Automated clinical-grade manufacturing of Vγ9Vδ2 T cells on the CliniMACS Prodigy®

Background

Gamma delta (γδ) T cells are a unique T lymphocyte subset crucial for immunosurveillance and tissue homeostasis.[1] The Vγ and Vδ chains of their T cell receptor (TCR) configuration enable MHC-independent recognition of diverse antigens.[1,2] This feature sets them apart from αβ T cells and makes them promising candidates for immunotherapy, particularly those using an allogeneic approach.[1–4]

The overall presence of γδ T cells in peripheral blood is low, with Vγ9Vδ2 T cells being the most common subset.[2] Using them in adoptive cell therapy requires establishing good manufacturing practice (GMP)–compliant methods to obtain sufficient cells for clinical doses. Vγ9Vδ2 T cells can be activated and expanded via exposure to natural or synthetic phosphoantigens, or aminobisphosphonate drugs such as zoledronic acid, in conjunction with cytokines.[1,5]

Often, GMP-compliant protocols to expand Vγ9Vδ2 T cells involve multiple manual steps that risk contamination and yield insufficient cell numbers for clinical applications. Furthermore, while genetic engineering can enhance γδ T cell cytotoxicity and targeting[1–3], their modification using common viral vector approaches remains challenging.

In this application note, we present data on the automated, clinical-grade manufacturing of Vγ9Vδ2 T cells using the CliniMACS Prodigy. The workflow developed consists of two processes (fig. 1). In the first process, TCRαβ$^+$ and CD19$^+$ cells are depleted from leukapheresis products to generate the starting material for subsequent expansion and optional transduction. In the second process, Vγ9Vδ2 T cells are preferentially activated and expanded from those depleted products using zoledronic acid. Additionally, activated Vγ9Vδ2 T cells can be successfully transduced within the same processing workflow using a baboon envelope pseudotyped (BaEV) lentiviral vector.

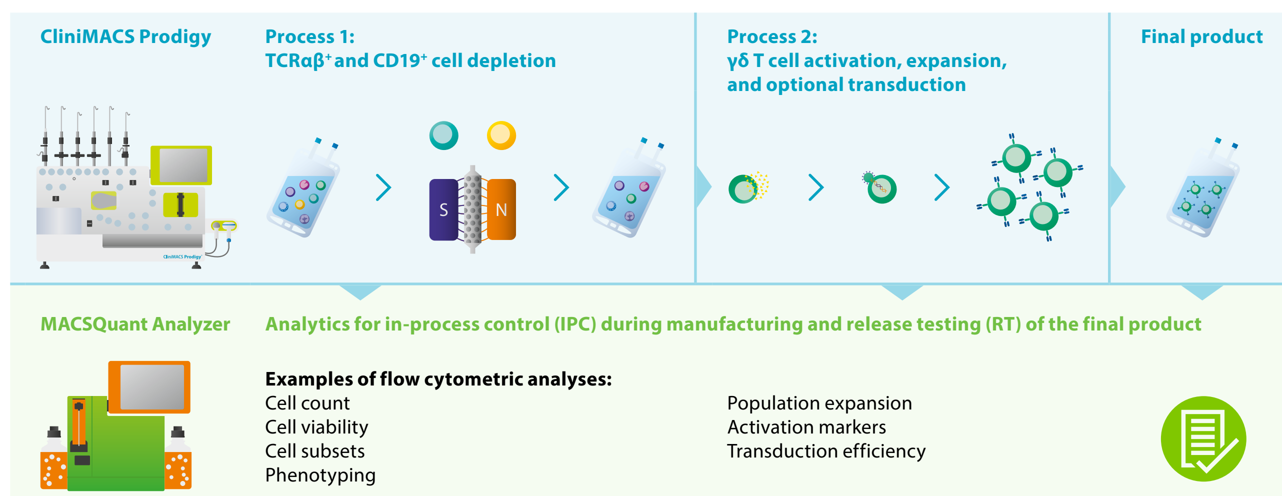

Figure 1: A two-process workflow for the generation of native or engineered γδ T cells from allogeneic starting materials and their analysis, including in-process control and release testing.

Materials and methods

Depletion of αβ T cells and B cells

Fresh, non-mobilized leukapheresis from healthy donors (n = 3) were depleted of αβ T and B cells using the CliniMACS Prodigy LP-TCRα/β-19-45RA System. Depletion was performed in the single-use CliniMACS Prodigy Tubing Set (TS) 320 with CliniMACS® TCR α/β-Biotin, CliniMACS Anti-Biotin Reagent, and CliniMACS CD19 Reagent.

Expansion of γδ T cells

The depleted cell fractions were transferred to the CliniMACS Prodigy TS 620 to proceed with Case 3 of CliniMACS Prodigy T Cell Transduction – Large Scale (TCT-LS) and a specific activity matrix for Vγ9Vδ2 T cell expansion. A total of 1×10^9 cells were seeded and cultured in stimulation medium consisting of TexMACS™ GMP Medium containing 5% human AB serum, 140 U/mL MACS® GMP Recombinant Human IL-15, 500 U/mL MACS GMP Recombinant Human IL-2, and 2 µg/mL zoledronic acid (pharmaceutical grade). As soon as the stimulation medium was consumed, medium without zoledronic acid was supplied, and the cells were maintained in culture for up to 14 days.

γδ T cell transduction and VCN determination

Another set of depleted cell fractions (n = 3) were genetically modified via viral transduction. The cells were seeded and stimulated as described above using a different activity matrix that included automated transduction. On day 3 of stimulation, the cells were incubated for 24 h with BaEV lentiviral particles containing a specific chimeric antigen receptor (CAR) construct and 10 µg/mL of MACS GMP Vectofusin®-1. Afterwards, the viral vector was washed out and the cells were expanded for up to 14 days. Vector copy number (VCN) was determined on day 14 using the MACS COPYcheck Kit, human, having isolated genomic DNA with the DNeasy® Blood & Tissue Kit (Qiagen).

QC testing by flow cytometry

The MACSQuant® Analyzer 10 was used to monitor T cell population growth and composition on days 0, 7, 10, and 14. Transduction efficiency was determined on days 7, 10, and 14 using the corresponding CAR Detection Reagent. The product table on the last page lists the antibodies and reagents used for analytical testing. For further details on the specific panels used, contact your local Technical Support.

Results

Efficient depletion of αβ T and B cells to boost safety of allogeneic starting material for γδ T cell expansion

αβ T and B cells must be removed from allogeneic material for γδ T cell therapy products to prevent graft-versus-host disease (GVHD) or B cell–triggered disorders.[6] We performed this depletion before γδ T cell expansion using an established automated process on the CliniMACS Prodigy, which resulted in a logarithmic depletion >4 for αβ T cells and >3 for B cells (table 1). Consequently, γδ T cells were partially enriched with a mean recovery of 87%. Given their low frequency in peripheral blood, however, that translates to 3.7% of the remaining cells. Other cells included monocytes and NK cells (table 1). On average, Vγ9Vδ2 T cells accounted for 72% of the γδ T cell population.

Strong expansion and favorable phenotype of Vγ9Vδ2 T cells

Cell fractions depleted of αβ T cells and B cells served as starting material for γδ T cell expansion on the CliniMACS Prodigy. We used zoledronic acid added to the culture medium to stimulate Vγ9Vδ2 T cells. The combination with TexMACS GMP Medium, IL-2, IL-15, and optimized feeding conditions achieved expansion rates exceeding 200-fold. After 14 days in culture, 90% of the cells were γδ T cells (98% of them were Vγ9Vδ2 T cells) with only low levels of αβ T cells and B cells (table 1). Despite donor variation regarding expansion capacity, an average of 6.63×10^9 γδ T cells were obtained by day 14, with up to 8.9×10^9 for one donor (fig. 2A). The expanded γδ T cells were highly viable (>97%) and exhibited a favorable central and effector memory phenotype (fig. 2B).

Parameter	Starting material depleted of αβ T and B cells (n = 3)	Final cellular product after expansion (n = 3)
αβ T cells (%)	0.007 ± 0.004	0.130 ± 0.180
γδ T cells (%)	3.670 ± 1.360	89.75 ± 5.520
B cells (%)	0.005 ± 0.003	0.007 ± 0.005
Monocytes (%)	59.37 ± 8.460	0.030 ± 0.008
NK cells (%)	24.58 ± 8.540	9.790 ± 5.580
$TCR\alpha\beta^+$ cell log depletion	4.590 ± 0.260	–
$CD19^+$ cell log depletion	3.960 ± 0.230	–

Table 1: Composition of starting material and final product of γδ T cell manufacturing using the CliniMACS Prodigy Platform. Values represent mean and standard deviation.

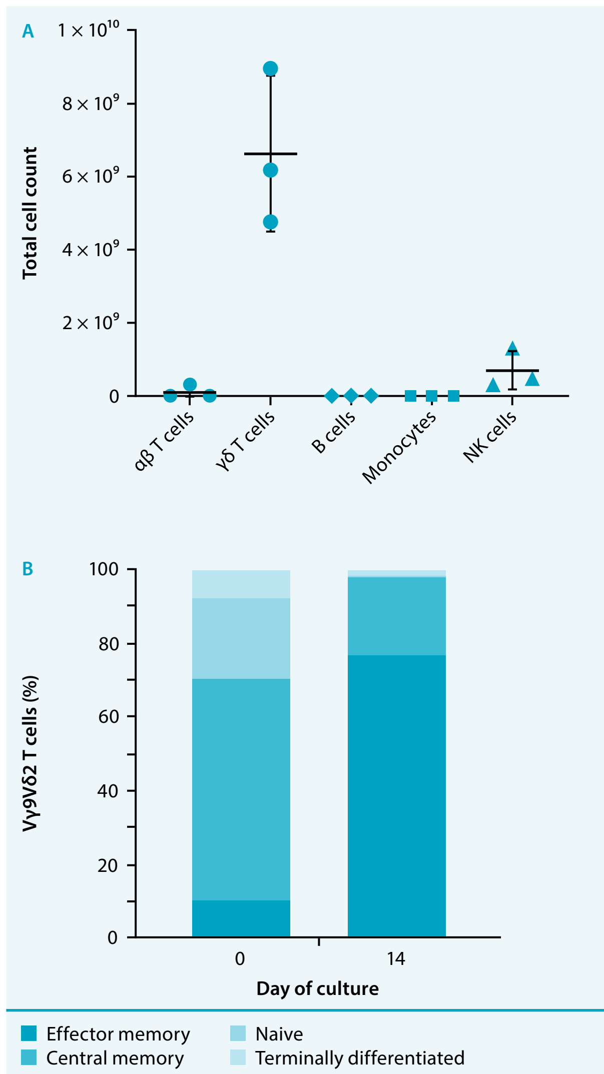

Figure 2: Vγ9Vδ2 T cells are efficiently expanded on the CliniMACS Prodigy Instrument and exhibit a central and effector memory phenotype. By day 14 in culture, absolute cell numbers from the starting material of three healthy donors averaged 6.63×10^9 total viable γδ T cells (A). The number of γδ T cells with effector memory ($CD27^-CD45^-$) and central memory ($CD27^+CD45RA^-$) phenotype increased through expansion, reducing the proportion of naive ($CD27^+CD45RA^+$) and terminally differentiated ($CD27^-CD45RA^+$) cells.

BaEV lentiviral vector efficiently generates CAR γδ T cells

In addition to establishing optimized expansion conditions for Vγ9Vδ2 T cells on the CliniMACS Prodigy, we evaluated the generation of CAR γδ T cells by adapting the predefined culture conditions to include a viral transduction step. We used BaEV lentiviral particles to introduce a specific CAR construct. This choice was based on previous observations that BaEV lentiviral particles significantly increase transduction rates of γδ T cells compared to the commonly used VSV-G pseudotype.[7]

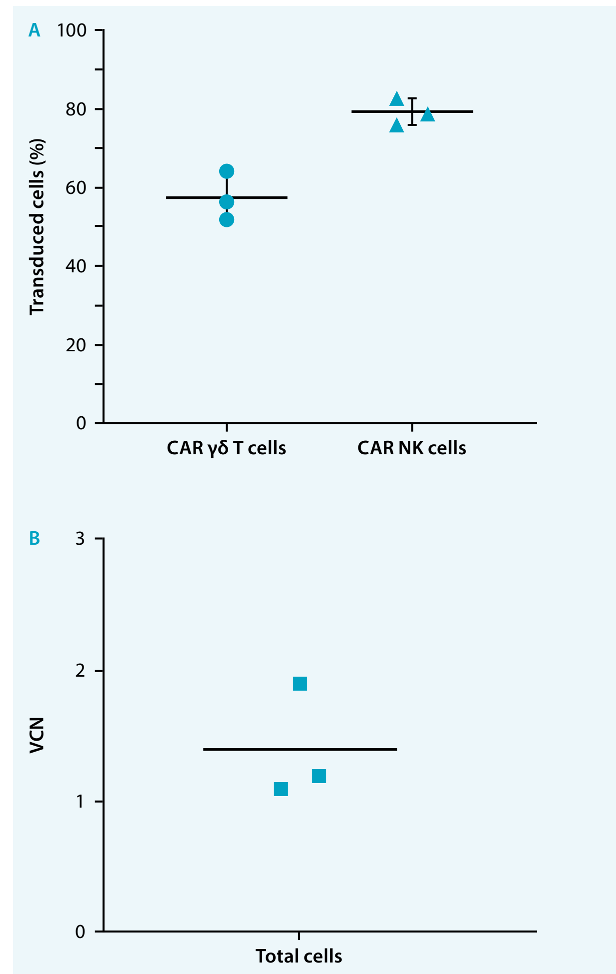

Figure 3: CAR γδ T cells are generated with high transduction efficiency and low VCN on the CliniMACS Prodigy.
The frequency of transduced γδ T cells and NK cells expressing a specific CAR was evaluated on day 14 of culture (A). Likewise, the VCN of the final cell product was determined on day 14. Shown are values from three healthy donors.

By day 14 in culture, an average of 57% of the expanded γδ T cells expressed the specific CAR (fig. 3A). Because many NK cells were still present when transduction took place on day 3, they were also efficiently transduced with BaEV lentiviral particles. Certain NK and CAR NK cells remained after the expansion period, contributing to the cytotoxic potential of the final cellular product (fig. 3A). After transduction, cells were expanded up to day 14. An average of 1.5×10^9 total γδ T cells (among those 8.2×10^8 CAR γδ T cells) were obtained with an average VCN of 1.4, suggesting a safe profile for the final cell product (fig. 3B).

Conclusions

This application note highlights the capabilities of the CliniMACS Prodigy in streamlining the manufacturing of Vγ9Vδ2 T cells to achieve clinically relevant doses for adoptive cell therapy, while enabling the effective depletion of αβ T cells and B cells for safer use in allogeneic approaches. Moreover, the integration of viral transduction using BaEV lentiviral particles into the processing workflow enables the efficient generation of CAR γδ T cells.

This automated and closed cell manufacturing workflow offers a promising avenue for the scalable and reproducible production of both unmodified and engineered Vγ9Vδ2 T cells for clinical applications, which represents a significant advancement for the development of novel cell-based immunotherapies.

References

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4. Yazdanifar, M. *et al.* (2020) γδ T cells: the ideal tool for cancer immunotherapy. Cells 9: 1305.
5. Bold, A. *et al.* (2023) An optimized cultivation method for future *in vivo* application of γδ T cells. Front. Immunol. 14:1185564.
6. Bethge, W. A. *et al.* (2021) Results of a multicenter phase I/II trial of TCRαβ and CD19-depleted haploidentical hematopoietic stem cell transplantation for adult and pediatric patients. Bone Marrow Transplant. 57:423–430.
7. Pinot, L. *et al.* (2025) Transduction of γδ T cells with baboon envelope pseudotyped lentiviral vector encoding chimeric antigen receptors for translational and clinical applications. Front. Immunol. 16: 1548630.

Product table

Product	Order no.
CliniMACS Prodigy Instrument with LP-TCRαβ-19-45RA Depletion application software and TCT-LS application software	200-075-301
CliniMACS Prodigy TS 320	Specific to regulatory variant required
CliniMACS TCRα/β-Biotin	
CliniMACS Anti-Biotin Reagent	
CliniMACS CD19 Reagent	
CliniMACS Prodigy TS 620	200-073-620
TexMACS GMP Medium, 2000 mL	170-076-306
MACS GMP Recombinant human IL-2	170-076-147
MACS GMP Recombinant human IL-15	170-076-114
MACS GMP Vectofusin-1	170-076-165
MACS COPYcheck Kit, human*	130-128-157
MACSQuant Analyzer 10	130-096-343
CD45 Antibody, anti-human, VioBlue®, REAfinity® (REA747)	130-110-637
TCRγ/δ Antibody, anti-human, APC, REAfinity (REA 591)	130-113-508
CD14 Antibody, anti-human, VioGreen™, REAfinity (REA599)	130-110-525
TCRα/β Antibody, anti-human, PE (BW242/412)	130-113-531
CD3 Antibody, anti-human, FITC, REAfinity (REA613)	130-113-138
CD56 Antibody, anti-human, PE-Vio® 615, REAfinity (REA196)	130-114-550
CD20 Antibody, anti-human, APC-Vio 770, REAfinity (REA780)	130-111-341
TCR Vδ2 Antibody, anti-human, VioBlue, REAfinity (REA771)	130-111-015
CD45RA Antibody, anti-human, VioGreen, REAfinity (REA562)	130-113-369
TCR Vδ1 Antibody, anti-human, FITC, REAfinity (REA173)	130-118-362
TCR Vγ9 Antibody, anti-human, APC-Vio 770, REAfinity (REA470)	130-128-218
CD27 Antibody, anti-human/mouse, PE, REAfinity (REA499)	130-113-640
7AAD Staining Solution	130-111-568
FcR Blocking Reagent	130-059-901
CAR Detection Reagents **	–

* Only compatible with the lentiviral vectors provided by Miltenyi Bioindustry and Miltenyi Biotec.
For more information on MACS antibodies, please visit **miltenyibiotec.com/antibodies**

** For information on CAR Detection Reagents, please visit **miltenyibiotec.com/CARdetection**

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