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

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Background / Introduction
- Chimeric antigen receptor T (CART) 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 CART T therapies requires leukapheresis to source starting material.
- Apheresis collection may represent a bottleneck to patient access due to limitation of available collection sites.
- We have developed a novel approach to isolate, transduce, and expand CD19+B cell CART 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) and whole blood (WB) collection process.
  - 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. CART T phenotype markers measured using NovoCyte Quantim.
  - Measurement of proliferative capacity by coculture of CART T cells with naïve cells for 14 days.
- Cytotoxicity of target CD19+ Nalm6 was captured via IncuCyte.

Results
- CD19 CART T cells were manufactured in a single cell suspension and at a ratio of 1:1 (stimulated) or not co-cultured with target cells as control (unstimulated).
- T cell proliferation was followed for 14 days after either target cell addition. Total CART and CAR+ population are shown as histograms for both SLE donors.
- Figure 6B: Phenotype of CAR T Cells
  - CD19 CAR T cells were manufactured successfully from the whole blood, material characterization and stability as well as process range of collection volumes from 80 to 200mL, with similar cytolytic activity, phenotype and proliferative ability.
- CD19 CART 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 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 function.
- To evaluate the potential to replace apheresis with whole blood collection in a routine blood 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.

Conclusions

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Conclusions
Whole blood collections were successfully used in lieu of leukapheresis material to produce CD19 41BB8 CART T cells (CABA-201), across a range of collection volumes from 80 to 200mL, with similar cytolytic activity, phenotype and proliferative ability.
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