

EXPERT INSIGHT

Optimal conditioning regimens for cord blood transplantation: advances and challenges

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Conditioning regimens of all intensities have proven effective for patients in need of umbilical cord blood transplantation. While total body irradiation (TBI) continues to play a prominent role in most adult and pediatric regimens, there are now highly effective regimens without TBI. Children with malignant and non-malignant disorders receive predominantly myeloablative conditioning regimens. The clinical need for less toxic regimens for adult patients, particularly those with co-morbidities, has prompted clinical research activity in reduced intensity and non-myeloablative regimens capable of facilitating engraftment of single or double umbilical cord blood grafts. Here, we will describe the evolution of many of these regimens and how they are currently being utilized in stem cell transplant centers throughout the world.

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Great progress has been made in the field of umbilical cord blood (UCB) transplantation since the first procedure was performed on a child with Fanconi anemia in 1988 [1]. Treatment related mortality (TRM) and graft failure rates have decreased, as has the time to hematopoietic recovery following UCB transplantation allowing for

outcomes that are comparable to other alternative graft sources [2]. A greatly expanded UCB graft inventory, improved UCB graft quality and clarification of cell dose thresholds have moved UCB transplantation from a primarily pediatric population to all patients irrespective of size or age. It is now well established that the incidence of chronic

graft-versus-host disease (GvHD) is lower among UCB transplant recipients compared to recipients of bone marrow grafts [3]. Recently published data from Seattle suggests that among patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) with minimal residual disease at time of transplant, outcomes are better

for those who receive double UCB grafts compared to unrelated adult donor grafts [4]. This difference was due to a significantly lower incidence of relapse suggesting a more potent graft-versus-tumor response from an UCB graft.

From the beginning, total body irradiation (TBI) has played a prominent role in preparing both pediatric and adult recipients for UCB transplantation. This remains the case today, however chemotherapy-based regimens are now performing quite well for recipients of all age groups. Effective non-myeloablative regimens are now available for older or more infirmed adult patients, expanding further the access of UCB transplantation to this large population [5]. The role of non-myeloablative regimens in the treatment of pediatric patients has yet to be defined. The use of serotherapy (anti-thymocyte globulin, alemtuzumab, etc.) in the UCB conditioning regimen remains controversial, given the known limitation of delayed cellular immune reconstitution following UCB transplantation.

Because of the divergent practice patterns and literature surrounding UCB transplantation in the pediatric and adult populations, this review will devote separate sections to each discipline. The literature is ripe with studies, large and small, describing novel UCB transplant regimens. The review will focus on the larger studies that have been adopted as standard of practice or in multicenter clinical trials (Tables 1 & 2).

PEDIATRIC MYELOABLATIVE REGIMENS

The choice of conditioning regimens for pediatric patients undergoing UCB transplantation should

consider the underlying disease (malignant vs non-malignant), the degree of immune suppression needed to decrease the risk of graft rejection, the baseline organ function of the recipient and the potential for late effects. Compared to adult patients, there are physiologic differences that affect the dosing and schedule of chemotherapy agents in children [6–8]. These differences are more pronounced in very young children and infants, but continue into adolescence [6]. Pediatric specific dosing guidelines should be followed to allow for appropriate drug exposure. Despite these differences, children most commonly receive myeloablative conditioning (MAC) regimens [9]. Children with inherited DNA-repair defects (i.e., Fanconi anemia and dyskeratosis congenita) are the exception and require reduced intensity approaches to avoid significant toxicities.

For pediatric patients with hematologic malignancies, TBI has been a longstanding component of myeloablative conditioning regimens. TBI has been shown to be effective in decreasing relapse and improving outcomes after HSCT for pediatric patients with acute lymphoblastic leukemia (ALL) [10–12], but the benefit in pediatric AML remains somewhat controversial [13–16]. Furthermore, TBI-based regimens are rarely used in infants and very young children due to concerns for late effects, especially neurocognitive. For older children and adolescents, however, TBI-containing preparative regimens are commonly used especially in ALL patients. In the first prospective UCB transplantation trial (COBLT), which enrolled from 1999 to 2003 [17], patients enrolled on the hematologic malignancy arm received total doses

► **TABLE 1****Pediatric myeloablative and non-myeloablative umbilical cord blood conditioning regimens.**

Conditioning regimen	Intensity	GvHD prophylaxis	Ref.
Fludarabine 75 mg/m ² , TBI 1200–1320 cGy, Cyclophosphamide 120 mg/kg	Myeloablative	Cyclosporine/MMF	[19,20]
TBI 1350 cGy, Cyclophosphamide 120 mg/kg, equine ATG	Myeloablative	Cyclosporine/methylprednisolone	[17]
Busulfan IV or oral*, Cyclophosphamide 200 mg/kg, rabbit or equine ATG	Myeloablative	Cyclosporine/Methylprednisolone or MMF	[19,65,66]
Busulfan 16 mg/Kg, Cyclophosphamide 120 mg/Kg, Melphalan 140 mg/m ²	Myeloablative	Cyclosporine/methylprednisolone	[67]
Fludarabine, Busulfan, Cyclophosphamide +/- Thiotepa	Myeloablative	Cyclosporine/MMF	[28,68]
Busulfan IV or oral*, Melphalan 135 mg/m ² , ATG	Myeloablative	Cyclosporine/methylprednisolone	[24]
Alemtuzumab 3 mg/kg, Hydroxyurea, Fludarabine 150 mg/m ² , Melphalan 140 mg/m ² , Thiotepa 200 mg/m ²	Non-myeloablative	Tacrolimus/MMF	[47]
Fludarabine 180 mg/m ² , Cyclophosphamide 60–200 mg/kg, rabbit ATG	Non-myeloablative	Tacrolimus/MMF	[69]
Hydroxyurea, alemtuzumab 45 mg, fludarabine 150 mg/m ² , thiotepa 8 mg/kg, melphalan 140 mg/m ²	Non-myeloablative	Tacrolimus or Cyclosporine/MMF	[48]

*Dosing based on weight or body surface area measurements and adjusted according to pharmacokinetic levels.
 ATG: Anti-thymocyte globulin; MMF: Mycophenolate Mofetil.

of TBI 1350 cGy, cyclophosphamide (Cy) 120 mg/kg, and equine ATG (90 mg/kg) for conditioning. The cumulative incidence of neutrophil engraftment at day 42 was 79.9% with primary graft failure occurring in 21 of the 191 transplanted patients. The probability of overall survival was 57.3% at 1 year and leukemic relapse occurred in 18.6% of patients. The probability of non-relapse mortality was 25.8% at 6 months. Based on data from a pilot study in adults [18], Fludarabine was added to the TBI/cyclophosphamide backbone in two subsequent multicenter prospective trials. Both studies randomized pediatric patients with acute leukemia to receive either single or double unit UCB transplantation after conditioning with fludarabine 75 mg/m², TBI 1200 [19] or 1320

[20] cGy and cyclophosphamide 120 mg/kg (Flu/TBI/Cy) [19,20]. In the trial conducted by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN; 0501 study), patients experienced low rates of TRM and very low rates of relapse (12 and 14% at 1-year for single and double unit, respectively) [20]. Michel *et al.* reported similar outcomes although a chemotherapy arm was also utilized in this study [19]. Eapen *et al.* further explored the Flu/TBI/Cy regimen in a recent analysis of patients (n = 496) broadly meeting the BMT CTN 0501 eligibility criteria who received a single unit UCB transplantation [21]. They compared outcomes of patients enrolled on the trial (n = 100) to those not enrolled on trial receiving Flu/TBI/Cy (n = 62) or an alternate regimen (n = 334) [21].

► **TABLE 2****Adult myeloablative and non-myeloablative umbilical cord blood conditioning regimens.**

Conditioning regimen	Intensity	GvHD prophylaxis	Ref.
TBI 1320 cGy, Fludarabine 75 mg/m ² , Cyclophosphamide 120 mg/kg	Myeloablative	Cyclosporine/MMF	[55, 18]
Thiotepa 10 mg/kg, Busulfan 9.6 mg/kg, Fludarabine 150 mg/m ² , Thymoglobulin 8 mg/kg	Myeloablative	Cyclosporine/Prednisone	[59]
TBI 1350 cGy, Fludarabine 160 mg/m ² , Thiotepa 10 mg/kg	Myeloablative	Tacrolimus/MMF	[58]
Busulfan, Fludarabine 100 mg/m ² , Clofarabine 30 mg/m ² , TBI 200 cGy, rATG	Myeloablative	Tacrolimus/MMF	[70]
Cyclophosphamide 120 mg/kg, TBI 1000–1200cGy, Cytarabine 6–12g/m ²	Myeloablative	Cyclosporine/methotrexate	[71,72]
Cyclophosphamide 50mg/kg, Fludarabine 150 mg/m ² , TBI 200 cGy	Non-myeloablative	Tacrolimus or Cyclosporine/MMF	[61]
Melphalan 80mg/m ² , Fludarabine 125 mg/m ² , TBI 400 cGy.*	Reduced intensity	Cyclosporine	[73]
Cyclophosphamide 50 mg/kg, Fludarabine 150 mg/m ² , Thiotepa 10 mg/kg, TBI 400 cGy	Reduced intensity	Cyclosporine/MMF	[63]

*Six subjects received Busulfan 8 mg/kg instead of Melphalan, and four subjects received TBI 800 cGy instead of 400 cGy.

Three-year survival was similar for patients receiving the Flu/TBI/Cy regimen but lower for those patients receiving an alternative regimen (70%, 50% and 53% for patients prepared with Flu/TBI/Cy, alternative TBI regimens and non-TBI regimens, respectively). Relapse rates were lowest with the Flu/TBI/Cy regimen, a finding that was recently supported in an analysis from Eurocord of pediatric ALL patients who received UCB transplantation [10]. Therefore, the use of Flu/TBI/Cy should be strongly considered when TBI-based approaches are indicated.

Chemotherapy-based conditioning regimens are well established and widely used across diagnoses and donor sources. The standard busulfan/cyclophosphamide regimen was originally described over 30 years ago using oral busulfan dosed every 6 hours [22] and

modified when the intravenous form became available [23]. Further successful modifications to the busulfan-containing backbone have included the addition of anti-thymocyte globulin (ATG), daily IV dosing and pairing busulfan with melphalan and/or fludarabine [24–28]. The availability of busulfan therapeutic drug monitoring has allowed for personalized dosing and the optimal AUC continues to be an area of interest [26,29,30]. Currently, myeloablative busulfan-containing regimens are considered standard of care for most pediatric UCB recipients with non-malignant diseases and in clinical settings where non-TBI approaches are indicated (e.g., young children with malignancies). The availability of therapeutic monitoring has allowed for decreased rates of busulfan-related toxicity in the early post-transplant period [26], but

it is unknown whether the long-term toxicities will also decrease, especially in young children who are at highest risk of developing these complications [31]. The use of ATG, initially added with the goal of decreasing rejection and GvHD, has also been controversial. Studies have demonstrated, in pediatric UCB recipients, less GvHD when myeloablative conditioning with ATG is given [32–34], but is also associated with delayed immune reconstitution without a survival benefit [33,35,36]. Further complicating the discussion is the variability in practice between centers. Different sources (rabbit vs horse), dosing ranges and timing of administration (e.g., earlier vs later in conditioning) may all influence these results. Admiraal *et al.* recently performed extensive pharmacokinetic studies to determine whether the timing and degree of ATG exposure influenced outcomes [37,38]. They demonstrated that higher pre-transplant ATG exposure was associated with less GvHD, whereas higher post-transplant exposure was associated with slower immune recovery and decreased overall survival. The impact of ATG on relapse after UCB transplantation remains controversial [10,33,34,39]. Given these complexities, pharmacokinetic monitoring for ATG and alternative dosing strategies warrant further investigation [38,40].

PEDIATRIC NON-MYELOABLATIVE REGIMENS

Given the concern for late effects, there has been considerable interest in developing non-myeloablative (NMA) regimens for pediatric

patients, primarily with non-malignant diseases, that would allow for donor engraftment with less potential regimen-related toxicity. Generally, these regimens are intermediate intensity, employ immunosuppressive or cytotoxic agents (e.g., fludarabine or cyclophosphamide) in combination with non-myeloablative doses of busulfan, melphalan or TBI. Studies investigating NMA approaches for pediatric patients have been most successful when bone marrow donors are used [41–45] with higher rates of primary graft failure reported in patients receiving UCB grafts [42,45,46]. There have been several recent reports using NMA regimens with UCB grafts that have been more promising in non-malignant diseases, sickle cell disease or severe aplastic anemia [47–49]. Parikh *et al.* treated 22 children with non-malignant diseases using the NMA backbone of alemtuzumab, fludarabine, melphalan with hydroxyurea and thiotepa [47]. The cumulative incidence of engraftment was 86.4% by day 42 with durable donor engraftment observed in the majority of patients. The 1-year OS and event-free survivals were 77.3 and 68.2%, respectively, with causes of death due to viral infections (n = 3), acute GvHD (n = 1) and transfusion reaction (n = 1). Abraham *et al.* recently reported outcomes of nine children with sickle cell disease after non-myeloablative conditioning using a similar approach (hydroxyurea, alemtuzumab, fludarabine, thiotepa and melphalan) followed by UCB transplantation [48]. Seven of the nine children achieved full donor engraftment. The overall survival and disease-free survival rates were 100 and 78% at 1-year,

respectively. Again, viral infections were common but all patients recovered. Most recently, Kudo *et al.* reported treatment of 27 pediatric patients with severe aplastic anemia with non-myeloablative conditioning followed by UCB transplantation [49]. The 5-year OS and failure-free survival of all patients were 69.5 and 59.3%, respectively. For the 17 patients transplanted after 2006, the 5-year OS was excellent at 93.8%. Interestingly, they observed decreased OS and failure-free survival when the regimens contained ATG. Therefore, NMA regimens may provide a viable approach for use with UCB grafts, but further investigation is needed.

ADULT MYELOABLATIVE REGIMENS

Since the first unrelated UCB transplant procedure, TBI has played a prominent role in the conditioning regimen [50]. Two large, registry-based studies of adult UCB published in 2004 show that myeloablative doses of TBI were used in 85% of the procedures in North America, and 65% of those in Europe [51,52]. These reports confirmed the feasibility and great potential of adult UCB transplantation, but also highlighted the many limitations. The North American study showed that when compared to recipients of matched unrelated adult donor stem cell grafts, UCB transplant recipients had slower hematopoietic recovery, higher TRM and lower overall survival. Another limitation that became apparent was the problem of delayed cellular immune recovery, which was particularly problematic in the adult population. Viral infections, including

Epstein–Barr virus were shown to be associated with the inclusion of ATG [53]. With its long half-life, ATG continues to lymphodeplete the host even after donor stem cell engraftment, thereby impeding lymphoid recovery.

The Minnesota group led a trend away from ATG-containing regimens, replacing it instead with another potent lymphodepleting agent, Fludarabine. A 2005 publication by Barker and colleagues of 23 adult UCB transplant recipients conditioned with TBI 1320/Fludarabine 75mg/m²/Cyclophosphamide 120mg/kg followed by infusion of two UCB grafts soon became a benchmark approach for adult UCB transplantation [54]. This report also introduced double UCB transplantation, suggesting that for those adults without an adequately sized single UCB graft, reliable engraftment could be achieved by transplanting two UCB grafts. All 21 evaluable patients in this study achieved donor stem cell engraftment at a median of 23 days. The 6-month TRM was 22%, a number comparable to TRM following myeloablative adult matched donor transplantation. One-year disease-free survival was 57%; however, it should be noted that the subjects in this study was relatively young with a median age of 24 years, and the median follow-up only 10 months.

The promising results of a TBI-based myeloablative approach paved the way for a prospective, multicenter Phase 2 study of adult double UCB transplantation using the same myeloablative regimen [55]. Ten centers participated in the study, which accrued 56 patients between 2007 and 2011 with high-risk acute leukemia or

myelodysplastic syndrome. The median age of the subjects was 35 years, which was 11 years older than the single center pilot study from Minnesota. The cumulative incidence of neutrophil engraftment was 89% at 100 days. With a median follow-up of 37 months, the overall survival at 1 and 3 years was a very encouraging 57% and 52%, respectively. The surprise from this study was an unacceptably high TRM of 39%, a direct or indirect reflection of the regimen intensity.

There has long been interest in defining a myeloablative yet reduced toxicity conditioning regimen that could be paired with either single or double UCB transplantation. Myeloablative doses of busulfan combined with fludarabine has long filled this niche for recipients of adult donor grafts [56]. However, this regimen appears to provide inadequate immunosuppressive activity resulting in a high incidence of graft failure when combined with an UCB graft [57]. At present, the published experience with reduced toxicity myeloablative regimens are limited to relatively small, single-center reports. The group at MD Anderson Cancer Center developed a reduced toxicity regimen consisting of busulfan, fludarabine, clofarabine and TBI 200 cGy. The group at Duke recently reported promising results using an approach that pairs double UCB transplantation with a TBI 1350 cGy, fludarabine and thiotepa [58].

While most centers in North America continued to study and modify TBI based myeloablative regimens, investigators in Europe were making progress with chemotherapy-based regimens. With the publication of BMT-CTN 0501 demonstrating equivalence of an

adequately sized single UCB graft compared to the double cord blood graft [20], many European centers were not compelled to adopt the myeloablative TBI-based double cord blood transplant package. The Valencia group developed and extensively tested a myeloablative regimen consisting of thiotepa, busulfan, fludarabine and thymoglobulin (TBF). Outcome of 88 patients (81% adults) who received UCB transplantation from nine centers in Spain using TBF was reported in 2012 [59]. All patients received a single UCB graft with a graft consisting of a total nucleated cell dose $>2 \times 10^7/\text{kg}$ and CD34^+ cells $>0.6 \times 10^5/\text{kg}$ or a graft with a total nucleated cell dose of $>1.5 \times 10^7/\text{kg}$ and CD34^+ cells $>1 \times 10^5/\text{kg}$. The cumulative incidence of myeloid engraftment was 94% at a median of 19 days. Non-relapse mortality at day 100 and 6 months was 14 and 23%, respectively. In a retrospective registry-based study, the European Blood and Marrow Transplant (EBMT) group compared the outcome of TBF single cord blood transplant approach with a radiation-based or busulfan-based single cord blood transplant approach, and a radiation based double cord blood approach [60]. The study focused on a relatively homogeneous patient population with acute leukemia in first complete remission, transplanted at European centers. Non-relapse mortality and relapse incidence were not statistically different among the three groups. However, leukemia-free survival was 48% for both the TBF single UCB transplant approach, and the TBI-based double UCB transplant approach. This was statistically significantly better than the 30% leukemia-free survival for the single

UCB transplantation using a TBI- or busulfan-based myeloablative conditioning regimen.

ADULT NON-MYELOABLATIVE REGIMENS

The menu of options for non-myeloablative UCB conditioning regimens for adult patients is quite short. Without question, the most widely used regimen was developed at the University of Minnesota, and consists of cyclophosphamide, fludarabine and TBI 200cGy followed by infusion of a double UCB graft. The original single center study was reported in 2007 and consisted of 110 patients with a variety of hematologic malignancies [61]. Neutrophil engraftment was achieved in 92% of patients at a median of 12 days. Transplant-related mortality was 26% at 3 years. Overall and event-free survival at 3 years were 45% and 38%, respectively. This regimen was tested in a multicenter trial conducted by the BMT-CTN [62], with the results proving to be quite similar to the single center trial. Patients with acute leukemia and lymphoma were eligible for participation. The regimen resulted in a cumulative incidence of neutrophil engraftment of 94%, with a median time to recovery of 15 days. Five patient experienced primary graft failure, and 1 with secondary graft failure.

Barker and colleagues from Memorial Sloan Kettering have developed a reduced intensity regimen consisting of cyclophosphamide, fludarabine, thiotepa and TBI 400cGy [63]. An update on the performance of this regimen was reported at the meeting of the *American Society of Hematology* in

2016 [64]. 110 patients (median age 51, range 18–65) with a variety of hematologic malignancies were transplanted, all but one of whom received a double UCB graft. Day 180 TRM was only 15%, with a relapse rate of 10% at 2 years. With a median follow-up of 2.7 years, the overall and progression-free survival was 72 and 69%, respectively.

CONCLUSION

Among the many factors responsible for the progress that has been observed in UCB transplantation, changes in the bone marrow conditioning regimen cannot be considered a major one. The reason being that, apart from the incorporation of the purine analog fludarabine into many of the regimens, the agents and doses that comprise the most widely used conditioning regimens are largely the same. From the inception of UCB transplantation, TBI has played a prominent role in the composition of the conditioning regimen. This remains the case today for both adult and pediatric patients. However, there is growing experience with highly effective regimens without TBI. These are the preferred regimens for the youngest of children. Chemotherapy based regimens are also preferred for children with myeloid malignancies, and for transplant centers where TBI is not readily available. The role of ATG in the UCB transplant conditioning regimen is controversial, with most centers tending to omit it due to the negative impact on immune reconstitution. Due to concerns of high TRM in adult patients conditioned with myeloablative doses of TBI, many centers are opting for a reduced toxicity approach.

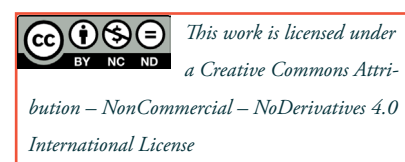
Non-myeloablative regimens are also highly effective, and employed primarily in older adults, or those with multiple co-morbidities. The fludarabine, cyclophosphamide, TBI 200cGy is far and away the most extensively studied regimen in this category.

In the coming years, we expect that outcome of both adult and pediatric UCB transplantation will continue to improve. However, this improvement will likely come from UCB graft manipulation such as stem cell expansion or stem cell homing strategies, reduction in relapse through novel maintenance strategies, and advances in the prevention and management of graft

versus host disease. Less important will be modifications to the conditioning regimen, perhaps focused on individualized dosing to optimize drug exposure.

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