# Closed and automated CAR T cell lentiviral transduction by spinoculation

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# Closed and automated CAR T cell lentiviral transduction by spinoculation

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## Abstract

There is a growing need to close and automate chimeric antigen receptor (CAR) T cell therapy manufacturing. CAR T workflows depend on an *ex vivo* gene transfer step for therapeutic efficacy. To streamline this step while keeping flexibility during process development, we introduced a stand-alone protocol software that uses existing Sepax C-Pro technology. SpinOculation C-Pro protocol software automates lentiviral vector (LVV) transduction and maintains a functionally closed system within a single-use disposable kit, and no viral entry enhancers are required. We present different user cases highlighting the flexibility of the SpinOculation C-Pro protocol software parameters to influence process development of LVV transduction efficiencies in combination with application-specific upstream processes and viral source.

## Introduction

Clinical and biopharmaceutical communities are undergoing an evolution in cancer treatment with the development and use of T cell immunotherapies. The approval of autologous CAR T cell therapies from Novartis (Kymriah™) and Gilead/Kite (Yescarta™) emphasizes the growing need for automated and closed solutions to industrialize cell and gene therapy manufacturing with enhanced risk management, scalability, and reproducibility. Although equipment is available for most workflow steps of CAR T cell therapy manufacturing, other steps, such as lentiviral transduction for gene transfer, need improved methods and technologies.

SpinOculation C-Pro protocol software, used in combination with Sepax™ C-Pro instrument and single-use disposable kit CT-60.1, is a new stand-alone solution developed to streamline and automate lentiviral transduction while maintaining flexibility during process development (Fig 1). This application allows users to adjust the input cell volume to reach a specific cell density during the transduction step. Also, it provides closed-system centrifugation to aid in gene transfer without the use of enhancers. The final output volume is adaptable to the customer-specific application. Table 1 outlines the options and ranges of parameters that users can customize to adapt the protocol software to their process. Here, we present two user cases as examples of using SpinOculation C-Pro protocol software for lentiviral vector transduction in different CAR T cell workflows (Fig 2).

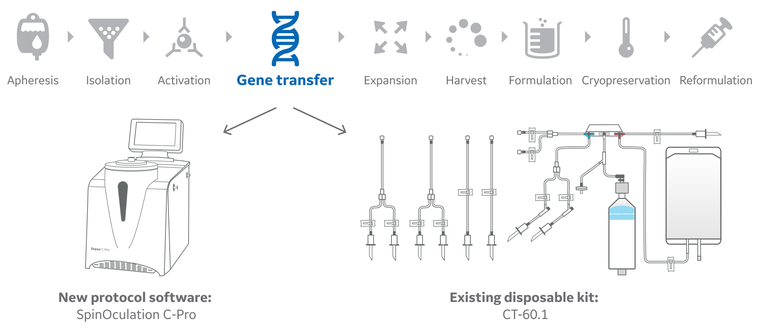


**Fig 1.** SpinOculation C-Pro protocol software, combined with Sepax C-Pro instrument and CT-60.1 single-use kit, offers a flexible, automated, and closed solution for T-cell lentiviral transduction. This protocol can be performed in CAR T workflows that include a gene transfer unit operation.

**Table 1.** SpinOculation C-Pro protocol software specifications

| Phase | Parameters | Range | Recommended value |
|---|---|---|---|
| **Concentration** | Initial volume | 20 to 880 mL | Initial volume |
| | Input bag rinse | Y/N | Y |
| | Input bag rinse volume | 25 to 100 mL | 50 mL |
| | Pause input bag rinse | Y/N | Y |
| | Optical cell detection | Y/N | Y |
| | Intermediate volume | 5 to 50 mL | 10 mL |
| **Concentration and washing** | G-force | 100 to 600 × g | 400 × g |
| | Time | 120 to 600 s | 240 s |
| | Wash cycles | 0 to 2 | 0 |
| **Spinoculation** | Pellet volume | 5 to 50 mL | Dependent on desired density during incubation |
| | Viral vector (VV) volume | 10 to 150 mL | Your VV volume |
| | Spinoculation time | 30 to 180 min | 90 min |
| | Spinoculation g-force | 500 to 1200 × g | 600 × g |
| **Final resuspension** | Final volume | 20 to 500 mL | Desired final volume |

## Material and methods

Case 1 used frozen apheresis units from one healthy donor. Case 2 used fresh apheresis units from three healthy donors. Case 1 included thawing and washing of the apheresis unit with closed, automated processes.

In both cases, T cell enrichment and activation were performed using manual, open processes on the first day of the workflow. Activation was continued in T-flask culture for the next 24 hours. Then, on culture day 1 in both cases, cells were split to perform parallel SpinOculation C-Pro process and manual transduction processes in static or shake flask for case 1 and in static flask for case 2. Table 2 shows the SpinOculation C-Pro protocol software parameters used in the two cases (case 2 included testing split donor material variable process parameter comparisons). Also, each case used a different LVV-eGFP manufacturing source at different multiplication of infection (MOI). After transduction, T cell expansion in both cases was performed in T-flasks for seven days. Xuri™ T Cell Expansion Medium contained 5% heat-inactivated human AB serum and 350 IU/mL IL-2 growth factor has been used in case 1 and RPMI 1640 with 10% fetal bovine serum and 250 IU/mL Xuri IL-2 growth factor in case 2. In case 1, two other expansion methods were tested for comparison: in shake flask and in the Xuri Cell Expansion System W25 (automated expansion) (1). At the end of the expansion phase, CAR T cells in case 1 were cryopreserved using the VIA Freeze™ instrument. Flow cytometry analysis was performed on process day 9 in case 1 and process day 8 in case 2 to evaluate transduction efficiency after T cell expansion.

**Table 2.** SpinOculation C-Pro protocol software parameters for each user case

| | User case 1 | User case 2 |
|---|---|---|
| Initial volume | 220 mL | 40 mL |
| Input bag rinse | Yes | Yes |
| Input bag rinse volume | 50 mL | 20 mL |
| Pause input bag rinse | No | Yes |
| Optical cell detection | No | Yes |
| Intermediate volume | 40 mL | 10 mL |
| G-force | 400 × g | 400 × g |
| Time | 300 s | 240 s |
| Wash cycles | 0 | 0 |
| Pellet volume | 20 mL | 20 mL |
| Viral vector volume | 20 mL | 10 mL |
| Spinoculation g-force | • 600 × g | • 600 × g<br>• 700 × g |
| Spinoculation time | • 90 min | • 30 min<br>• 60 min<br>• 90 min |
| Final volume | 100 mL | 40 mL |

* 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. Thaw-Large Volume is 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).

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

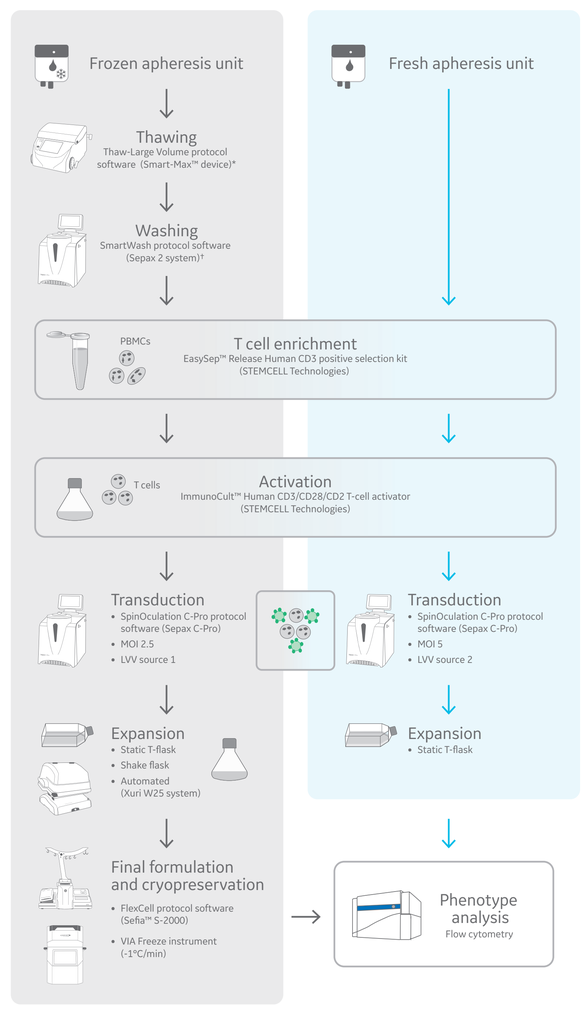


**Fig 2.** Overview of the two user cases. Schematic of the two workflows, highlighting the differences.

## Results

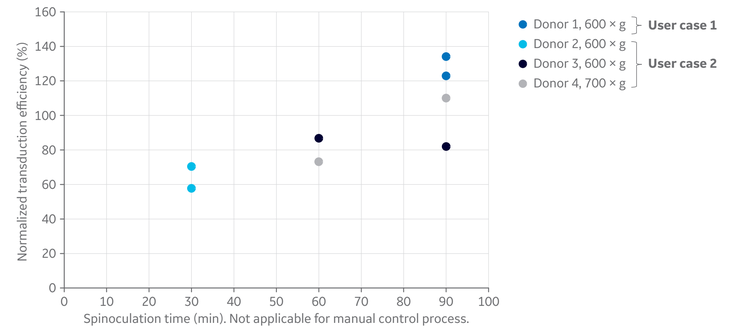


**Fig 3.** Normalized transduction efficiency of SpinOculation C-Pro versus manual performance at end of expansion phase. Transduction efficiencies from four donors were normalized by comparing the different SpinOculation C-Pro processes to their respective split donor control arm, which was manually processed in an open T-flask. Donor 1 material was processed using the user case 1 workflow in a nine-day process. Donor 2, 3, and 4 materials were processed using the user case 2 workflow in an eight-day process. Transduction efficiencies evaluated at the end of the processes between the two cases spanned a range of 30.5% up to 74.8%. This absolute transduction level is highly dependent on the viral construct source, the workflow used, the combination of protocol parameters selected, and the donor material.

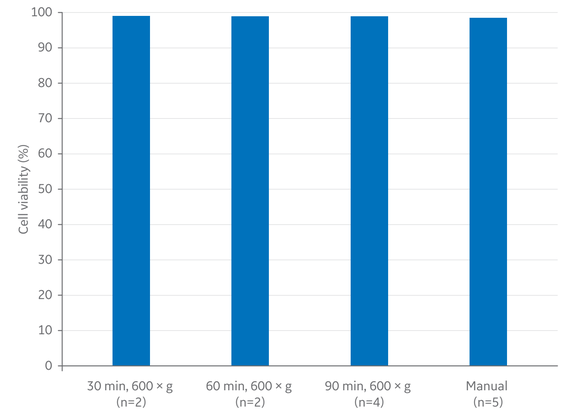


**Fig 4.** Average of cell viability (%) obtained at the end of the expansion phase after manual versus automated processes. Manual and automated (SpinOculation C-Pro) processes across donors and variable parameters maintained high cell viabilities, all above 97.5%.

## Conclusions

As these user cases demonstrate, SpinOculation C-Pro protocol software offers a solution for a closed, automated LVV transduction process step where transduction efficiency can be comparable to a manual, open process. The open, flexible software parameters provide a wide potential for process development and optimization by allowing users to vary multiple parameters (Table 1). It is important to consider that many factors influence the performance that can be achieved (Fig 3, Fig 4). Notably, transduction efficiency is highly dependent on LVV source and the MOI. Moreover, the upstream preparation of T cells (enrichment phase), as well as the method and reagents used to expand transduced T cells, can impact performance (Fig 5). Process steps downstream to transduction, such as T cell expansion methods and culture vessels, can also affect the efficiency of the final therapeutic product (Fig 5). SpinOculation C-Pro protocol software completes our solutions to meet the growing need for fully automated workflow solutions for chimeric antigen receptor (CAR) T cell therapies to help standardize processes and reduce the impact of donor variability.

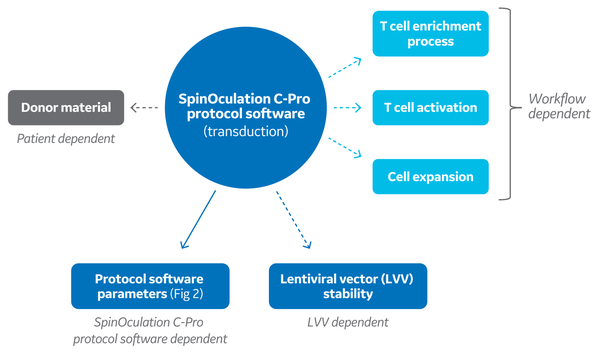


**Fig 5.** A simple solution for a complex model. Overview of all factors that could influence transduction performance. While most of them can be standardized, the donor material is subject to variability.

## *References*

1. Application note: Closed and semi-automated processing of CAR T cells, GE Healthcare, KA8037280619AN (2019).

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