Protocol for expansion and optional transduction of Gamma Delta (γδ) T cells using CliniMACS Prodigy®

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Contents

Important note

This protocol represents a verified variation of the already existing CliniMACS Prodigy T Cell Transduction - Large Scale (TCT-LS) System. The respective user manual of the CliniMACS Prodigy TCT-LS System has to be followed. Miltenyi Biotec as the provider of the CliniMACS Prodigy TCT-LS System does not provide any recommendations regarding the use of the manufactured cells for therapeutic purposes and does not make any claim regarding a clinical benefit.

1. Description

1.1 Purpose

Gamma Delta (γδ) T cells can be *ex vivo* expanded and genetically modified using viral vectors to enhance their anti-tumor capabilities for improved adoptive γδ T cell therapy approaches.

This protocol describes the process to expand γδ T cells, with optional transduction, in a GMP compliant and closed system using the CliniMACS Prodigy TCT-LS System. The protocol requires the use of fresh leukapheresis products that have been enriched on γδ T cells via depletion of αβ T cells and $CD19^+$ cells, by using systems intended for TCRα/β and CD19 depletion on CliniMACS® Plus Instrument or CliniMACS Prodigy. The enriched γδ T cell fraction, containing also monocytes and natural killer (NK) cells, is stimulated using zoledronic acid which induces a selective activation and preferential expansion of Vδ2 T cells. Optionally, γδ T cells can be genetically engineered with viral vectors.

1.2 Reagent and instrument requirements

- CliniMACS Prodigy instrument
- T Cell Transduction-Large Scale (TCT-LS) software (process version 2.0)
- CliniMACS Prodigy TS 620 (1 set)
- TexMACS™ GMP Medium (five 2L bags)
- Human AB-serum (GMP-grade)
- Zoledronic acid (pharmaceutical-grade)
- MACS GMP Recombinant Human IL-2 (2 vials, 500 µg/vial)*
- MACS GMP Recombinant Human IL-15 (3 vials, 25 µg/vial)*
- Viral vector (optional)
- MACS® GMP Vectofusin®-1 (optional, 1 vial)
- Formulation solution (e.g. CliniMACS Formulation Solution)
- Sterile Tubing Welder
- Sterile water
- Luer spike interconnector
- Triple sampling adapter (optional)
- UV light protection bag (provided with TexMACS 2 L bags)
- Uninterruptable power supply
- CO_2 and compressed air supply
- 5 L Collection Bag (for Waste bag exchange)

* The number of vials needed may vary depending on lot-specific activity of the MACS GMP Recombinant Cytokine.

2. Protocol

2.1 Preparation of material and equipment

▲ All preparations should be performed under sterile conditions under a laminar flow hood.

▲ It is recommended to prepare the culture medium and the stimulation medium one day prior to starting activation and expansion of the cells. Store medium bags at 2-8 °C until use.

Preparation of MACS GMP Recombinant Cytokines

Reconstitute both MACS GMP Recombinant Human IL-15 and MACS GMP Recombinant Human IL-2 to 1×10^6 U/mL in sterile water.

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Preparation of MACS GMP Vectofusin-1 (optional, for transduction)

Note: If transduction is performed, MACS GMP Vectofusin-1 can be optionally added to the vector to enhance transduction efficiency.

Reconstitute one vial of MACS GMP Vectofusin-1 in 1mL of sterile water.

Preparation of culture medium

1. If transduction is performed, remove an appropriate amount of TexMACS GMP Medium for viral vector dilution prior to adding any additives. It is recommended to use a 1:1 vector dilution when using MACS GMP Vectofusin-1.
2. For optimal γδ T cell proliferation, supplement five bags (= 10 L) of TexMACS GMP Medium with 5% human AB serum, 140 U/mL of MACS GMP Recombinant Human IL-15 and 500 U/mL of MACS GMP Recombinant Human IL-2.

Preparation of stimulation medium

For the efficient stimulation of γδ T cells from peripheral blood after TCRα/β and CD19 depletion, supplement one bag (= 2 L) of culture medium with 4 mg of zoledronic acid.

Tubing set installation and priming

- ▲ For details about handling the CliniMACS Prodigy instrument, refer to the corresponding User Manual (Instrument).
- ▲ For details about handling the CliniMACS Prodigy TCT-LS System, refer to the corresponding User Manual (Application).
- ▲ The TCT-LS software guides the user through all steps of the process.

1. Switch on the CliniMACS Prodigy with the ON/OFF switch located on the rear panel of the instrument.

Note: the instrument needs to be connected to a CO_2 and compressed air supply source.

2. After the instrument initialization phase the main screen is shown. Use the buttons on the menu and tool bar of the touchscreen to operate the instrument.
3. Select the process "TCT-LS". Press <run> to confirm the selection.
4. Enter the requested parameters in the TCT-LS software. All entered parameters are saved in a process-related protocol.
5. Read the bar code on the tubing set label with the bar code reader. Alternatively, press edit to enter part number (P/N) and batch code (LOT) manually with the keyboard shown on the screen. The components of the CliniMACS Prodigy TS 620 are shown in figure 1.
6. Choose Case 3 "T cell cultivation with TS installation".
7. Proceed with the tubing set installation and priming. Check all tubing, fittings, valves, chamber, and columns for leaks or kinks that may block the fluids.

Note: In case there is any leakage, restart the process with a new tubing set, medium and buffer.

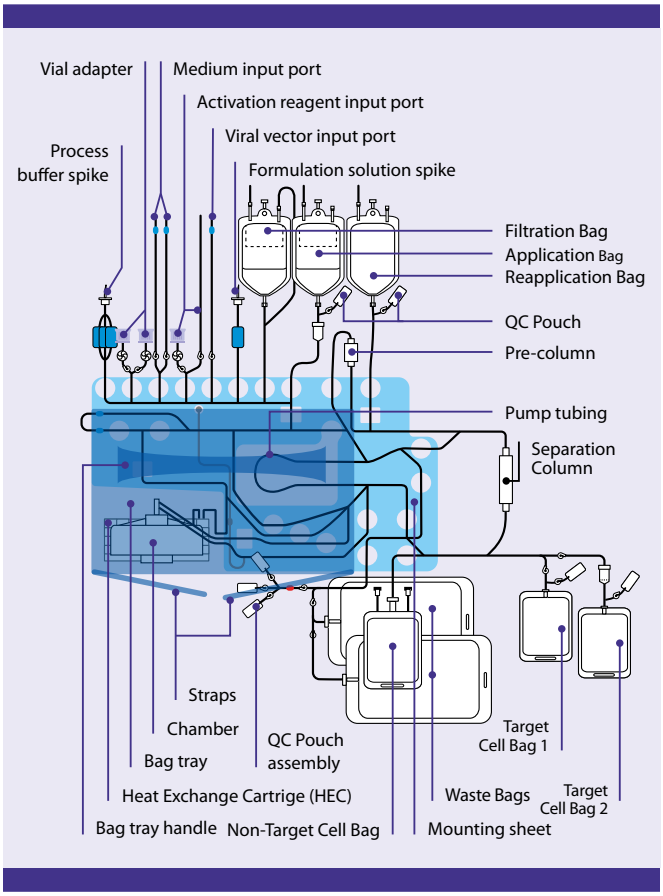

Figure 1.: Components of the CliniMACS Prodigy TS 620

2.2 Culture set up

- ▲ Use a fresh leukapheresis product as starting cell product, that has been depleted from TCRα/β^+ cells and $CD19^+$ cells.

1. Transfer the starting cell product to the Reapplication Bag at valve 10.
2. Take a sample of the starting cell product from the sampling pouch connected to the Reapplication Bag.
3. Determine the viability, and cell concentration of the starting cell product.
4. Enter the cell concentration of the starting cell product and the total number of cells to be seeded. It is recommended to seed a total number of 1×10^9 white blood cells. This corresponds to an approximate cell seeding density of 7.14×10^6 cells per cm^2.

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5. Protect the bag of the stimulation medium from light exposure by covering the bag with the protection bag provided.
6. Connect the stimulation medium bag to the tubing set on valve 3 by sterile welding. Use a sterile tubing welder placed next to the instrument.
7. Place the stimulation medium bag on bag hanger B and make sure the clamp of the bag is open, The bag must be positioned at a suitable height to prevent severe bending or overstretching of the tubing.
8. Read the barcode label on the medium bag with the barcode reader. Alternatively, tap <edit> to enter part number (P/N) and batch code (LOT) manually with the keyboard shown on the screen.
9. Seal off or clamp off the parts of the tubing set that are not needed for cultivation to avoid diffusion of solutions or cells to these parts: bags on valve 1, valve 7 and valve 8, vial adapters on valve 2, the pre-column and the separation column of the tubing sets.
10. Connect the gas tubing and enter 5% for CO_2 concentration.
11. Temperature parameters will be set automatically to +37 °C (+99 °F), if temperature offset values are detected on the instrument.

Note: If the instrument has no temperature offset values, a warning will appear stating that no temperature offset values could be detected. In this case, the temperature parameter will be set automatically to +39 °C (+102 °F), which equals a cultivation temperature of +37 °C (+99 °F).

12. Enter a required activity matrix for γδ T cell manufacturing (Table 1 or Table 2). To enter a new activity matrix, select "Empty matrix" and press "save as".
13. Rename the new activity matrix with a maximum of 30 digits (e.g., "gd T cell protocol") and give it an individual description with a maximum of 300 digits. Special characters are not allowed.
14. Proceed with entering the cultivation parameters in the activity matrix. If only stimulation and expansion are required, enter the cultivation parameters described in Table 1. Additionally, if transduction is also required, enter the recommended parameters described in Table 2.

Note: The activity matrix described in Table 1 has been only verified for the use of zoledronic acid as stimulation reagent and for a cultivation time of up to 14 days. If using a different stimulation reagent, or different duration of cultivation, the activity matrix may need to be modified or a new activity matrix may need to be defined by the user.

Day	Activity (parameter)	Actions and volumes
0	Feed	Add 120 mL
1	Media exchange	Remove 260 mL; add 260 mL
2	Media exchange	Remove 260 mL; add 260 mL
2	Medium bag exchange	Attach one bag (2L) of culture medium
3	Media exchange	Remove 260 mL; add 260 mL
4	Media exchange	Remove 260 mL; add 260 mL
5	Media exchange	Remove 260 mL; add 260 mL
6	Media exchange	Remove 260 mL; add 260 mL
7	Media exchange	Remove 260 mL; add 360 mL
7	Activate Shaker	Shaker type 6
7	Medium bag exchange	Attach one bag (2 L) of culture medium
8	Media exchange	Remove 360 mL; add 360 mL
9	Media exchange	Remove 360 mL; add 460 mL
10	Media exchange	Remove 460 mL; add 560 mL
10	Activate Shaker	Shaker type 8
11	Media exchange	Remove 560 mL; add 560 mL
11	Activate Shaker	Shaker type 9
11	Medium bag exchange	Attach one bag (2L) of culture medium
12	Media exchange	Remove 300 mL; add 300 mL
12	Media exchange	Remove 560 mL; add 560 mL
13	Media exchange	Remove 300 mL; add 300 mL
13	Media exchange	Remove 560 mL; add 560 mL
13	Medium bag exchange	Attach one bag (2L) of culture medium
13	Waste bag exchange	Remove full waste bags and attach new waste bag
14	Media exchange	Remove 300 mL; add 300 mL
14	End of culture (harvest)	Harvest type 1

Table 1: Activity matrix with required culture parameters for the stimulation and expansion of γδ T cells starting from a TCRα/β and CD19 depleted fresh leukapheresis.

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Day	Activity (parameter)	Actions and volumes
0	Feed	Add 120 mL
1	Media exchange	Remove 260 mL; add 260 mL
2	Media exchange	Remove 260 mL; add 260 mL
2	Medium bag exchange	Attach one bag (2L) of culture medium
3	Media exchange	Remove 260 mL; add 230 mL
3	Transduction	Reagent volume 10 mL
4	Culture wash	1 cycle
4	Volume reduction	Target volume 300 mL
5	Media exchange	Remove 260 mL; add 260 mL
6	Media exchange	Remove 260 mL; add 260 mL
7	Media exchange	Remove 260 mL; add 360 mL
7	Activate Shaker	Shaker type 6
7	Medium bag exchange	Attach one bag (2L) of culture medium
8	Media exchange	Remove 360 mL; add 360 mL
9	Media exchange	Remove 360 mL; add 460 mL
10	Media exchange	Remove 460 mL; add 560 mL
10	Activate Shaker	Shaker type 8
11	Media exchange	Remove 560 mL; add 560 mL
11	Activate Shaker	Shaker type 9
11	Medium bag exchange	Attach one bag (2L) of culture medium
12	Media exchange	Remove 300 mL; add 300 mL
12	Media exchange	Remove 560 mL; add 560 mL
13	Media exchange	Remove 300 mL; add 300mL
13	Media exchange	Remove 560mL; add 560 mL
13	Medium bag exchange	Attach one bag (2L) of culture medium
13	Waste bag exchange	Remove full waste bags and attach new waste bag
14	Media exchange	Remove 300 mL; add 300 mL
14	End of culture (harvest)	Harvest type 1

Table 2: Activity matrix with required culture parameters for the stimulation, transduction and expansion of γδ T cells starting from a TCRα/β and CD19 depleted fresh leukapheresis

Note: The activity matrix described in Table 2 has been tested using baboon envelope pseudotype lentiviral vectors. It includes recommended non-verified parameters for transduction. Further parameter optimization may be required.

15. When the activity matrix is created and all editing completed, proceed to the final check of entered parameters.
16. Once final setup check is performed, proceed with automated culture set up.

Note: The culture setup is performed automatically and includes the rinsing of the tubing set, transfer of the respective amount of starting cellular material to the chamber, washing of the cells with stimulation medium and the setup of aeration and temperature. Afterwards, an automated feed of stimulation medium fills the culture volume to 300 mL.

17. Attach the culture medium bags when requested by the activity matrix.

Note: The automated culture will be performed until day 14. For each time a feeding step takes place, make sure that enough culture medium is connected to the tubing set on valve 3. Two medium bags can be connected to the tubing set at the same time. If two bags are connected, hang one bag lower than the other. Weld off the higher bag once emptied. Follow the software instructions for programmed medium bag exchange. If the Waste Bag is full, follow software instructions for programmed Waste Bag exchange.

The Activities "Medium bag exchange", "Waste bag exchange"and "End of culture" require user interaction on day 2, 7, 11,13 and 14. Additionally, samples can be taken for analysis independently of the activity matrix.

Changes to the activity matrix which are made during culture will only be effective for the current run. For available activities that can be modified, refer to the TCT-LS System user manual.

2.3. Transduction (optional)

Preparation of vector

- The transduction will not start without confirmation by the user.
- It is recommended to prepare the viral vector one hour before transduction. The viral vector must be prepared under sterile conditions in a bag that can be connected to the tubing set via sterile welding. The viral vector must be in a volume between 5 mL and 250 mL. It is recommended to use concentrated viral vector in a volume of 10 mL. If the vector is in a much larger volume, consider reducing the volume of the culture prior to transduction using the activity "volume reduction".

Optional addition of MACS GMP Vectofusin-1:

1. Prepare a bag containing the same volume of TexMACS GMP Medium as the vector volume and add 10 µg/mL of MACS GMP Vectofusin-1. The medium should not contain any additives.

Note: The final concentration of 10 µg/mL of MACS GMP Vectorusin-1 is calculated for the volume after transduction (culture volume + 2× Vector volume + 20 mL).

2. Add the vector to the bag at the same volume as the medium containing MACS GMP Vectofusin-1 (1:1 ratio).
3. Mix the contents of the bag gently and incubate for 10 minutes.

Note: The bag should not be filled completely as some medium will be added to the bag before the contents is drawn.

4. Connect the bag containing the viral vector to the tubing on valve 5 using a sterile tubing welder.

Note: The transduction is an activity defined within the culture parameters of the activity matrix described in Table 2 and will be performed automatically.

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2.4. Harvest

- The harvest will not start without confirmation by the user. Immediately start the harvest when asked in the pop-up window of the software. When the programmed time for "End of culture" is reached, user interaction is required.
- The user can choose between four harvest types. The process has been verified using "Harvest type 1". If a different harvest type is required, it needs to be edited in the activity matrix during culture set up.

1. Connect the formulation solution (e.g., CliniMACS Formulation Solution) to the spike port on valve 6 or via sterile welding below the filter.

Note: When a protein containing formulation solution is used (e.g., CliniMACS Formulation Solution), ensure to weld below the filter to remove it from the tubing set. Protein might cause clogging of the filter and impede sufficient fluid flow.

2. Read the bar code label on the formulation solution bag or press <edit> to enter the information manually.
3. Tap <ok> to continue.

Note: Cells can be filtered prior to harvest if required. This is possible via the pre-assembled in-line filter connected to use of the two designated Target Cell Bags. If using the in-line filter on the Target Cell Bag, make sure that the in-line filter is in an upright position. If filtration of the cells is not required, choose the alternative Target Cell Bag. If another specific Target Cell Bag is required, sterile connect this bag between valve 22 and the in-line filter.

4. Make sure that the clamp of the Target Cell Bag that will be used for harvest is open and liquid can flow freely. Close the clamp of the other Target Cell Bag that will not be used.
5. Follow the instructions from the software to prepare the Target Cell Bag. The volume of the cell product will be 100 mL.

Note: Make sure that the Waste Bag is not full at this point. If required, connect a new empty Waste Bag via sterile welding.

The automated rebuffering and harvesting step will take about 45 minutes. The cells in the chamber are washed twice with the formulation solution before transfer to the chosen Target Cell Bag on valve 22.

6. Once the harvest process is finished, weld off the target cell bag above the connection of the QC pouch so that the QC pouch stays on the target cell bag.

Note: At the end of the process, the program guides through the final process steps such as deinstallation of the tubing set. Follow the instructions in the CliniMACS Prodigy for cleaning and disinfection of the instrument. Copy the log file of the run and switch off the instrument via the shutdown function of the CliniMACS Prodigy.

Disclaimer

The CliniMACS System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485. In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Set TS and CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application, Investigational Device Exemption (IDE) or FDA approval.

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