Autologous CD19 CART manufacturing from whole blood collection for the treatment of autoimmune disease

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Background / Introduction

- Chimeric antigen receptor T (CAR T) cells targeting B-cells have now demonstrated promising clinical responses in refractory autoimmune diseases, including, but not limited to, systemic lupus erythematosus (SLE), myositis, systemic sclerosis, and myasthenia gravis.
- Clinical manufacturing for currently approved autologous CAR T therapies requires leukapheresis to source starting material.
- Apheresis collection may represent a bottleneck to patient access due to limitation of available collection slots.
- We have developed a novel approach to isolate, transduce, and expand CD19 41BBz CAR T cells (CABA-201) from whole blood collections to generate a potent cell therapy product.

Materials and Experimental Design

- Three key studies included:
  - Small-scale split runs with our standard leukapheresis (LUK) process and 80mL whole blood collection (WB).
  - Large-scale runs to determine amount of product from current process starting with 200mL of WB.
  - Small-scale runs using 100mL WB collected from SLE patients.
- Analytics included:
  - Cell counts and viability measured using NC200.
  - CAR T phenotype markers measured using NovoCyte Quanteon.
  - Measurement of proliferative capacity by coculture of CAR T cells with Nalm6 cells for 14 days.
  - Cytotoxicity of target CD19+ Nalm6 was captured via IncuCyte.

Figure 1: Overview of manufacturing processes.
Day –X: Fresh leukopaks (LUK) were washed on the Sepax C-Pro and cryopreserved. Fresh whole blood collections (WB) were processed using the NeatCell ficoll program on Sepax and resulting PBMCs were cryopreserved. All collections were sourced from CGT.
Day 0: Cryopreserved material was thawed then enriched on Prodigy using CD4 and CD8 selection reagents. Positive Fraction was seeded in the Prodigy or in G-Rex 6M in cytokine supplemented media and activated with TransAct.
Day 1: Cells were transduced with LVV.
Day 3: Culture was washed on Prodigy (large-scale) or via centrifugation (small-scale) and resuspended in cytokine and human serum supplemented media, then transferred to either G-Rex 100M or G-Rex 6M.
Day 9: Cells were harvested and cryopreserved. Day 6 in-process samples were collected in small-scale studies only.

Figure 2A: LUK vs WB growth curve

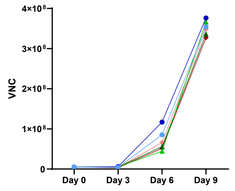


Figure 2B: LUK vs WB CAR expression

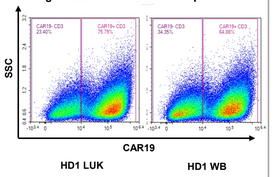


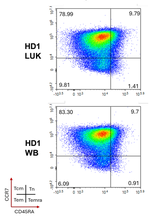
Figure 2: CD19 CAR T cells manufactured at small-scale with split collection of whole blood (WB) and leukapheresis (LUK) from 3 healthy donors (HD) show comparable cell expansion and CAR expression.
A: Cell growth during manufacturing is shown as viable nucleated cells (VNC) over time for three donors. **B:** Representative plots showing CAR expression of split collection of whole blood and leukapheresis from 1 donor. **C:** Percentages of CAR+ population and VNC of small-scale healthy donor split runs are shown for three donors. All cells have high CAR+ population and similar fold expansion.

Figure 2C: %CAR+ and total CAR+ cells

	%CAR19+	Fold expansion	D9 CAR19+ Cells
HD1 LUK	75.8	70.8	2.68E+08
HD1 WB	64.9	75.2	2.44E+08
HD2 LUK	82.1	73.2	3.00E+08
HD2 WB	69.9	67.2	2.35E+08
HD3 LUK	79.4	69.6	2.76E+08
HD3 WB	68.2	65.4	2.23E+08

Results

Figure 4A: Memory phenotype of CAR T cells



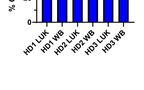


Figure 4B: Phenotype of CAR T cells

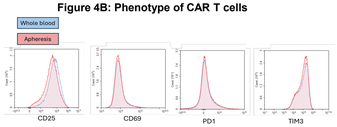


Figure 4C: Proliferation of CAR T cells

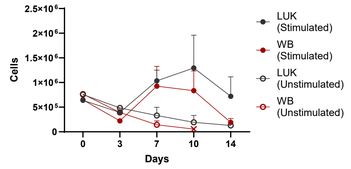


Figure 4. CD19 CAR T cells show comparable T-cell phenotype and long-term proliferation between leukapheresis sourced process and whole blood collection of three healthy donors. **A:** T cell memory phenotype of manufactured cells. Representative flow plot is shown from 1 donor (left), and percentages of each population are shown for 3 donors (right). T cell memory subsets are categorized based on the expression level of CCR7 and CD45RA relative to FMO controls via flow cytometry. Analysis were performed on live CD3+ CAR+ single cell populations. **B:** Activation (CD25, CD69) and exhaustion (PD-1, TIM-3) phenotype of CD3+ CAR+ cells. Representative histograms from 1 donor are shown. **C:** CD19 CAR T cells were co-cultured with target Nalm6 cells at E:T ratio of 1:1 (stimulated) or not co-cultured with target cells as control (unstimulated). T-cell proliferation was followed for 14 days after initial target cell stimulation. Total CAR+ cell numbers are shown as mean + SD across 3 donors. No statistical differences observed between LUK and WB for all time points.

Figure 3A: Nalm6 cytotoxicity (E:T = 1:1)

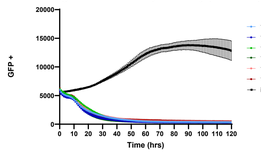


Figure 3B: Nalm6 cytotoxicity (E:T = 0.125:1)

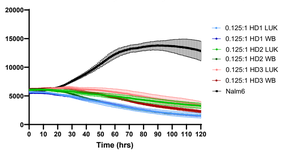


Figure 3: CD19 CAR T cells manufactured from split collection of whole blood and leukapheresis from 3 healthy donors show comparable cytotoxicity. CD19 CAR T cells were co-cultured with target Nalm6 cells at indicated effector to target (E:T) ratios for 120 hours. **A, B:** Cytolysis of wild-type CD19 positive Nalm6 cells was measured using an imaging-based IncuCyte cytotoxicity assay, and GFP+ count is shown as mean ± SD in triplicates. E:T ratio is based on transduced T-cell number. **C:** Area under the curve (AUC) is shown as mean ± SD from the graphs shown in A and B and for all E:T ratios and donors tested.

Figure 3C: Nalm6 cytotoxicity

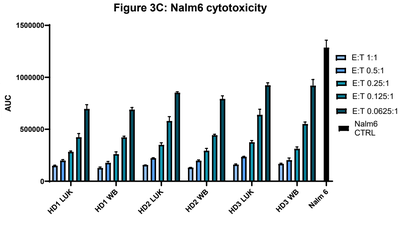


Figure 5A: Growth curve

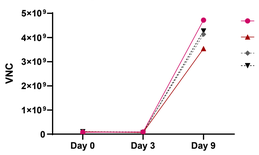


Figure 5C: %CAR+ and total CAR+ cells

	%CAR19+	Fold expansion	D9 CAR19+ Cells
HD4 WB	80.8	60.5	3.81E+09
HD5 WB	66.3	35.8	2.35E+09
HD6 LUK	84.7	41.3	3.50E+09
HD7 LUK	78.4	42.7	3.35E+09

Figure 5B: Nalm6 cytotoxicity

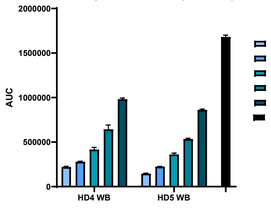


Figure 5. CD19 CAR T cells manufactured at clinical-scale from two healthy donor whole blood collections. **A:** Comparison of 200mL whole blood starting material with platform process. Donors are not matched between whole blood sourced and platform process. Cell growth during manufacturing is shown as viable nucleated cells (VNC) over time. **B:** CD19 CAR T cells were co-cultured with target Nalm6 cells at indicated E:T ratios for 120 hours. Cytolysis of target cells was measured using an imaging-based IncuCyte cytotoxicity assay, and AUC is shown as mean ± SD. E:T ratio is based on transduced T-cell number. Both whole blood conditions demonstrated efficient killing across a variety of E:T ratios. **C**: CAR+ percentages and VNC of clinical-scale runs are shown. Whole blood runs demonstrated similar %CAR and total number of cells compared to platform process runs.

Figure 6A: CAR expression and Nalm6 cytotoxicity

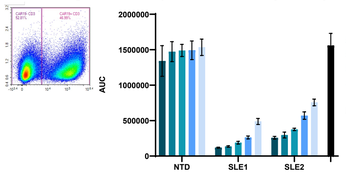


Figure 6C: Phenotype of CAR T cells

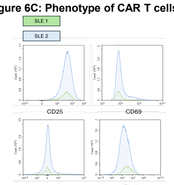


Figure 6B: Memory phenotype of CAR T cells

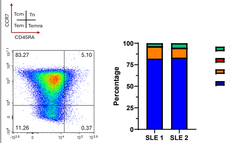


Figure 6. Small-scale runs from 100mL whole blood collection from SLE patients show effective cytotoxicity and expected phenotype. **A:** Representative CAR expression is shown for 1 SLE donor (left). Effector cells [CD19 CAR T and nontransduced (NTD)] were co-cultured with target Nalm6 cells at indicated E:T ratios for 120 hours. Cytolysis of target cells was measured using an imaging-based IncuCyte cytotoxicity assay, and AUC is shown as mean ± SD. E:T ratio is based on transduced T-cell number (right). **B:** T cell memory phenotype of CD3+CAR+ manufactured cells. Representative flow plot is shown for 1 donor (left) and percentages of each population are shown for 2 donors (right). **C:** Activation (CD25, CD69) and exhaustion (PD-1, TIM-3) phenotype of CD3+ CAR+ cells are shown as histograms for both SLE donors.

Conclusions

- Whole blood collections were successfully used in lieu of leukapheresis material to produce CD19 41BBz CAR T cells (CABA-201), across a range of collection volumes from 80 to 200mL, with similar cytolytic activity, phenotype and proliferative ability.
- CD19 CAR T cells produced in a small-scale process using 3 donor matched leukapheresis and whole blood split runs demonstrated similar growth, viability, memory phenotype and cytotoxicity.
- Cells from the same runs yielded comparable long-term proliferation and expressed comparable memory, activation, and exhaustion expression pre- and post-stimulation.
- Large scale runs using 200mL whole blood collections yield similar amounts of CD19 CART cells as platform process runs using leukapheresis material and demonstrated similar cytotoxicity across a range of E:T ratios.
- CD19 CART cells were manufactured successfully from the whole blood sourced from SLE patients and showed expected T-cell memory subtype and cytotoxic functionality.
- To evaluate the potential to replace apheresis with whole blood collection in a routine blood test lab setting, additional studies are being performed to explore donor variability, reliability of PBMC recovery from whole blood, material characterization and stability as well as process robustness.