Reducing CAR-T Manufacturing Costs and Duration Using Next-generation Cell Therapy Manufacturing Platform.

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Introduction.

Chimeric Antigen Receptor T-cell (CAR-T) therapies have shown remarkable success in treating B-cell malignancies, yet access remains limited due in large part to manufacturing hurdles. Current CAR-T cell manufacturing relies on outdated methods and technologies, resulting in high costs, variable product quality, and limited scalability. Oribiotech has launched a new cell therapy manufacturing platform (IRO®) specifically to address these manufacturing challenges while improving biological performance.

Figure 1: IRO cell therapy manufacturing platform.

IRO's core innovations include a bellows-based bioreactor, which allows for customizable mixing and the OriConnect™ tubeless sterile connection technology enabling automated fluid handling. This case study demonstrates how a novel mixing protocol in IRO can be used to improve transduction efficiency, significantly reducing viral vector input to achieve an equivalent CAR-T cell yield, shorten processing times and ultimately lower cost of goods (COGs).

Materials and Methods.

Three different Multiplicity of Infections (MOIs) of 1.0, 0.5 and 0.25 (two biological replicates for each MOI) were tested using CD19 CAR lentivirus (scFv-41BB-CD3ζ, FMC63) in IRO and a widely used, first generation manufacturing platform (control).

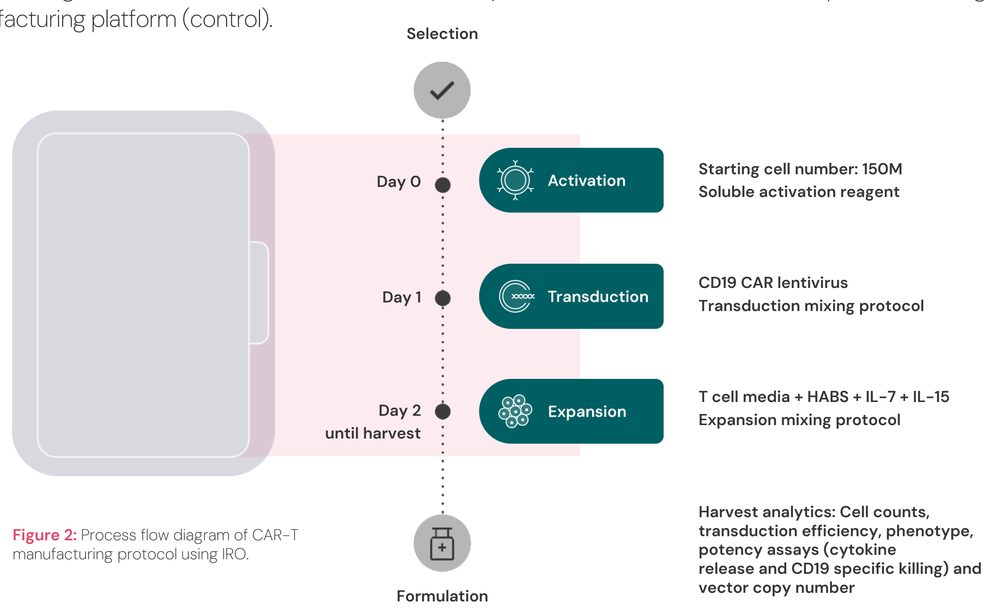

Figure 2: Process flow diagram of CAR-T manufacturing protocol using IRO.

Results.

IRO achieved a higher average CAR-T cell yield using MOI 0.25 and 0.5 compared to the control using MOI 1.0

The IRO platform, with its proprietary transduction mixing protocol, achieved a higher transduction efficiency compared to the "gold-standard" platform (control) across three different MOI's (Figure 3B). The transduction mixing regime has been optimized to promote cell-cell and virus-cell interaction, which accounts for the higher transduction efficiency. IRO also enabled cell culture to reach higher cell densities (Figure 3A) and, together with improved transduction, meant the CAR-T cell yield at harvest was higher than the control. Figure 3C shows that IRO achieves a higher yield of CAR-T cells using MOI 0.25 and 0.5 compared to the control using MOI 1.0. This demonstrates the possibility of reducing the amount of lentiviral vector required to achieve a target CAR-T cell yield and ultimately reducing COGs. The improved transduction efficiency and cell expansion allows the target CAR-T cell yield to be achieved earlier in IRO thereby providing the opportunity for reduced process duration to capture further COGs savings (Figure 3D).

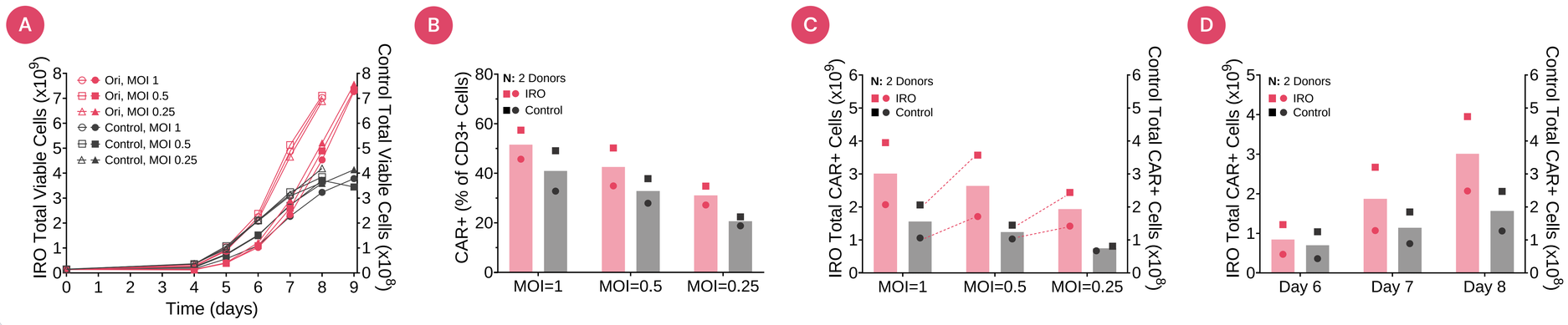

Figure 3: Biological performance of CD19 CAR-T production. **A)** Cell growth profile showing total viable cells in IRO and control transduced at three different MOIs each (1.0, 0.5 and 0.25). Open and closed symbols represent different donors. **B)** Transduction efficiency at different MOIs assessed using flow cytometry. **C)** Total CAR+ cell yield in IRO and control at Day 8. Dotted lines show 40-75% increase in total CAR-T cells using IRO compared to the control while using 50% less viral vector. **D)** Total CAR+ cell yield in IRO and control at Day 6, 7 and 8 using MOI 1.0. Note: Data in Fig. 3B, C and D shows mean of the two donors (circle and square represent donor 1 and 2 respectively). A scale-down vessel (10x smaller) of a widely used cell expansion system was used as a control.

IRO produces CAR-T with potent anti-tumor response: CAR-T cells generated using MOI 0.25 and 0.5 in IRO performed comparably to an MOI 1.0 in control

The IRO platform generated potent CAR-T cells that killed CD19+ tumor cells (Figure 4A) and produced IFNγ, IL2 and TNFα following co-culture with CD19+ tumor cells (Figure 4B). Minimal non-specific killing or cytokine release was measured following co-culture with CD19- K562 (Figure 4A, cytokine data not shown). CAR-T cells generated using lower MOI in IRO (0.5 shown in Figure 4A+4B; and 0.25, data not shown), performed comparably to CAR-T cells generated using MOI 1.0 in control. Furthermore, product phenotype was comparable, with no statistically significant differences across platforms, with cells consisting of majority Tcm phenotype (Figure 4C). Vector copy number (VCN) remained below 3 VCN per CAR+ cell across MOI tested, as per FDA guidance; with VCN positively correlating with MOI used.

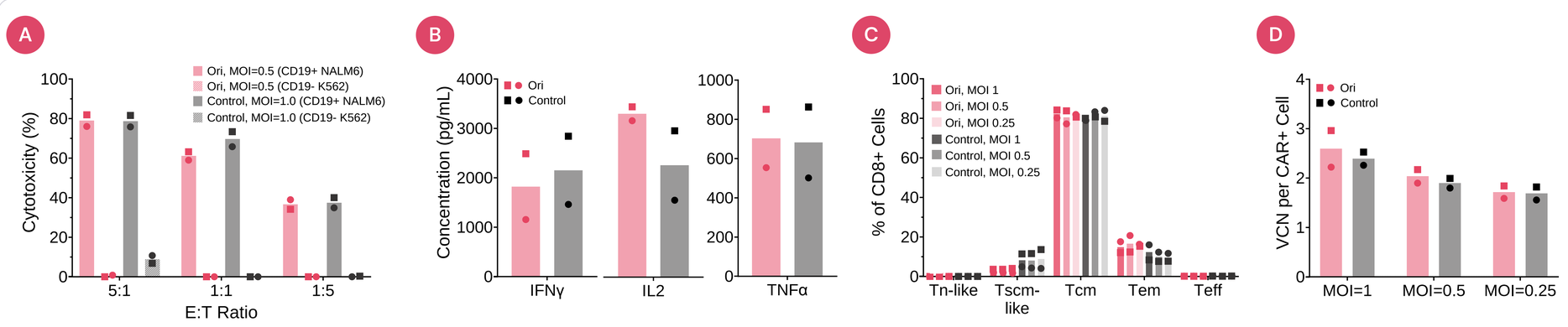

Figure 4: CD19 CAR-T product characterization. CD19 CAR-T (scFv-41BB-CD3ζ, FMC63) cultured in IRO using MOI 0.5 and cultured in Control using MOI 1.0, were assessed in potency assays (A+B). **A)** CD19 CAR-T cells were analyzed using an impedance-based killing assay (xCelligence, Agilent), with different effector to target (E:T; CAR-T:tumor cell) ratios. CAR-T were co-cultured with CD19+ NALM6 or CD19- K562. Killing (%) was assessed at 24hrs post effector cell addition. **B)** CD19 CAR-T were co-cultured 1:1 with CD19+ NALM6 and cell culture supernatant collected after 24hrs. Cytokine concentrations were assessed using Ella immunoassay. **C)** T cell memory phenotype was assessed using flow cytometry (BD FACSLyric). CD45RO, CCR7, CD95 were used to define memory subsets. Tn-like (naïve-like), Tscm-like (stem cell memory like), Tcm (central memory), Tem (effector memory), Teff (effectors) **D)** Vector copy number (VCN) was assessed using ddPCR assay. Note: Data shows mean of the two donors (circle and square represent donor 1 and 2 respectively).

Conclusion.

IRO could reduce process COGs by ~40%

Optimizing transduction efficiency with IRO can reduce the lentiviral vector requirement and the time needed to achieve the target CAR-T yield. When considering these advantages along with the full suite of benefits that the automated IRO platform provides, Ori expects IRO to deliver a 30-50% reduction in COGs (actual cost reductions are process dependent). Reducing the COGs in cell therapy manufacturing is crucial to making these life-saving treatments more accessible and affordable for patients, supporting broader adoption and improving patient outcomes.

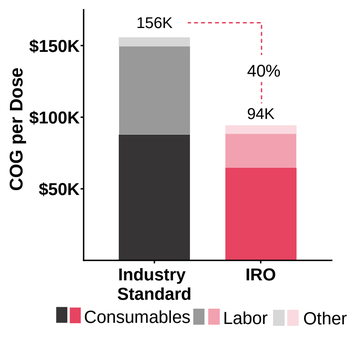

Figure 5 : Model of the COGs per dose between the industry standard and IRO. The cost model assumes using an MOI of 0.5 for the transduction step.

Acknowledgments

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