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# Expanded umbilical cord blood T cells used as donor lymphocyte infusions after umbilical cord blood transplantation

SOFIA BERGLUND[1,2], JENS GERTOW[2], MICHAEL UHLIN[1,2] & JONAS MATTSSON[1,2]

[1]*Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Stockholm, Sweden, and*
[2]*Department of Laboratory Medicine, Division of Therapeutic Immunology, Karolinska Institutet, Stockholm, Sweden*

**Abstract**

*Background.* Umbilical cord blood (UCB) is an alternative graft source for hematopoietic stem cell transplantation and has been shown to give results comparable to transplantation with other stem cell sources. Donor lymphocyte infusion (DLI) is an effective treatment for relapsed malignancies after hematopoietic stem cell transplantation. However, DLI is not available after UCB transplantation. *Methods.* In this study, *in vitro*–cultured T cells from the UCB graft were explored as an alternative to conventional DLI. The main aim was to study the safety of the cultured UCB T cells used as DLI because such cell preparations have not been used in this context previously. We also assessed potential benefits of the treatment. *Results.* The cultured UCB T cells (UCB DLI) were given to 4 patients with mixed chimerism ($n = 2$), minimal residual disease ($n = 1$) and graft failure ($n = 1$). No adverse reactions were seen at transfusion. Three of the patients did not show any signs of graft-versus-host disease (GVHD) after UCB DLI, but GVHD could not be excluded in the last patient. In the patient with minimal residual disease treated with UCB DLI, the malignant cell clone was detectable shortly before infusion but undetectable at treatment and for 3 months after infusion. In 1 patient with mixed chimerism, the percentage of recipient cells decreased in temporal association with UCB DLI treatment. *Conclusions.* We saw no certain adverse effects of treatment with UCB DLI. Events that could indicate possible benefits were seen but with no certain causal association with the treatment.

**Key Words:** *cell culture, DLI, T-cell expansion, umbilical cord blood*

## Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) with umbilical cord blood used as a stem cell source has been shown to give results comparable to bone marrow or peripheral blood stem cells [1–3]. Umbilical cord blood transplantation (UCBT) is associated with fewer incidences of graft-versus-host disease (GVHD) and permissiveness for greater human leukocyte antigen (HLA) disparity between donor and recipient [3,4]. However, the smaller cell dose combined with the naivety of the immune cells results in delayed engraftment and immune reconstitution [5–8].

Donor lymphocyte infusion (DLI) is a treatment for relapsed or residual malignancies after HSCT. DLI has also been successfully used to treat threatening graft rejection and mixed donor-recipient chimerism [9–11]. Patients receiving UCBT lack the possibility of receiving DLI [12]. *In vitro*–cultured cord blood DLI (UCB DLI) presents an attractive option for patients with complications after UCBT [13–16]. Sufficient numbers of T cells for DLI can be obtained with the use of an aliquot of a clinical UCB graft by means of Good Manufacturing Practice–grade *in vitro* culture through cluster of differentiation (CD)3/CD28 cross-linking and clinical-grade interleukin-2 (IL-2) [13,16]. The cultured T cells have an activated phenotype and respond functionally to re-stimulation with non-specific mitogenic stimuli *in vitro* [13,16,17]. Addition of clinical-grade interleukin-7 (IL-7) to the culture protocol has been explored and was found to enhance proliferation and reduce apoptosis [18,19].

The present study was performed with the aim of evaluating the safety of the *in vitro*–produced UCB DLI as an alternative to conventional DLI. To our knowledge, cell infusions prepared in this manner have not been administered to patients in this context previously. We also studied potentially beneficial effects of the infusions.

## Methods

We performed a preliminary safety study of treatment with cultured umbilical cord blood T cells, in

Correspondence: **Sofia Berglund**, MD, Division of Therapeutic Immunology, F79, Karolinska University Hospital Huddinge, 14186 Stockholm, Sweden.
E-mail: sofia.berglund@ki.se

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accordance with the European hospital exception rules. The regional ethics committee approved the study (2009/4:1), including the extraction of aliquots from clinical cord blood units, the culture procedure and use of the UCB DLI. All patients, or the legal guardians in the case of minors, gave their informed consent before inclusion.

### *Patients and cord blood units*

Five percent of the volume of every UCB graft was collected at transplantation for T-cell expansion of UCB DLI at the Centre for Allogeneic Stem cell Transplantation (CAST), Karolinska University Hospital Huddinge, Stockholm, Sweden, and for two grafts at the Department of Hematology at Sahlgrenska University Hospital, Gothenburg, Sweden. In total, cultures were performed for 40 CB units (31 patients, 33 UCBTs). The expansions were given consecutive numbers as they were collected. The median total nucleated cell count in the aliquot was 60.5 million (range, 20–154).

### *Cell culture*

The expansion of UCB-derived T cells has been described previously [16,18]. Briefly, T cells were positively selected and stimulated with anti-CD3/CD28 paramagnetic beads (Life Technologies [Invitrogen], Grand Island, NY, USA). The median yield of T cells after separation was 2.7 million (range, 0.25–28). T cells were cultured at a concentration of $3 \times 10^5$ cells/mL in complete medium: 1640 Roswell Park Memorial Institute medium (Life Technologies [Gibco]) supplemented with 10% pooled human AB serum (Department of Transfusion Medicine at Karolinska University Hospital), 100 IU/mL of penicillin G, 100 mg/mL of streptomycin, 0.25 mg/mL amphotericin B (Life Technologies [Gibco]) and 2 mmol/L L-glutamine (Sigma Aldrich Inc, St Louis, MO, USA). Recombinant IL-2 (PeproTech, Rocky Hill, NJ, USA) was added at 600 IU/mL until expansion number 33. From expansion number 34, 100 IU/mL of IL-2 was combined with IL-7 (20 ng/mL) (PeproTech). Cell culture was performed at 37°C at 5% $CO_2$. Viable cells were counted with the use of trypan blue exclusion on days 4, 5, 6 and 7, and cell concentration was maintained at $<3 \times 10^5$ cells/mL. On days 7–11, depending on expansion rate, T cells were harvested and remaining beads were removed by means of magnet separation. Cell harvest was performed when the number of cells was sufficient for cryopreservation of UCB DLI doses of at least $1 \times 10^6$/kg patient weight. The median T-cell count at harvest was $639 \times 10^6$ T cells (range, 2.2–2240). T cells were then cryopreserved in complete medium supplemented with 10% dimethyl sulfoxide (Wak-Chemie Medical GmbH, Steinbach, Germany), divided in suitable DLI doses of $5 \times 10^3$/kg to $5 \times 10^6$/kg patient weight.

### *UCB DLI composition*

Aliquots of the cryopreserved UCB DLI were thawed and analyzed by means of flow cytometry to determine the phenotype. The T cells had an activated phenotype as described in detail previously [16,20]. The expression profile of the four DLI products used is shown in Table I.

### *Conditioning*

Patients received myelo-ablative conditioning with the use of 16 mg/kg of busulphan [21] combined with cyclophosphamide 120 mg/kg, with cyclophosphamide 120 mg/kg and fractionated total body irradiation 12 Gy, or with clofarabine 200 mg/m$^2$, thiotepa 20 mg/kg and melphalan 140 mg/m$^2$ (Table I).

### *GVHD prophylaxis, diagnosis and treatment*

The GVHD prophylaxis was either cyclosporine A combined with prednisone [22] or tacrolimus combined with sirolimus. The treatment strategy has been described previously [5]. All patients received ATG (Thymoglobulin; Sanofi-Aventis, Bridgewater, NJ, USA) [23]. Acute GVHD was graded from 0–IV [24]. Chronic GVHD was classified as limited or extensive and mild, moderate or severe [25]. Acute GVHD grades I–IV were primarily treated with prednisone [5,22].

### *Supportive care*

Patients were treated either in isolation in the stem cell transplantation ward until engraftment or in home care from day +1 [26]. The supportive care has been described previously [26,27].

### *Monitoring*

The patients were monitored for signs of rejection and relapse by chimerism analysis of peripheral blood every second week after engraftment (defined as a neutrophil count of $>0.5 \times 10^9$/L) up to 3 months and at 6, 9, 12, 18 and 24 months and annually thereafter. Polymerase chain reaction amplification of variable numbers of tandem repeats was used to evaluate donor and recipient chimerism in CD3+, CD19+ and CD33+ cells enriched from