Automated tumor-infiltrating lymphocyte expansion on a GMP-compliant cell manufacturing platform

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Background

Tumor-infiltrating lymphocytes (TILs) are crucial components of the tumor microenvironment. They play a significant role in tumor growth, cancer progression, and therapy response[1], and several studies have associated the presence of activated, proliferating T cells in primary tumors with improved survival[2]. The inherent ability of TIL populations to recognize unique cancer cell targets addresses issues with tumor heterogeneity and immune escape mechanisms limiting cell therapies. Given these advantages, TILs have been deployed in therapeutic settings with remarkable results in recent years[3–6] and a first FDA approval in early 2024 (lifileucel).[7,8]

Despite progress, broad clinical implementation faces numerous challenges, including regulatory barriers, clinical protocol optimization, patient-specific variability, patient access, drug availability, manufacturing process optimization, and production costs.[9–11] As a provider of cell manufacturing solutions, Miltenyi Biotec strives to improve the efficiency and effectiveness of TIL production processes. The key to success lies in standardized and automated protocols that are scalable and reliably generate high-quality products.

The CliniMACS Prodigy® Tumor Reactive T Cell (TRT) Process streamlines and automates GMP-compliant TIL manufacturing. This application note presents data from Singula Bio Ltd. on the manufacture and analysis of fully functional neoantigen-reactive TILs derived from ovarian cancer tissues. Furthermore, we demonstrate the reproducibility of the CliniMACS Prodigy TRT Process by comparing runs from further manufacturing sites (Miltenyi Biotec, DE; Miltenyi Bioindustry, US) using different starting materials (melanoma and large-cell lung cancer tissues).

Methods

Preparation and analysis of starting material

Fresh tumor material from ovarian cancer (advanced stage, primary presentation) and melanoma (advanced stage) patients was harvested perioperatively and processed on the same day. The tissues were dissociated in an enzymatic cocktail using the gentleMACS™ Octo Dissociator with Heaters according to the manufacturer's instructions. The resulting single-cell suspensions were characterized using flow cytometry. A large-cell lung cancer (LCLC; advanced stage) tumor digest was obtained from a third-party vendor.

Epitope prediction and neoantigen simulation

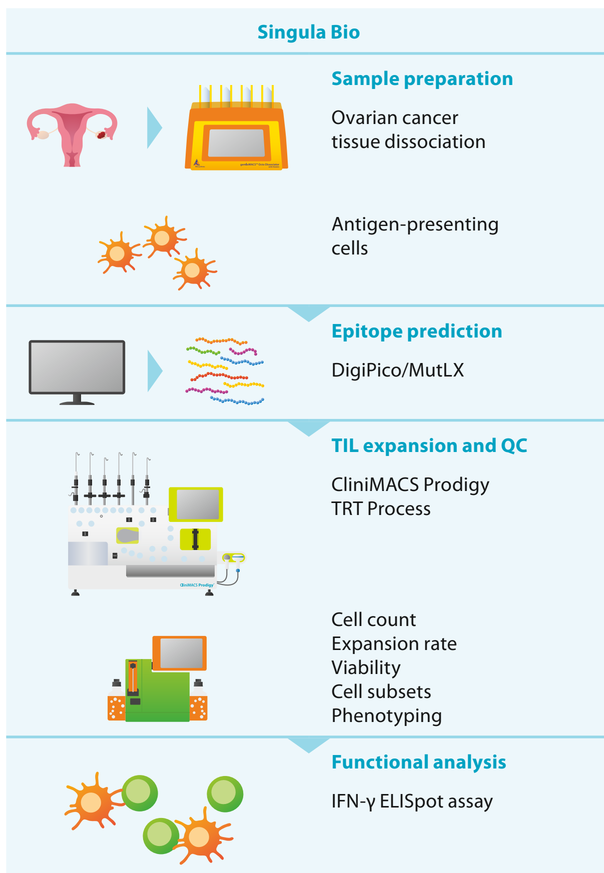
Single-cell suspensions of ovarian cancer tissue were sorted and sequenced using Singula Bio's proprietary method. This method combines their novel digital whole-genome sequencing of picogram quantities of DNA (DigiPico) with a machine-learning algorithm to detect rare mutations from small numbers of cells (Mutation Learning or MutLX).[12]

Automated TIL expansion using the CliniMACS Prodigy TRT Process

TILs were expanded for 14 days using the rapid expansion protocol of the CliniMACS Prodigy TRT Process (case 3; see User Manual).[13] In this application note, four independent manufacturing runs processing digests of different tumor entities were performed at three sites (fig. 1). For all runs, TexMACS™ GMP Medium was supplemented with 3% human AB serum, IL-2 (3,000 IU/mL), and antibiotics (Penicillin/Streptomycin 1%) to minimize contamination risk. TILs were activated with MACS® GMP CD3 pure in the presence of 4×10^8 irradiated feeder cells.

Generation of feeder cells

Growth-arrested feeder cells were derived from peripheral blood mononuclear cells (PBMCs) of a healthy-donor leukapheresis (LP), irradiated with 50 Gy, and resuspended in TexMACS GMP Medium.



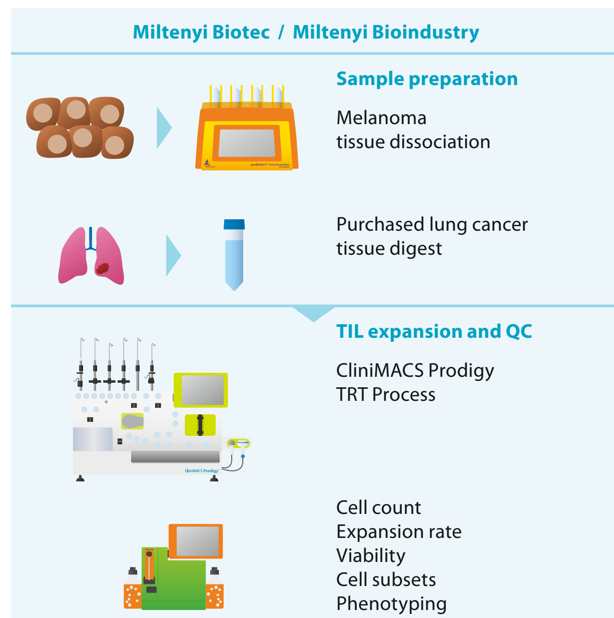


Figure 1: Overview of the experimental setup. Experiments were conducted at three sites and using different tumor entities as starting material. Tumor tissue was processed with the gentleMACS Technology, except at Miltenyi Bioindustry where a tumor digest was purchased from an external vendor. TILs were expanded for 14 days on the CliniMACS Prodigy Instrument. Singula Bio additionally carried out neoantigen-derived peptide prediction and synthesis via single-cell sequencing and AI-driven prediction (DigiPico/MutLX). They then prepared antigen-presenting cells for an IFN-γ ELISpot assay to assess the reactivity of the expanded TILs toward the predicted neoantigens.

In-process control (IPC) and release testing (RT) via flow cytometry

Samples were collected at different time points (start, during, and end of culture) during the 14-day expansion. The immune cell composition of samples was analyzed on the MACSQuant® Analyzer 10 using the StainExpress™ Immune Cell Composition Cocktail, human, and CAR T Cell Express Mode Package or using custom antibody panels. T cell subsets were characterized in the final cell product derived from lung cancer (data not shown) and melanoma tissue, and their expression of T cell exhaustion markers (TIM-3, LAG-3, and PD-1) was evaluated with dedicated panels.

IFN-γ ELISpot assay

An IFN-γ ELISpot assay was used to assess if the predicted neoantigens trigger a response by the expanded TILs.[14,15] Briefly, pulsed antigen-presenting cells were co-cultured with harvested ovarian cancer–derived TILs in three technical replicates for 18–48 hours. T Cell TransAct™, human, was used as a positive control. Spots were counted on an automated system (CTL Analyzer, Cellular Technology).

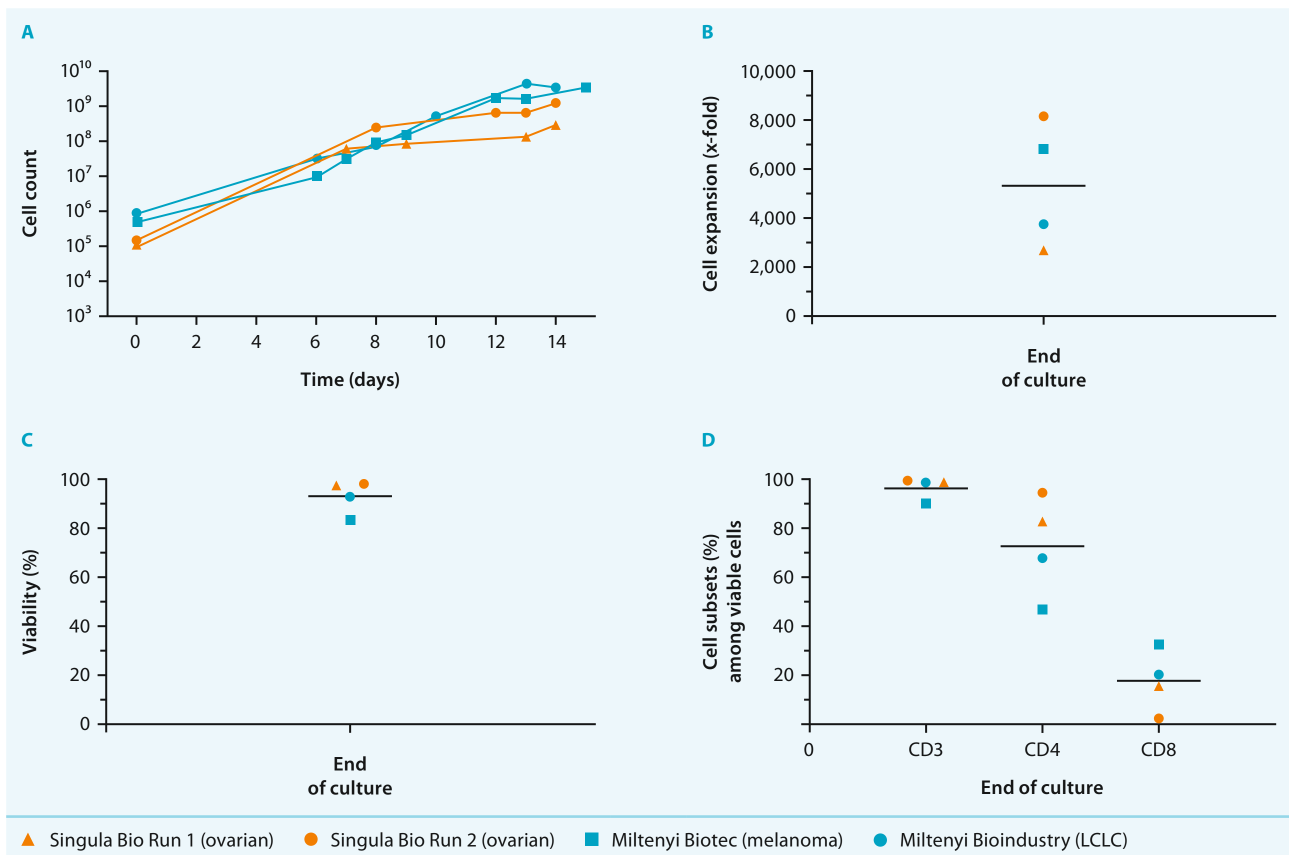


Figure 2: Flow cytometry analysis of T cell expansion, viability, and subsets. The CliniMACS Prodigy TRT Process consistently expanded TILs over 14 days regardless of tumor source or manufacturing site (A). Each line represents a separate manufacturing run. Expansion rates for the automated cultivation on the CliniMACS Prodigy Instrument ranged between 2,676 and 8,150-fold (B). The cell products showed high viability (C) and variable cell subsets after the 14-day expansion period (D).

Results

High-rate expansion of viable TILs regardless of manufacturing site or tumor source

Low cell counts of 1.05–8.00×10^5 were successfully expanded in 14 days to up to 3.41×10^9 cells using the rapid expansion protocol of the CliniMACS Prodigy TRT Process (fig. 2A, table 1). The high-fold expansion (fig. 2B) produced consistently viable cell populations (fig. 2C) comprised of up to 99.8% T cells ($CD3^+$) with variable percentages of $CD8^+$ and $CD4^+$ T cells (fig. 2D).

Site	Tumor source	Starting cell number	Viability at end of culture [%]	Cell number in final product
Singula Bio Run 1	Ovarian cancer	1.05×10^5	97.38	2.81×10^8
Singula Bio Run 2	Ovarian cancer	1.50×10^5	98.00	1.22×10^9
Miltenyi Bioindustry	Large-cell lung cancer	8.00×10^5	92.46	3.00×10^9
Miltenyi Biotec	Melanoma	5.00×10^5	83.23	3.41×10^9

Table 1: Performance data for four independent runs to expand TILs derived from tumor material using the CliniMACS Prodigy TRT Process.

TILs derived from melanoma consist predominantly of effector memory cells with low LAG-3 and PD-1 expression

The final product of melanoma-derived TILs had predominantly an effector memory and central memory phenotype (fig. 3A). Based on the low expression of LAG-3 and PD-1, as well as the low co-expression of these markers plus TIM-3, the expanded TILs did not show an exhausted phenotype (fig. 3B).

TILs derived from ovarian cancer show neoantigen reactivity and tumor-specific killing

The reactivity of expanded ovarian cancer–derived TILs was assessed *in vitro*. The production of IFN-γ upon stimulation by neoantigen-presenting cells was assessed with an IFN-γ-ELISpot assay. Induction of higher IFN-γ production using mutants was confirmed compared with wildtype controls (data not shown), demonstrating that functional neoantigen-specific TILs were expanded. The killing ability of the final cell product was also confirmed (data not shown).

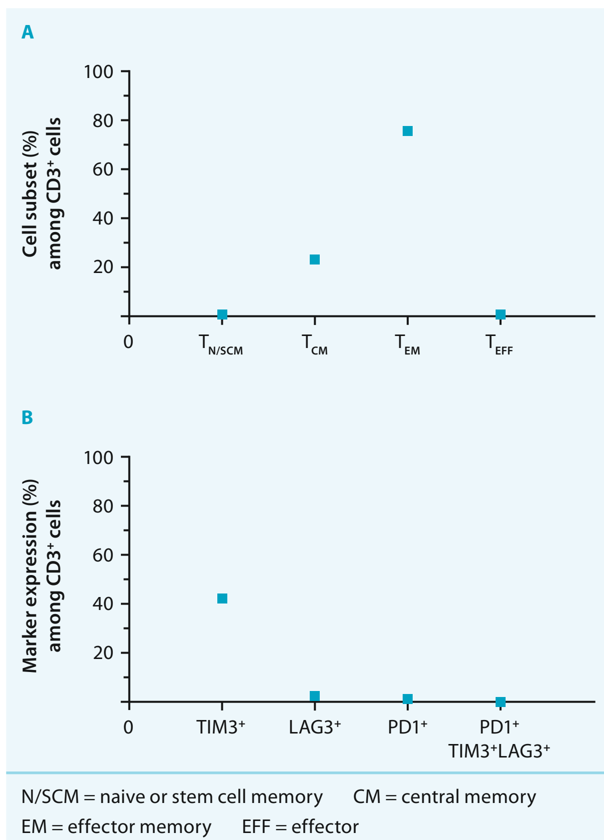


Figure 3: Flow cytometry analysis of melanoma-derived and expanded TILs. After expansion with the CliniMACS Prodigy TRT Process, the harvested cells showed an effector memory and central memory phenotype (A) and low exhaustion levels (B).

Conclusions

The present application note underscores the robustness of the CliniMACS Prodigy TRT Process for TIL manufacturing. Independent of starting material and manufacturing site, comparably rapid expansion to high numbers of viable TILs was observed. Central to that performance is the high degree of automation and the closed operation of the CliniMACS Prodigy Platform. The final products comprised up to 99.8% T cells (CD3⁺). Analysis of the melanoma-derived TILs showed a predominantly effector memory phenotype with low exhaustion levels. TILs derived from ovarian cancer samples and enriched for a specific activation marker showed strong killing capacity *in vitro*. These data corroborate the suitability of the CliniMACS Prodigy TRT Process to expand fully functional tumor-reactive TILs for cancer immunotherapy.

Product	Order no.
Tumor Dissociation Kit, human	130-095-929
gentleMACS Octo Dissociator with Heaters	130-096-427
CliniMACS Prodigy Instrument	200-075-301
CliniMACS Prodigy TS 520	200-073-613
CliniMACS® PBS/EDTA Buffer	200-070-022
TexMACS GMP Medium	170-076-306
T Cell TransAct, human	130-111-160
MACS GMP CD3 pure	170-076-124
MACS GMP Recombinant Human IL-2 (500 µg)	170-076-147
MACSQuant Analyzer 16	130-109-803
StainExpress Immune Cell Composition Cocktail, human	130-127-637
CAR T Cell Express Mode Package	160-002-376

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