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REVIEW ARTICLE

Small-molecule nicotinamide for ex vivo expansion of umbilical cord blood

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Umbilical cord blood transplant is an alternative graft source for patients lacking a human leukocyte antigen–matched donor; however, delayed engraftment times have historically resulted in transplant-related morbidity and mortality from complications such as infections and ineffective hematopoiesis. Recent advances in ex vivo expansion techniques have successfully augmented the initial cell dose delivered from an umbilical cord blood graft, leading to improved immune reconstitution, durable hematopoiesis, decreased transplant-related morbidity and mortality, and better outcomes. Herein we review the data for existing and developing ex vivo expansion techniques, with a focus on the preclinical and clinical data for nicotinamide-mediated cord blood expansion across both malignant and benign hematologic indications. © 2019 Published by Elsevier Inc. on behalf of ISEH – Society for Hematology and Stem Cells.

Allogeneic hematopoietic stem cell transplantation is an effective therapy for many hematologic malignancies; however, it is associated with significant transplant-related morbidity and mortality (TRM) regardless of graft source [1]. Umbilical cord blood (UCB) is an attractive graft source for patients who lack a suitable human leukocyte antigen (HLA)–matched donor; however, it has historically been fraught with delayed engraftment times and increased TRM [1]. To compare treatment endpoints including survival, disease-free survival (DFS) and engraftment among commonly used transplant grafts, Eapen et al. conducted a retrospective analysis of 1,525 adult patients with acute leukemia, of whom 165 had received a single-unit umbilical cord blood (UCB) transplant, 888 a matched–unrelated donor (MUD) peripheral blood stem cell transplant, and 472 a MUD bone marrow transplant [1]. Overall TRM was higher for single-unit UCB-transplanted patients, which was due in part to delayed engraftment times when compared with MUD transplants. Median times to neutrophil and platelet recoveries after peripheral blood MUD transplant were 14 and 19 days, respectively, and 19 and 28 days after bone marrow MUD transplant [1]. Comparatively, median times to neutrophil and platelet recoveries after UCB transplant were 24 and 52 days, respectively [1]. The increased risk of infection and TRM with delayed engraftment is well known [2]; thus, initial outcomes with single-unit UCB transplant in adults were poor [3]. Survival improved following the observation that engraftment was critically dependent on initial graft cell dose, leading to optimization of UCB unit selection. Ultimately, Barker and colleagues [4,5] at the University of Minnesota pioneered a strategy of double-unit UCB transplantation to augment cell graft dose, which made UCB transplantation an option for adults previously felt to be too large for a single-unit UCB transplant. This approach, combined with the use of reduced-intensity conditioning, confirmed UCB as a viable graft source, with reported DFS between 30% and 50% [4,5]. However, issues surrounding delayed engraftment and poor immune cell reconstitution remain an active area of research. Building on the success of double-unit UCB transplantation, the remainder of this review focuses on ex vivo expansion techniques aimed at augmenting the initial cell dose.

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Pioneering ex vivo hematopoietic stem cell/progenitor cell expansion

The use of two UCB units for transplantation offers investigators the unique opportunity to manipulate one UCB unit through ex vivo expansion techniques while maintaining an unexpanded unit as a "backup" in the event of engraftment failure of the experimental unit; furthermore, as UCB units are genetically distinct, it is possible to monitor engraftment and lineage differentiation of each individual UCB unit [6]. A variety of expansion techniques have been explored in cell culture and early-phase clinical trials, including a Notch1 ligand, an aryl hydrocarbon receptor antagonist, mesenchymal progenitor cells (MPCs), and nicotinamide, the focus of this review. Though these studies vary widely in their in vitro culture conditions, conditioning regimens, patient population, and disease characteristics, comparisons of common endpoints can be made.

Delaney et al. [7] pioneered ex vivo expansion by manipulating the Notch1-mediated hematopoietic stem cell (HSC) differentiation pathway. The investigators cultured CD34+ HSCs with an engineered Notch-1 ligand (Delta1) combined with fibronectin fragments and cytokines (stem cell factor [SCF], Fms-related tyrosine kinase 3 ligand [FLT-3L], thrombopoietin [TPO], and interleukin [IL]-3, and IL-6), observing a 222-fold increase in the number of CD34+ cells through arrest of HSC differentiation after 17 days of cell culture. Preliminary results of a phase I trial of 10 adult patients reported a significantly increased CD34+ cell dose following Delta1-enrichment, with an average CD34+ cell dose of 6×10^6 CD34+ cells/kg (0.93×10^6 to 13×10^6) versus 0.24×10^6 CD34+ cells/kg (0.06×10^6 to 0.54×10^6) from the unmanipulated cord blood graft [7]. Of note, no mature T cells were infused in the expanded graft. Myeloid recovery, defined as an absolute neutrophil count (ANC) >500 cells per microliter, was shortened by a median of 10 days, though long-term engraftment at 6 months was predominantly of the unexpanded UCB unit [7].

De Lima et al. [8] sought to recreate physiological HSC proliferation cues of the stem cell niche through co-culture with mesenchymal stromal cells. This resulted in a 30-fold expansion of CD34+ hematopoietic stem and progenitor cells. In 31 patients transplanted with an expanded unit and an unexpanded UCB unit, the median time to neutrophil recovery was 15 days (9–42) versus an average of 23 days (6–52) in historical controls [8]. At 6 months, the expanded UCB unit was present in only 13% of patients; at > 1 year, hematopoiesis was derived solely from the unexpanded UCB unit [8]. This suggests early hematopoietic recovery without sustained hematopoiesis from MSC-enriched units [8].

The Minnesota group was the first to test a novel compound identified by Novartis, StemRegenin-1 (SR-1), which directly binds and inhibits the aryl hydrocarbon receptor, arresting HSC differentiation during cytokine-driven expansion culture (SCF, FLT-3, TPO, and IL-6) [9]. Investigators noted significantly greater numbers of CD34+ cells in the manipulated UCB unit, with a median of 17.5×10^6 (1.4×10^6 to 48.3×10^6) CD34+ cells/kg actual body weight in expanded units in contrast to a median of 0.2×10^6 CD34+ cells/kg in unexpanded UCB units. In a phase I trial of 17 recipients of an SR-1–expanded UCB unit with a concurrent unexpanded unit, neutrophil recovery was achieved in all patients at a median of 15 days (6–30), while only 86% of patients in the control group recovered at a median of 24 days [9]. The SR-1–expanded UCB unit contributed to sustained hematopoiesis in 11 of 17 patients, contrasting with both de Lima et al. [8] and Delaney et al. [7]. Of note, mature CD3+ T cells were infused in this protocol, suggesting an immunologic mechanism for sustained engraftment.

Ex vivo expansion using nicotinamide

Omidubicel, previously known as NiCord, is an expanded UCB product that was developed by Gamida Cell Ltd. and has borne out successful outcomes in early-phase UCB transplant trials [10–12]. The active agent in the Omidubicel culture system, nicotinamide (NAM), is a form of vitamin B3 critical to several cell cycle functions, including HSC differentiation and bone marrow homing [10]. The mechanism of action of NAM is still a topic of active investigation. A detailed description of current theories on the mechanism is beyond the scope of this review. NAM and a similar compound, nicotinamide riboside, govern hundreds of cellular pathways (Figure 1). As a precursor of NAD+ and an inhibitor of NAD catabolism, NAM functions as a ubiquitous and essential metabolic cofactor of enzymes that participate in a variety of cellular responses. This includes energy metabolism, DNA repair, transcription, and mitochondrial functions [13–19]. NAM has been extensively studied in embryonic stem cell culture systems and appears to coordinate diverse cellular responses including proliferation, differentiation, and apoptosis. Culture systems depleted of nicotinamide result in enhancement of apoptosis and an inability to reprogram somatic cells to a pluripotent state. In relation to hematopoietic stem cells, NAM appears to maintain "stemness" during ex vivo culture through epigenetic modification. At the same time, it serves to extend the replicative life span of stem cells through reduction of reactive oxygen species.

To produce Omidubicel, a single UCB unit is selected for CD133+ cells using immunomagnetic beads and then cultured in medium containing NAM and stimulatory hematopoietic cytokines for a 21-day expansion period [11]. The expanded unit is re-cryopreserved and available for future use as needed, which allows for flexibility in transplant planning and allows for the

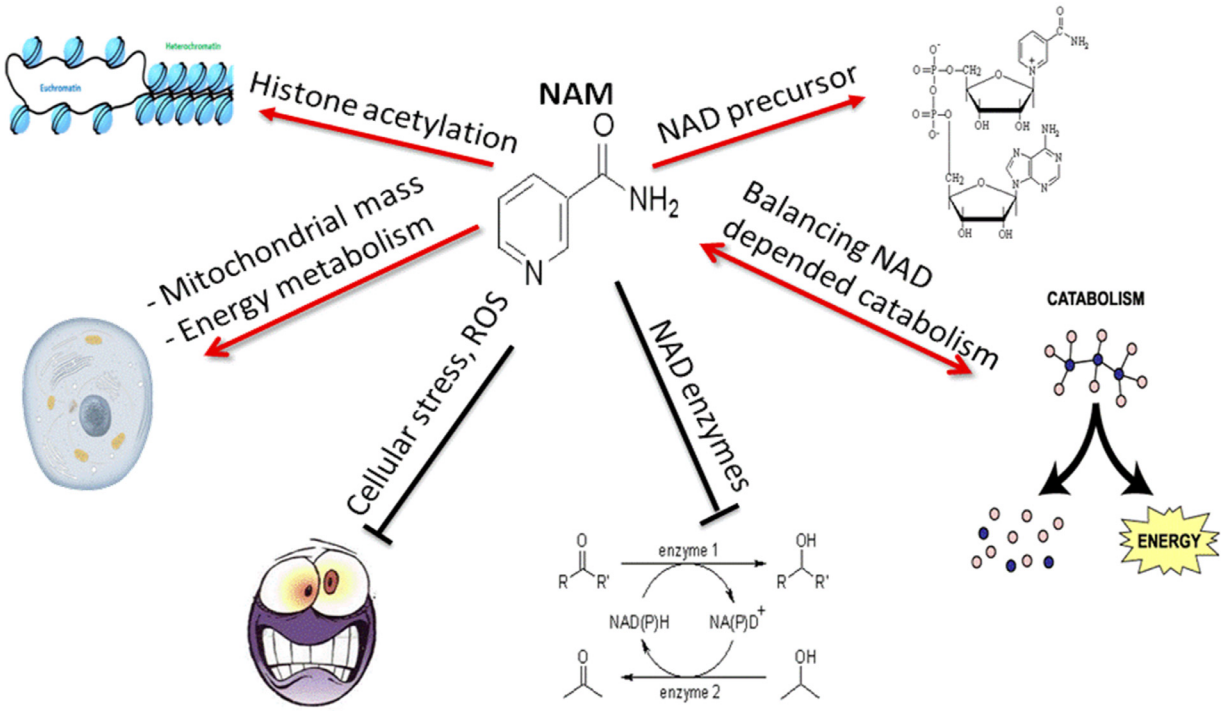

Figure 1. Nicotinamide (NAM) and a similar compound, nicotinamide riboside, govern hundreds of cellular pathways. *ROS*=reactive oxygen species.

possibility of additional therapy should the patient's clinical status change. The CD133− fraction−containing mature lymphoid cells is retained separately and also re-cryopreserved [11]. Both these measures ensure safer, more reliable transport of product from the centralized manufacturing facility to the transplant center.

The clinical utility of Omidubicel was first studied in a single-center, phase I clinical trial in which 11 adult patients with hematologic malignancies underwent co-infusion of a single expanded UCB unit and a single unexpanded UCB unit following myeloablative conditioning with total body irradiation (TBI) and fludarabine (Flu), with or without cyclophosphamide at the discretion of the investigator [11]. Outcomes were compared with those for a set of 17 matched historical controls [11]. Expansion resulted in a median 72-fold (16- to 186-fold) increase in CD34+ cells as reported by the cord blood bank before cryopreservation [11]. This resulted in a median infused dose of 3.5×10^6 (0.9×10^6 to 18.3×10^6) CD34+ cells/kg versus 0.07×10^6 (0.03×10^6 to 0.48×10^6) CD34+ cells/kg in the unexpanded unit [11]. The median time to neutrophil recovery was 13 days for the 8 patients who engrafted with the Omidubicel unit (7−18 days) versus 25 days for historical controls [11]. The investigators noted upfront that Omidubicel culture conditions do not support lymphoid maturation and, based on experience from previous studies [7,8], surmised this would place the Omidubicel-expanded unit at an immunologic disadvantage for sustained engraftment. As such, the CD133− non-cultured re-cryopreserved fraction containing UCB-immunocompetent lymphoid cells was co-infused during transplantation [11]. Omidubicel was the first ex vivo expanded cord blood product to be co-infused with the T-cell fraction. Complete or partial Omidubicel engraftment was observed in 8 of the 11 patients at 100 days, and Omidubicel-derived engraftment remains stable in all patients, now out to a follow-up period of 7 years [12].

This study provided the safety data and clinical basis for a multicenter, multinational, nonrandomized, prospective phase I/II clinical trial of transplantation of a single, stand-alone graft of ex vivo expanded UCB in 36 adult patients with hematologic malignancies matched against historical controls [11,12]. These patients underwent one of three possible myeloablative conditioning regimens before infusion with a single Omidubicel-expanded UCB unit. The unexpanded CD34+ cell count as reported by the cord blood bank before cryopreservation was 0.13×10^8 (0.08×10^8 to

0.25×10^8), which increased by 33-fold to a median of 4.5×10^8 (1.6×10^8 to 13.1×10^8) [12]. This resulted in a median CD34+ infusion cell dose of 6.3×10^6/kg ($1.4\ 10^6$/kg to 14.9×10^6/kg) [12]. Median time to neutrophil recovery was 11.5 days (9–14) versus 21 days (20–23) for the control cohort [12]. Furthermore, whole-blood chimerism analysis at 100 days post transplant revealed that 25 of 26 (96%) patients had ≥95% donor engraftment and 1 of 26 (4%) had 57% donor whole-blood chimerism [12]. One patient experienced primary graft failure, and 2 had secondary graft failure resulting from viral infections [12]. The study was the first example of a successful single-unit ex vivo expanded UCB transplantation with long-term, durable hematopoiesis; it established the safety and efficacy of this approach, and demonstrated reductions in time to engraftment, bacterial infections, and length of hospitalization [12,20].

It is hypothesized that with the higher infused stem cell dose of an ex vivo expanded UCB graft, faster lymphoid immune recovery would occur. Thus, de Koning et al. performed an in-depth analysis of full immune reconstitution (IR) in 27 Omidubicel recipients compared with 27 unexpanded UCB transplant recipients and 20 bone marrow transplant recipients, given data supporting CD4+ IR is a better predictor of event-free survival than neutrophil engraftment [21,22]. Of note, the comparator cohort were all under 21 years of age. Ninety-one percent of Omidubicel-infused patients achieved successful CD4+ IR within 100 days of transplant at a rate comparable across study groups [22]. Interestingly, reconstitution of natural killer (NK) cells, as well as follicular B cells, memory B cells, and plasma cells, was much faster after Omidubicel transplant [22]. These findings will need to be confirmed in an ongoing prospective, multinational, multicenter, phase III trial comparing single-unit Omidubicel-expanded UCB with standard myeloablative UCB transplant for patients with leukemia, lymphoma, or myelodysplastic syndromes, with time to neutrophil engraftment as the primary endpoint (NCT02730299).

Applications for NAM-expanded cell products are being explored in other areas of hematology. For example, the larger cell dose provided by the Omidubicel graft justifies investigation into the use of this graft among transplant candidates with myeloproliferative disorders and aplastic anemia—two indications that historically have not been well suited for cord blood transplant. The group at the National Institutes of Health recently reported on the use of single-unit Omidubicel in 2 patients with refractory severe aplastic anemia in a phase II clinical trial, with rapid neutrophil recovery (6.5 days) and sustained engraftment, in comparison to historical controls with a single-unit cord blood transplant for whom neutrophil recovery required a median of 10 days [23]. The Minnesota group is exploring the use of NAM for cytolytic NK cell culture. Adoptive NK cell transfer is currently used as cellular therapy for a variety of hematologic malignancies, although barriers include early elimination and impaired effector function of infused NK cells. Bachanova et al. [24] reported on the use of ex vivo NAM-expanded NK cells (NAM-NK) in 5 patients with refractory non-Hodgkin's lymphoma (NHL) and 2 patients with refractory multiple myeloma (MM) [24]. Patients received lymphodepleting chemotherapy followed by NAM-NK and low-dose interleukin-2 infusion concurrently with rituximab or elotuzumab for NHL and MM, respectively [24]. At a follow up of 2 months, 3 NHL patients were in a metabolic complete remission, 1 MM patient had stable disease, and 1 MM patient had progressive disease; the authors plan for dose escalation followed by an expansion cohort at the maximum tolerated dose in a phase I study of lymphoma and MM patients (NCT03019666). Finally, Parikh et al. [25] recently reported on the use of Omidubicel in 11 pediatric sickle cell disease (SCD) patients in a phase I/II multicenter, nonrandomized study of patients receiving both an Omidubicel-expanded UCB graft with the CD133− T cell–containing fraction and an unexpanded UCB graft following myeloablative chemotherapy, matched against historical pediatric controls [25]. All 11 patients engrafted neutrophils at a median time of 7 days (range, 6–20; 10/11 with the Omidubicel graft), although long-term engraftment was predominantly (63%) derived from the unexpanded unit [25].

Future directions

Outcome of UCB transplantation has improved significantly since its inception nearly 30 years ago. The improvements have been most striking for adult patients. Historical barriers to success, including delayed engraftment, incomplete immune reconstitution, increased infection, and early transplant-related morbidity and mortality, remain active areas under investigation. Early studies of multiple ex vivo expansion platforms have suggested that these barriers can be overcome; however, ongoing larger trials are needed for confirmation [8–12]. As we gain experience in Omidubicel and other ex vivo expanded products, we will need to refine logistics to make the product more attainable, accessible, and transportable, given current logistical barriers and operating costs. With active research in this area, a commercially available ex vivo expanded UCB product will likely be available in the near future. It is anticipated that once approved by the U.S. Food and Drug Administration (FDA), UCB transplantation using Omidubicel will be performed among patients with similar disease states using a similar

conditioning regimen intensity. However, efforts will be put forth to manufacture Omidubicel from UCB units containing a lower total nucleated cell or CD34+ dose, thus increasing the availability of better matched units for patients. Commercial availability will also facilitate the development of studies to test the efficacy of Omidubicel transplantation following reduced or nonmyeloablative conditioning. Lastly, head-to-head studies with other alternative graft sources, such as partial HLA-mismatched unrelated and haplo-identical matched related donors, will be needed to establish the safety, efficacy, and long-term outcomes of UCB in comparison to other available approaches.

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