**Supplementary Materials:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table S1: T cell recovery following bead removal | | | | |
|  | Day 3 | | Day 5 | |
|  | **cell count x106/mL** | | **cell count x106/mL** | |
|  | Pre-debeading | Post-debeading | Pre-debeading | Post-debeading |
| #1 | 2.61 | 2.04 | 1.8 | 1.75 |
| #2 | 3.14 | 3.17 | 1.81 | 1.81 |
| #3 | 2.74 | 2.6 | 2.7 | 2.5 |

**Fig. S1**

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**Fig. S1 Frequency of CD8+ T cells subsets over time.** Data represents healthy donors and ALL subjects. Data are plotted as mean +/- SD of 9 donors.

**Fig. S2**

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**Fig. S2 Absolute counts of CD4+ T cells subsets over time.** Data represents healthy donors and ALL subjects. Data are plotted as mean +/- SD of 9 donors.

**Fig. S3**

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**Fig. S3 Early harvested CART19 cells show enhanced effector function in ALL subjects.** **A**) Tumor Cytotoxicity of CART19 cells from AII patiants assessed by luciferase assay after 16 hours. CART19 cells were co-cultured with target cells engineered to express luciferase. Graph shows the bioluminescence present in cell lysates at indicated E:T ratios. Mean value from four independent experiments are shown. Paired student t test was used, \*P < 0.05. (**B**) CART19 cells from individuals with ALL and CLL were co-cultured with beads coated with anti-idiotype CD19 for 24 hours. Cellular supernatants were collected after 24 hours of stimulation. Indicated cytokine levels were measured by luminex analysis. Unpaired student t test was used, \*P < 0.05.

**Fig. S4**

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**Fig. S4 Cytokine production is similar in d3, d5 and d9 CART19 cells in healthy donors.** CART19 cells from healthy donors were co-cultured with K562-19 cells or K562-wild type for 24 hours. Cellular supernatants were collected after 24 hours of stimulation. Indicated cytokine levels were measured by luminex analysis. Results represent at least five independent donors. Horizontal line is the mean of each group.

**Fig. S5**

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**Fig. S5 Early harvested CART19 cells show enhanced effector function in healthy**

**donors.** CART19 cells from healthy donors were co-cultured with (A) NALM-6 cells or

(B) chronic lymphocytic leukemia (CLL) patient cells for 24 hours. Cellular supernatants

were collected after 24 hours of stimulation. Indicated cytokine levels were measured by

luminex analysis. Data are plotted as mean ± SD of 3 replicates. (C) CART19 cells from

healthy donors expanded and harvested on day 5 and 9, were co-cultured with NALM-6

cells or CLL patient cells for 120h at a 1:1 E:T ratio. Graph shows total live T cell counts.

Results represent 3 independent donors.

**Fig. S6**

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**Fig. S6 Early harvested cells show superior tumor control.** Graphs show the

duration of response of mice receiving CART19 cells (3x106 and 0.5x106 per mouse)

cultured for 3, 5 or 9 days, as described in Fig 3A.

**Fig. S7**

**Macintosh HD:Users:sabaghassemi:Dropbox:3D CultureManuscript:3d manufacturing:CancerImmunologyResearch_2:20180219_Revision Round 2:Final:The ultimate final version for submission:Figures:3d manufacturing Fig S7.ai**

**Fig. S7 Early harvested CART19 cells show increased potency and persistence in vivo.** Serial quantification of leukemic disease burden by bioluminescence imaging following CART19. NSG mice were injected with Nalm6 leukemic cells on day 0 as described in Fig 3A. Different doses of CART19 cells (3x106, 1x106 and 0.5x106 per mouse) cultured for 5 or 9 days were injected intravenously on day 5 following Nalm6 injection. The leukemic burden was assessed semi-weekly by BLI. Each line represents an individual mouse in the treatment group.

**Fig. S8**

**Macintosh HD:Users:sabaghassemi:Dropbox:3D CultureManuscript:3d manufacturing:CancerImmunologyResearch_2:20180219_Revision Round 2:Final:The ultimate final version for submission:Figures:3d manufacturing Fig S8.ai**

**Fig. S8** **An abbreviated CAR-T culture approach is technically feasible in a GMP large-scale production facility.** (A) Ex vivo expansion of patient-derived T cells following stimulation with anti-CD3/CD28 microbeads using a large-scale cGMP process as described in the materials and methods. Cells were transduced with a CAR directed towards mesothelin (13, 35) and mesoCAR-modified T cells (CART-meso) were harvested on either day 5 or day 9 for further analysis. (B) MesoCAR abundance was measured by qPCR analysis at day 5 and day 9. (C). The surface expression of MesoCAR as measured by flow cytometry at specified time-points. (D) CART-meso cells from patients were co-cultured with mesothelin-expressing target cells. Cellular supernatants were collected after 24 hours of stimulation. Indicated cytokine levels were measured by Luminex analysis. Unpaired student t test was used, \*\*P < 0.01, \*P < 0.05. (E) Tumor Cytotoxicity of CART-meso cells assessed by luciferase assay after 16 hours. CART-meso cells were co-cultured with target cells engineered to express luciferase. Graph shows the bioluminescence present in cell lysates at indicated E:T ratios. Results represent three independent donors. (F) Surface phenotype of CART-meso cells at harvest (day 5 or day 9) by flow cytometric analysis, using the gating strategy shown in panel A of Figure 1.