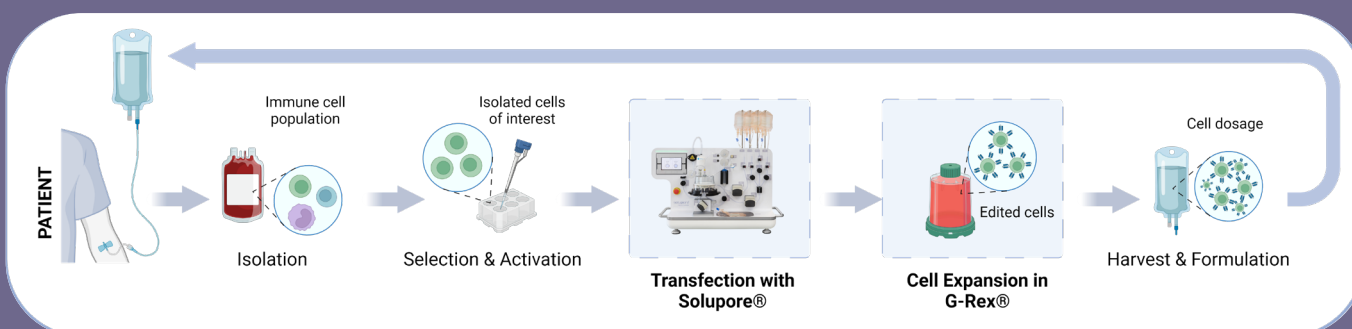


Enhanced Stem Cell Memory and Cytotoxic Function in Engineered T Cells Expanded for 7 days to $>1.3 \times 10^9$

Using Solupore® Transfection and IL-7 & IL-15 in the G-REX® 100M System



The Solupore® transfection system developed by Avectas offers an automated, closed, reproducible single-use solution for delivering a wide variety of cargo (RNA, DNA, protein) to primary immune cells used in clinical cell therapy applications. Solupore offers a gentler transfection approach, focusing on the health and function of the transfected cell population. This results in retaining effector cells that are both healthy and highly functional. Moreover, when cultured in a G-REX® (Wilson Wolf Manufacturing, LLC) system, these cells are comparable to their untransfected counterparts.

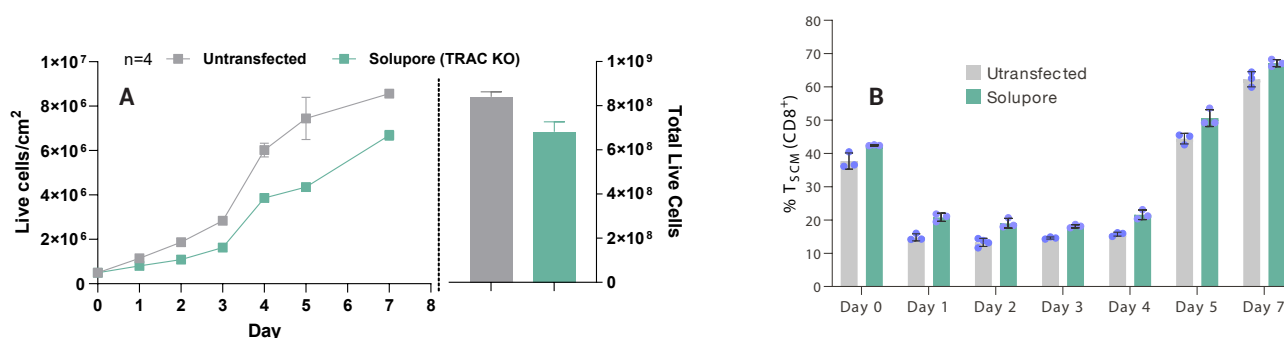


Figure legend: A) T cell proliferation in 6M G-REX plates comparing untransfected control to Solupore transfected cells (Cas9 RNP targeting TRAC). B) Stem cell memory subpopulation (TSCM) measured by flow cytometry. C) G-REX 100M cell yield between untransfected cells and Solupore transfected cells after 7 days using IL-7 and IL-15.

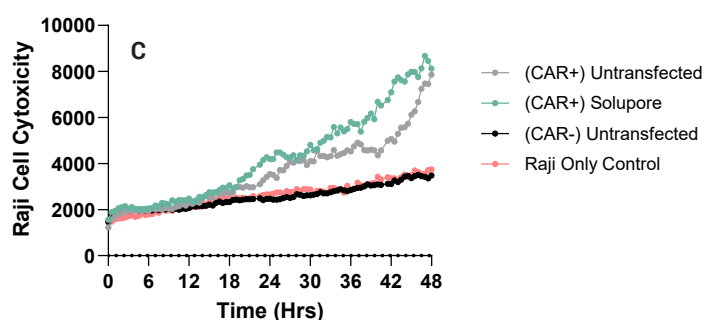


Figure legend: C) Cells were harvested from the 6M G-Rex plates on day 2, seeded in a 96-well plate, and were transduced with a lentiviral vector for the expression of anti-CD19 CAR for 24 hours. The CAR-T cells (80% CAR+ on Day 3) were then co-cultured with Raji cells at a ratio of 1:1 and the Raji cell cytotoxicity was measured using the Incucyte live cell imaging system for 48 hours.

Condition	Starting Input	Fold increase over 7D	Final output of live cells	Final % T _{SCM}	Final output of TRAC KO cells
Untransfected*	1.0 x 10 ⁸	16.8x	1.68x10 ⁹	62%	NA
Solupore	1.0 x 10 ⁸	14.4x	1.36x10 ⁹	67%	8.3x10 ⁸

Summary: Solupore transfection of cas9 RNP targeting TRAC for knock-out yielded healthy, highly functional cells when stimulated for 7 days with IL-7 and IL-15 in G-REX systems. With improved retention of TSCM cells and a yield of over 800 million TRAC knocked-out cells, we demonstrate more potent cellular cytotoxicity when compared to untransfected cells.

