POSTER #1024

# CLN-978, a novel CD19/CD3/HSA-specific T cell engager with extended serum half-life, is effective against target cells expressing very low levels of CD19

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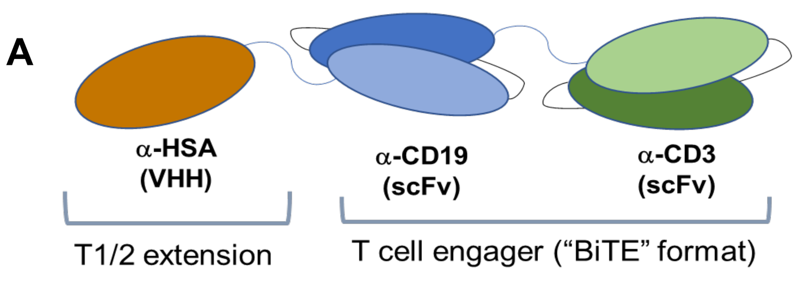
## Background

- T cell therapies targeting the B cell antigen CD19 have profoundly changed the way we treat various B cell malignancies.
- Both the CD19/CD3-bispecific T cell-engaging bispecific antibody construct blinatumomab and the various autologous CD19-specific CAR-T cell therapies have exhibited robust clinical activity in relapsed/refractory acute lymphocytic leukemia (ALL) and non-Hodgkin lymphoma (NHL).
- However, both therapies fail in patients where resistance mechanisms select for tumor cells with low levels of CD19 target expression that are no longer recognized for target cell lysis.
- Recent data suggests that blasts that appear to have lost CD19, as measured by immunohistochemistry (IHC), may still exhibit low levels of CD19 expression (ie, <3000 copies of CD19 per cell) when measured by more sensitive techniques like flow cytometry[1].
- CLN-978 is a single-chain fusion protein consisting of two tandemly arranged humanized single chain Fv domains with specificities for CD19 and CD3, and one humanized VHH domain with specificity for serum albumin.
- CLN-978 was designed for high-affinity binding to CD19 to enable killing of low-CD19 expressing target cells, and for long serum half-life.

## Results

### Figure 1: Design and characterization of CLN-978

A



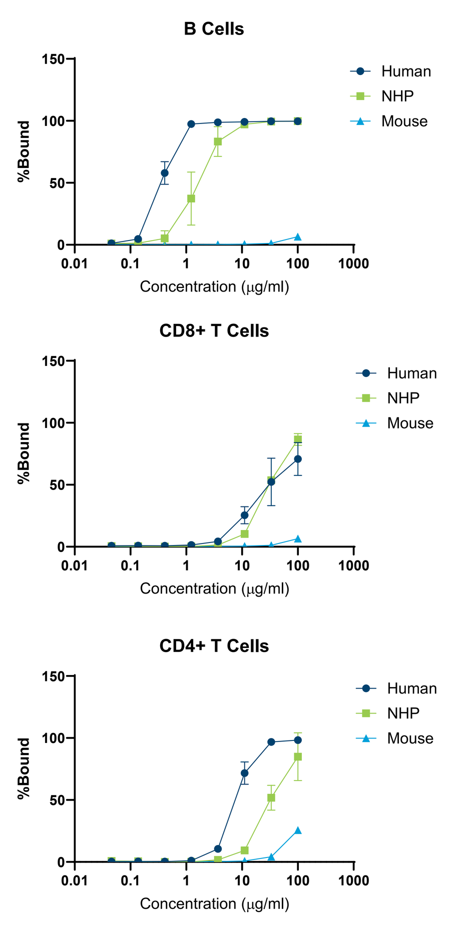

B SPR Binding

| Species | Protein | Serum Albumin | ka (1/Ms) | KD (nM) |
|---|---|---|---|---|
| Human | CD19 | - | 1.90 x $10^5$ | 0.0738 |
| | | + | ND | ND* |
| | CD3 | - | 6.98 x $10^5$ | 7.18 |
| | | + | 2.23 x $10^5$ | 16.79 |
| | Serum Albumin | NA | 4.50 x $10^{-4}$ | 0.6 |
| Cynomolgus | CD19 | - | 9.26 x $10^4$ | 19.22 |
| | | + | 3.08 x $10^4$ | 44.98 |
| | CD3 | - | 7.30 x$10^5$ | 6.04 |
| | | + | 2.05 x $10^5$ | 16.45 |
| | Serum Albumin | NA | 4.44 x $10^{-4}$ | 0.70 |
| Mouse** | Serum Albumin | NA | 6.48 x $10^{-3}$ | 4.40 |

*The kinetics of CLN-978 were not amenable to the investigation of the effects of albumin on the CLN-978-human CD19 interaction

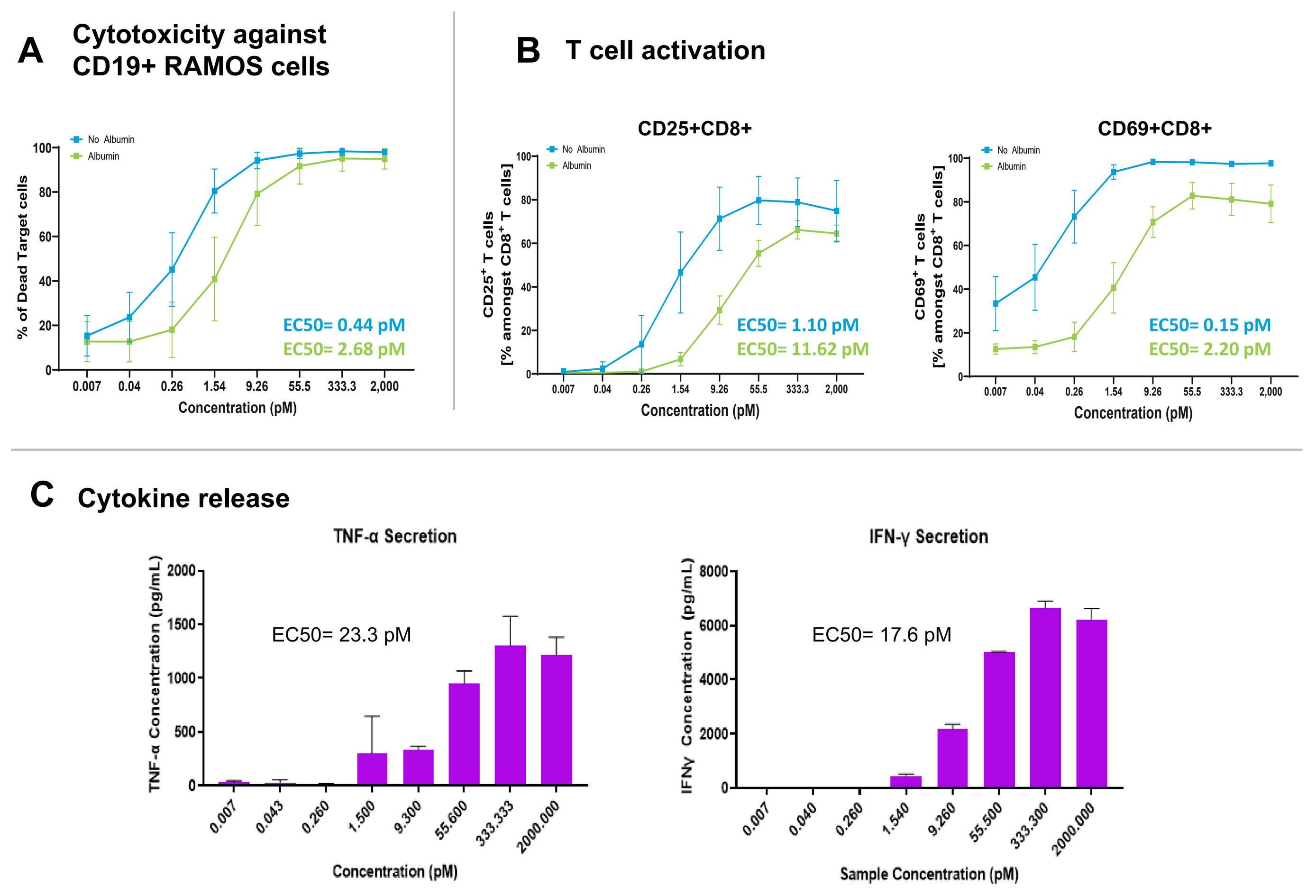
**Murine CD19 and CD3 were not evaluated due to low homology and lack of binding confirmed by on-cell binding

C On-Cell Binding to PBMC



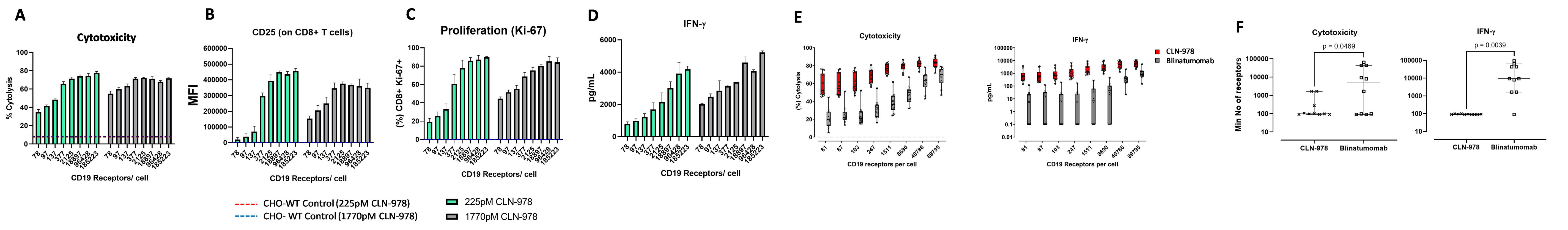

A) Design of CLN-978. B) Summary of Biacore binding data of CLN-978 against targeted proteins in the presence or absence of albumin. C) Binding of CLN-978 to human, cynomolgus monkey and mouse peripheral blood mononuclear cells (PBMC; n=3 donors).

### Figure 2: CLN-978 potently induces T cell activation and T cell directed cytotoxicity



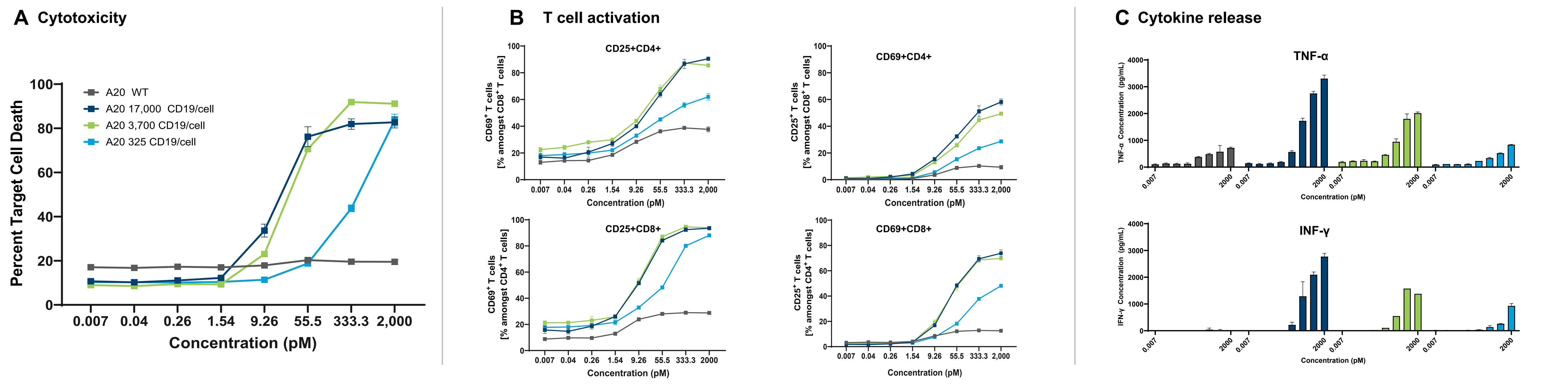

RAMOS cells were co-cultured with PBMC at an E:T ratio of 10:1 and the indicated concentrations of CLN-978 in the presence or absence of human serum albumin for 48 h (n=10). Shown are A) lysis curves, as assessed by flow cytometry for 7-AAD uptake in RAMOS cells and B) CD25 and CD69 expression on CD4+ and CD8+ T cells. C) Supernatants from co-cultures of RAMOS cells and PBMC in the presence of albumin were analyzed for the indicated cytokines by Luminex.

### Figure 3: CLN-978 is active against an inducible CHO cell line expressing low copy numbers of CD19



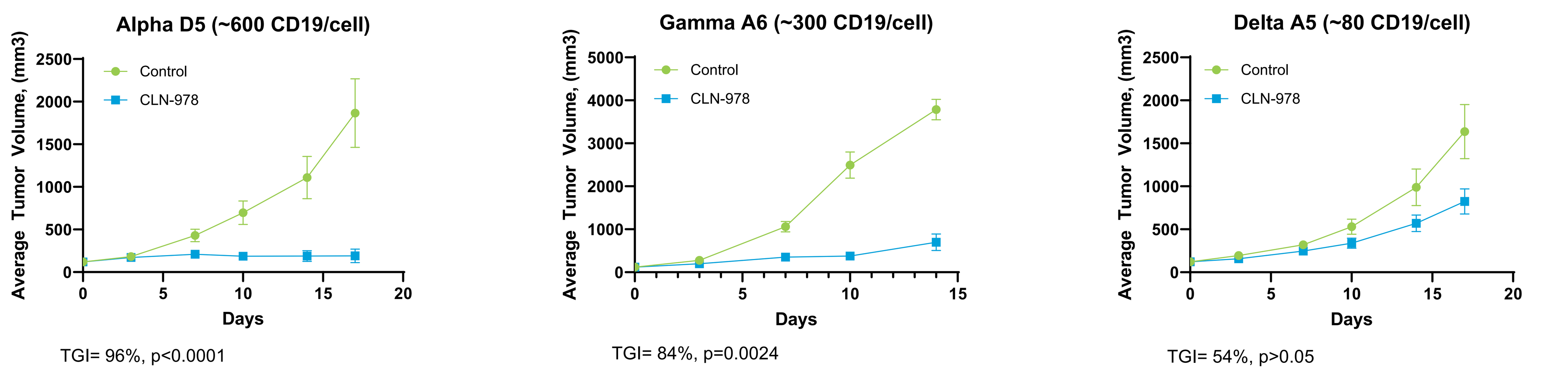

Chemical inducer-regulated CD19-expressing CHO cells were co-cultured with purified T cells at an E:T ratio of 10:1 in the presence or absence of the indicated concentrations of CLN-978 for 68 h (n=4). Shown are: A) target cell lysis curves, as assessed by flow cytometry assessed for 7-AAD uptake in CD19-expressing CHO cells. B) CD25 and C) Ki67 expression profiles as marker for activation and proliferation of CD8+ T cells. D) Supernatants from co-cultures of CD19-expressing CHO cells and PBMC as in 4A-C were analyzed for IFNγ. E) Cytotoxicity or IFNγ production at 225 pM concentration of CLN-978 or blinatumomab at 72 hr (n=10). F) Receiver operating characteristic (ROC) analysis determined minimal receptor number required for biological effect; Wilcoxon signed-rank test.

### Figure 4: CLN-978 is active against lymphoma cell lines engineered to express very low copy numbers of CD19




Human CD19-expressing murine A20 cells were co-cultured with purified T cells at an E:T ratio of 10:1 in the presence of the indicated concentrations of CLN-978 and human albumin for 48 h (n=2). Shown are A) target cell lysis curves, as assessed by flow cytometry for 7-AAD uptake B) CD25 and CD69 expression profiles as marker for activation of CD4+ and CD8+ T cells C) Supernatants from co-cultures of hCD19-A20 cells and PBMC were analyzed for the indicated cytokines by Luminex.

### Figure 5: In vivo efficacy of CLN-978 in lymphoma models engineered to express low copy numbers of CD19




Human CD3ε-expressing BALB/c mice were inoculated with syngeneic A20 cells expressing varying levels of human CD19. Mice were treated once weekly IV with 0.1 mg/kg CLN-978 or control when tumor volume reached ~100mm$^3$. Copy numbers reflect measurement of ex vivo tumor samples.

## Conclusions

- CLN-978 binding affinities for CD19 and CD3 were optimized for high potency and wide therapeutic index.
- CLN-978 redirects T cells to lyse B cell lymphoma cells expressing very low levels of CD19 in vitro.
- CLN-978 elicited potent in vivo efficacy against target cells expressing very low levels of hCD19.
- The characteristics of CLN-978 may translate into high response rates and longer response duration in NHL patients expressing normal and very low levels of CD19 on malignant B cells.
- CLN-978 is currently in Phase 1 clinical trials (NCT05879744).

References: 1. Spiegal JY, et al. *Nature Medicine*, 2021