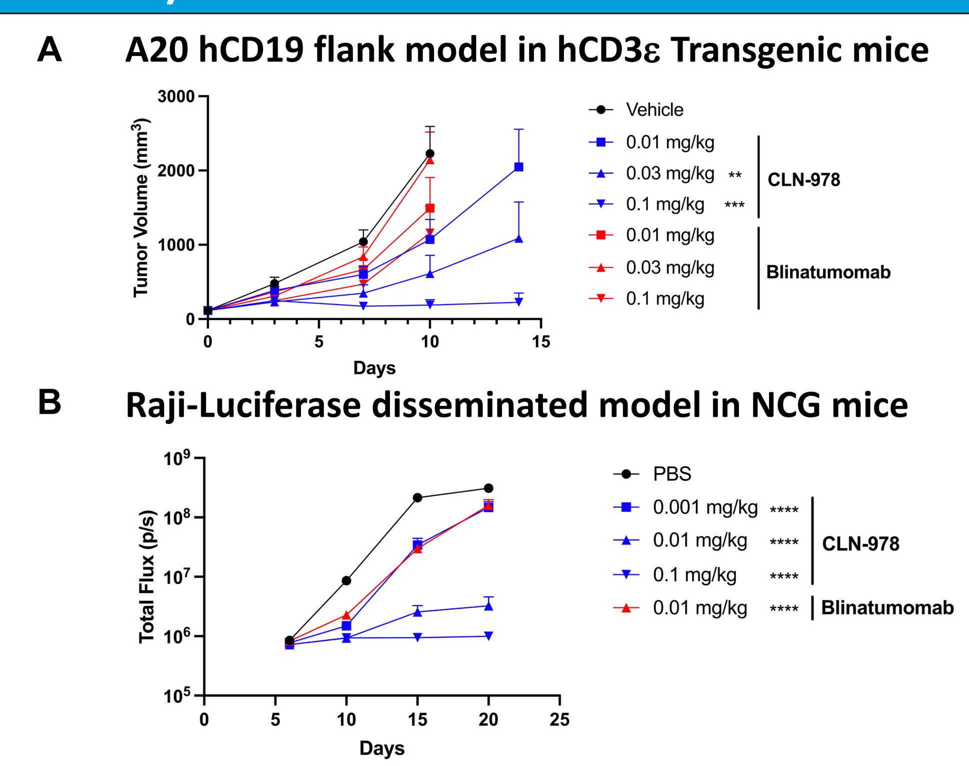


Immunodeficient NCG mice were IV engrafted with $2x10^5$ Raji cells. On the following day (Day 1), $2x10^7$ PBMCs from a healthy donor were implanted IP. CLN-978 was dosed either IV or SC weekly starting on Day 1. All treatment conditions imparted statistically significant responses relative to controls by ANOVA at d14 (p < 0.0001).



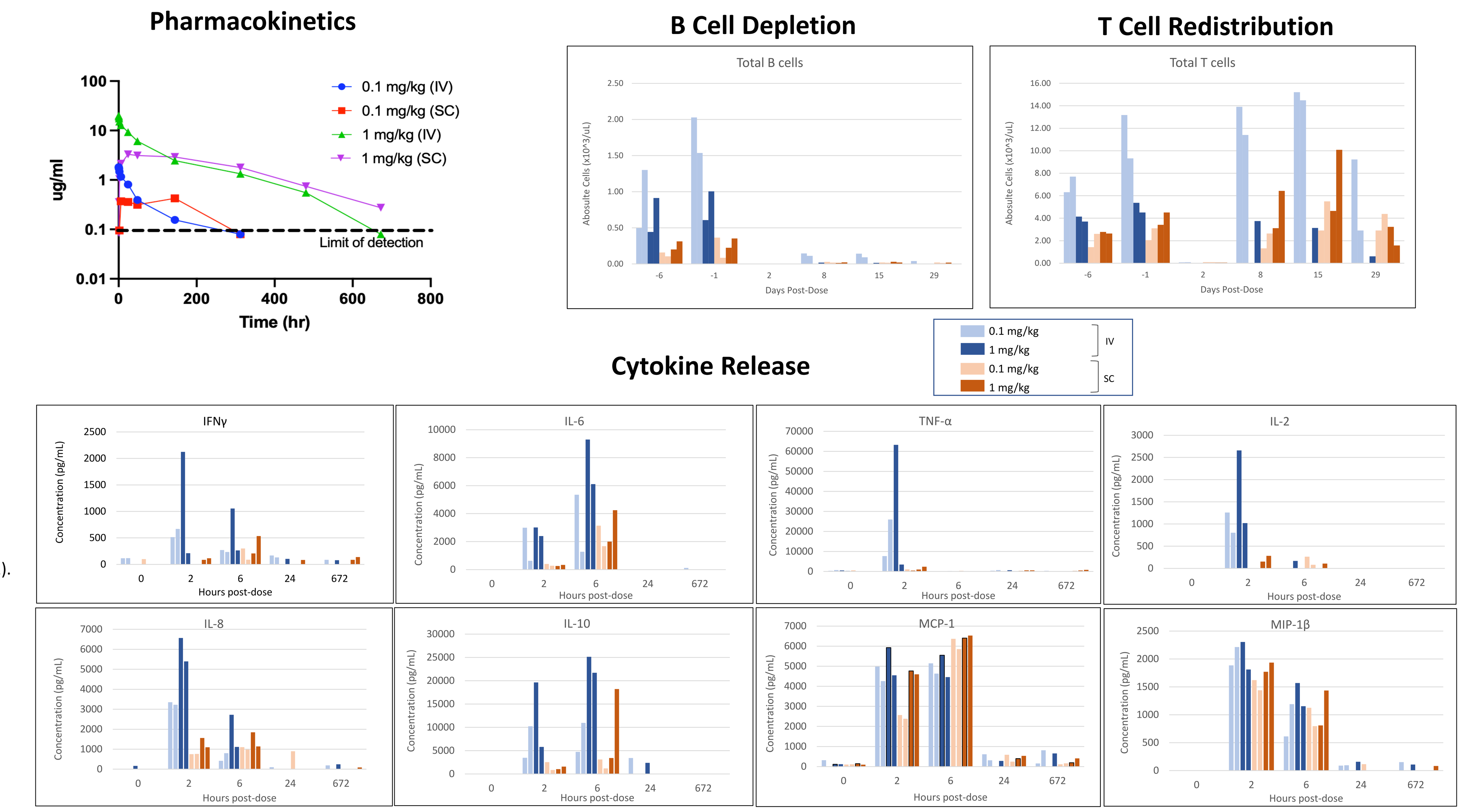
On Day 12 of the above efficacy study, Raji cells and B cells in blood were quantified by flow cytometry. Statistics were calculated versus PBS using ANOVA with multiple comparisons test.

Figure 6: Comparison of CLN-978 with blinatumomab for *in vivo* efficacy in two mouse models



A) hCD3ε-expressing BALB/c mice were inoculated with A20 cells expressing hCD19. Mice were treated once IV when tumor volume reached ~100mm³. B) Immunodeficient NCG mice were IV engrafted with $2x10^5$ Raji cells, then implanted IP with $2x10^7$ PBMCs the following day. Mice were treated IV weekly starting on day 1. Statistics were calculated versus vehicle or PBS using ANOVA with multiple comparisons test on d10 in A and d15 in B.

Figure 7: Pharmacokinetics, B cell depletion, T cell redistribution and cytokine release in Cynomolgus monkeys in response to a single IV- or SC-administered CLN-978 dose



Female monkeys (n=2) were dosed at 0.1 or 1 mg/kg either IV (blue bars) or SC (orange bars). Blood samples were collected at predetermined timepoints for PK, cytokine, immunophenotyping, clinical chemistry and hematology. One animal in the 1 mg/kg IV dose group was euthanized on Day 1 due to cytokine release-like symptoms.

Conclusions

- CLN-978 is designed to be an off-the-shelf CD19-targeted therapy with improved properties relative to existing therapies
- CLN-978 is differentiated from blinatumomab (BLINCYTO®):
  - Higher affinity to CD19 enables redirected lysis of low CD19 expressing target cells
  - Extended serum half-life of CLN-978 enables a convenient dosing regimen
- CLN-978 affinities to CD19 and CD3 were optimized to maximize therapeutic index
- CLN-978 potently triggers redirected lysis of CD19-expressing target cells by cytotoxic T cells in cell coculture assays, mouse models and non-human primates
- Subcutaneous delivery of CLN-978 promises a wider therapeutic window than IV delivery
- CLN-978 has the potential to treat patients having relapsed from therapies with BLINCYTO® and CD19 CAR-T cells due to reduced CD19 target expression
- CLN-978 is currently in IND-enabling studies