#P0824

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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 demonstrated promising clinical responses in refractory autoimmune diseases, including, but not limited to, systemic lupus erythematosus (SLE), myositis, systemic sclerosis, and myasthenia gravis.
- CAR T cell dose in autoimmune diseases are lower as compared to oncologic indications, opening the possibility that leukapheresis may not be required.
- 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

- 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 100-200mL of WB.
  - Stability of WB stored at various conditions with different anticoagulants.
- Analytics included:
  - Cell counts and viability measured using NC200.
  - CAR T phenotype markers measured using MACSQuant16.
  - Cytotoxicity of target CD19+ Nalm6 was captured via IncuCyte and luciferase-based cytotoxicity assays.

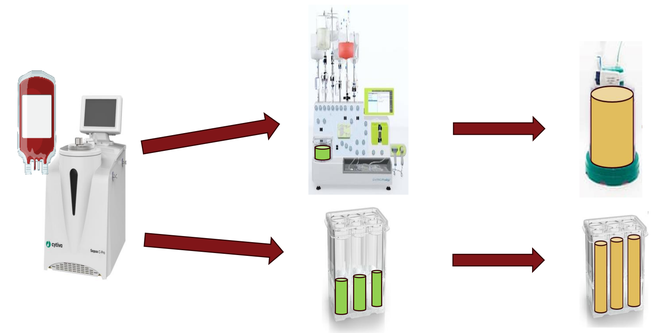
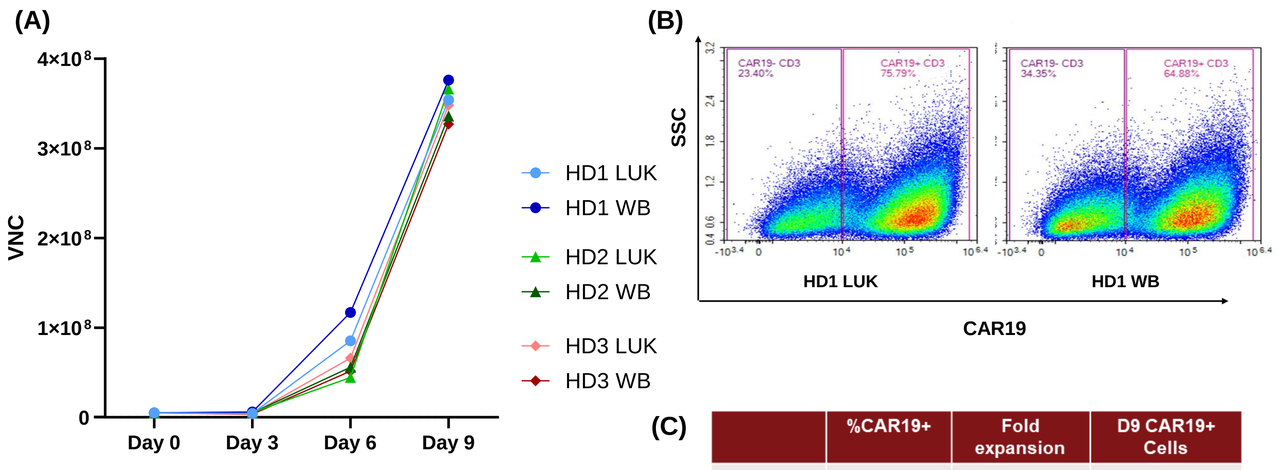


Figure 1: Overview of manufacturing processes

Day –X: Fresh leukopaks (LUK) were washed on the Sepax C-Pro and cryopreserved. Fresh WB collections were processed using the NeatCell ficoll program on Sepax and resulting PBMCs were cryopreserved. All collections were sourced from CGT Global.
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 lentiviral vector (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.

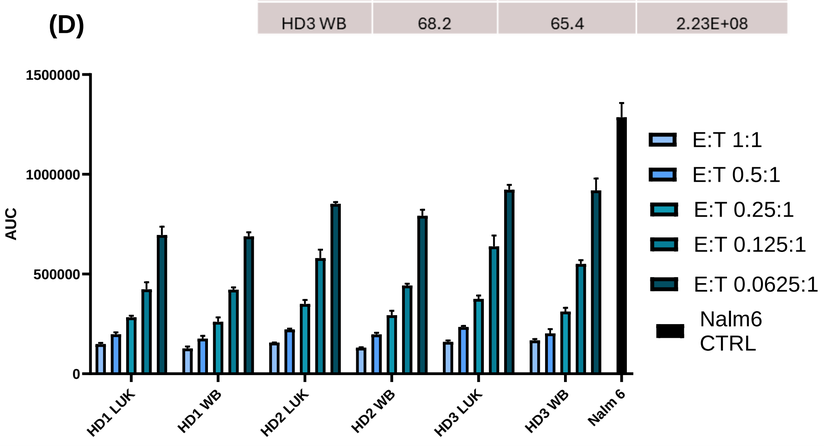
Results

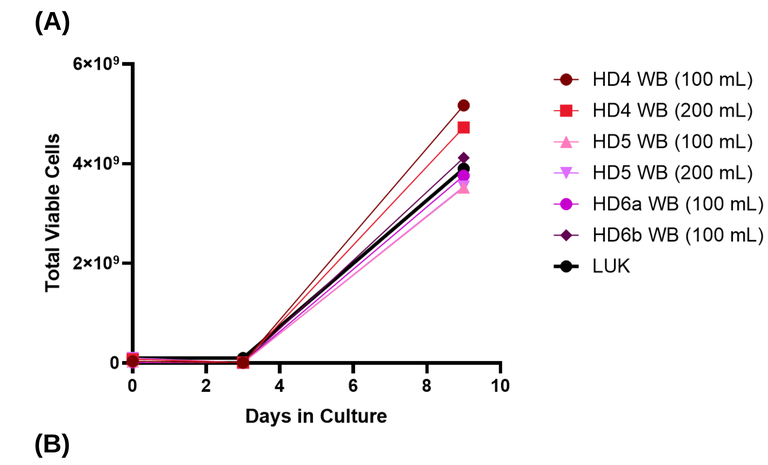


(C)

	%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

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 total number of transduced cells of small-scale healthy donor split runs are shown for three donors. All cells have high CAR+ population and similar fold expansion. **D:** Cells manufactured as shown in A, B, and C were placed in IncuCyte cytotoxicity experiments against a GFP+ CD19+ Nalm 6 cell line. Cytolytic capacity shown as area under the curve (AUC) as mean ± SD.

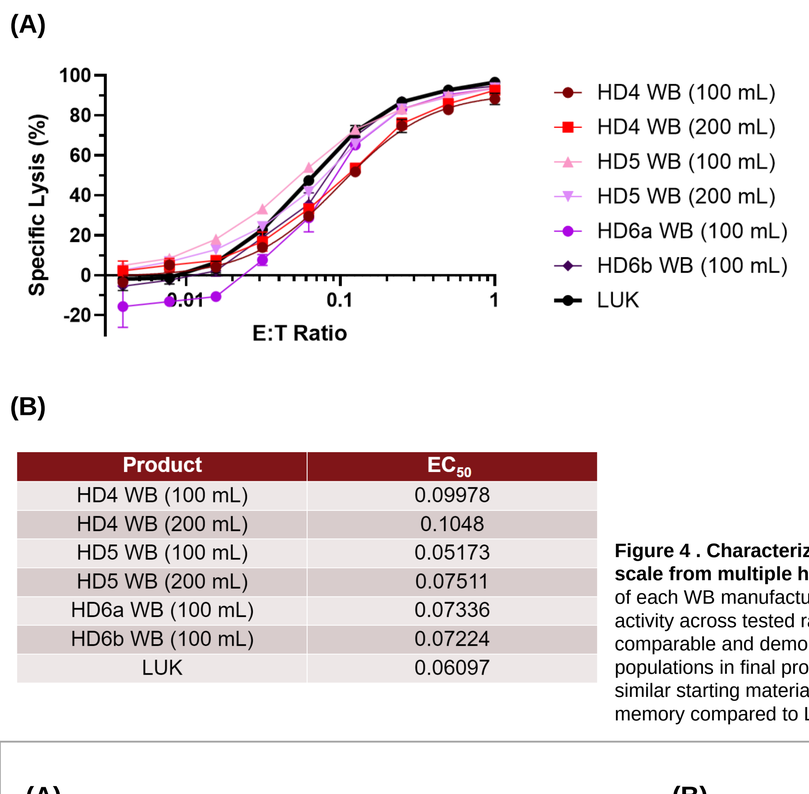




(B)

	%CAR19+	Fold expansion	D9 CAR19+ T cells
HD4 WB (100 mL)	67.5	159.3	3.44e9
HD4 WB (200 mL)	80.8	56.7	3.57e9
HD5 WB (100 mL)	44.9	104.3	1.36e9
HD5 WB (200 mL)	66.3	25	1.64e9
HD6a WB (100 mL)	27.5	298.4	8.97e8
HD6b WB (100 mL)	32.8	250.8	1.09e9
LUK	67.8	22.3	1.77e9

Figure 3. CD19 CAR T cells manufactured at clinical-scale from multiple healthy donor whole blood collections. A: Comparison of 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. Growth was comparable across all runs. **B:** Percentages of CAR+ population and total number of transduced cells are shown for products made using 100-200 mL WB from three healthy donors. Product made from lower volumes of WB have higher rates of expansion and lower transduction rates. **C:** Memory phenotyping of transduced CD4+ and CD8+ T cells is more differentiated in WB product compared to platform process. CD45RA and CCR7 expression was assessed (as shown in Figure 4C).



(B)

Product	EC_{50}
HD4 WB (100 mL)	0.09978
HD4 WB (200 mL)	0.1048
HD5 WB (100 mL)	0.05173
HD5 WB (200 mL)	0.07511
HD6a WB (100 mL)	0.07336
HD6b WB (100 mL)	0.07224
LUK	0.06097

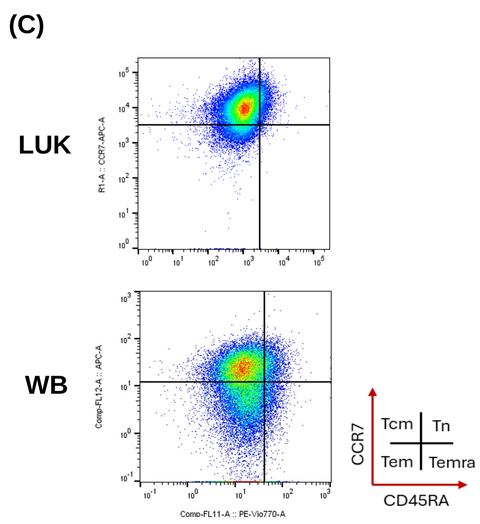


Figure 4 . Characterization of CD19 CAR T cells manufactured at clinical-scale from multiple healthy donor whole blood collections A: Lysis curves of each WB manufactured product with LUK control demonstrated similar activity across tested range. **B**: Calculated EC50 values for each run were comparable and demonstrated good killing. **C:** Representative plots of memory populations in final product. Similar populations are seen across runs with similar starting material. WB runs have more effector memory and less central memory compared to LUK product.

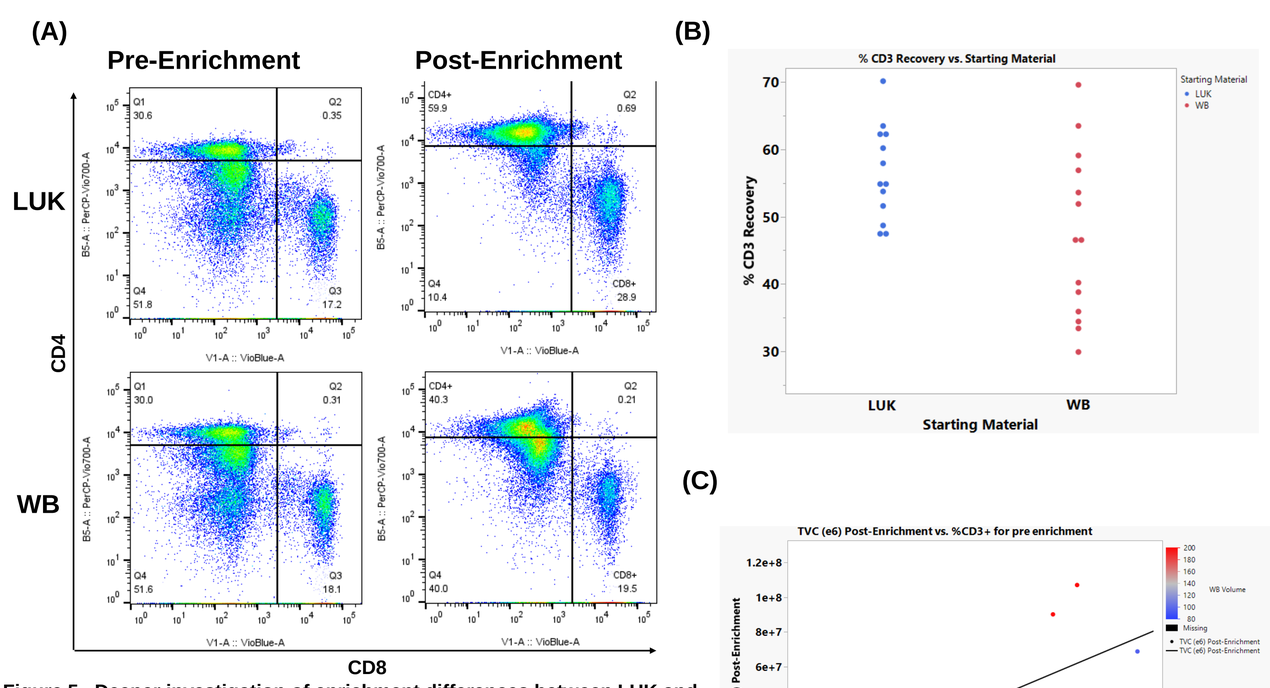


Figure 5 . Deeper investigation of enrichment differences between LUK and WB A: Enrichment of LUK material demonstrates good clearance of $CD4^{-}CD8^{-}$ population from pre to post enrichment. WB retains a significant $CD4^{dim}$ population post enrichment. **B and C**: Relationships between starting material characteristics and enrichment performance were explored, looking at the impact of initial cell number and purity on the enrichment process. CD3+ recovery is lower and more variable in WB compared to LUK. Variability in starting materials between donors directly impacts cell numbers post enrichment.

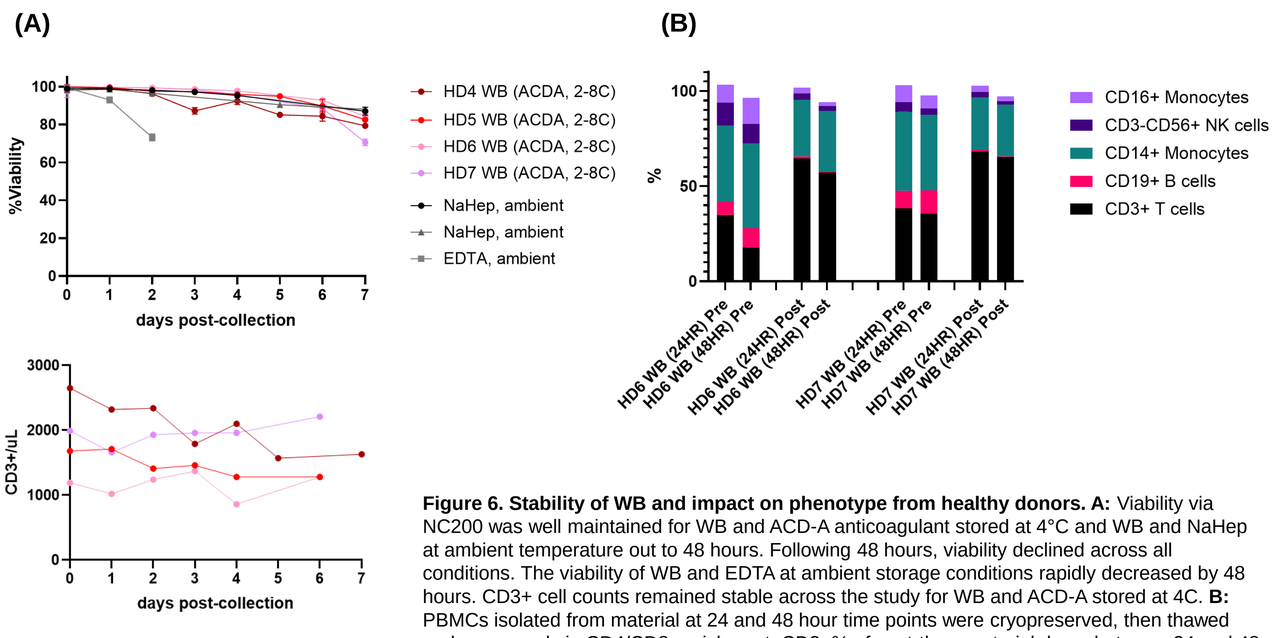


Figure 6. Stability of WB and impact on phenotype from healthy donors. A: Viability via NC200 was well maintained for WB and ACD-A anticoagulant stored at 4°C and WB and NaHep at ambient temperature out to 48 hours. Following 48 hours, viability declined across all conditions. The viability of WB and EDTA at ambient storage conditions rapidly decreased by 48 hours. CD3+ cell counts remained stable across the study for WB and ACD-A stored at 4C. **B:** PBMCs isolated from material at 24 and 48 hour time points were cryopreserved, then thawed and processed via CD4/CD8 enrichment. CD3+% of post-thaw material drops between 24 and 48 hour storage, which is also observed in the post-enrichment phenotyping.

Conclusions

- Donor-matched starting material from three split apheresis/whole blood collections was used to manufacture CD19 41BBz CAR T cells (CABA-201) using a scaled down model of CABA's platform process with similar proliferative ability, transduction and cytolytic activity.
- 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 CAR T cells produced at clinical scale using 100 mL of WB demonstrated higher expansion with a slightly more differentiated memory phenotypic profile compared to the platform process and lower levels of transduction, with higher variability. These products generated at clinical scale show similar cytolytic activity to historical data from product made using the platform process with apheresis starting material and material derived from 200mL of WB.
- Further investigation into CD4/CD8 enrichment performance revealed quantity and/or purity of starting material can impact characteristics of the starting culture at D0 of manufacture, which impact characteristics in the final product.
- Despite maintaining viability, preliminary stability data show that CD3+ populations may not be maintained in WB with ACD-A anticoagulant after being stored for >24 hours at 4C then processed via ficoll, as observed in immunophenotyping of pre- and post-enriched cells.
- 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 healthy and diseased donor variability, reliability of PBMC recovery from whole blood, material characterization and stability as well as process robustness.