Development of a High Yield, Fully Automated ONCT-808 Autologous ROR1 CAR T Cell Clinical Manufacturing Process

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Background: The six FDA approved autologous CAR-T cell therapies for hematological cancers have shown great effectiveness and the potential of T cell-based immunotherapy in treating cancers that are resistant to standard chemotherapy and radiation therapy. However, critical manufacturing challenges still exist, including scale translation and transfer and the inherent process variability due to the inclusion of multiple manual operation steps. These challenges can lead to contamination, operator errors, production failures, and extended vein-vein times. Consequently, many eligible patients cannot be treated.

Methods: To address these issues, Oncternal has developed a fully closed, robust and automated manufacturing process for ONCT-808, an autologous ROR1 CAR T cell product, using the CliniMACS Prodigy platform. ONCT-808 cells are genetically modified via ex vivo transduction with lentivirus vector to express a CAR construct containing a ROR1-directed single chain variable fragment (scFv) derived from Oncternal's investigational anti-ROR1 mAb zilovertamab. For process development, manufacturing was started with $CD4^+$ and $CD8^+$ cells enriched from frozen or fresh apheresis materials from healthy donors as well as a late-stage solid cancer patient and chronic lymphocytic leukemia patient. A number of critical parameters were evaluated during the ONCT-808 manufacturing process development and verification, including seeding cell number, research vs GMP grade lentivirus vector, lentivirus MOI, culture medium exchange scale and frequency, presence or absence of human AB serum, and manufacturing sites.

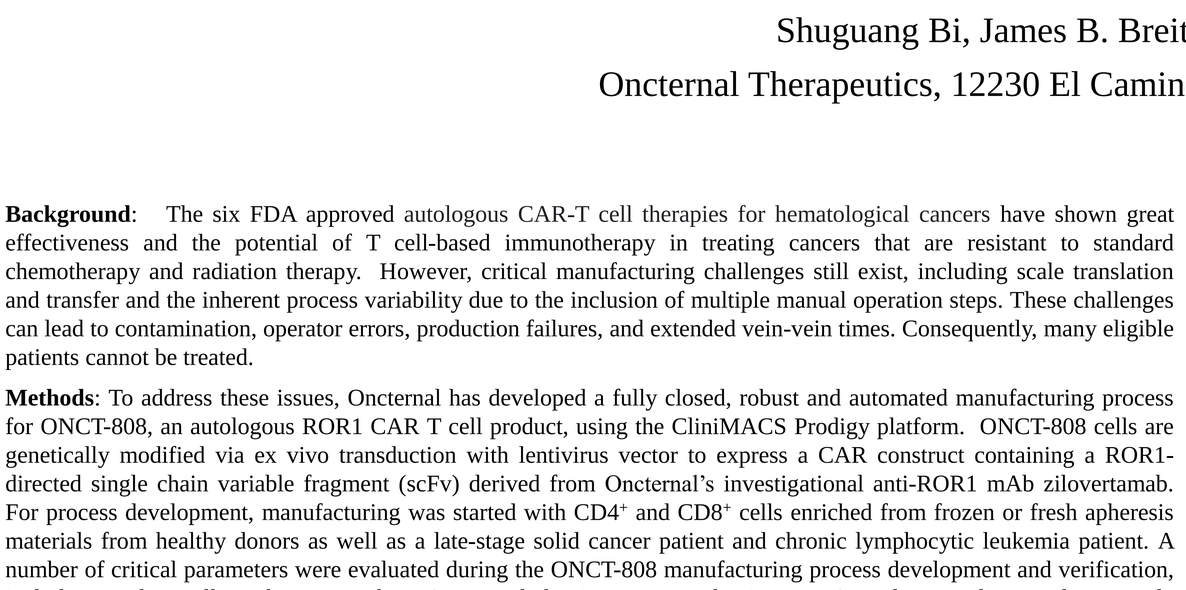
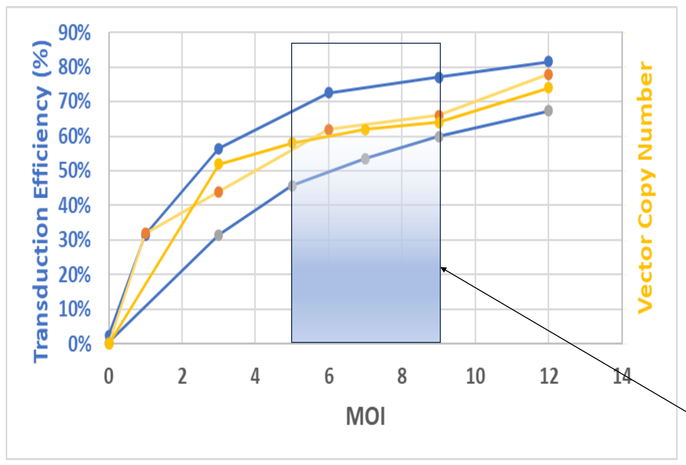
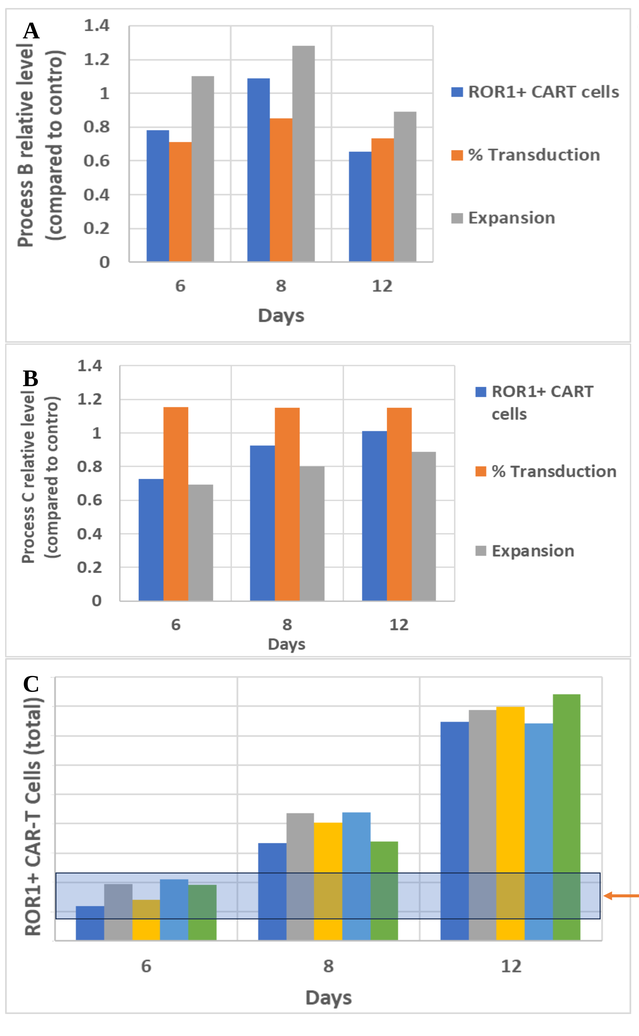


Figure 1: Four stages of ONCT-808 process development.
Starting default variables include standard CliniMACS T cell transduction (TCT) program, cytokine concentration and type, medium, and a 12 day process.



- 2 lentivirus lots: 4-L GMP and 50-L GMP
- Healthy donor leukopak (2 donors)
- Target: transduction efficiency > 45% and VCN < 3.5 copies/transduced cell above target cell count and viability
- Target MOI range identified between minimum transduction % and maximum VCN

Figure 2: Stage 1- Establishment of Target Multiplicity of Infection (MOI)
To establish target MOI range, effect of MOI on transduction efficiency and vector copy number (VCN) was determined. Primary T cells were enriched from apheresis material from healthy donor, followed by T cell seeding and activation, transduction with different volumes of lentivirus, and T cell expansion. T cell transduction rate was determined using flow cytometry to detect expression of ROR1+ CAR in the product T cells. Vector copy number was determined with qPCR. Two different lots of the lentivirus were used for the study.



- 12 days process with partially modified Miltenyi TCT as control
  - Four runs; 2 healthy donors
  - All runs achieve > 5 billion viable cells with >98% viability and > 50% transduction
  - Modify TCT program along with serum concentration
  - Higher expansion and viable cell count by day 8
  - Lower overall yield by day 12
- 12 days process with partially modified Miltenyi TCT as control
  - Two runs; 1 heathy donor
  - All runs achieve > 4.5 billion viable cells with >97% viability and > 70% transduction
  - Newer TCT program with media exchange
  - Higher % transduction with equivalent cell count with modified process
  - However, higher VCN and differentiated cell population
- 6-8 day process achieves sufficient viable cell counts and transduction efficiencies for planned clinical study
  - Reducing the culture duration would also reduce vector copy number and increase concentration of juvenile-like cell phenotype
  - **Decision: Revise process to 8 days**

Figure 3: Stage 2 and 3 Development
(A): The standard TCT program was modified to initiate the process development runs. T cells enriched from apheresis material from each of the two healthy donors were seeded into two CliniMACS Prodigy systems, and manufactured simultaneously using the same TCT program except that the human AB serum concentration was varied;
(B) New TCT program with more aggressive medium exchange plan was used for CAR T cell manufacturing. This run has two arms. Each arm used the same TCT program but the human AB serum concentration was varied;
(C) Cell expansion time was reduced to 8 days and TCT program was further optimized.

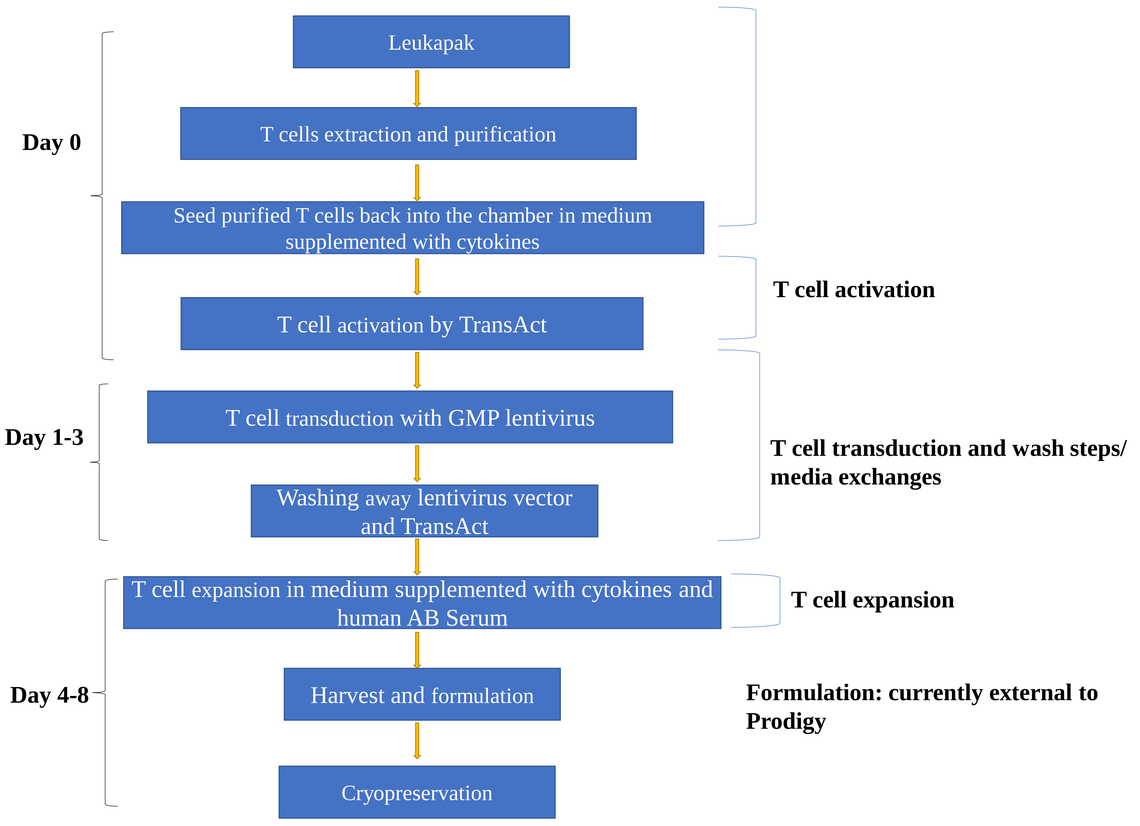


Figure 4: Optimized GMP CAR T manufacturing process for clinical manufacturing

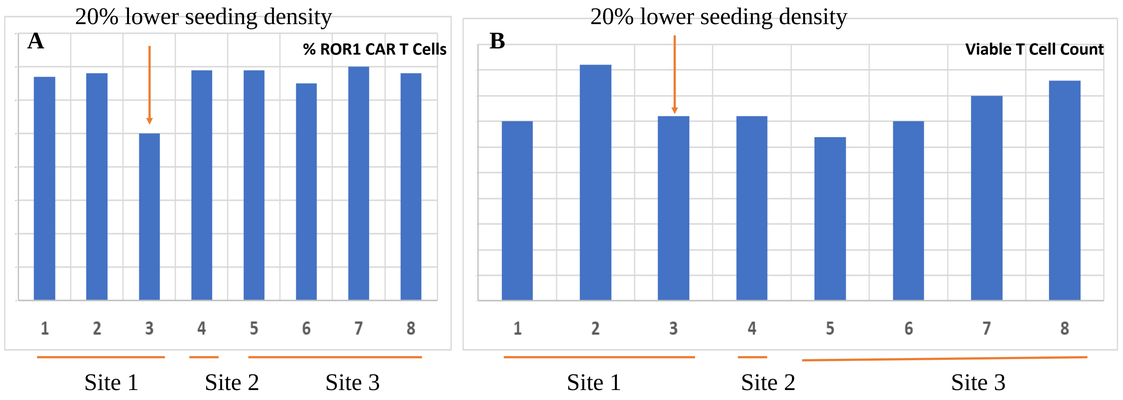


Figure 5: Stage 4 - Process Development and Confirmation
Manufacturing runs at the same production scale were conducted at 3 independent manufacturing sites with apheresis from 7 healthy donors (men, women, large age and BMI range).
(A) Percentage of ROR1 CAR T cells in the final product manufactured at three different sites at same Prodigy scale was within 5% difference, except for run 3 that differed by 10% due to a lower seeding density;
(B) Total viable T cell counts and vector copy numbers of the final product manufactured at three different sites were within 16% difference of each other, while viability was within 3% difference of each other.

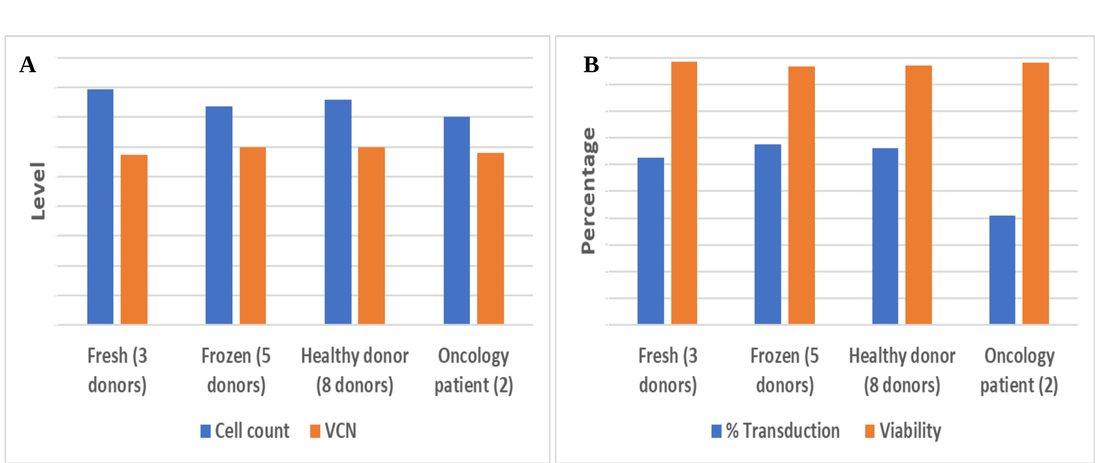


Figure 6: Impact of Fresh vs Frozen Apheresis and Healthy Donor vs Cancer Patient
ONCT-808 manufacturing was not significantly impacted by starting apheresis material (fresh or frozen), or from a healthy donor or cancer patient.
(A) Status of apheresis, fresh or frozen, had little impact on the final product yield or VCN;
(B) Whether the apheresis was fresh or frozen, or whether the donor was healthy donor or cancer patient had no impact on the cell viability. Use of apheresis material from a cancer patient led to lower % transduction, but still above specifications.

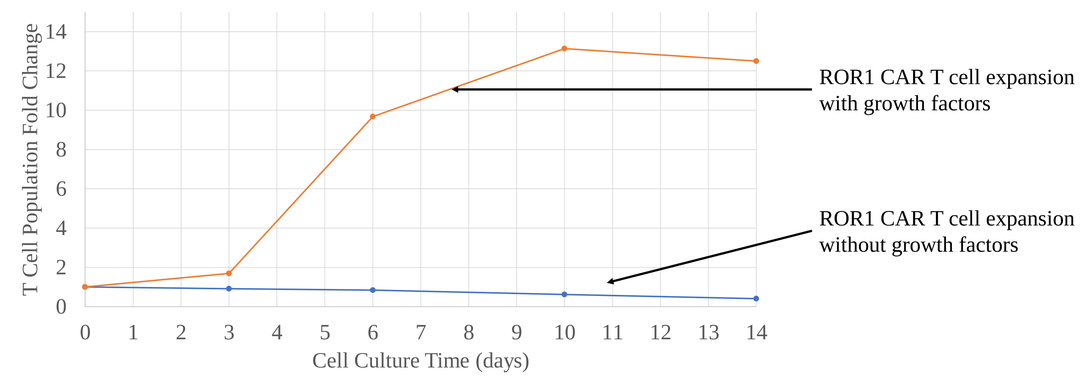


Figure 7: Transformation Study with Final ONCT-808 Process
Cell expansion relied on growth factors. Without growth factors, ONCT-808 cells could not divide. Therefore, ONCT-808 cells were not transformed.

Results: With the 8-day automated manufacturing process, greater than 3 billion T cells, including approximately 2 billion ROR1 CAR-positive T cells of mainly memory phenotypes were harvested from healthy donor apheresis. For manufacturing started with late-stage solid cancer patient and CLL patient apheresis, greater than 2 billion T cells, including approximately 1-2 billion ROR1 CAR-positive T cells were harvested. Analysis of ONCT-808 manufacturing from healthy donors showed that only a small fraction of the CAR T cells were terminally differentiated with fewer than 5 vector copies per transduced T cell, and undetected RCL. The majority of product T cells exhibited phenotypes with potential for favorable persistence and expansion in vivo.

Conclusion: A closed, high yield, robust and high-performance CAR T manufacturing process was confirmed.