SUPPLEMENTARY METHODS:

Cell Lines

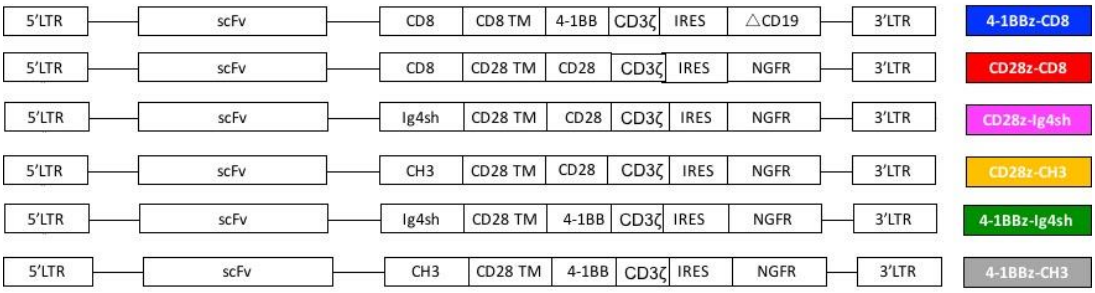
We calculated CLL-1 expression in each cell line with mean fluorescence intensity (MFI) ratio which is calculated by the MFI of the cell line/MFI of isotype (BV421 Mouse Anti-Human CD371 (Clec12A) Clone 50C1, BD Biosciences, San Jose, CA Purified Mouse $IgG2_{alpha}$ Isotype control BD Biosciences, San Jose, CA). We acquired flow cytometric data from a Gallios (Beckman Coulter, Indianapolis, IN) which we analyzed using FlowJo (version 10, Tree Star): AML cell lines THP-1 (acute myeloid leukemia cell monocytic), HL-60 (acute myeloid leukemia cell line-promyelocytic), MOLM-13 (acute myeloid leukemia-monocytic). THP-1 was maintained in culture in RPMI 1640 (Gibco-BRL, San Francisco, CA) supplemented with 10% FBS (Hyclone, Waltham, MA) and 2mM L-gulutamine (Gibco-BRL, San Francisco, CA) and penicillin-streptomycin (Pen Strep; Gibco-BRL San Francisco, CA). HL-60 and MOLM-13 was maintained in culture in Iscove's modified Dulbecco's medium (IMDM;Gibco-BRL, San Francisco, CA) supplemented with 20% FBS (Hyclone, Waltham, MA) and 2mM L-gulutamine (Gibco-BRL, San Francisco, CA) and penicillin-streptomycin (Pen Strep; Gibco-BRL, San Francisco, CA).

Co-culture and Sequential Killing Assays

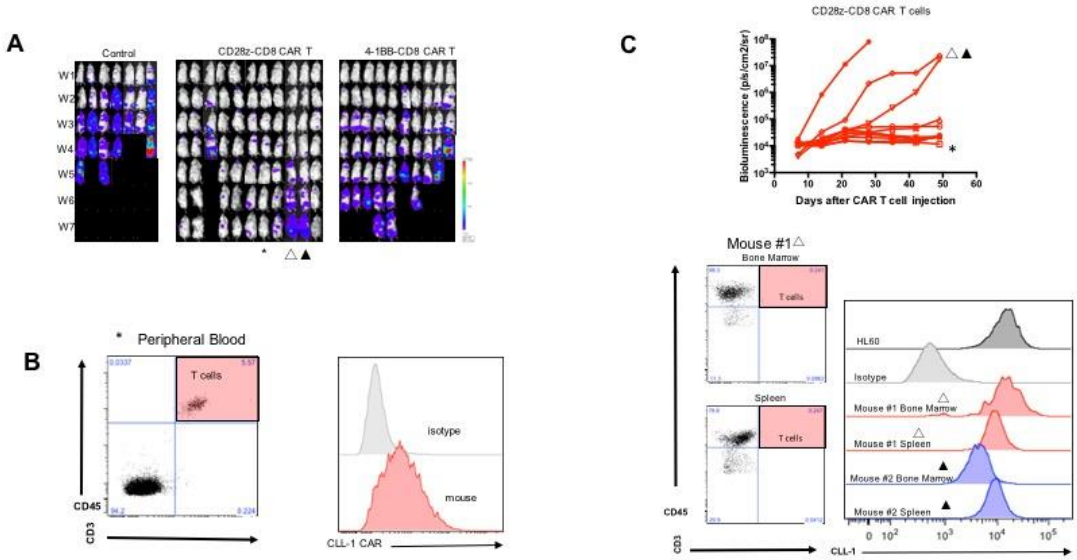
Transduced or non-transduced T cells (1 x 10^5/well) were co-cultured with each of the tumor cell lines described (THP-1, HL-60, Molm-13) (4x10^5/well) at an E:T ratio of 1:4 in 96 well plates in the absence of exogenous cytokines. We identified tumor cells by GFP expression. We stained dead cells by measuring the population positive for 7-aminoactinomycin D (7-AAD) (Thermo Fisher Scientific, Waltham, MA) and counted them using CountBright Beads (Thermo Fisher Scientific, Waltham MA).

In sequential/recursive killing assays, T cells were collected every 3 days and counted by flow cytometry using CountBright Beads (Thermo Fisher Scientific, Waltham MA). We replated and rechallanged T cells with fresh tumor cells at the same E:T ratio.

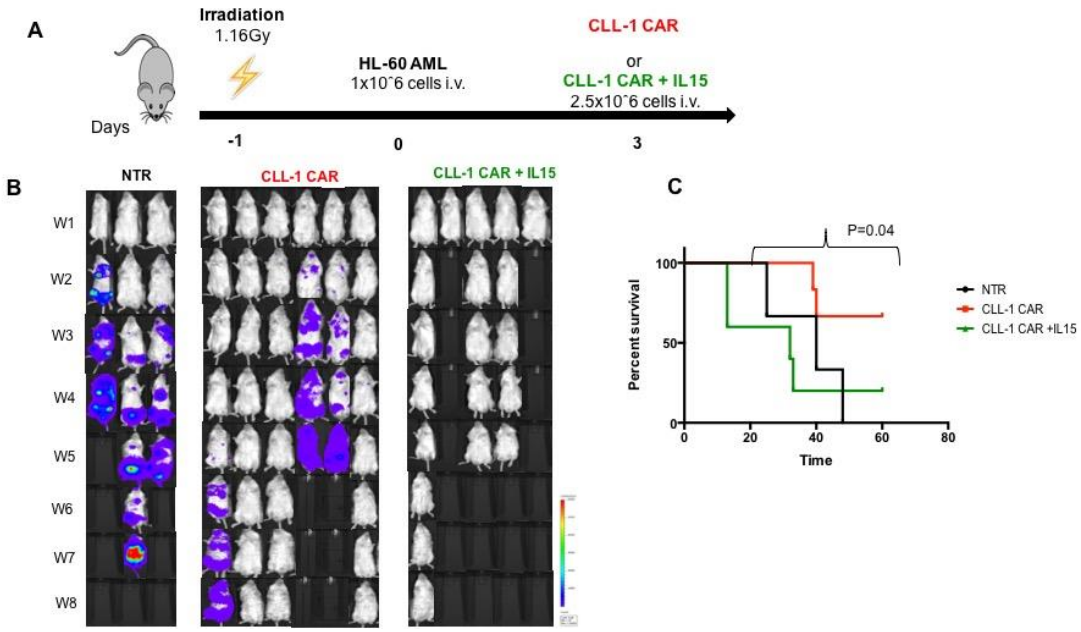
Ataca Atilla P, *et al. J Immunother Cancer* 2020; 8:e001229. doi: 10.1136/jitc-2020-001229



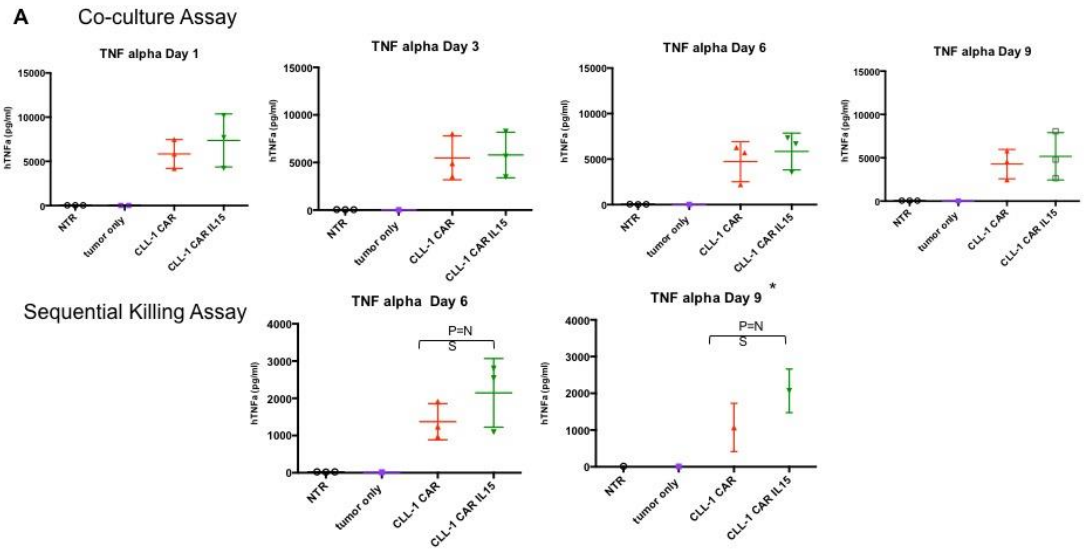
Supplementary Figure 1. Panel of various CLL-1 CAR constructs

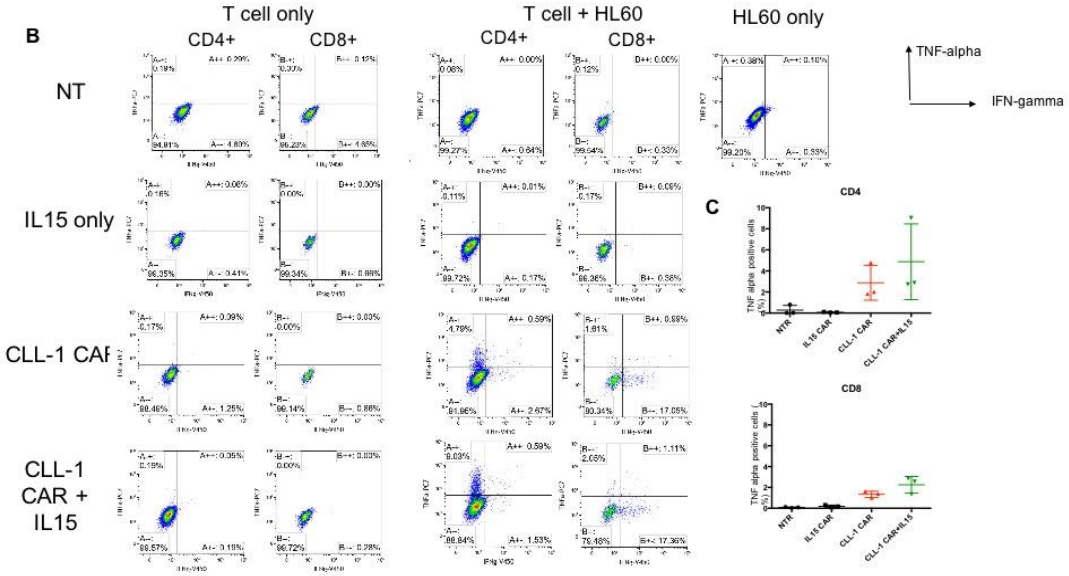


Supplementary Figure 2. Comparison of CLL-1 CARs CD28z.CD8 vs 4-1BB.CD8 in aggressive HL60 disease model. A. Representative images showing leukemia progression in groups from week 1 to week 7. **B.** The T cells and CLL-1.CD28z.CD8 CAR T cells in peripheral blood of mouse marked with (*) at week 7. **C.** Tumor bioluminescence signals measured at specified time points of mice that received CLL-1.CD28z.CD8 CAR T cells. The bone marrow and spleen tissues were harvested in mice marked with (△) and (▲). The T cells were not persistent and also the CLL-1 expression was retained.



Supplementary 3. Transgenic IL15 in HL-60 model. A. Schematic figure of HL 60 in vivo model comparing CLL-1 CAR *vs* CLL-1 CAR+IL15. **B.** Representative images showing leukemia progression in groups from week 1 to week 8. **C.** Kaplan-Meier curve showing the survival of mice in each experimental group. P values were determined by log-rank Mantel-Cox test (NTR:Non Transduced).

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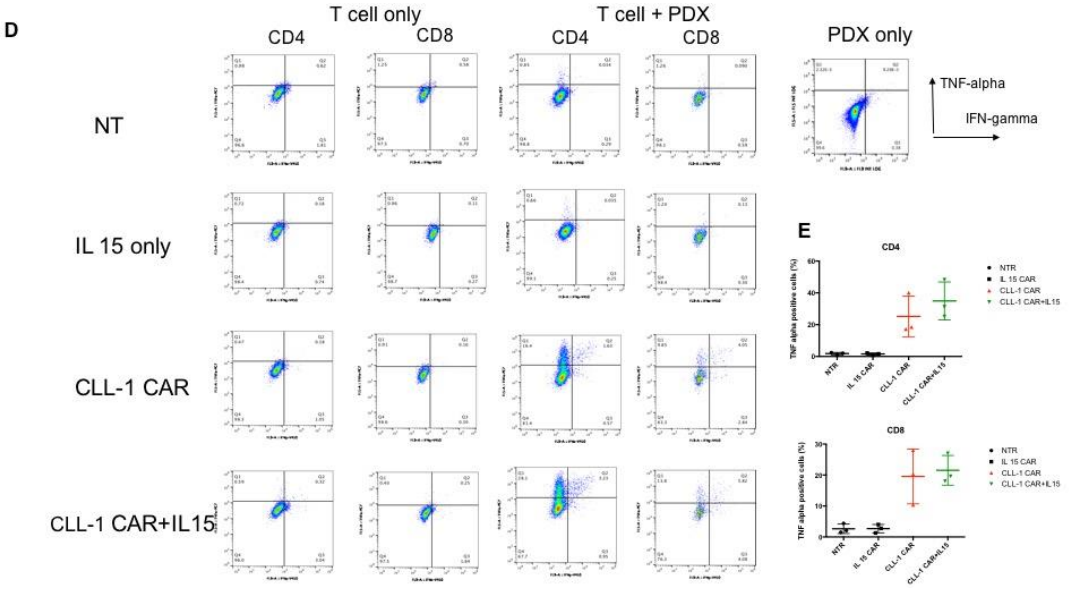
T cell + HL60
HL60 only
CD4+
CD8+
NT
IL15 only
CLL-1 CAR
CLL-1 CAR + IL15
TNF-alpha
IFN-gamma
C
CD4
CD8
TNF alpha positive cells (%)
NTR
IL15 CAR
CLL-1 CAR
CLL-1 CAR+IL15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T cell + PDX
PDX only
CD4
CD8
NT
IL 15 only
CLL-1 CAR
CLL-1 CAR+IL15
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CD4
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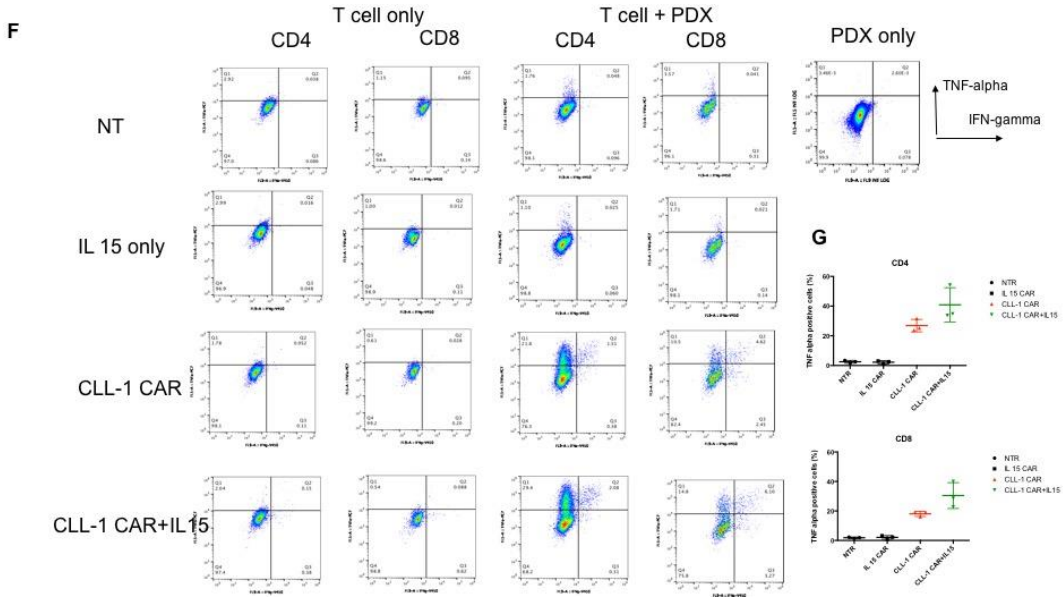
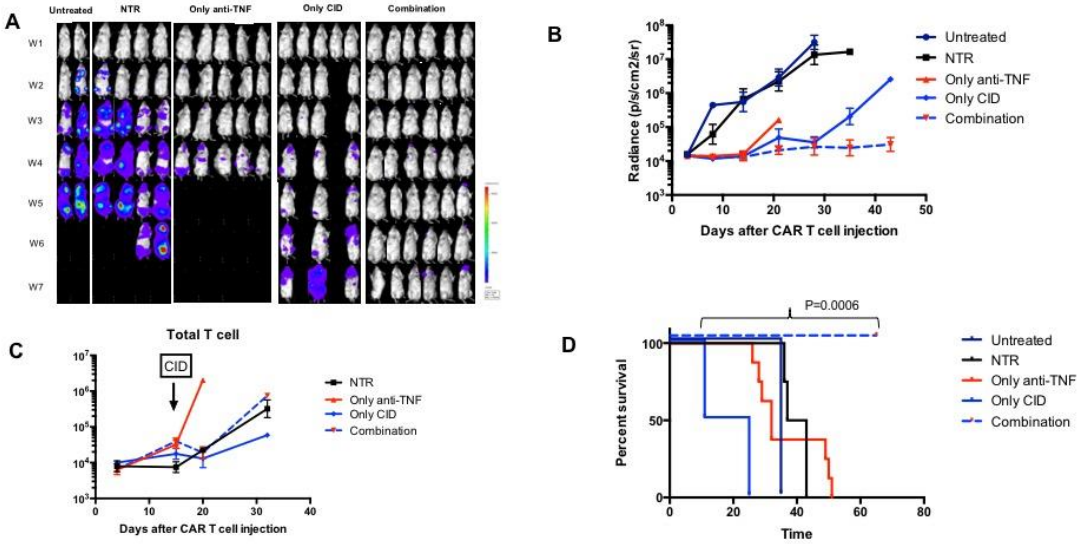
CLL-1 CAR+IL15



Supplementary 4. Cytokine Release in vitro. A. TNF alpha release in co-cultures with HL60 (E:T=1:4) at day 1, 3, 6, 9 (top) and sequential killing assays at day 6 and 9 (bottom) (n=3). Sequential killing assay at day 9 marked with (*) done in 2 donors. CLL-1 CAR is compared with CLL-1 CAR transgenic IL15 (CLL-1 CAR IL15) in culture (NS: not significant). P value was determined by unpaired Student's t test. **B.** Flow plots of intracellular staining of IFN-gamma and TNF-alpha in CD4 and CD8 T cells with/without HL60 (E:T=1:4) and HL 60 only in a representative donor **C.** TNF-alpha expressing CD4 and CD8 T cells when CAR T cells co-cultured with HL-60 (E:T=1:4) (n=3). **D.** Flow plots of intracellular staining of IFN-gamma and TNF-alpha in CD4 and CD8 T cells with/without PDX cells (E:T=1:4) and PDX only in a representative donor. **E.** TNF-alpha expressing CD4 and CD8 T cells when CAR T cells co-cultured with PDX cells (E:T=1:4) (n=3). **F.** Flow plots of intracellular staining of IFN-gamma and TNF-alpha in CD4 and CD8 T cells with/without PDX cells (E:T=1:10) and PDX only in a representative donor. **E.** TNF-alpha expressing CD4 and CD8 T cells when CAR T cells co-cultured with PDX cells (E:T=1:10) and PDX only in a representative donor. **G.** TNF-alpha expressing CD4 and CD8 T cells when CAR T cells co-cultured with PDX cells (E:T=1:10) (n=3).



Supplementary 5. Effect of Anti-TNF and/or CID in HL60 model. A. Representative images showing leukemia progression in groups from week 1 to week 7. Only Anti-TNF alpha group received Anti-TNF alpha 1.5mg/kg on day 7, 14 and 22; Combination group received Anti-TNF alpha (1.5 mg/kg) administration on days 7, 14, 22 and following CID (100ug/mouse) on day 15; Only CID group received CID (100ug/mouse on day 15). **B.** The average tumor bioluminescence signals measured at specified time points of mice in each group. **C.** Average of Total T cell counts in each group, CID on day 15 is marked with black arrow. **D.** Kaplan-Meier curve showing the survival of mice in each experimental group. P values were determined by log-rank Mantel-Cox test (NTR:Non Transduced).