Miltenyi Biotec

CliniMACS® Technology in stem cell transplantation

Graft manipulation

Balance of GVT versus GVHD

Fighting infections

Towards complete automation

CliniMACS® System – applications

Benefits of passive T cell depletion

Prevention of GVHD in allogeneic HSCT

Graft-versus-host disease (GVHD) is one of the main complications after allogeneic hematopoietic stem cell transplantation (HSCT).[1] To overcome the risk of GVHD, Miltenyi Biotec has developed several strategies for graft engineering, based on clinical-grade antibodies for different epitopes. In Europe, the CliniMACS® System components are available as CE-marked medical devices.

CD34⁺ cell enrichment – effective passive T and B cell depletion

In vitro CD34⁺ cell enrichment with the CliniMACS System is a very potent technology for effective T cell depletion and results in a 10^4 to 10^5-fold depletion of T cells from the graft. A high *ex vivo* T cell depletion enables effective prevention of GVHD.[2–4] This technique has been widely used for almost two decades.

Single GVHD prophylaxis – FDA approved

In 2014, the CliniMACS CD34 Reagent System received approval by the US Food and Drug Administration (FDA), based on the results of a phase II single-arm multicenter clinical trial (BMT CTN 0303) (see figure 1). It was registered as sole GVHD prophylaxis in allogeneic HSCT from an HLA-identical sibling donor in adult patients with acute myeloid leukemia (AML) in first complete remission. Particularly striking is the very low incidence of chronic GVHD at 2 years (19%), and the high rate of GVHD-free survival at two years (46%). These data suggest the benefit of the therapy is achieved, without negatively affecting engraftment, relapse, overall survival, or disease-free survival.[5]

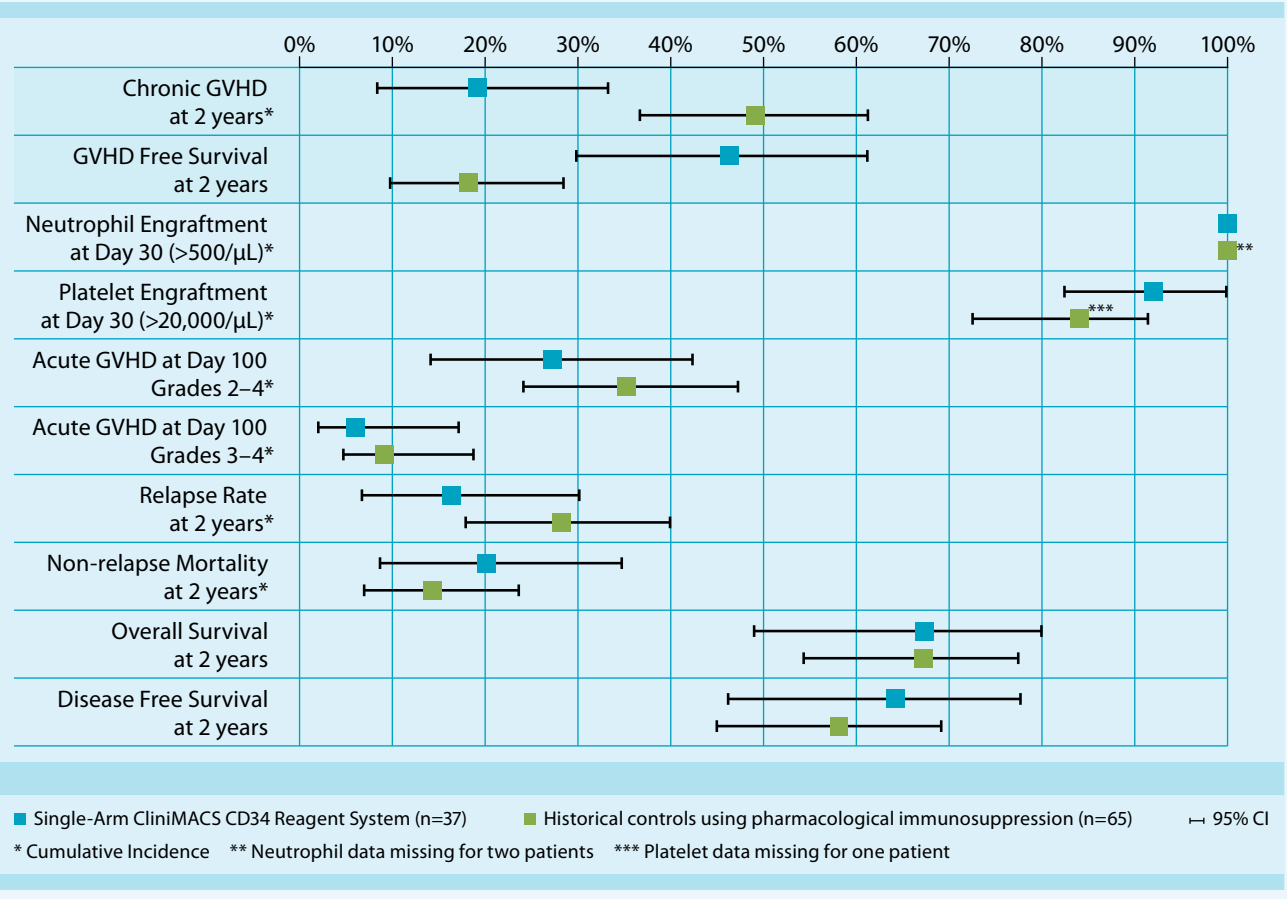

Figure 1: Comparative analysis.[5]

Stem cell boost – rescue therapy for Poor Graft Function (PGF)

PGF is a complication after allogeneic HSCT associated with increased morbidity and mortality due to severe infections, hemorrhagic complications, and organ failure caused by iron-overload. The syndrome is defined as transfusion-dependent cytopenia in one or more hematopoietic cell lines for at least 2 consecutive weeks beyond day +14 post-transplant or at any time point after achieving engraftment. A boost of selected CD34+ stem cells from the initial donor without any further GVHD prophylaxis has been described as an option to overcome persistent PGF. Table 1 summarizes published diagnosis criteria for PGF. Partially, the absence of severe GVHD, relapse, CMV reactivation, and myelosuppression are diagnostic criteria, too. Clinical results show a 72–100% rapid and sustained lineage recovery with low rates of GVHD. 3 years overall survival rates of 40–63% were reported.[6–9]

Genua	Hamburg/ Marseille	Tübingen
2–4 cytopenic cell lines	>2 cytopenic cell lines	Any cytopenic cell line
Hb<10 g/dL	Hb<8.5 g/dL	Hb<8.0 g/dL
Neutrophil count $<1.0\times10^9$/L	Neutrophil count $<1.5\times10^9$/L	Neutrophil count $<0.5\times10^9$/L
Platelet count $<30\times10^9$/L	Platelet count $<30\times10^9$/L	Platelet count $<20\times10^9$/L
Complete donor chimerism	Complete donor chimerism	Complete donor chimerism

Table 1: Summarized criteria for PGF from different sites.

The Cord Haplo Approach

CD34+ cell enrichment has also been applied in combined cord–haplo transplantations in which a CD34+ cell-enriched haploidentical transplant bridges the gap until the primary engraftment of the cord blood transplant takes place. The idea of a combined cord-haplo tranplantation is based on the observation that engraftment after haploidentical transplantation occurs faster than after cord blood transplantation. To provide the benefit of faster engraftment, the patient receives a bridging haploidentical transplant together with the cord blood at the same time. After several weeks chimerism shows 100% cord blood–derived cells. Clinical data show that with this approach even cord blood units containing very low stem cell numbers can be transplanted successfully and lead to sustained engraftment.[10–11]

CD34+ cell enrichment in autologous HSCT

CD34+ cell enrichment was originally developed for passive tumor cell depletion from autologous stem cell grafts. Currently, the technique is used for some tumors of early childhood[12–13] or non-Hodgkin lymphoma and other diseases in adults[14–15]. Furthermore, CD34+ cell enrichment has also been used to deplete autoreactive cells from stem cell grafts for severe refractory autoimmune diseases like systemic lupus erythematosus and systemic sclerosis.[16–18]

CliniMACS® System – concepts

Modern approaches to active T cell depletion

Active T and B cell depletion

The graft after active T and B cell depletion contains CD34$^+$ stem cells, CD34$^-$ stem cells, other progenitor cells, natural killer (NK) cells, and other members of the innate immune system that might have engraftment-facilitating effects. Based on the encouraging results of studies using stem cell grafts after depletion of CD3$^+$/CD19$^+$ cells, further depletion strategies for distinct T cell subsets have been developed.[19–21]

Active depletion of TCRα/β$^+$/CD19$^+$ cells

One possibility for active T cell depletion is the CliniMACS® TCRα/β$^+$/CD19$^+$ cell depletion system (TCR: T cell receptor). The process results in the depletion of alloreactive TCRα/β$^+$ T cells. This approach retains stem cells and immune effector cells, such as NK cells and TCRγ/δ$^+$ T cells, in the cellular product. Both cell populations are reported to induce graft-versus-leukemia/tumor (GVL/T) effects while the potential risk of inducing GVHD may be reduced.[22]

TCRγ/δ$^+$ T cells – a fascinating cell type

Their unique set of functions includes the ability to directly lyse infected or stressed cells, the production of cytokines and chemokines, and antigen presentation comparable to dendritic cells. Furthermore, they are thought to be highly effective against tumor cells and common infections.[23–25]

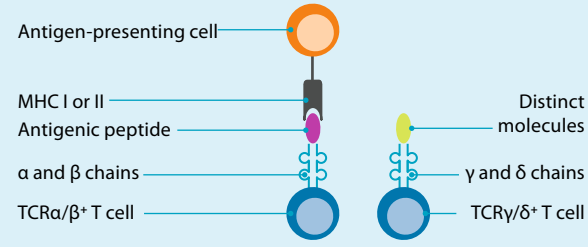

Figure 2: T cell receptors of TCRα/β$^+$ and TCRγ/δ$^+$ T cells. One major difference lies in the T cell receptor which is composed of different chains, α and β or γ and δ. TCRγ/δ$^+$ T cells are not activated by MHC-presented antigens which might contribute to the low alloreactivity of TCRγ/δ$^+$ T cells. These cells become activated by structures of bacterial walls or heat shock proteins, for example.

Results from clinical applications

First clinical results using stem cell grafts after depletion of TCRα/β$^+$ or TCRα/β$^+$/CD19$^+$ cells were published in 2011 by Lang *et al.*, from the Children's Hospital, University Tübingen, Germany.[26] Since then the number of publications increased tremendously. The technique has been applied[27–31] in:

- malignant and non-malignant diseases,
- myeloablative and reduced intensity conditioning regimens,
- pediatric and adult patients,
- settings with reduced or no post-transplant immune suppression,
- haploidentical and matched unrelated HSCT.

Table 2 summarizes the graft composition in haploidentical HSCT from 3 different sites.

	Tübingen[27]	Rome[28]	Parma[29]
CD34$^+$ stem cells $\times 10^6$/kg	14.9	15.8	12.4
TCRαβ$^+$ T cells $\times 10^3$/kg	16.9	40	11
TCRγ/δ$^+$ T cells $\times 10^6$/kg	11.0	9.4	5.3
NK cells $\times 10^6$/kg	81.3	38.2	30
CD20$^+$ B cells $\times 10^4$/kg	n.a.	4	6,8
No. of patients	41	23	16

Table 2: Graft composition.

The observed rates for primary engraftment are described as fast in various publications with 10 to 16 days for neutrophil recovery.[27–31] Also the immune reconstitution data are rated as remarkably fast as described by Balashov *et al.*[30] Bertaina *et al.* published the clinical results from 23 children with non-malignant disorders receiving haploidentical HSCT and reported a 2-year probability of disease-free survival of 91.1%.[28] In an early study in an adult patient population suffering from high risk leukemias, partially in active disease, it was shown that infusion of grafts depleted of TCRα/β$^+$/CD19$^+$ cells was safe and effective, resulting in rapid donor hematopoietic engraftment and early expansion of donor-derived T lymphocytes.[29]

CliniMACS® System – applications

Tailored concepts in cell therapy

Memory T cell DLI – a versatile concept

Early mouse models showed that GVHD starts in the secondary lymphoid organs caused by naive T cells homing to these regions.[32–33] This observation and others gave rise to the clinical concept of depleting naive T cells from stem cell grafts in order to prevent GVHD in allogeneic HSCT. Additionally, the remaining cells would provide the patient with engraftment-facilitating cells and memory T cells against a broad spectrum of infections[34]. So far, different clinical strategies have been followed and first clinical data are available.

The CliniMACS® CD45RA Depletion System is a potent tool to effectively remove naive T cells from cellular products.[34–35] The antigen CD45RA is present on a variety of white blood cells. Using the CliniMACS CD45RA System Teschner *et al.* showed a median log depletion of 4.4 for CD45RA$^+$ cells and a detailed subset analysis confirmed the profound depletion of naive T cells. Furthermore, the authors reported the removal of almost the entire fraction of B cells.[35]

Graft engineering approach on the CD34 platform

The intention of this strategy is to provide a graft consisting of purified stem cells and memory T cells. The CliniMACS Platform allows the combination of CD34$^+$ cell enrichment and subsequent CD45RA$^+$ cell depletion from the CD34-negative fraction of the same mobilized leukapheresis products, see figure 3.[34]

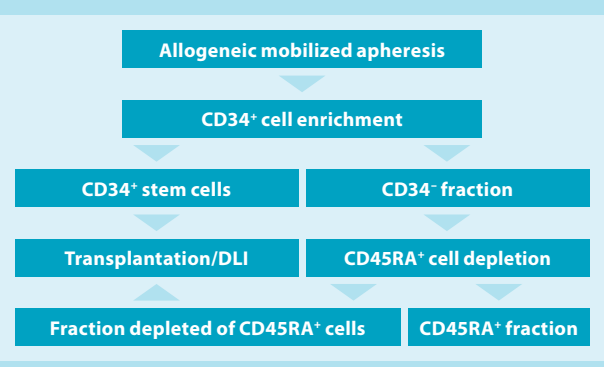

Figure 3: Process for the manufacture of cell products consisting of a pure CD34$^+$ cell fraction combined with highly purified CD45RA$^-$ cells, based on the CliniMACS System. As CD45RA is not a specific T cell marker, a substantial part of CD34$^+$ cells in mobilized products also expresses this antigen, therefore requiring this stepwise approach[36].

In 2015, Bleakley *et al.* (Seattle, USA) published the outcome for 35 adult high-risk leukemia patients after allogeneic transplantation from an HLA-matched related donor. Patients received CD34$^+$ cell–enriched and CD45RA$^+$ cell–depleted transplants (fig. 3) after a myeloablative conditioning regimen. GVHD prophylaxis consisted of tacrolimus being tapered after day 50 in the absence of GVHD. As a result, a remarkably low occurrence of chronic GVHD (9%, 3 patients) at 2 years was reported. 2–year overall survival of 78% was rated successful by the authors especially due to the fact that 54% of the patients had either minimal residual disease and/or were in 2nd-3rd remission or had active disease at time of transplant[37]. This graft engineering approach was also applied in a pediatric haploidentical setting for very young children suffering from combined immune deficiencies. Touzot *et al.* (Paris, France) published the outcome for five children with chronic infections at time of transplant. Successful engraftment was achieved in four children, and all their infections could be resolved. Only one child developed grade I GVHD. As proof of concept in both studies, *in vivo* activity of the transfused cells against pathogens could be demonstrated.[38]

DLI – memory T cells post-transplant

DLIs depleted of CD45RA$^+$ cells have been infused post-transplant either as infection prophylaxis or as rescue therapy to treat drug-resistant infections. This approach might be applied in combination with any kind of GVHD prophylaxis, such as *ex vivo* or *in vivo* T cell depletion strategies. Published results from the children's hospital in Lund, Sweden describe patients having received a haploidentical transplant and afterwards suffering from severe, drug-resistant multiple infections. After infusions of memory T cell DLI, viral clearance or significant reduction in copy numbers could be observed. Most children received CD45RA$^+$ cell–depleted infusions at doses of 2.5×10^4 cells/kg.[39–40] The children's hospital in Moscow, Russia, reported on prophylactic infusions of memory T cell DLI depleted of CD45RA$^+$ cells. In this study, 19 patients at high risk for CMV infections (donor and recipient serum positive for CMV) received CD45RA$^+$ cell–depleted infusions in a stepwise approach. The infusion plan consisted of up to 3 infusions in monthly intervals (table 3). For the 15 patients (75%) with detectable CMV DNA at time of transfusion, significantly increasing numbers of CMV-reactive cells post-infusion could be demonstrated.[41]

Donor type	Cell doses
Haploidentical	25×10^3/kg, 50×10^3/kg, 100×10^3/kg
MUD	100×10^3/kg, 200×10^3/kg, 300×10^3/kg

Table 3: Infusion plan.[41]

CliniMACS® Plus Instrument

Two decades of clinical-scale cell separation

Since 1989 Miltenyi Biotec has played an essential role in the cell therapy community, providing sophisticated tools for the isolation of particular cell types. For example, in the mid-1990s, the CliniMACS® Plus Instrument was introduced for the clinical-scale positive enrichment of beneficial cells from grafts, such as CD34⁺ cells for repopulating the immune system after immune ablation. Furthermore, it allows the depletion of unwanted cells from cellular products. Over the years, more than 25,000 leukemia patients have been treated with cellular products manufactured with the CliniMACS System, and strategies addressing cellular therapy have continually been refined.

Key features

- Clinical-scale cell separation
- Automated procedure
- Compatible for use in a GMP setting
- Functionally closed, CE-marked system
- Enrichment of cell populations by positive selection of target cells or depletion of undesired cells
- Reproducible high purity and excellent yield

Modular platform

- Variable cell source
- Variable target cell type
- Focused separation strategies

Figure 4: The CliniMACS Plus Instrument

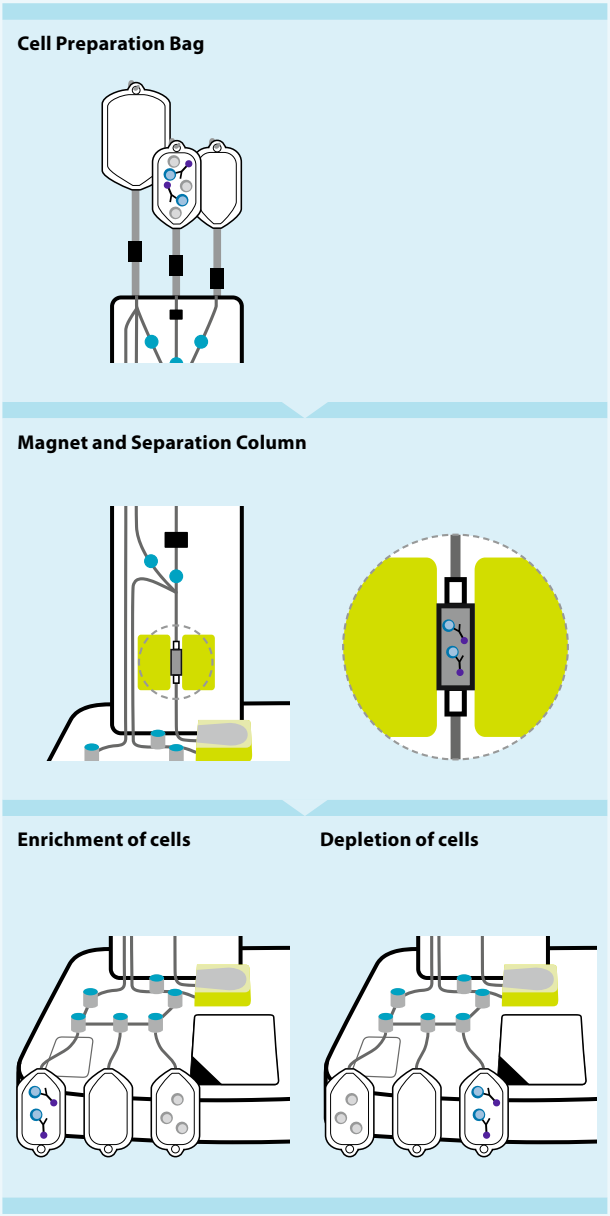

Figure 5: Workflow on the CliniMACS Plus Instrument

CliniMACS Prodigy® System

Mastering the complexity of cell processing

The CliniMACS Prodigy® integrates all cell processing steps, including sample preparation, cell washing, density gradient centrifugation, magnetic cell separation, cell activation, genetic modification, cell culture, and final cell product formulation. The fully automated, sensorcontrolled processes provide a high level of standardization and reproducibility. Hands-on time is reduced substantially.

As all steps are performed in single-use, closed tubing sets, the instrument also reduces cleanroom requirements. In combination with the wide variety of GMP Products manufactured by Miltenyi Biotec, the CliniMACS Prodigy facilitates the implementation of GMP-compliant cell processing.

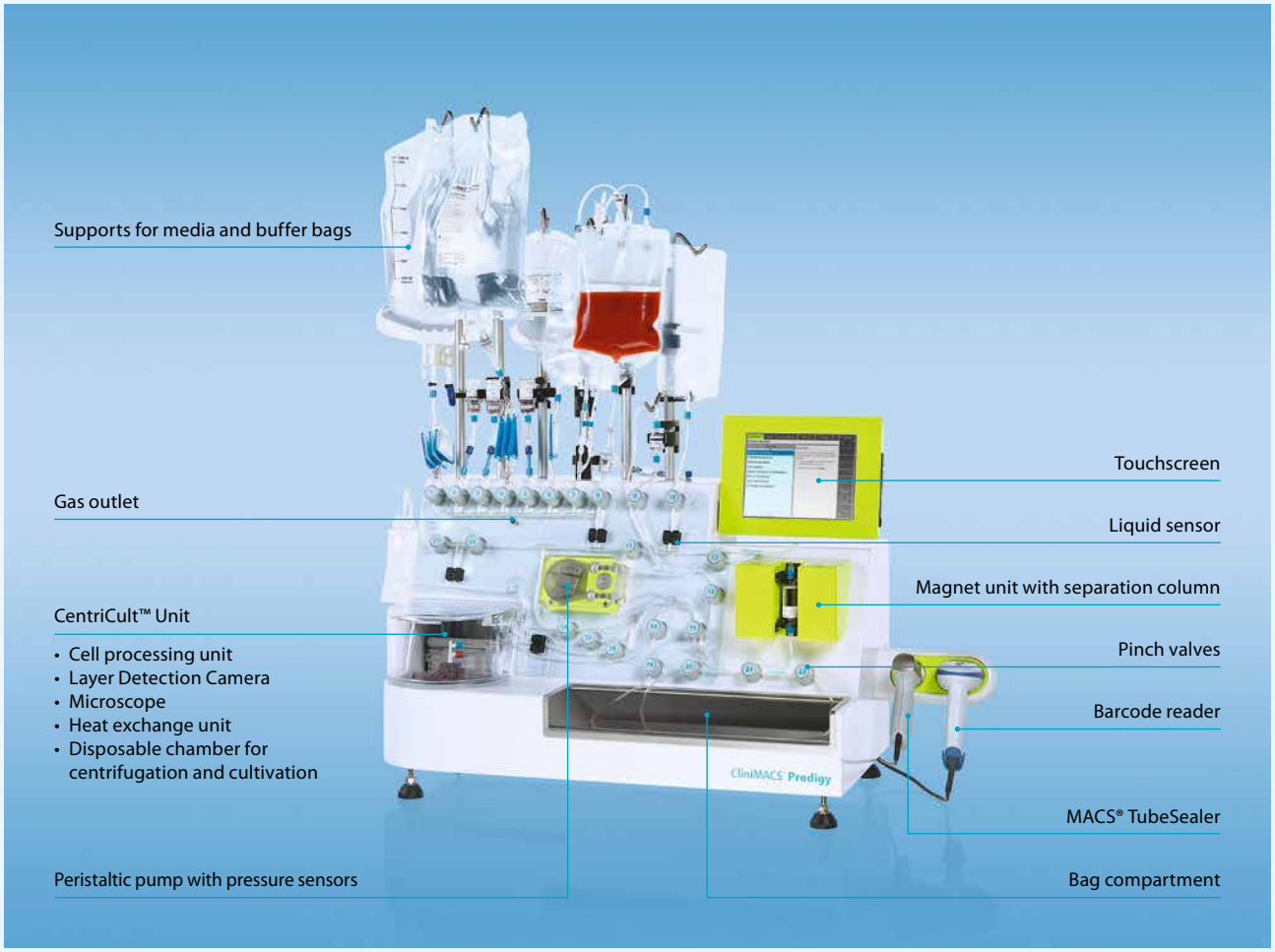

Figure 6: The CliniMACS Prodigy Instrument

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