Closed and semi-automated processing of CAR T cells

There is considerable excitement surrounding the treatment potential of T cell immunotherapies. Despite the clinical success of chimeric antigen receptor (CAR) T cells, there remain challenges associated with routinely offering these products as treatment alternatives. These challenges include the costly manufacturing process relying on lengthy and complex open workflows with high manual labor requirements that influence product variability. This application note describes the details of a robust CAR T cell manufacturing workflow that can be adapted for cGMP compliance in commercial production of CAR T cells. This semi-automated, closed CAR T process achieves 1×10^{10} expanded T cells with more than 80% enhanced green fluorescent protein (eGFP) transduction efficiency across an 8-day manufacturing process.

Introduction

In 2019 alone, an estimated 1.7 million new cases of cancer are expected to be diagnosed in the US and about 600 000 Americans are expected to die of cancer (1). While chemotherapy combined with drugs continues to be the standard of care, an increasing number of adults and children are refractory to conventional treatment modalities, motivating the “next generation” of approaches for cancer therapy.

Unlike conventional chemotherapy, novel approaches are now being developed based on a deeper understanding of immune-oncology. Among these approaches, adoptive T cell therapy has gained significant attention for its curative potential. Approval of autologous CAR T cell therapies from Novartis (Kymriah™) and Gilead/Kite (Yescarta™) (2) underscores both the promise and challenges ahead in order to make these therapies available to patients.

There is now a growing need for end-to-end workflow solutions supporting industrial scale-up and scale-out of CAR T cell therapies. To address these challenges, we have investigated individual CAR T unit operations to identify commercially available reagents and modular equipment that drive process closure and automation as a method to improve workflow efficiency and product consistency.

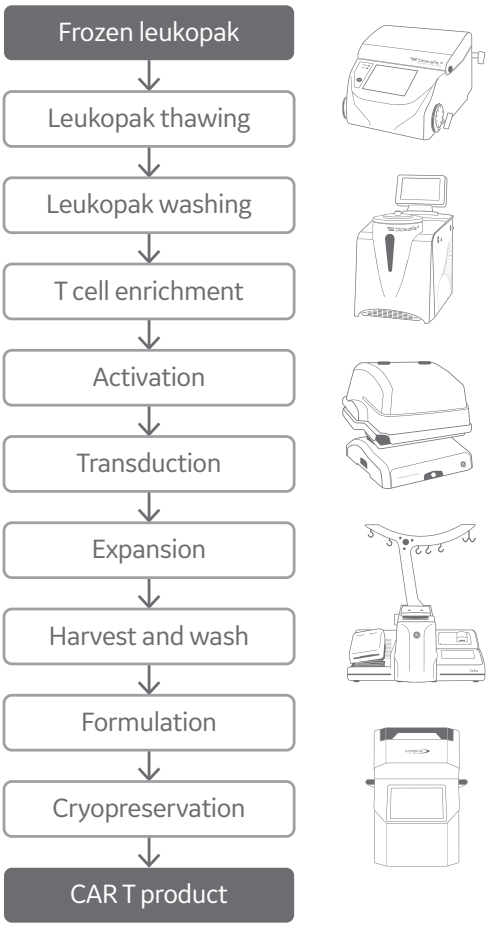

Fig 1. Workflow for the semi-automated, closed CAR T cell manufacturing process. The cell material at the start and end of the process is highlighted in gray and the unit operations in white.

The CAR T workflow is shown in Figure 1. We evaluated commercially available T cell enrichment and activation reagents, closed small-scale culture vessel options, alternative solutions to enhance transduction, and the specific timing of process steps. The aim was to develop a modular platform process that is robust and flexible for the varied needs of CAR T developers.

Materials and methods

Comparison of thawing and washing methods

Cells from three donor leukopaks (HemaCare) were either thawed directly in a 37°C water bath followed by manual cell washes, or thawed and processed in an automated manner using the Thaw-Large Volume*‡ Protocol Software on the Smart-Max™ AS-310 instrument (GE Healthcare Life Sciences) and SmartWash Protocol Software on the Sepax™ 2† Cell Separation Device (GE). In both cases, T cells were subsequently enriched using the EasySep™ Release Human CD3 Positive Selection Kit (STEMCELL Technologies) according to the manufacturer's protocol.

Comparison of T cell enrichment and activation methods

Leukopaks from three donors were processed using the Thaw-Large Volume Protocol Software on Smart-Max AS-310 and SmartWash Protocol Software on Sepax 2. T cells were then enriched using either EasySep Release Human CD3 Positive Selection Kit, CD3 Microbeads run through an LS Column using the QuadroMACS™ Separator (Miltenyi Biotec), or a combination of CD14 and CD19 Microbeads run through an LD Column using the QuadroMACS Separator. The fractions containing the enriched T cells were activated by ImmunoCult™ Human CD3/CD28/CD2 T cell Activators (STEMCELL Technologies). All other listed materials are from Miltenyi Biotec.

All subsequent T cell expansion was done using cell culture medium prepared by supplementing Xuri™ T Cell Expansion Medium (GE) with 5% heat-inactivated human AB serum (Gemini) and 350 IU/mL Xuri IL-2 growth factor (GE). Cells were inoculated into Nunc™ tissue culture-treated multiwell plates (Thermo Fisher Scientific) at a density of 1×10^6 cells/mL, counted every day after day 3, and diluted to 5×10^5 cells/mL.

Comparison of T cell transduction methods

Impact of selection method and timing of lentivirus (LV) addition
A subset of the activated T cells from the various enrichment methods mentioned above were transduced either the same day as activation, or 1 or 2 days after activation to gauge the impact of enrichment method and time prior to viral addition on transduction efficiency. T cells were transduced with LV-eGFP vector (Tailored Genes) at a multiplicity of infection (MOI) of 2.5 in Nunc tissue culture-treated multiwell plates. Volumes were doubled with fresh media to improve cell health 16 to 20 h after transduction. Transduction efficiency was measured on the CytoFLEX™ Flow Cytometer (Beckman Coulter) 48 h after media addition.

Impact of transduction method
To compare several methods of transducing T cells, leukopaks from two donors were processed using the Thaw-Large Volume Protocol Software on Smart-Max AS-310 and SmartWash Protocol Software on Sepax 2. T cells were then enriched using EasySep Release Human CD3 Positive Selection Kit and activated using the ImmunoCult Human CD3/CD28/CD2 T cell Activators. Cells were inoculated into Nunc tissue culture-treated T-flasks (Thermo Fisher Scientific) at a density of 1×10^6 cells/mL and cultured in an incubator (37°C, 5% CO_2) for 1 day to allow them to activate before transduction.

Activated T cells were transduced with LV-eGFP at a MOI of 2.5 by inoculating the virus and cells in either Nunc T-flasks, shake flasks (Corning) or RetroNectin™-coated plates (Takara). A subset of activated T cells were transduced in a closed automated manner using the SpinOculation‡ C-Pro Protocol Software on the Sepax C-Pro instrument (GE) at the same MOI.

The cells in T-flasks and RetroNectin-coated plates were cultured in an incubator (37°C, 5% CO_2). The cells transduced with LV-eGFP using the SpinOculation C-Pro Protocol Software were inoculated into Erlenmeyer shake flasks, and both sets of shake flasks were cultured on a MaxQ™ CO2 Plus Shaker (Thermo Fisher Scientific) in an incubator (37°C, 5% CO_2, 90 rpm). Volumes were doubled with fresh media to improve cell health 16 to 20 h after transduction. Cells were cultured for an additional 2 or 3 days to allow them to activate before further manipulations were performed. After a total of 4 or 5 days of culture, cells were counted and diluted to 5×10^5 cells/mL every day for an additional 4 days. At the end of culture, transduction efficiency was measured on the CytoFLEX Flow Cytometer.

Comparison of T expansion methods

Leukopaks from four donors were processed using the Thaw-Large Volume Protocol Software on Smart-Max AS-310 and SmartWash Protocol Software on Sepax 2. T cells were then enriched using EasySep Release Human CD3 Positive Selection Kit and activated by ImmunoCult Human CD3/CD28/CD2 T cell Activators. Cells were inoculated into Nunc tissue culture-treated T-flasks or Erlenmeyer shake flasks and seeded at a density of 1×10^6 cells/mL. Shake flasks were placed on a MaxQ CO2 Plus Shaker at 90 rpm while the T-flasks remained static. Cells were cultured in an incubator (37°C, 5% CO_2) for 3 days to activate before manipulations were performed. After 3 days, cells were counted and diluted to 5×10^5 cells/mL. Cells were maintained for 5 days and then phenotypic analysis was performed on the CytoFLEX Flow Cytometer.

Large-scale transduced T cell production

Thaw and wash
A frozen leukopak was thawed on Smart-Max AS-310 using the Thaw-Large Volume Protocol Software. The thawed leukopak was connected via a spike port to a CS-600.1 Sepax Cell Separation Kit (GE) and run on Sepax 2 using the SmartWash Protocol Software. Although this configuration was used for generating this data set, a more flexible equivalent alternative is available using a CT-60.1 Sepax C-Pro cell processing kit (GE) on Sepax C-Pro cell processing instrument (GE) using the CultureWash C-Pro Protocol Software (Table 1).

Cells were diluted with HyClone™ Dulbecco's phosphate-buffered saline without calcium or magnesium (DPBS-/-, GE) supplemented with 2% heat-inactivated human serum AB and 1 mM EDTA (Sigma). A wash was performed, and cells were extracted in the starting volume of wash buffer.

* For upstream thawing, we used the Thaw-Large Volume Protocol Software on the Smart-Max instrument. An alternative would be to use the VIA Thaw instrument for both upstream and downstream processing.
† Sepax 2 Cell Separation Device was used in this study, however the Sepax C-Pro is recommended as the equivalent instrument for manufacturing purposes.
‡ Coming soon. There is no guarantee regarding the release of any products (GE Healthcare reserves the right to change plans and timing in regards to the release of any products).

Table 1. Equivalent CultureWash C-Pro parameters to mimic SmartWash parameters[1]

Parameter	SmartWash on Sepax 2	CultureWash C-Pro on Sepax C-Pro
Input volume	50–880 mL (70 mL)	20–1200 mL (70 mL)
Detect initial volume	N/A (No)	Flag (No)
Input bag rinsing	N/A (Yes)	Flag (Yes)
Input bag rinse volume	N/A (50 mL)	25–100 mL (50 mL)
Pause input bag rinsing	N/A (No)	Flag (No)
Final volume	50–200 mL (70 mL)	8–500 mL (70 mL)
Dilution ratio	0.0–2.0 (2.0)	0.0–3.0 (2.0)
Intermediate volume	N/A (10 mL)	5–50 mL (10 mL)
Optical cells detection	N/A (Yes)	Flag (Yes)
Dilution speed	N/A (17 mL/min)	10–120 mL/min (17 mL/min)
G-force	N/A (400 g)	100–800 g (400 g)
Sedimentation time	N/A (240 s)	120–600 s (240 s)
Wash cycles	Standard (1) or High wash (2) (Standard)	0–3 (1) and 0–3 (0)
Hang bag validation	N/A (Yes)	Flag (Yes)
Manual extraction	N/A (No)	Flag (No)
Product filling speed	N/A (60 mL/min)	17–120 mL/min (60 mL/min)
Waste extraction speed	N/A (75 mL/min)	17–120 mL/min (75 mL/min)

[1] The selected parameters are in brackets. If the parameter is not available (N/A), the brackets are the default parameters.

T cell enrichment and activation

$CD3^+$ cells were enriched using the EasySep Release Human CD3 Positive Selection Kit according to the manufacturer's instructions. Cell culture medium was prepared as described in "Comparison of T cell enrichment methods". T cells were activated using ImmunoCult Human CD3/CD28/CD2 T cell Activators according to the manufacturer's protocol. Cells were inoculated into 1 L Erlenmeyer cell culture flasks (Corning) at a density of 1.5×10^6 cells/mL. Cells were cultured in an incubator (37°C, 5% CO_2, 90 rpm) on a MaxQ CO2 Plus Shaker for 1 day to activate before further manipulations were performed.

Transduction and culture

For large-scale transduction, cells were counted 1 day after activation and 220 mL of cell suspension was transferred to a 600 mL transfer pack (Fresenius Kabi). A volume of LV-eGFP corresponding to a MOI of 2.5 was diluted in 20 mL of culture medium. The medium was the same formulation used in activation. The virus was transferred to a sterile syringe (BD Biosciences) and kept on ice. The cells and virus were welded onto the CT-60.1 Sepax C-Pro cell processing kit and run on the Sepax C-Pro instrument using the SpinOculation C-Pro Protocol Software (Table 2).

Cells were counted and inoculated back into 1 L Erlenmeyer cell culture flasks at the original density prior to transduction in fresh culture medium. Cells were cultured for 16 to 20 h in an incubator under the same conditions used for activation. After 16 to 20 h, culture medium was added to double the volume. Cells were cultured for an additional 2 days.

Cell expansion

A 2 L Xuri Cellbag™ bioreactor (GE) was placed on a Xuri Cell Expansion System W25 (GE) filled with 5% CO_2, and a reservoir containing culture medium was aseptically welded on. The medium was the same formulation used in small-scale culture. An initial volume of 200 mL culture medium was added to the bioreactor and allowed to equilibrate overnight. The system parameters were set at 37°C, gas flow rate of 0.05 L/min, and a rocking rate of 10 rpm at a 6° angle.

After a total of 4 days in Erlenmeyer shake flasks, cells were inoculated into the bioreactor and culture medium added, giving a total of 500 mL of culture in the bioreactor. Once initiated, the bioreactors were left overnight while fresh medium was added to the reactor at 1 L/d to reach a total volume of 1 L.

From day 5 onwards, 1 L of medium/d was continuously perfused to control lactate, ammonium, and glucose levels. Daily sampling was performed for cell number, viability and biochemical analysis (lactate, ammonium, glucose, and pH). Cells were maintained in culture until day 8.

Table 2. SpinOculation C-Pro parameters

Parameter	Value
Initial volume	220 mL
Input bag rinsing	Yes
Input bag rinse volume	50 mL
Pause input bag rinsing	No
Optical cell detection	No
Intermediate volume	40 mL
Wash cycles	0
Concentration g-force	400 g
Concentration time	300 sec
Virus volume	25 mL[1]
Spinoculation time	90 min
Spinoculation g-force	600 g
Final volume	100 mL
Spinoculation volume	60 mL

[1] Though the volume of lentiviral vector in culture medium was 20 mL, a value of 25 mL was entered here to avoid a hold-up volume in the line.

Phenotypic analysis and transduction efficiency
T cell populations were analyzed by flow cytometry on days 0, 4, and 8 of culture. Briefly, 1×10^6 cells were washed and blocked with human FcR Blocking Reagent (Miltenyi Biotec) prior to staining with CD3-BV510, CD4-APC-H7, CD8-BV650, CD45RO-BV421, CD62L-PE, CD25-BV786, CD279-APC and 7AAD (all from BD Biosciences). Transduction efficiency was measured using eGFP detection on the fluorescein isothiocyanate (FITC) channel simultaneously with the phenotypic analysis. The stained cells were analyzed on the CytoFLEX Flow Cytometer.

Harvest and cryopreservation
T cells were harvested using the Sefia™ S-2000 Cell Processing instrument, FlexCell Protocol Software and CT-800.1 Cell Processing kit (GE) (Table 3). Briefly, 1×10^{10} T cells were reduced in volume to 50 mL at 75 mL/min flow rate. Two wash cycles were performed using PLASMA-LYTE A (Baxter) supplemented with 10% human serum albumin (Gemini). Washes were performed at 400 × g for 5min. Cells were then extracted at 1×10^8 cells/mL in PLASMA-LYTE A with 50% CryoStor™ CS10 (BioLife Solutions) and 5% human serum albumin into three CryoMACS™ 50 freezing bags (Miltenyi Biotec). The three cryogenic bags were loaded into the VIA Freeze™ Quad freezer (GE) with a cooling rate of -1°C/min until the temperature reached -100°C. After freezing, all cells were transferred to liquid nitrogen storage.

Table 3. Parameters used in the FlexCell Protocol Software

Parameter	Value
Initial volume	Input volume of 1×10^{10} cells (mL)
Detect initial volume	No
Enable initial bag weight sensor	No
Enable waste bag weight sensor	Yes
G-force, concentration	400 g
Sedimentation time, concentration	300 s
Intermediate volume, concentration	50 mL
Pump speed	75 mL/min
Wash cycles	2
G-force, washing	400 g
Sedimentation time, washing	300 s
Intermediate volume, washing	20 mL
Final volume bag 1	20 mL
Final volume bag 2	20 mL
Final volume bag 3	20 mL
Switch bag for resuspension	No
Dead volume extraction	No
Enable process temperature	No
Process temperature	20°C
Enable final product temperature	Yes
Final product temperature	4°C
Final product conditioning time	0 s
Enable final dilution	Yes
Final dilution injection rate	17 mL/min

Results and discussion

Comparison of thawing and washing methods

Open manual methods are available for thawing and washing cells from leukopaks. However, these do not comply with evolving GMP requirements and might introduce risks associated with operator variability and contamination. Alternatively, a closed, automated approach can be used to thaw and wash the cells. Such an approach can reduce manual labor requirements and product variability.

The thaw and wash using the Thaw-Large Volume Protocol Software on Smart-Max AS-310 together with SmartWash Protocol Software on Sepax 2 resulted in cell recovery and viability results comparable to a manual method ($p \geq 0.05$ by 2-sample t-test, n = 3) (Fig 2A). Cells from the automated method also gave comparable T cell recovery, purity, and viability ($p \geq 0.05$ by 2-sample t-test, n = 3) (Fig 2B).

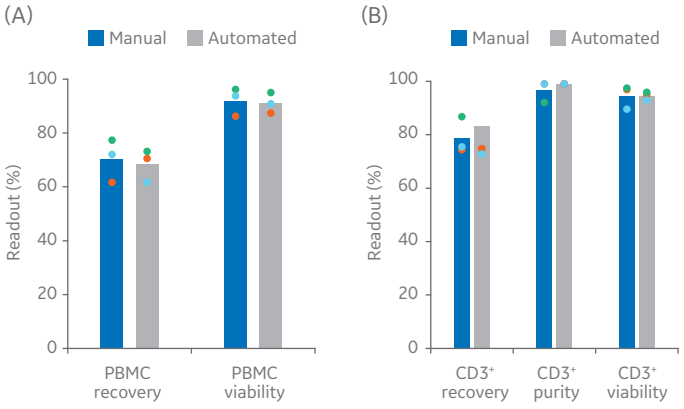

Fig 2. Recovery and viability after manual and automated thaw and wash. (A) Viable peripheral blood mononuclear cell (PBMC) recovery and viability immediately after manual or automated thaw and wash; (B) recovery, purity and viability of enriched T cells after EasySep Release Human CD3 Positive Selection. Bars represent the average of the biological triplicates with individual donor values shown by dots.

Comparison of T cell enrichment methods

There are several commercially available methods for enriching T cell populations. Two common systems are $CD3^+$ selection and $CD14^+/CD19^+$ depletion. In order to determine the optimal protocol for enriching T cells, two $CD3^+$ selection methods and one $CD14^+/CD19^+$ depletion method were evaluated.

As expected, depletion of the $CD14^+$ and $CD19^+$ populations resulted in significantly lower T cell purities than either of the $CD3^+$ selection methods ($p < 0.001$ by one-way ANOVA, n = 3) (Fig 3B). T cell enrichment using the EasySep Release Human CD3 Positive Selection Kit resulted in significantly higher recoveries than selection with the QuadroMACS system ($p = 0.01$ by one-way ANOVA, n = 3) (Fig 3B). However, there were no statistically significant differences in the viability and 5-day expansion kinetics between any of the enriched T cell populations ($p \geq 0.05$ by one-way ANOVA, n=3) (Fig 3C).

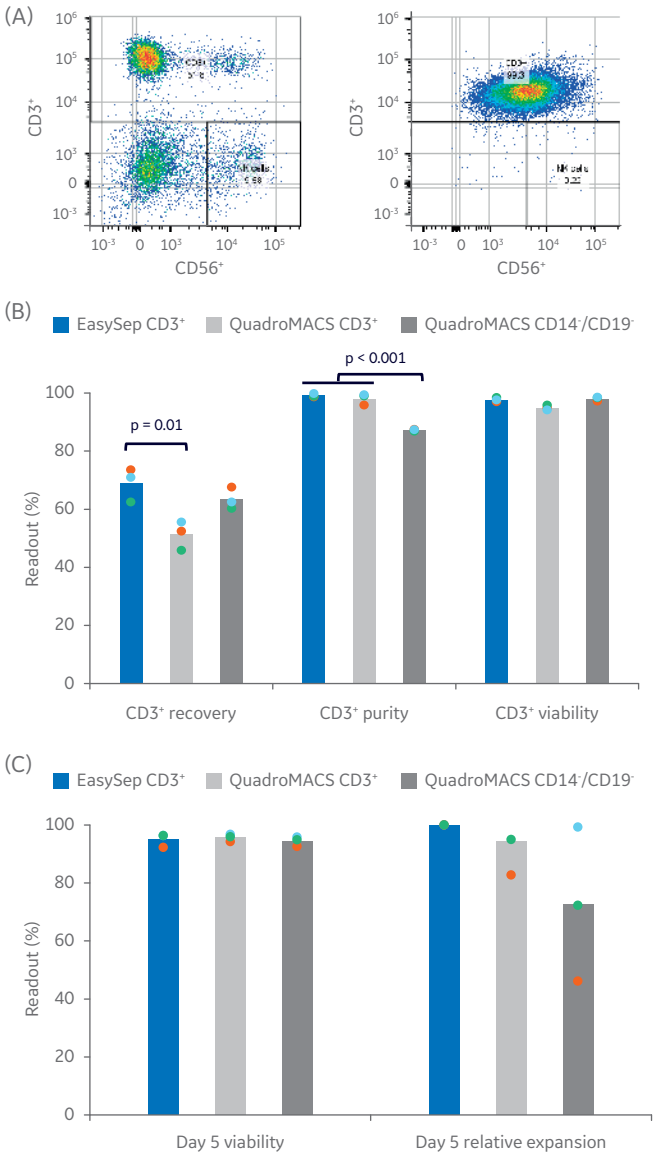

Fig 3. Comparison of enrichment methods. (A) FlowJo™ representation of gated cells before and after EasySep CD3+ selection; (B) recovery, purity, and viability of T cells after either EasySep CD3+ selection, QuadroMACS CD3+ selection, or QuadroMACS CD14+/CD19+ depletion; (C) day 5 viability and cumulative fold expansion relative to the EasySep CD3+ enriched T cell growth of the respective donor. Bars represent the average of the biological triplicates with individual donor values shown by dots.

Comparison of T cell transduction methods

In order to optimize transduction, the timepoint to perform transduction after the initial activation of the T cells was evaluated. In order to maintain as short a process duration as possible, earlier timepoints were prioritized. Several methods for transducing T cells are currently in use. However, a number of these rely on open and manual processing in plates or flasks, which are unlikely to be amenable to GMP requirements. Alternatively, we tested the automated, functionally closed SpinOculation C-Pro Protocol Software on the Sepax C-Pro instrument to transduce the T cells with LV-eGFP.

Figure 4 shows results from the determination of optimal day for transduction. T cells enriched through CD3+ selection had higher transduction efficiencies than the depletion method when transduced on day 0 ($p = 0.000$ by one-way ANOVA, $n = 3$) and day 1 ($p < 0.001$ by one-way ANOVA, $n = 3$). However, no differences were seen on cells transduced 2 days after activation ($p \geq 0.05$ by one-way ANOVA, $n = 3$). Overall, the highest transduction efficiency was observed by transducing CD3+ selected cells 1 day after activation ($p < 0.02$ by one-way ANOVA, $n = 3$).

As described in the methods, various manual transduction approaches currently used by numerous groups (T-flasks, shake flasks, and RetroNectin) were compared to the SpinOculation C-Pro Protocol Software on the Sepax C-Pro instrument. The automated closed transduction method with the SpinOculation C-Pro on the Sepax C-Pro resulted in both comparable viability (Fig 5A) and transduction efficiency at the end of culture (Fig 5B).

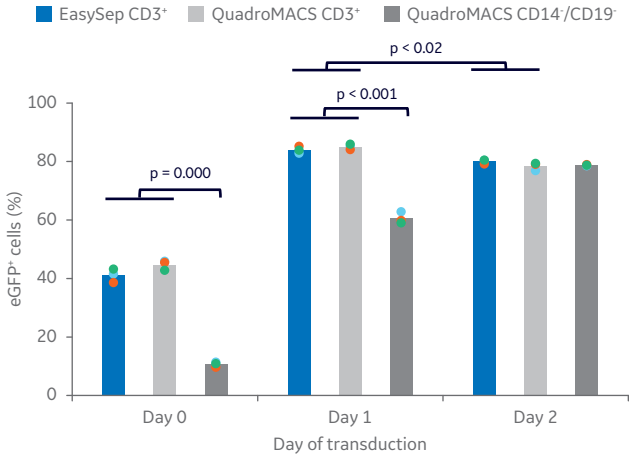

Fig 4. Determination of optimal day for transduction. Percentage of eGFP-positive T cells 72 h after transduction on various days after activation. Bars represent the average of the biological triplicates with individual donor values shown by dots.

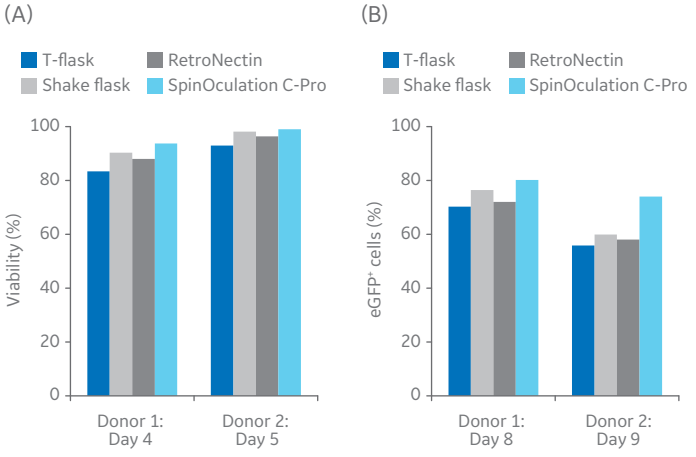

Fig 5. Comparison of transduction methods. (A) Viability of cells using various transduction methods after activation phase of culture (3 days or 4 days after transduction); (B) percentage of eGFP-positive T cells at the end of culture (7 or 8 days after transduction). Bars represent the average of technical triplicates that were run for each of two biological donors.

Comparison of T cell expansion methods

There are several open methods for culturing T cells at small-scale prior to entering a bioreactor, with culture in T-flasks being the more common method. There are also closed methods such as gas permeable bags and G-Rex™, but in our previous evaluations, these were not able to reach the same growth kinetics observed in T-flasks (data not published). We investigated Erlenmeyer shake flasks as a closed culturing alternative that could match the fold expansion of T cells inoculated in T flasks.

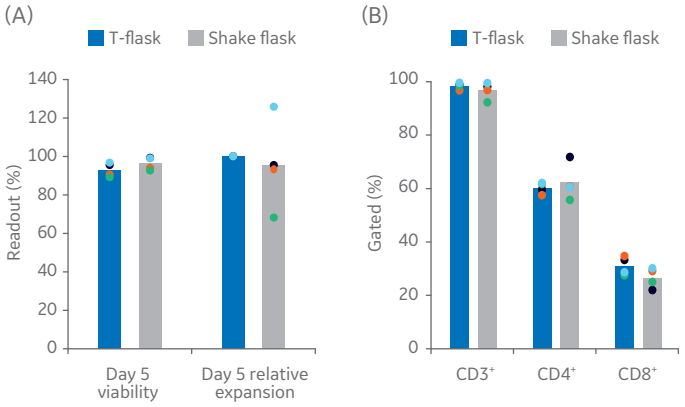

Fig 6. Comparison of small-scale culturing methods. (A) Day 5 viability and cumulative fold expansion relative to the T cell growth in T-flask of the respective donor; (B) phenotypic analysis of T cells and subpopulations $CD4^+$ and $CD8^+$ cells by flow cytometry at the end of culture. Bars represent the average of four biological replicates with individual values shown by dots.

No statistically significant differences were found in the T cell viability or expansion ($p \geq 0.05$ by 2-sample t-test, n = 4) (Fig 6A). Phenotypic comparison of cells at the end of culture are shown in Figure 6B. No statistically significant differences were found in T cell purity or $CD4^+$ and $CD8^+$ subpopulations ($p \geq 0.05$ by 2-sample t-test, n = 4).

Large-scale transduced T cell production

The automated thawing and washing of leukopaks using the Thaw-Large Volume Protocol Software on Smart-Max AS-310 and SmartWash Protocol Software on Sepax 2 were implemented along with T cell enrichment using the EasySep Release Human CD3 Positive Selection Kit, SpinOculation C-Pro Protocol Software on the Sepax C-Pro instrument, and small-scale activation and expansion in shake flasks and Xuri Cell Expansion System W25 (Fig 7).

From three large-scale production runs, we were able to get over 75% cell recovery from the thaw and wash steps using the setup described, with above 90% viability (Fig 8A). Populations of enriched T cells were obtained by magnetic separation using the EasySep system, which increased T cell purity starting from 53% to 62% in the initial input population to above 95% in the output, regardless of different starting populations of cells (Fig 8B).

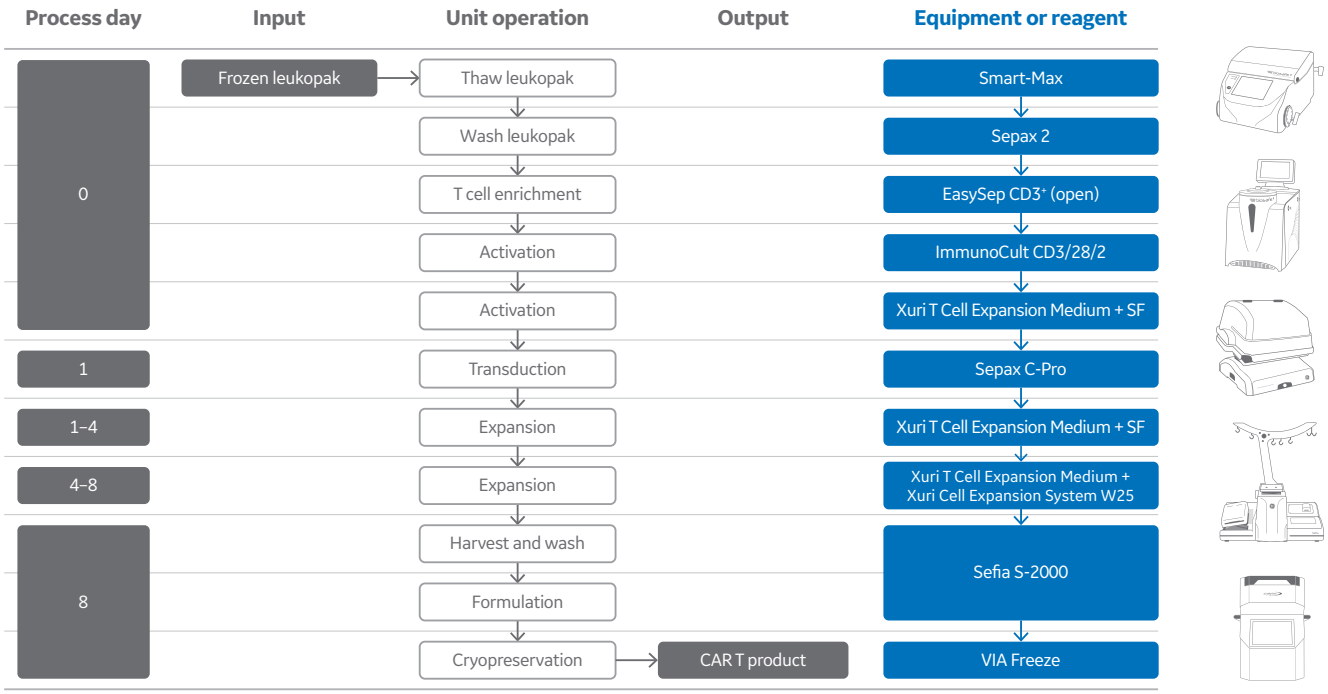

Fig 7. Process workflow. A diagram of the steps and systems used to prepare a clinically relevant dose of CAR T cells from frozen leukapheresis units. Most steps were functionally closed and automated, and process is compatible with GMP guidelines. Leukopak wash was tested on Sepax 2, but an equivalent product, Sepax C-Pro, is commercially available. SF = shake flask.

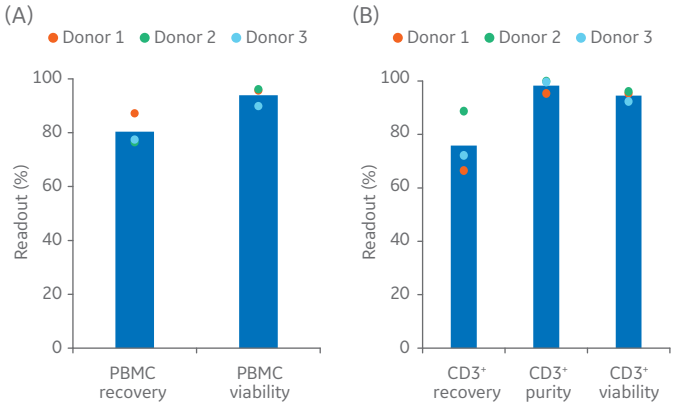

Fig 8. Purity and recovery of T cells after enrichment. (A) Viable PBMC recovery and viability after SmartWash Protocol Software on Sepax 2; (B) Recovery, purity, and viability of enriched T cells after EasySep Release Human CD3 Positive Selection. Bars represent the average of the biological triplicates with individual donor values shown by dots.

After isolation, cells were activated, transduced and expanded in shake flasks for 4 days before being transferred into the Xuri Cell Expansion System W25. After 4 days, over 68% of the T cell population was transduced (Fig 9A). T cells were expanded in the Xuri Cell Expansion System W25 by over 78-fold from the starting cell population (Fig 9B). Transduction was maintained above 84% throughout the culture (Fig 9B) resulting in over 1×10^{10} transduced T cells on day 8, on average (Fig 9C).

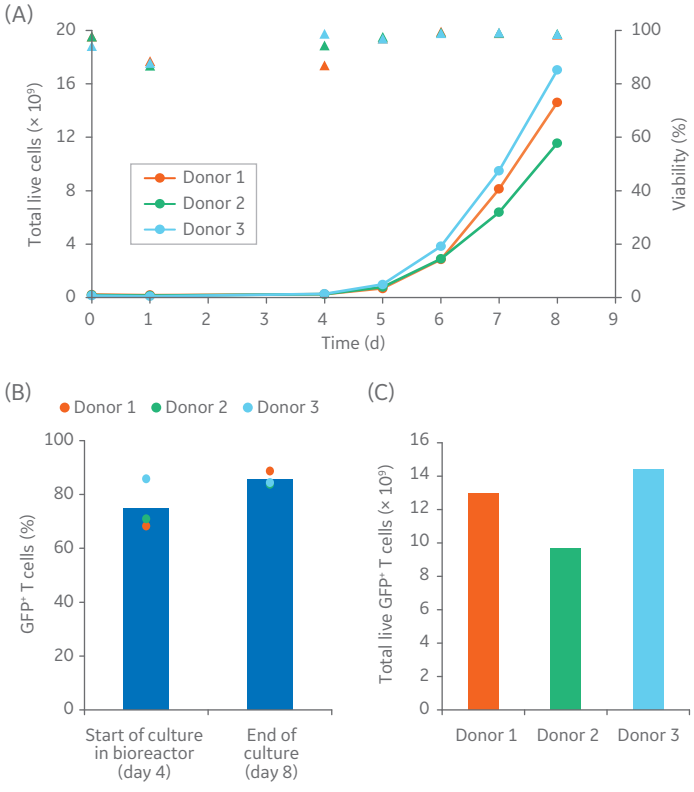

Fig 9. Culture growth and transduction efficiency. (A) Cumulative growth and viability of T cells. Lines represent the expansion profile and triangles represent the viability during the CAR T process; (B) percentage of transduced GFP⁺ T cells after activation in shake flasks and after culture in the Xuri Cell Expansion System W25. Bars represent the average of the biological triplicates with individual donor values shown by dots; (C) cumulative GFP⁺ T cells on day 8 for donors 1, 2, and 3.

After culture, expanded transduced T cells were harvested using the FlexCell Protocol Software on the Sefia S-2000 Cell Processing instrument prior to cryopreservation. After volume reduction, wash, and cryoformulation, total live cell recovery was over 95% without a decrease in viability (Fig 10A). Cryogenic bags of cells were cryopreserved using the VIA Freeze Quad before being transferred to liquid nitrogen storage.

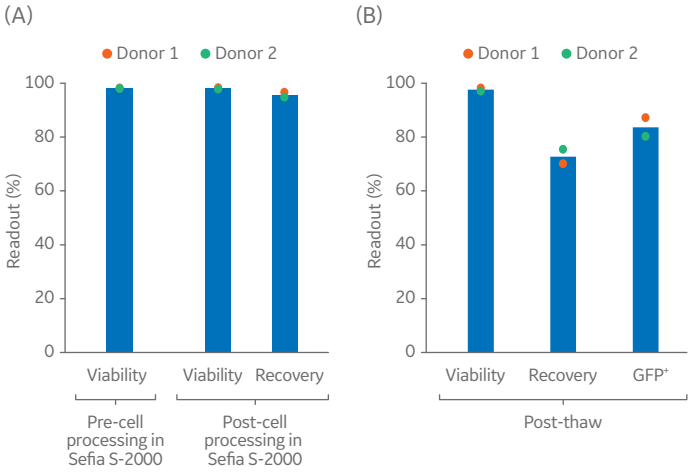

Fig 10. Recovery and viability of T cells. (A) Recovery and viability of cultured T cells, from donor 1 and 2 after harvest using FlexCell Protocol Software on the Sefia S-2000 instrument; (B) post-thaw recovery and viability of transduced T cells cryopreserved after cryoformulation using FlexCell Protocol Software on the Sefia S-2000 instrument.

Figure 10B shows the viability and recovery post-thaw using VIA Thaw™ CB1000 (GE) after manual washing, centrifugation, and buffer exchange to evaluate the cells after cryopreservation (as a surrogate to the product ultimately infused into the patient). As shown, over 70% of the cells were recovered with above 97% viability. The results also show that transduction was maintained above 80% through the cryopreservation process.

Conclusions

The workflow used in this study is suitable for industrialized, autologous CAR T cell production as it incorporates several automated and functionally closed unit operations. This large-scale transduced T cell production provided clinically relevant doses (1×10^{10} cells per donor) with high transduction efficiency (> 80% eGFP⁺). Phenotypically, the final product demonstrated characteristics of an activated T cell population. Future work could focus on fine-tuning each step to reduce costs while maintaining a robust, scalable process. For example, bioprocess parameters such as agitation and rocking speed, as well as perfusion strategy, could be further improved by reducing the amount of media exchange and optimizing rocking speed and angle.

Overall, the methodology presented here is compatible with industrial workflows for CAR T manufacturing. This application note shows the feasibility of a semi-automated T cell expansion and closure of key unit operations. In particular, the small-scale expansion in shake flasks demonstrates the possibility of an alternative to conventional static culture in gas-permeable bags or closed flasks.

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2. Approved Cellular and Gene Therapy Products, United States Food and Drug Administration. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products. [Online.] Accessed 22 May 2019.

Ordering information

Product	Product code
Smart-Max AS-310 instrument	3500
Thaw-Large Volume Protocol Software*‡	-
Sepax 2 Cell Separation Device†	14000
SmartWash Sepax 2 Protocol Software	14305
CS-600.1 Sepax Cell Separation Kit	10006
Sepax C-Pro Cell Processing Instrument	29264741
CultureWash C-Pro Protocol Software	29264736
SpinOculation C-Pro Protocol Software‡	-
CT-60.1 Sepax C-Pro Cell Processing Kit	29264739
Sefia S-2000 Cell Processing Instrument	29285527
FlexCell Sefia Protocol Software	16301
CT-800.1 Sefia Cell Processing Kit	20001
Dulbecco's Phosphate Buffered Saline (DPBS)	SH30028.03
Xuri T Cell Expansion Medium 1000 mL	29185231
Xuri IL-2 (1 mg)	29062790
Xuri Cell Expansion System W25	29064568
Xuri Cell Expansion System Cellbag Perfusion, pH and DO, 2 L	29105498
VIA Freeze Quad	VFQ_30010
50 mL bag SBS plate for VIA Freeze freezers	ASY_30038
VIA Thaw CB1000	TBA_30001

* For upstream thawing, we used the Thaw-Large Volume Protocol Software on the Smart-Max instrument. An alternative would be to use the VIA Thaw instrument for both upstream and downstream processing.

† Sepax 2 Cell Separation Device was used in this study, however the Sepax C-Pro is recommended as the equivalent instrument for manufacturing purposes.

‡ Coming soon. There is no guarantee regarding the release of any products (GE Healthcare reserves the right to change plans and timing in regards to the release of any products).

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