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Simplified protocol for clinical-grade tumor-infiltrating lymphocyte manufacturing with use of the Wave bioreactor

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Abstract

Background aims. The high level of complexity of current Good Manufacturing Practice–compliant methods of manufacturing hampers rapid and broad application of treatment with tumor-infiltrating lymphocytes (TILs). *Methods.* To ensure higher applicability of TIL production to laboratory routine, a practical and simple protocol of TIL manufacturing with the use of a closed-system bioreactor was developed and implemented at our institution. *Results.* This protocol enabled significant work load reduction during the most labor-intense step of TIL expansion, and allowed generation of high-quality TIL products, which mediated clinical regression in patients with metastatic melanoma. *Conclusions.* Implementation of simplified methods of TIL expansion will speed up dissemination of TIL methods worldwide and will increase patient access to this highly effective treatment.

Key Words: *metastatic melanoma, rapid expansion, tumor-infiltrating lymphocytes, Wave bioreactor*

Introduction

Adoptive T-cell therapies (ACTs) are expected to enter the mainstream of standard treatment for advanced cancers in the next few years (1). Current data documenting the efficacy of the unarguably most successful form of ACT—based on tumor-infiltrating lymphocytes (TILs)—support the use of clinical-grade products manufactured in close proximity of the clinical center, which are infused immediately after release (2–6).

However, to date, only a handful of ACT programs that are based on TILs have been initiated at highly specialized cancer treatment institutions worldwide, mostly because of the complexity and high labor intensity of current TIL manufacturing methods. Previous studies suggest that broad application of TIL methods can be achieved through the implementation of a blood-banking model, with specialized facilities for TIL production integrated into existing blood banks at selected cancer hospitals (7). Therefore, practical protocols simplifying TIL manufacturing to support widespread application of ACTs are needed.

The use of the Wave (Cardiff, UK) for TIL or other T-cell product manufacture has been described previously by others (8–10). This is a closed-system bioreactor that uses a rocking platform inducing "waves" into the media and therefore providing an optimal oxygenation to the expanding TILs, as well as active perfusion to generate high cell numbers in minimal volumes. This greatly simplifies clinical-scale manufacturing of TILs under optimal conditions. Several advantages over classic expansion in static conditions (with the use of flasks or gas-permeable bags) have been identified, such as reduced working volume, culture manipulations and work load; high level of automation with automatic media exchange, reducing the accumulation of waste products into culture medium; closed nature of the system, which makes it easily compliant to current Good Manufacturing Practice (cGMP) regulations (8,11).

In the present study, we developed and implemented into a phase II clinical trial performed at the Center for Cancer Immune Therapy, Herlev, Denmark, a simplified protocol for T-cell expansion to be used in the second step of TIL manufacturing (protocol, see Supplementary Materials). Implementation of this protocol into routine practice of blood bank–based production of TILs is warranted.

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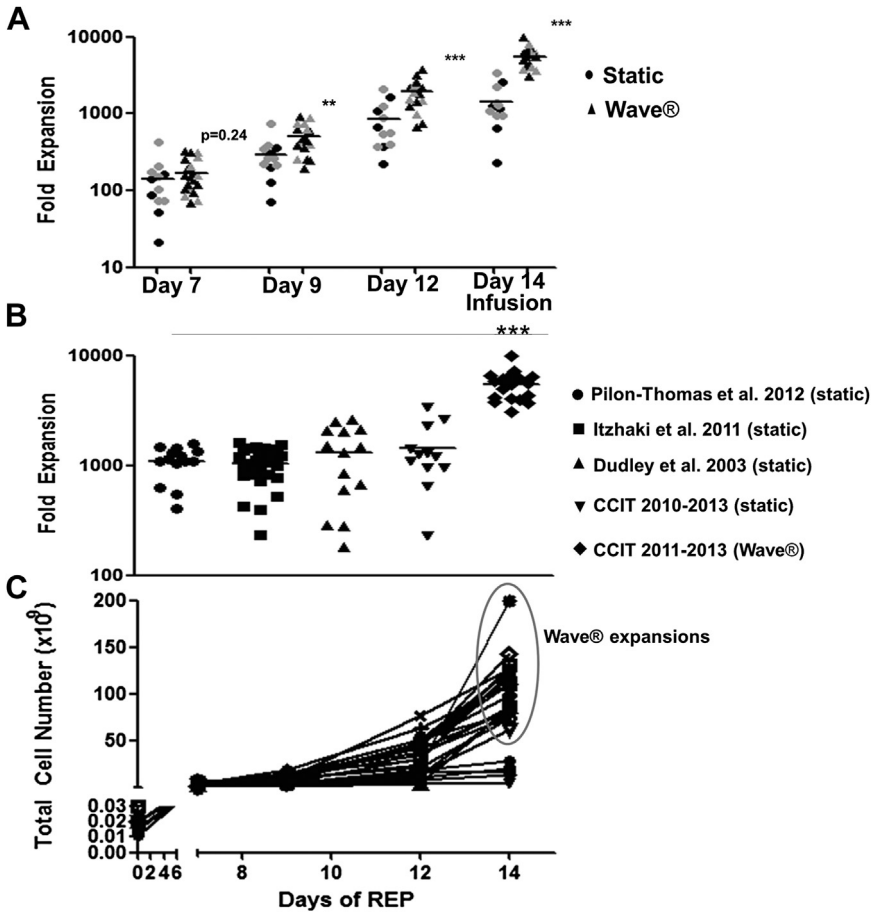

Figure 1. Rapid expansions with standard static conditions or with use of the Wave. (A) Fold expansions in REPs performed at the Center for Cancer Immune Therapy, Herlev, Denmark. Black dots indicate consecutive REPs; gray dots indicate REPs performed in parallel with static conditions or with use of the Wave. (B) Comparison of fold expansions in REPs performed at different centers. (C) Total cell numbers for infusion in the clinical trial NCT00937625 produced at the Center for Cancer Immune Therapy (n = 24 infusion products) with the use of static conditions of expansion or with use of the Wave. $^{**}P < 0.01$; $^{***}P < 0.001$.

TILs were initially isolated by plating 1- to 3-mm^3 fragments of melanoma metastases in individual wells of 24-well plates and culturing in high doses of interleukin-2 with either the "Standard" or "Young" TIL method, as previously described (12). Cryopreserved or freshly prepared TILs were subjected to a standard 14-day Rapid Expansion Protocol (REP) with anti-CD3 antibodies, a 200-fold excess of allogeneic irradiated peripheral blood mononuclear feeder cells and high doses of interleukin-2, as described (13).

REPs were initiated with 1×10^6 TILs per T175 flask (the amount was corrected to obtain 1×10^6 T cells when the culture contained less than 65% T cells), for a total of 10–30 flasks.

At day 7 of REP, TIL expansion was continued into static conditions (T175 flasks or gas-permeable bags, PL732 Polyolefin bags, 1000 mL, Fenwal Baxter, Mont Saint Guibert, Belgium) or TILs were transferred to the Wave, and in the latter case our novel investigational protocol was used. This protocol allows minimal cell manipulation after Wave start, with operator-dependent actions taken only at day 8–9 and 12 before harvest at day 14 (protocol, see Supplementary Materials), as opposed to static protocols requiring cell counting every 2–3 days as well as manual culture splitting or media addition to maintain appropriate cell densities, and opposed to previous Wave protocols requiring daily pH, glucose and ammonia control to adjust the automatic media perfusion rate (8).

In our studies, cell harvesting (day 14) was carried out by gravity-dependent transfer into 500-mL centrifuge containers (Corning) inside a laminar flow cabinet with subsequent washing by means of centrifugation, which introduces a final open step. However, fully closed methods of cell harvesting and washing are possible with connection of Wave cell bag ports to appropriate pumping systems and volume reduction with automated apheresis, as described (14).

Importantly, in our protocol, all operator-dependent manipulations are taken during working (week) days—provided REP is started on a Wednesday—therefore