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**Supplementary Fig. 1: Antibody-mediated blockade of IFNg in CAR T cells.**

T cells isolated from healthy donors were stimulated with anti-CD3/CD28 Dynabeads for 24 hours before transducing with a lentiviral vector to express anti-CD19 CAR with a 4-1BB costimulatory domain. **a**, Transduction was confirmed by flow cytometry, *representative data*. b, CAR-T were activated with PMA/Ionomycin in varying doses of aIFNg or isotype control (0.25 – 20ug/ml) and % inhibition of IFNg was calculated based on ELISA data from Fig. 1d, *n=5*. **c-d**, IFNgR1 and pSTAT1 expression in Nalm6 (**c**) and Raji (**d**) cells treated with (+) or without (-) 10ng/ml rh IFNg +/- aIFNg blocking antibody (mg/ml) as shown by percent positive cells, *n=3 experiments*. **e**, Gating strategy for IFNgR1 expression on untransduced (UTD) and CAR-T following a 24hr incubation with IFNg blocking antibody, *representative;* *n=5*. **f**, IFNgR1 expression on cancer cell lines +/- IFNg blockade as shown by MFI, *representative;* *n=3 experiments*. **g**, CAR-T viability following incubation with aIFNg blocking antibody at 6 hours post-PMA/Ionomycin, 18 hours post-Nalm6 and 24 hours on resting T cells, *n=5*. **h**, Tumor cell lines were incubated in aIFNg blocking antibody for 24 hours prior to assessing cell viability by flow cytometry, *n=3 experiments*. Data are shown as mean ± s.e.m. with *P* values by one-way ANOVA. *P:* \*\*<0.01, \*\*\*\*<0.0001, ns=not significant.

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**Supplementary Fig. 2. Generation of IFNg knockout CAR-T.**

**a,** SMZ-1 cells were activated with 4mg/ml PMA/Ionomycin (P/I) and assessed for IFNg production by flow cytometry, *representative; n=2 experiments*. **b,c**, Four guides targeting IFNg and a mock GFP guide from the Brunello library were assessed for their ability to knockout IFNg in the SMZ-1 T cell line as shown by FACS plots (**b**) and percent expression compared to untransduced (UTD) cells (**c**), *n=1*. **d**, The top two guides (1 and 2) that knocked out IFNg after P/I stimulation were chosen, incorporated into CAR constructs, and tested for successful knockout in primary human T cells, *n=4-6*. **e,f**, KO and IFNgKO CAR-T (using guide 1) were generated from healthy donors with a similar transduction efficiency (**e**) and efficient knockdown of TCR (CD3) expression (**f**). **g,h,** CD3- CAR-T were sorted and IFNg production determined by flow cytometry following either P/I (**g**) or antigen-specific (**h**) stimulation, *representative; n=3*. **i-k,** KO/IFNgKO CAR-T were assessed by flow cytometry for memory (**i**), chemokine receptors (**j**), and IFNgR1 expression (**k**), *n=3-4*. **l**, KO and IFNgKO CAR-T viability following activation for 6 hours with PMA/Ionomycin or 18 hours with Nalm6 cells, *n=4*. **m-o**, NanoString analysis of BBz KO and IFNgKO CAR-T following a 5-day incubation with Nalm6 cells showing gene heatmap (**m**), PCA plots by donor (top) and CAR (bottom) (**n**) and volcano plot for fold change IFNgKO vs KO (**o**), *n=3*. Two adjusted *P*-value cutoffs are shown at <0.05 (bottom line), <0.01 (top line). Data are shown as mean ± s.e.m. with *P* values by one-way ANOVA. *P:* \*\*<0.01, ns=not significant.

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**Supplementary Fig. 3: IFNg may be differentially required for effective CAR-T therapy of hematologic and solid malignancies.**

**a,b,** Healthy donor T cells were used to generate KO/IFNgKO CAR-T with a CD28 costimulatory domain (**a**) and specific lysis of Nalm6 target cells was performed using the Incucyte (**b**), *n=5*. **c,** CAR constructs targeting BCMA and using a 4-1BB costimulatory domain, with or without guide sequences to TRAC and/or IFNg, were created. **d,** BCMA BBz CAR-T were incubated overnight with the multiple myeloma cell line RPMI-8226 at varying E:T ratios and IFNg, GM-CSF, TNFa, and Granzyme B expression was assessed by ELISA, *n=5*. **e,f**, Luciferase-based killing assay of BCMA BBz with aIFNg blocking antibodies (**e**; *n=5*) or BCMA KO/IFNgKO BBz CAR-T (**f**; *n=3*) for both MM.1S and RPMI-8226 cell lines. **g-i**, Mesothelin-specific KO/IFNgKO BBz CAR-T were generated using the constructs shown in **g** and killing of tumor cell lines BxPC-3 and Capan-2 was observed using luciferase-based (**h**) and real-time Incucyte (**i**) assays, *n=5*. **j,k,** BBz CAR-T targeting the EGFR antigen were generated (**j**) and assessed by luciferase-based killing assays in the absence or presence of aIFNg blocking antibodies (**k**), *n=5*. Data are shown as mean ± s.e.m. with *P* values by one-way ANOVA (antibody blockade) or unpaired t-tests (genetic knockout). For antibody blockade, statistics shown as BBz vs. BBz + 5mg/ml aIFNg (turquoise), BBz vs. BBz + 20mg/ml aIFNg (red), or BBz vs. both aIFNg concentrations (black). *P:* \*<0.05, \*\*<0.01, \*\*\*<0.001, \*\*\*\*<0.0001, ns=not significant.

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**Supplementary Fig. 4: IFNg deletion reduces immune checkpoint proteins and yields greater 28z CAR-T proliferation.**

**a-c,** BBz KO/IFNgKO CAR-T were generated from healthy donors and combined with Nalm6 cells at an effector-to-target (E:T) ratio of 1:10 plus or minus donor-matched GMCSF-activated macrophages (1E:10T:0.02M) for five days prior to assessment of CAR-T proliferation (**a**; Incucyte) and immune checkpoint proteins by flow cytometry (**b**-representative MFI, **c**-percent positive cells), *n=3-5*. **d-f,** 28z KO/IFNgKO CAR-T were generated from healthy donors and combined with Nalm6 cells at a 1:10 E:T ratio plus or minus donor-matched GMCSF-activated macrophages (1E:10T:0.02M) for five days prior to assessment of CAR-T proliferation (**d**; Incucyte) and immune checkpoint proteins by flow cytometry (**e**-representative MFI, **f**-percent positive cells), *n=5*. Data are shown as mean ± s.e.m. with *P* values by one-way ANOVA. *P:* \*<0.05, \*\*<0.01, \*\*\*<0.001, ns=not significant.

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**Supplementary Fig. 5: Increased IFNg, IL-6, IL-10, IP-10, MCP-1, and MIP-1b correlates with clinical CRS and neurotoxicity.**

Serum was collected from lymphoma patients 2-5 days after receiving tisagenlecleucel or axicabtagene ciloleucel CAR-T products and analyzed by Luminex. Individual data in **a,b** is graphed by color to denote CRS status: white (no CRS), green (grade 1; G1), yellow (grade 2; G2) and red (grade 3; G3) as well as by pattern to denote neurotoxicity (NT): no stripes (no NT), stripes (NT). NG=no grade. **c**,**d**, Patient data was combined and graphed as mean value with black arrows denoting the markers that were upregulated in CRS/NT patients (bottom row) compared to control patients who did not develop CRS or NT (top row), *n=6 patients/treatment*.

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**Supplementary Fig. 6:** **GMCSF-activated macrophages best mimic CRS *in vitro*.**

**a-c**, PBMC and monocytes were isolated from healthy donors and used directly, or monocytes were differentiated into macrophages (M0, GMCSF-activated, M1, M2). Cytokine/chemokine profiles were determined at baseline (**a**) or 48 hours after treatment with supernatant from BBz CAR-T/Nalm6 (**b**) or 28z CAR-T/Nalm6 (**c**) cultures, *n=5*. **d**, Serum collected from lymphoma patients 2-5 days post-treatment with tisagenlecleucel or axicabtagene ciloleucel CAR-T products was added to healthy donor GMCSF-activated macrophages *in vitro* and functional profile was determined using Luminex 48 hours later. Data is shown as mean values with black arrows indicating cytokines/chemokines that were upregulated in macrophage cultures treated with CRS/NT serum (bottom row) compared to control patients with no CRS/NT (top row). Data are shown as mean ± s.e.m. with *P* values by one-way ANOVA. *P:* \*<0.05, \*\*<0.01, \*\*\*<0.001, \*\*\*\*<0.0001, ns=not significant.

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**Supplementary Fig. 7: Loss of IFNg yields decreased macrophage function.**

**a,** BBz and 28z KO CAR-T were combined with Nalm6 target cells with or without donor-matched GMCSF-activated macrophages at a 1E:1T or 1E:1T:0.02M ratio for 48 hours and supernatant was analyzed by Luminex, *n=3*. **b-d,** BBz and 28z KO and IFNgKO CAR-T were generated from healthy donors, combined at a 1E:1T ratio with Nalm6 target cells (**b,c**) or with donor-matched GMCSF-activated macrophages and Nalm6 cells at a 1E:1T:0.02M ratio (**d,e**), and supernatant was collected and analyzed by Luminex at 6, 24, 48, and 72 hours, *n=5*. Data are shown as mean ± s.e.m. with *P* values by unpaired t-tests. *P:* \*<0.05, \*\*<0.01, \*\*\*<0.001.

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**Supplementary Fig. 8: IFNgKO CAR-T reduce macrophage activation in multiple models.**

**a,b,** KO/IFNgKO CAR-T from Fig. 5c were generated from healthy donors and combined with Nalm6 cells for 24 hours prior to supernatant collection and addition to donor-matched GMCSF-activated macrophages. 48 hours later, macrophages were fixed and stained for CD86, pJAK2, and pSTAT1 for both BBz (**a**) and 28z (**b**) cultures, *representative; n=2* (magnification 63x). Scale bars = 10mm. **c,d,** Serum from Nalm6-bearing NSG mice treated with KO or IFNgKO CAR-T (Fig. 6) was added to donor-matched GMCSF-activated macrophages *in vitro*. 48 hours later, macrophages were fixed and stained for CD86, pJAK2, and pSTAT1 for both BBz (**c**) and 28z (**d**) cultures, *representative; n=2* (magnification 63x). Scale bars = 10mm.

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**Supplementary Fig. 9: IFNg blockade reduces function in both macrophages and monocytes.**

**a,b**, CAR-T were combined with Nalm6 cells for 24 hours prior to supernatant collection and addition to donor-matched GMCSF-activated macrophages +/- blocking antibodies against IFNg, IL-1Ra, and IL-6R and cytokines were assessed 48 hours later for BBz (top) and 28z (bottom), *n=3*. **c**, Healthy donor monocytes were expanded into M0, M1, M2, and GMCSF-activated subsets and combined with donor-matched KO/IFNgKO CAR-T and Nalm6 target cells at a 1E:1T:0.02M ratio (individual macrophage groups or mixed macrophage--MM--containing an equal number of each subset). Supernatant was collected after 48 hours and assessed by ELISA, *n=1*. **d,e,** CAR-T were combined with Nalm6 cells for 24 hours prior to supernatant collection and addition to donor-matched monocytes +/- blocking antibodies against IFNg, IL-1Ra, and IL-6R and cytokines were assessed 48 hours later for BBz (top) and 28z (bottom), *n=3*. Data are shown as mean ± s.e.m. with *P* values by one-way ANOVA. *P:* \*<0.05, \*\*<0.01, \*\*\*<0.001, \*\*\*\*<0.0001.

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**Supplementary Fig. 10: IFNg blockade creates a distinct transcription profile in macrophages compared to anti-IL1Ra and anti-IL6R blocking antibodies.**

Serum from patients receiving tisagenlecleucel or axicabtagene ciloleucel CAR-T products was collected 2-5 days post-CAR treatment, added to healthy donor-derived GMCSF-activated macrophages +/- blocking antibodies to IFNg, IL-1Ra and IL-6R and were assessed 48 hours later. **a**, Cultures receiving serum from tisagenlecleucel patients were assessed by Luminex. **b-g,** Macrophages were analyzed by NanoString, and data is shown by direct global significance score (compared to no treatment (NT)) (**b**), PCA analysis (**c**), heatmap of all data (**d**), volcano plot (**e**) and normalized gene counts (**f,g**). Experiments above were repeated using serum from axicabtagene ciloleucel (**h-n**). Data are shown as mean ± s.e.m.