Supplementary figure 1

(A) HD PBMCs were CTV labelled and left unstimulated or in the presence of 100ng/mL CD3xCD19 DART or aCD3/aCD28 antibodies. CD71 expression was measured after 4 days. (n=4) (B-C) FACS analysis of lysis of CTV labelled CD19+ cell-lines cocultured with healthy donor T cells in presence of CD3x19 DART. Lysis of Daudi (n=2) and JeKo-1 (n=4) cell-line in 4:1 E:T ratio after 4 days. Figures B and C represent different T cell donors. (D-G) %Dead cells of CTV labelled primary CLL cocultured with healthy donor T cells in presence of CD3xCD19 or CD3xFITC DART. Figure represents raw data from figures 2A-B (D-E) Primary CLL cells cocultured in different 1:1 (D) or 4:1 (E) E:T ratios and with different concentrations of the CD3xFITC and CD3xCD19 DART molecules for 24h(n=3) (F-G) Primary CLL cells cocultured in different 1:1 (F) or 4:1 (G) E:T ratios and with different concentrations of the CD3xFITC and CD3xCD19 DART molecules for 4 days (n=3). (H-I) FACS analysis of lysis of CTV labelled primary CLL cocultured with healthy donor T cells in presence of CD3xCD19 DART. (H) Lysis of unmutated IGHV (n=5), mutated IGVH (n=4), chemorefractory (n=6) and TP53 mutated/17p deleted (n=4) CLL by HD T cells in 4:1 E:T ratio after 4 days. Figure represents different T cell donor than used in Fig 2C. (I) Lysis of venetoclax refractory (n=1) and ibrutinib refractory (n=2) CLL by HD T cells in 4:1 E:T ratio after 4 days. Donor A and B represent two different T cell donors. (J) HD* or CLL PBMCs were CTV labelled and left unstimulated or in the presence of 100ng/mL CD3xCD19 DART or aCD3/aCD28 antibodies. CD71 expression was measured after 4 days. (n=4) *Data from HD PBMCs equal to data in figure S1A.
Supplemental Figure 1

A

\%CD1+ \\

CD4 CD8

B

\% Specific Lysis

Daal Jeko-1

C

\% Specific Lysis

Daal Jeko-1

D

CD3\times CD19 1:1

\% Dead Cells

ng/mL

E

CD3\times CD19 4:1

\% Dead Cells

ng/mL

F

CD3\times CD19 1:1

\% Dead Cells

ng/mL

G

CD3\times CD19 4:1

\% Dead Cells

ng/mL

H

CD3\times FITC 1:1

\% Specific Lysis

ng/mL

I

CD3\times FITC 4:1

\% Specific Lysis

ng/mL

J

CD4

CD8

J Immunother Cancer


Supplementary figure 2

(A) CD40 activation of primary CLL was confirmed by measuring resistance to treatment with 0.01μM ABT-199 for 24h (n=6). (B) Knock-out of BAX and BAK was confirmed by western blot in 4 JeKo-1 clones. (C-D) Viability was assessed of 3T3 (dashed bars) or 3T40 treated (empty bars) CLL cells treated for 24h with ABT-199 and QVD (n=3) (C) or for 48h with CD3xCD19 DART and QVD with HD T cells in a 4:1 ratio (D) (n=2-3). (E) Specific lysis of JeKo-1 cells treated for 24h with 10μM ABT-199 in presence or absence of QVD or co-cultured with HD T cells in 4:1 ratio and 100ng/mL CD3xCD19 DART for 24h in presence or absence of QVD (n=3-6).

Supplemental Figure 2

A

B

C

D

E