Cryopreservation of donor lymphocyte clinical products with conventional 10% DMSO results in reduced T-cell viability, proliferation and cytotoxicity compared with alternative conventional methods. Results of a multicenter trial.

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**BACKGROUND**

Peripheral blood mononuclear cells (PBMCs) collected from leukaphereses of allogeneic hematopoietic stem cell (HSC) donors are often given to transplantation recipients as a prophylactic or therapeutic immunomodulatory therapy. This product has been generically named donor-derived leukocyte infusion (DLI).

Unmanipulated or minimally manipulated PBMCs are given to speed immune recovery after transplantation with T-cell depleted grafts, and are also used to treat leukemia relapse after transplantation. Independently of the extent of manipulation and specific application to therapy, the adaptive immunity response depends on T-cell specific cytoketic effects after activation. The success of DLI exhibits the potential to harness the Graft-versus-Tumor (GvT) effect of the human immune system for clinical benefit. CD4+ and CD8+ cells with reactivity to both the host and to leukemic cells exist and may be essential for GvT effect.

T-reg infusions have also been shown to have an effective role in immune reconstitution, decreased cytomegalovirus (CMV) reactivation and decreased graft versus host disease (GVHD).

For decades, the reference cryoprotectant for these products was 10% dimethylsulfoxide (DMSO). However, several studies on HSC engraftment have indicated that combinations of 4-5% DMSO and hydroxyethyl starch provide similar (or even better) post-thaw viability/function.

Whether the functional response of different T-cell populations is similar or not is unknown.

**HYPOTHESES**

Cryopreservation may induce significant T-cell viability and functional defects which may differ for different T cell populations.

Different T cell populations may differ in their optimal cryopreservation medium requirements.

**RESULTS**

Figure 1. Lymphocyte population viability. DMSO 10% viability is decreased for CD4+ and T-reg populations but not for CD8+ cells. Data are presented as mean ± SD. *p<0.01

Figure 2. Lymphocyte cell cycle (BrdU uptake/7-AAD). DMSO 10% shows accumulation in G2/M and decreased numbers in S-phase. Cells in culture in phytohemagglutinin and 2 U/mL IL-2 for 2 days. Data are presented as mean ± SD. *p<0.01

Figure 3. Cytokine release in culture. Normalized levels of GM-CSF, IFNγ, IL-10, IL-12, IL-1β, TNFα in cell supernatant of cultured lymphocytes. Levels of IL-5, IL-6, IL-7 and IL-8 were not significantly affected by the cryopreservation method. Culture in phytohemagglutinin and 2 U/mL IL-2 for 2 days. Data are presented as mean ± SEM. *p<0.01

1. 10% DMSO cryopreservation results in decreased viability of CD4+ and T-reg cells.
2. Decreased proliferative response of T-lymphocytes cryopreserved in 10% DMSO
3. Cytotoxic cytokine profile of 10% DMSO cryopreserved lymphocytes is significantly impaired.