Rapid Assembly and Screening of Multivalent Immune Cell-Redirecting Therapies for Leukemia

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SUPPORTING INFORMATION

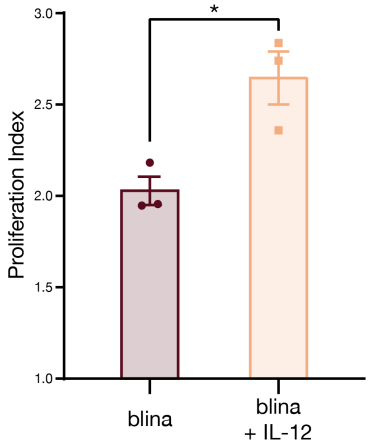


Figure S1. Recombinant IL-12 improves blinatumomab-induced T cell proliferation. Proliferation index of primary human $CD8^+$ T cells co-cultured with $CD19^+$ NALM-6 leukemia cells following treatment with blina (7 ng/mL) with or without IL-12 (3.5 ng/mL) as measured by flow cytometry (E:T 1:1, 72h). Values represent mean±SEM (n=3 donors). *p<0.05.

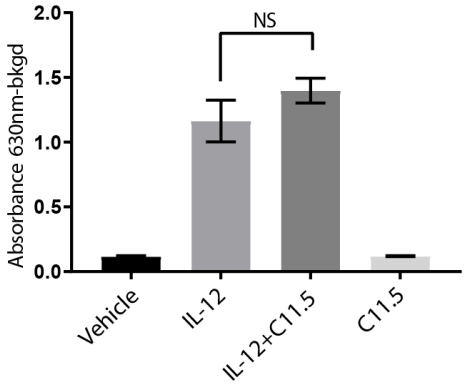


Figure S2. Anti-IL-12 monoclonal antibody, clone C11.5, is non-neutralizing. Soluble IL-12 activity (100 ng/mL, 24 h) in the presence or absence of αIL-12 as measured using IL-12 reporter cells.

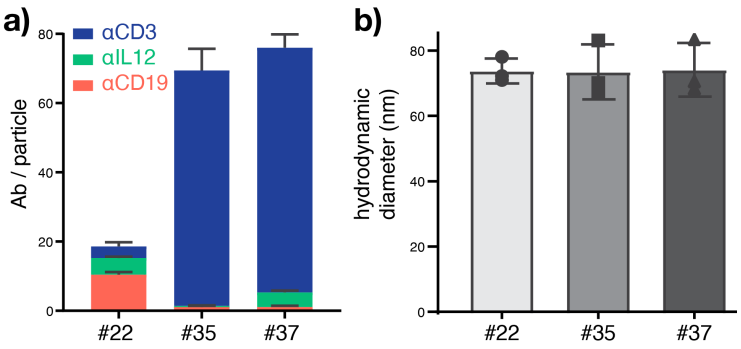


Figure S3. BiTEokine test compound composition and size are reproducible batch-to-batch. a) Composition of independent batches of test compounds #22, #35, and #37 as measured by antibody fluorescence intensity. b) Hydrodynamic size of test compounds in (a) demonstrating both reproducibility and size consistency across selected library compounds. Values in (a,b) represent mean±SD of 3 independent preparations.

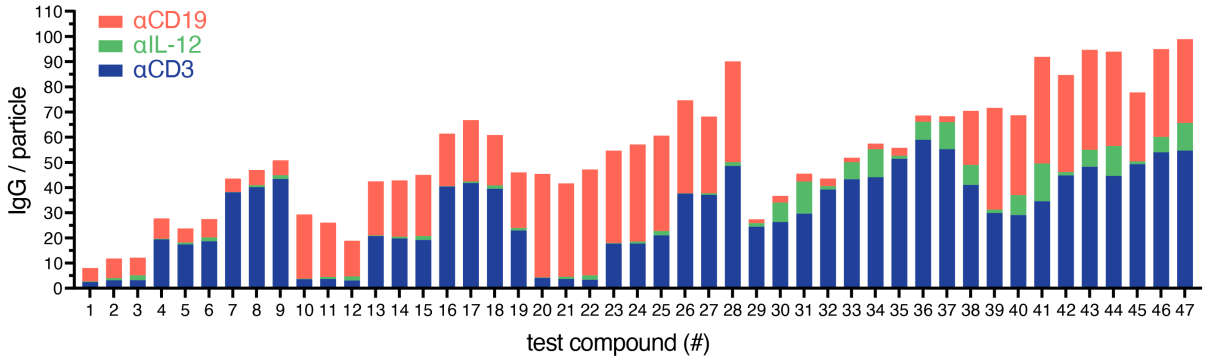
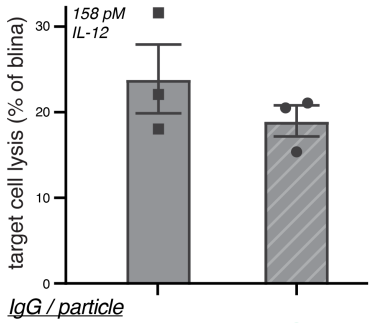


Figure S4. Composition of the CD19 x CD3 x IL12 BiTEokine test compound library as measured by antibody fluorescence intensity.



IgG / particle		
αIL12	**1**	**0**
isotype	52	49
αCD19	3	2
αCD3	71	76
total	126	128

Figure S5. Impact of cytokine tethering on BiTEokine-induced leukemia cell lysis. Lysis of NALM-6 target cells by primary human CD8+ T cells as measured by flow cytometry (E:T 1:1, 72 h, 130 pM BiTEokine). Values represent mean±SEM of 3 T cell donors. T cells used in these studies were obtained from donors distinct from those used elsewhere in this work.

Gating Strategy

Figure 1. Gating is presented as (b) time>singlets>FS/SS above debris>live/dead NIR+CFSE+ (NALM-6), (c) time>cells excluding count beads by far red+>cells excluding debris by FS/SS> CellTrace Violet+> live/dead NIR+), (d) time>cells excluding count beads by far red+>cells excluding debris by FS/SS> NIR-/CellTrace Violet- live T cells> CellTrace Yellow+ dividing cells.

Figure 4. Gating is presented as (a) time>cells excluding count beads by far red+>cells excluding debris by FS/SS> live cells by live/dead NIR-> CellTrace Yellow+/CellTrace Violet- (T cells) or CellTrace Yellow-/CellTrace Violet+ (Nalm6 cells) > anti-CD3 AF594, anti-CD19 AF700, or anti-IL-12 AF488 and (b) time>cells excluding count beads by far red+>cells excluding debris by FS/SS> CellTrace Violet+> live/dead NIR+.

Figure 5. Gating is presented as (a,b) low aspect ratio> CellTrace Violet+>LAMP-1 AF488+.