Alternative Donor Transplantation for Acute Myeloid Leukemia

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Abstract: Allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative therapy for adult patients with acute myeloid leukemia (AML), but its use for consolidation therapy after first remission with induction chemotherapy used to be limited to younger patients and those with suitable donors. The median age of AML diagnosis is in the late 60s. With the introduction of reduced-intensity conditioning (RIC), many older adults are now eligible to receive allo-HCT, including those who are medically less fit to receive myeloablative conditioning. Furthermore, AML patients commonly have no human leukocyte antigen (HLA)-identical or medically suitable sibling donor available to proceed with allo-HCT. Technical advances in donor matching, suppression of alloreactivity, and supportive care have made it possible to use alternative donors, such as unrelated umbilical cord blood (UCB) and partially HLA-matched related (haploidentical) donors. Outcomes after alternative donor allo-HCT are now approaching the outcomes observed for conventional allo-HCT with matched related and unrelated donors. Thus, with both UCB and haploidentical donors available, lack of donor should rarely be a limiting factor in offering an allo-HCT to adults with AML.

Keywords: AML; alternative donor; UCB; Haploidentical; Transplantation
1. Introduction

Allogeneic hematopoietic cell transplantation (HCT) is widely used as a curative therapy for acute myeloid leukemia (AML). The use of reduced intensity conditioning (RIC) extended eligibility of HCT to older adults and those with comorbid conditions [1–4]. However, donor availability for many adults with AML still remains a significant challenge because HLA-identical matched sibling donors (MSD) or adult unrelated donors (MUD) are available for only about 60% of patients [5]. As the age cutoff for RIC HCT eligibility has increased, there has been a critical need for alternative donors for those who may not have a suitable HLA-matched MSD or MUD donor. Moreover, because high-risk AML is more common among the elderly, the time it takes to secure a MUD [1,2] increases the risk of leukemia relapse in this group who need to proceed to HCT promptly. Thus, in recent years unrelated umbilical cord blood (UCB) or haploidentical grafts have been studied as alternative donor types for adults with acute leukemia [1–4,6–10]. In this manuscript, we review the outcomes of HCT with these two alternative donor types in the management of adults with AML.

2. Umbilical Cord Blood Transplantation

UCB has been increasingly used for the past two decades as an alternative donor type given its rapid availability, less restrictive HLA-selection criteria, no donor risk, and relative low risk of graft-versus-host disease (GVHD). The introduction of double UCB (dUCB) transplantation extended access of UCB to most adults with hematological malignancies, including acute leukemia. However, barriers to UCB transplantation include limited stem cell content, delayed engraftment accompanied by increased risks of infectious complications, and cost. Several strategies have been used recently to improve the clinical outcome of UCB transplantation, such as achieving faster engraftment and further minimizing the incidence of GVHD without compromising immune reconstitution. Such promising strategies include the expansion of cord blood progenitor cells by various techniques [11–13], intra-bone marrow injection of cord blood cells [14–16] to improve hematopoietic engraftment, and use of Tregs to reduce risk of GVHD after UCB transplantation [17].

3. Myeloablative Single UCB Transplantation

Initial reports on the use of UCB as a donor type for hematopoietic cell transplantation (HCT) were based on the use of single UCB grafts and largely limited to pediatric patients [18–24]. At that time, the largest barrier to the use of UCB in adults was the weight of these patients relative to the limited cell dose available in individual UCB units. The first study to focus on UCB transplantation of adults reported on 68 patients [25] who were heavily pretreated and had high-risk hematological malignancies, 19 of whom had AML. The nucleated cell dose used in that study was inadequate considering today’s standard; however, the cell dose used was based on available data largely from pediatric studies. Not unexpectedly, hematopoietic recovery was slow and treatment-related mortality (TRM) was high (47% at three months), resulting in poor leukemia-free survival (LFS) (26% at 40 months). That study found better outcomes among patients who received higher total nucleated (TNC) (≥2.4 × 10^7/kg) and higher infused CD34+ (≥1.2 × 10^5/kg) cell doses. In addition, the adult cohort of the Cord Blood Transplantation (COBLT) prospective study observed poor outcomes, mainly owing to inadequate
TNC dose and the use of UCB as a “last resort” effort for very high-risk patients [26]. Despite limitations, these studies demonstrated the feasibility of UCB allografting in adults and set the stage for future studies seeking to improve outcomes among UCB recipients.

While many centers started using double UCB transplantation (reviewed below) to achieve an adequate cell dose for adult patients who are heavier than pediatric patients, many centers remained interested in single UCB transplantation either per institutional or country policy. Moreover, in recent years the availability of a larger inventory of UCB units has further improved the chances of finding adequate single-UCB unit grafts for adult transplantation. Takahashi et al. identified that, despite a delay in hematopoietic recovery, UCBT was associated with a markedly lower rate of chronic GVHD as compared to MRD transplantation [27]. More recently, the Valencia group reported their experience with single UCB transplantation after myeloablative conditioning in adults with higher-risk AML [28,29]. They used busulfan (BU)-based chemotherapy as a conditioning regimen and a UCB graft selection strategy based on improved cord blood banking standards that take into account the CD34+ cell count at the time of cryopreservation. They observed that median neutrophil engraftment occurred at 19 to 20 days and that disease-free survival (DFS) at five years was approximately 40%. Notably, patients with AML in first complete remission (CR1) who received a TNC dose \( \geq 2 \times 10^7/\text{kg} \) had a DFS at four years of 75%. In the most recent report, they also showed that patients receiving less well-HLA-matched UCB grafts had a lower risk of relapse and superior LFS [30]. Another important advance in single UCB transplantation is the strategy of delivering the graft, often with a TNC dose below current standards, directly into the bone marrow (known as intra-bone marrow infusion, IBMI) [14].

4. Myeloablative Double UCB Transplantation

The University of Minnesota pioneered the use of double UCB transplantation, which was developed to overcome the limitation of infused cell dose in adults and serve as a platform for graft manipulations [19]. The first series of double UCB recipients included 23 adult patients with high-risk leukemia using a myeloablative conditioning regimen consisting of cyclophosphamide (Cy; 120 mg/kg), fludarabine (Flu; 75 mg/m²), and total body irradiation (TBI; 1320cGy) [31]. In this case series, double UCB transplantation led to improvements in median infused TNC dose (3.5 \times 10^7/\text{kg}), sustained neutrophil engraftment (median of 23 days), and DFS at one year (57%). This success was in part due to no graft failure events and a low rate of TRM (22%). While the risk of acute GVHD (65%) with double UCB was higher than that seen in single UCB transplantation, it was largely due to an increase in grade II acute GVHD. The risk of chronic GVHD, however, was still low. Thus, the strategy of double UCB unit infusion became widely used, with other transplant centers investigating different preparative regimens and post-transplant immunosuppression [32–35]. While some preparative regimens were found not to support this treatment platform [32], variations of the myeloablative Cy/Flu/TBI regimen resulted in similar clinical outcomes, allowing many transplant centers worldwide to utilize double UCB transplantation for many adults with AML who required myeloablative conditioning [4,31,34,35]. The dissemination of this strategy, at least in part, was due to its simplicity, as any center technically able to thaw and infuse single UCB grafts was able to take advantage of the double UCB platform to extend transplantation to larger patients.
5. UCB Transplantation with Reduced Intensity Conditioning Regimen

The introduction of RIC extended the use of allogeneic HCT to older, less clinically fit, and extensively pre-treated patients, such as those who had previous autologous transplant. This transplant approach is particularly important for patients with AML as it typically presents in their late 60s, an age in which the morbidity and mortality of a conventional myeloablative regimen would be excessive. Furthermore, older patients may lack an HLA-matched sibling donor who is healthy enough to donate, making alternative donor transplantation necessary for this group of patients. Moreover, high-risk AML subtypes, such as secondary AML, for which allogeneic transplantation is the only potentially curative treatment option, is more frequent among older patients as well [36,37]; for such patients, long-term survival with chemotherapy alone is generally poor [37]. Thus, the advantage of RIC HCT using UCB for older patients is its rapid availability, which helps to avoid further delay in proceeding with a potentially curative HCT.

One of the most commonly used platforms for RIC HCT using UCB is the one developed at University of Minnesota that consists of Cy 50 mg/m², Flu 200 mg/m² divided in five days, and TBI 200 cGy with cyclosporine A (CSA) and mycophenolate mofetil (MMF) for immune suppression [38–43]. Variations on this platform, which have led to promising results, include the use of treosulfan by the Seattle group [44] and thiotepa (Thio) by the MSKCC group [45]. The backbone of the conditioning platform (Cy 50 mg/m², Flu 200 mg/m², TBI-200) has been used to support single and double UCB transplantation according to various institutional practice criteria and has been shown to result in sustained donor engraftment in >90% of recipients, TRM between 20%–30%, and long-term DFS in 25%–50% of patients depending on disease stage and the presence of co-morbid conditions prior to transplantation [4]. The Boston group has also reported on an equally promising regimen that includes the combination of Flu, melphalan (Mel), and rabbit ATG [3,46], and when sirolimus/tacrolimus was used for immune suppression, a very low risk of GVHD was observed [46]. In addition, these overall encouraging results of RIC UCB HCT have been recently reproduced by two multicenter phase II studies by the Blood and Marrow Transplant Clinical Trials Network (BMT-CTN) [7] and the Société Française de Greffe de Moelle Osseuse et Therapie Cellulaire and Eurocord [47–49].

6. Double versus Single UCB Graft

Several reports demonstrate that clinical outcomes among recipients of double and single UCB grafts are similar [42,43,47,50]. A recent registry study in patients with acute leukemia (n = 409; 285 AML) compared adults who received an adequate single UCB graft defined as a TNC dose of ≥ 2.5 × 10⁷/kg vs. double UCB grafts [42]. This study showed no difference in outcomes between one or two unit grafts. However, this conclusion has not been uniformly supported for relapse and acute GVHD. In some studies, a higher rate of AML relapse with single UCB transplantation was reported [43,51,52], but in other studies, no such association between number of infused UCB units and relapse or long-term treatment failure was observed [42,47,50,53,54]. In addition, although a higher risk of acute GVHD with double UCB was reported in one study [55], no difference in the risk of acute GVHD between single and double UCB transplantation was seen in other studies [53,54]. This discrepancy may in part be explained by differences in the patient population and use of ATG as part of the conditioning
regimen. Additional evidence supporting the comparability of single and double UCB transplantation includes a recently reported prospective, multicenter, randomized, phase III study comparing single vs. double UCB grafts in children [56]. Outcomes were similar between the two groups. This study demonstrated that if a suitable single unit is available, there is no advantage in using a double UCB graft. However, an adequately dosed single-unit UCB graft cannot be frequently found for adults. Most double-unit UCB recipients would not have been eligible for UCB transplantation if an adequately dosed graft could not be generated with two UCB units. Thus, in adults who rarely have an adequate single UCB unit that meets the minimum cell dose criteria, a double UCB graft remains the standard of care.

7. UCB Grafts versus Other Donor Sources

Many retrospective studies have compared the outcomes of UCB to those of matched and mismatched URD in various settings including single [57] and double UCB [58] allografting and myeloablative [27,57–61] and RIC regimens [39,62–67] (Table 1). Notably, most studies have shown similar long-term outcomes between UCB and URD [27,58,61,67,68]. These studies demonstrated that UCB recipients had slower hematopoietic recovery [27,57–60,66], higher TRM [59,64–66,69], and often lower rates of acute grade II-IV and chronic GVHD than URD recipients [39,58,60]. Atsuta et al. reported higher TRM (30% vs. 19%, \( p = 0.004 \)) and inferior survival (HR = 1.5; 95% CI 1.0–2.0; \( p = 0.028 \)) among UCB recipients than URD recipients. As compared to other reports, this discrepancy can be explained in part by the majority of UCB recipients receiving a median TNC dose of \( 2.5 \times 10^7/\text{kg} \), an inadequately dosed UCB graft by today’s standards [69]. In summary, the delay in hematological recovery and early TRM among UCB recipients observed in most studies was at least in part offset by lower risk of chronic GVHD and its complications and, in some series, remarkably lower risk of relapse, resulting in survival rates similar to other donor types. Novel strategies to improve the safety and efficacy of UCB transplantation are under way and have been reviewed elsewhere [70].
## Table 1. Comparative allo-HCT studies of UCB with other donor types for acute leukemia in adults.

| Reference       | Malignancy | Donor Type | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10⁹/L | aGVHD (II-IV) CI (%) | cGVHD CI (%) | TRM | Relapse | DFS |
|-----------------|------------|------------|----------------|-------------------|---------------------------|-----------------------------------|----------------------|-------------|-----|---------|-----|
| **Myeloablative conditioning** |
| Laughlin 2004   | Hematologic Malignancy | UCB | 150 | (16–60) | 27 days | 60 days | 0.81 | 1.62 | 1.89 | 0.73 | 1.48 |
|                 | 200 AML    | URD (BM)  | 367 | (16–60) | 20 days | 29 days | 0.66 | 1.12 | 0.99 | 0.85 | 0.94 |
|                 | MM URD (BM)| 83        | (16–60) | 18 days | 29 days | 1.0    | 1.0   | 1.0   | 1.0   | 0.69 |
|                 |            |           |           |       |         |        |       |       |       |       |       |
| Rocha 2004      | Hematologic Malignancy | UCB | 94 | (15–55) | 26 days | --     | 0.57 | 0.64 | 1.13 | 1.02 | 0.95 |
|                 | 362 AML    | URD BM    | 584 | (15–59) | 19 days | --     | 1.0   | 1.0   | 1.0   | 1.0   | 1.0 |
| Takahashi 2004  | Hematologic Malignancy | UCB | 68 | (16–53) | 36      | 22 days | 40 days | 0.61 | 0.60 | 0.32 | 0.75 | 0.27 |
|                 | 54 AML     | URD BM    | 45  | (16–50) | 26      | 18 days | 22.5 days | 1.0  | 1.0  | 1.0   | 1.0   | 1.0 |
| Takahashi 2007  | Hematologic Malignancy | UCB | 92 | 38 | 22 days | 40 days | 1.09 | 0.49 | 0.49 | 0.72 | 0.74 |
|                 | 88 AML     | MRD       | 71  | 40 | 17 days | 22.5 days | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Gutman 2009     | AML/ALL 53 AML | UCB | 31 | 22 | -- | -- | 80.6% | -- | 20.6% | 3.2% | 76.2% |
|                 |            | MUD       | 31 | 25 | -- | -- | 67.7% | -- | 17% | 25.8% | 57.1% |
|                 |            | MM URD    | 31 | 25 | -- | -- | 87.1% | -- | 29.2% | 23% | 47.8% |
| Atsuta 2009     | AML        | UCB | 173 | 38 | -- | -- | 32% | 28% | 30% | 31% | 36% |
|                 |            | URD       | 311 | 38 | -- | -- | 35% | 32% | 19% | 24% | 54% |

*Note: Median Time to ANC ≥500/µL and Median Time to Platelet >20 × 10⁹/L are in days. TRM (Truncal Relapse Mortality) and DFS (Disease-Free Survival) are percentages.*

*p-values indicated for statistical significance.*
Table 1. Cont.

| Reference          | Malignancy     | Donor Type | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10⁹/L | aGVHD (II-IV) CI (%) | cGVHD CI (%) | TRM | Relapse | DFS |
|--------------------|----------------|------------|----------------|--------------------|-----------------------------|-------------------------------------|-----------------------|--------------|-----|---------|-----|
| **Myeloablative conditioning** |                |            |                |                    |                             |                                     |                       |              |     |         |     |
| Eapen 2010 AML/ALL 880 AML |                | UCB        | 165            | 28                 | 24 days                     | 52 days                             | 1.0                   | 1.0          | 1.0 | 1.0     | 1.0 |
|                     |                | URD BM     | 332            | 39                 | 19 days                     | 28 days                             | 0.78                  | 0.63         | 1.69 | 0.85    | 1.15 |
|                     |                | MM URD BM  | 140            |                    |                             |                                     | 0.59                  | 0.59         | 1.06 | 0.84    | 0.93 |
|                     |                | URD PB     | 632            | 33                 | 14 days                     | 19 days                             | 0.57                  | 0.38         | 1.62 | 0.85    | 1.12 |
|                     |                | MM URD PB  | 256            |                    |                             |                                     | 0.49                  | 0.46         | 0.95 | 0.91    |      |
| Brunstein 2010     | Hematologic    | DUCB       | 128            | 25                 | 26 days                     | 53 days                             | 1.0                   | 1.0          | 1.0 | 1.0     | 1.0 |
|                     | Malignancy     | MRD        | 204            | 40                 | 16 days                     | 20 days                             | 1.08                  | 1.58         | 0.31 | 3.67    | 1.09 |
|                     | 476 acute leukemia | URD      | 152            | 31                 | 19 days                     | 21 days                             | 1.83                  | 1.71         | 0.61 | 3.05    | 0.85 |
|                     |                | MM URD     | 52             | 31                 | 18.5 days                   | 21 days                             | 2.35                  | 2.07         | 0.38 | 2.50    | 1.12 |
| Ponce 2011         | Hematologic    | DUCB       | 75             | 37                 | 24 (10)                     | 51 (38)                             | 43%                   | 28%          | 21% | 20%     | 55% |
|                     | Malignancy     | MRD        | 108            | 47                 | 11 (11)                     | 17 (12)                             | 27%                   | 31%          | 8%  | 19%     | 66% |
|                     | 133 AML        | URD        | 184            | 48                 | 11 (10)                     | 18 (17)                             | 39%                   | 44%          | 13% |         |     |
| Raiola 2014        | Hematologic, 232 acute leukemia | UCB | 105            | 40 (18–64)        | 23 days                     | 40 days                             | 19%                   | 23%          | 35% | 30%     | 33% |
|                     |                 | MRD        | 176            | 47 (15–69)        | 18 days                     | 160 days                            | 31%                   | 29%          | 24% | 40%     | 32% |
|                     | 69% MAC        | 8/8 URD    | 43             | 42 (19–66)        | 17 days                     | 100 days                            | 21%                   | 22%          | 33% | 23%     | 36% |
|                     |                 | 7/8 URD    | 43             | 47 (17–62)        | 16 days                     | 110 days                            | 42%                   | 19%          | 35% | 30%     | 34% |
|                     |                 | Haplo      | 92             | 45 (17–69)        | 18 days                     | 118 days                            | 14%                   | 15%          | 18% | 35%     | 43% |
| D50 (median)       |                | D100       | 1 yr           | D180               | 2 yr                        | 2 yr                                |                       |              |     |         |     |
| (p = 0.084)        |                | (p < 0.001) |                |                    |                             |                                     |                       |              |     |         |     |
### Table 1. Cont.

| Reference             | Malignancy                  | Donor Type | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10⁹/L | aGVHD (II-IV) CI (%) | cGVHD CI (%) | TRM | Relapse | DFS |
|-----------------------|-----------------------------|------------|----------------|--------------------|-----------------------------|------------------------------------|----------------------|--------------|-----|---------|-----|
|                        |                             |            |                |                    |                             |                                    |                      |              |     |         |     |
| Brunstein 2006        | AML                         | UC B       | 43             | 53                 | 88%                         | --                                 | 51%                  | --           | 28%| 35%     | 31% |
|                        |                             | Sib PBSC   | 21             | 54                 | 100%                        | --                                 | 62%                  | --           | 38%| 35%     | 32% |
|                        |                             |            |                |                    |                             |                                    |                      |              |     |         |     |
| Majhail 2008          | Hematologic malignancies    | UC B       | 43             | 59                 | --                          | --                                 | 49%                  | 17%          | 28%| --      | 34% |
|                        |                             | MRD        | 47             | 58                 |                             | p = 0.20                          | p = 0.02              | p = 0.23     |    |         | 0.98|
| Majhail 2012          | AML/MDS                     | UC B       | 60             | 61                 | --                          | --                                 | 45%                  | 33%          | 25%| 47%     | 22% |
|                        |                             | MRD        | 38             | 63                 |                             | p = 0.19                          | p = 0.04              | p = 0.43     |    |         | 0.23|
|                        |                             |            |                |                    |                             |                                    |                      |              |     |         |     |
| Brunstein 2012        | AML/ALL                     | DUCBT-TCF  | 121            | 55 (23–68)         | 1                            | --                                 | 1.91                 | 0.43         | 0.92| 1.26    | 1.13|
|                        |                             | 8/8 PBCT   | 313            | 59 (23–69)         | 0.21                         | --                                 | 1.44                 | 0.45         | 0.57| 1.15    | 0.88|
|                        |                             |            |                |                    |                             |                                    |                      |              |     |         |     |
|                        |                             | 7/8 PBCT   | 111            | 58 (21–69)         | 0.21                         | --                                 | 0.06                 | p = 0.013  |    |         |     |
|                        |                             | DUCBT-other| 40             | 48 (21–67)         | --                           | --                                 | 1                    | p = 0.06   |    |         |     |
| Chen 2012             | Hematologic malignancies,   | DUCBT      | 64             | 53 (19–67)         | 21.5                         | 41                                 | 14.1%                | 21.9%        | 26.9%| 42.7%   | 30% |
|                        | 95 AML                      | URD        | 221            | 58 (19–73)         | 13                            | 19                                 | 20.3%                | 53.9%        | 10.4%| 49.8%   | 40% |
|                        |                             |            |                |                    |                             |                                    |                      |              |     |         |     |

Reduced intensity conditioning

1 yr 2 yr 2 yr OS

D100 3 yr D180 3yr

D100 2yr 2yr 2yr 2yr

D100 2yr 2yr 2yr 2yr

p < 0.0001 p < 0.0001 p < 0.0001 p < 0.0001 p < 0.0001
Table 1. Cont.

| Reference          | Malignancy         | Donor Type | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10⁹/L | aGVHD (II-IV) CI (%) | cGVHD CI (%) | TRM | Relapse | DFS |
|--------------------|--------------------|------------|----------------|-------------------|-----------------------------|-------------------------------------|---------------------|----------------|------|---------|-----|
| Le Bourgeois 2013  | Hematologic malignancies | DUCB      | 39             | 56 (22–69)       | 16                          | 38                                  | 26%                 | 26%           | 26.5% | 23%     | 50.5% |
|                    |                    | PBSC       | 52             | 59 (22–70)       | 17                          | 0                                   | 31%                 | 35%           | 6%    | 35.5%   | 59%  |
| Weisdorf 2014      | AML                | UCB        | 205            | 59 (50–71)       |                             |                                     | 97%                 | 91%           | p < 0.0001 | p < 0.0001 | p < 0.69 | p < 0.001 | p < 0.05 | p < 0.95 | p < 0.01 | p < 0.001 | p < 0.001 | p < 0.0001 | p < 0.01 | p < 0.13 | p < 0.39 |
|                    |                    | 8/8 URD    | 441            | 58 (50–75)       |                             |                                     | 36%                 | 43%           | 53%    | 27%     | 35%  |
|                    |                    | 7/8 URD    | 94             | 58 (50–72)       |                             |                                     | 44%                 | 59%           | 41%    | 26% at 34% at |
| Malard 2015        | AML                | UCB        | 205            | 49 (19–69)       |                             |                                     | 75%                 | 56%           | 1      | 1       | 1    |
|                    |                    | 10/10 URD  | 347            | 57 (19–70)       |                             |                                     | 96%                 | 84%           | 1.72   | 2.15    | 1.05 |
|                    |                    | 9/10 URD   | 99             | 55 (19–68)       |                             |                                     | p < 0.001           | p < 0.001     | p < 0.08 | p < 0.85 | 0.60 |
|                    |                    |            |                |                   |                             |                                     | p < 0.001           | p < 0.001     | p < 0.007 | p < 0.23 | p < 0.13 | p < 0.07 | p < 0.07 | p < 0.29 |
8. Haploidentical Transplantation

Allogenic HCT using a haploidentical (haplo) donor historically has been an attractive alternative approach given that donors are readily available for almost all patients. However, the initial experience with T-cell replete haplo-HCT was disappointing because of unacceptably high non-relapse mortality (NRM) and incidence of severe GVHD occurring in about half of patients [71–73]. In contrast, when *ex vivo* T-cell depletion platforms were utilized with the intention of minimizing GVHD, it led to an excessive increase in graft failure and infectious complication rates [74,75]. Novel strategies such as post-transplantation cyclophosphamide (PT-Cy), CD34+ “mega dose”, and α/β+ T–cell depletion have improved clinical outcomes and broadened the use haplo-HCT in recent years. The advantages of this alternative donor type include immediate donor availability, motivation of family donors, simplicity of use (at least in the context of PT-Cy), and low cost [76–79]. This is particularly important in developing countries where ease of access to international unrelated donors and UCB may be limited by cost and local policies. The main limitation of haplo-HCT is still a high risk of disease relapse, which in part can be addressed by the use of more intensive conditioning regimens. Ongoing studies are investigating post-transplantation maintenance therapy methods. Delayed immune reconstitution has also been a major limitation in some haplo-HCT platforms. Thus, several strategies were undertaken to minimize GVHD after haplo-HCT without significantly affecting immune reconstitution. Most of these strategies are still in the developmental stage, including those directed towards augmentation of immune reconstitution after T-cell-depleted haplo-HCT, such as infusion of pathogen-specific T-cells [80–84], suicide-gene expressing T-cells [85–87], regulatory T-cells (Tregs) [88–90], or *ex vivo* photodepletion of alloreactive donor T-cells [91,92]. Selective allodepletion in T-cell replete haplo-HCT was another strategy explored that includes *ex vivo* T-cell tolerance induction via co-stimulation blockade [93,94], *ex vivo* selective depletion of T-cells [95–98], and use of PT-Cy [9,76,99–101].

9. T-Cell Depleted Haploidentical Graft

Infusion of a mega-dose (>10 × 10⁶ cells/kg) of CD34+ selected cells is a strategy developed to overcome the poor hematopoietic engraftment of T-cell-depleted haploidentical grafts after myeloablative conditioning [8,102,103]. The Perugia group conducted a phase II trial of mega-dose infusion of CD34+ cells after intensive conditioning with Thio/Flu/TBI (8Gy) and rabbit ATG in 104 patients with acute leukemia [104]. Despite achievement of successful engraftment in over 90% of patients, the rate of NRM was still excessive (36.5%), mainly owing to infectious complications from delayed immune reconstitution. Similarly, EBMT reported unacceptable TRM (36%–61% ± 10% at two years) due to serious infections and poor immune reconstitution after T-cell-depleted myeloablative conditioning in 266 patients with acute leukemia [103]. However, this strategy led to a low incidence of GVHD and appeared promising. By using α/β/CD19+ T-cell depletion, Locatelli and colleagues recently reported that myeloablative haplo-HCT using Thio/Flu/TBI and ATG-based conditioning yielded acceptable engraftment, a low rate of GVHD, and faster immune reconstitution [98]. Although clinical outcomes of haplo-HCT appear to be improving with the use of these novel
techniques, future studies will need to carefully weigh the cost and benefits of these approaches relative to other alternative donor choices.

10. Unmodified Haploidentical Graft with Post-Transplant Cyclophosphamide

The PT-Cy approach was pioneered by the John Hopkins group, and in recent years has become widely used at many transplant centers given its lower cost and ease of use. With this strategy, the bone marrow or peripheral blood stem cell graft is unmodified when it is infused into the patient, allowing alloreactive T-cells to proliferate until days +3 and +4 post-transplant. The patient then receives 50 mg/kg/day cyclophosphamide for in vivo T-cell depletion. As shown in animal models, and recently in humans, this dose of cyclophosphamide kills actively proliferating T-cells, but does not harm the hematopoietic progenitor and stem cells that are critical for blood count recovery and engraftment [78]. This strategy results in low risk of acute and chronic GVHD, which has been explained by the use of in vivo alloreactive T-cell depletion with high-dose Cy [78]. In addition, several recent studies suggest improvement of immune reconstitution with preserved memory T-cells when PT-Cy is used in T-cell-replete haplo-HCT [76,100]. Luznik and colleagues reported incidence rates of sustained engraftment, grades II-IV acute GVHD, and chronic GVHD of 87%, 34%, and <25%, respectively, among 67 RIC haplo-HCT recipients with hematological malignancies [105]. NRM at one year was acceptably low at 15%; however, the relapse rate was higher at 51%, resulting in two-year event-free survival (EFS) of only 26%. Similar results were observed in their most updated report of a phase II study involving 210 patients with hematological malignancies receiving RIC conditioning followed by bone marrow haplo-HCT and PT-Cy: the cumulative incidence rates of grade II-IV acute GVHD, chronic GVHD, five-year relapse, and EFS were 27%, 13%, 55%, and 27%, respectively [76]. Another recent study by Ciurea and colleagues compared the clinical outcomes of 65 haplo-HCT recipients with T-cell-replete haplo-HCT/PT-Cy versus T-cell-depleted peripheral blood HCT. They demonstrated the superiority of T-cell-replete haplo-HCT/PT-Cy in terms of one-year NRM (16% vs.42%, p = 0.03), chronic GVHD (8% vs. 18%, p = 0.03), PFS (45% vs. 21%, p = 0.03), and OS (66% vs. 30%, p = 0.02) [99]. Bashey and colleagues identified comparable rates of relapse, DFS, and OS between haplo-HCT/PT-Cy and MSD or adult URD HCT [79]. Although in this study the UCB group had higher TRM and inferior survival compared to haplo-HCT, myeloablative conditioning with Thio/Bu/Flu or Bu/Cy was the most common regimen (83%) used for UCB allograft, which likely contributed to higher TRM and inferior survival in this group. A recent CIBMTR study examined the clinical outcomes of 2174 adults with AML receiving haplo-HCT/PT-Cy (n = 192) or matched URD (n = 1982) allograft and identified similar two-year survival rates for these two donor groups after both myeloablative conditioning and RIC [77]. The BMT-CTN conducted two parallel multicenter phase II trials of RIC haplo-HCT/PT-CY versus UCB HCT. Both of these alternative donor approaches produced comparable survival rates; however, a lower risk of TRM among recipients of RIC haplo-BMT/PT-CY was offset by a higher risk of relapse as compared to UCB HCT recipients [7]. A recent collaborative study by French and Italian groups retrospectively compared the clinical outcomes of haplo-HCT and UCB transplantation in 150 patients with various hematological malignancies [106]. While haplo-HCT in this study was
mostly performed for a lymphoma diagnosis (84%), the UCB group in contrast was enriched with acute leukemia patients (63%) and those undergoing alloHCT with a significantly higher disease risk index (44% vs. 20%). These findings most likely contributed to a higher cumulative incidence of disease relapse and lower DFS after UCB transplantation as compared to haplo-HCT. However, these results had no impact on overall survival, and TRM was similar between the groups. A more recent and larger retrospective study by EBMT examined differences between haplo-HCT (32% PT-Cy-based) and UCB (49% Cy/Flu/TBI-based) allografts in 1446 patients with acute leukemia and identified delayed engraftment and a lower rate of chronic GVHD with UCB transplant, but otherwise similar long-term clinical outcomes with both donor types [107]. Newer strategies are being tested to further improve the outcomes of haplo-HCT/PT-Cy. Grosso and colleagues recently reported an encouraging two-year DFS of 74% among 30 patients with hematological malignancies who received myeloablative haplo-HCT with 1200 cGy TBI followed by infusion of fixed-dose donor T-cells, 48 hours later by high-dose Cy, and 24 hours later by selected donor CD34+ cell infusion [108]. In conclusion, clinical research to improve the outcomes of haplo-HCT has witnessed dramatic successes within the past decade (Table 2), allowing many adults with leukemia who do not have an available HLA-identical relative to receive allogenic HCT using readily available UCB or haploidaliental donors.
### Table 2. Haploidentical transplantation for acute leukemia in adults.

| Reference | Malignancy       | Conditioning Regimen | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10⁹/L | aGVHD (II-IV) CI (%) | cGVHD CI (%) | TRM | Relapse | DFS |
|-----------|------------------|----------------------|----------------|--------------------|-----------------------------|--------------------------------------|---------------------|---------------|-----|--------|-----|
| Aversa 2005 | Acute leukemia 67 AML | Thio/Flu/TBI/ATG     | 104            | 33 (9–64)          | 11 days                     | 15 days                             | 8%                  | 7%            | 36.5% | 25% at 6mo | 39% at median 22mo |
| Ciceri 2008 | Hematologic Malignancy 173 AML | TBI-based; 74% CR1/CR2 CR1/CR2 36 (16–63) advanced | 173            | 37 (17–66)         | 12 days                      | --                                  | 5%                  | 10%           | 36% CR1 | 16% CR1 | 23% CR2 | 1% advanced |
| Chang 2009 | Hematologic Malignancy 43 AML | Bu/Cy +ATG          | 133            | 15 (2–18)          | 12 days                      | 15 days                             | --                  | --            | -- | -- | -- |
| Chang BBMT 2009 | Hematologic Malignancy 100 AML | Bu/Cy/ cytarabine/ Semustine/rATG | 348            | 24 (2–54)          | 13 days                      | 16 days                             | --                  | --            | -- | -- | -- |
| Luznik 2008 | Hematologic Malignancy 27 AML | NMA Flu/Cy/TBI      | 68             | 46 (1–71)          | 15 days                      | 24 days                             | 34% at Day 200      | 5%–25% at 1-yr | 15% at 1-yr | 51% at 1-yr | 26% at 2-yr |
| Kazamon 2010 | Hematologic Malignancy 49 AML | NMA Flu/Cy/TBI      | 185            | 50 (1–71)          | --                          | --                                  | 31%                 | 15%           | 15% at 1-yr | -- | 35% at 1-yr |
| Munchel 2011 | Hematologic Malignancy 43 AML | NMA Flu/Cy/TBI      | 210            | 52 (1–73)          | 15 days                      | 24 days                             | 27%                 | 13%           | 18% at 5-yr | 55% at 5-yr | 27% at 5-yr |
| Solomon 2012 | Hematologic Malignancy 12 AML | MA Flu/Bu/Cy        | 20             | 44 (25–56)         | 16 days                      | 27 days                             | 30%                 | 35%           | 10% at 1-yr | 40% at 1-yr | 50% at 1-yr |
Table 2. Cont.

| Reference         | Malignancy            | Conditioning Regimen | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10^9/L | aGVHD (II-IV) CI (%) | eGVHD CI (%) | TRM | Relapse | DFS |
|-------------------|-----------------------|----------------------|----------------|-------------------|----------------------------|--------------------------------------|----------------------|--------------|-----|--------|-----|
| **Haplo-HCT with PT-Cy** |                       |                      |                |                   |                           |                                      |                      |              |     |        |     |
| Ciurea 2012       | Hematologic Malignancy | TCR-Haplo/PT-Cy:     | 65             | 45 (20–63)        | 18 days                  | 26 days                              | 20%                  | 7%           | 16% at 1-yr | 34% at 1-yr | 50% at 1-yr |
|                   | 42 AML/MDS            | 26 MA & 6 NMA        |                |                   |                           |                                      |                      |              |     |        |     |
|                   |                       | TCD-Haplo/ATG: MA    |                | 36 (18–56)        | 13 days                  | 12 days                              | 11%                  | 18%          | 42% at 1-yr | 36% at 1-yr | 21% at 1-yr |
| Castagna 2014     | Hematologic Malignancy| NMA                  | 46 BM          | 44 (19–68)        | 21 days                  | 29 days                              | 25%                  | 13%          | 22% at 2-yr | --           | 62% at 2-yr |
|                   | 4 AML/MDS             | Flu/Cy/TBI           |                |                   |                           |                                      |                      |              |     |        |     |
|                   |                       | 23 PB                |                | 54 (25–65)        | 20 days                  | 27 days                              | 33%                  | 13%          | 12% at 2-yr | --           | 62% at 2-yr |
| **Haplo-HCT with intensive immunosuppression** |                       |                      |                |                   |                           |                                      |                      |              |     |        |     |
| Huang 2006        | Hematologic Malignancy| MA                   | 171            | 25 (2–56)         | 12 days                  | 15 days                              | 55%                  | 47% at 2-yr | 19%–31% at 2-yr | 12%–39% at 2-yr | 42%–68% at 2-yr |
|                   | 51 AML                | Bu/Cy/ARA-C/         |                |                   |                           |                                      |                      |              |     |        |     |
|                   |                       | Semustine            |                |                   |                           |                                      |                      |              |     |        |     |
|                   |                       | IS: ATG/CSA/MTX/MMF  |                |                   |                           |                                      |                      |              |     |        |     |
| Huang 2009        | Acute leukemia        | MA                   | 250            | 25 (2–56)         | 12 days                  | 15 days                              | 46%                  | 23% at 3-yr | 19%–51% at 3-yr | 12%–49% at 3-yr | 25%–71% at 3-yr |
|                   | 108 AML               | Bu/Cy/ARA-C/         |                |                   |                           |                                      |                      |              |     |        |     |
|                   |                       | Semustine            |                |                   |                           |                                      |                      |              |     |        |     |
|                   |                       | IS: ATG/CSA/MTX/MMF  |                |                   |                           |                                      |                      |              |     |        |     |
| Di Bartolomeo 2013| Hematologic Malignancy| 80%MA /20%RIC       | 80             | 37 (5–71)         | 21 days                  | 28 days                              | 24%                  | 6% at 2-yr | 36% at 1-yr | 21% at 1-yr | 38% at 3-yr |
|                   | 45 AML                | Thio/Bu/Flu          |                |                   |                           |                                      |                      |              |     |        |     |
|                   |                       | IS: ATG/CSA/MTX/MMF/Basiliximab | | | | | | | | | |
| Reference     | Malignancy                 | Conditioning Regimen | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10^9/L | aGVHD (II-IV) CI (%) | cGVHD CI (%) | TRM | Relapse | DFS |
|---------------|---------------------------|----------------------|----------------|--------------------|----------------------------|--------------------------------------|----------------------|--------------|-----|--------|-----|
| Fu 2014       | Hematologic Malignancy    | TBI/Cy/simustine/ATG | 38             | 20 (13–46)        | 13 days                   | 19 days                              | 32%                  | 61% at 1-yr  | 13% at 1-yr | 27% at 2-yr | 58% at 2-yr |
|               |                           | Bu/Cy/simustine/ARA-C/ATG | 77            | 24 (8–51)        | 12 days                   | 16 days                              | 48%                  | 53% at 1-yr  | 16% at 1-yr | 32% at 2-yr | 57% at 2-yr |
| Comparative studies with Haplo-HCT |
| Lu 2006       | Hematologic malignancies  | MA                   | 135            | 24 (3–50)         | 12 days                   | 15 days                              | 32%                  | 55%          | 22%          | 18%          | 64%          |
|               |                           | Haplo (Bu/Cy/ATG)    |                |                    |                           |                                      |                      |              |              |              |              |
|               |                           | MRD (Bu/Cy)          | 158            | 37 (5–50)         | p < 0.001                 | p = NS                               | N = 0.13             | p = 0.90     | p = 0.10     | p = 0.40     | p = 0.27     |
| Brunstein 2011| Hematologic malignancies | RIC                  | 50             | 48 (7–70)         | 16 days                   | 24 days                              | 32%                  | 1-yr         | 7%           | 45%          | 48%          |
|               |                           | Haplo PT-Cy          |                |                    |                           |                                      |                      |              |              |              |              |
|               |                           | dUCB                 | 50             | 58 (16–69)        | 15 days                   | 38 days                              | 40%                  | 25%          | 24%          | 31%          | 46%          |
| Bashey 2013   | Hematologic malignancies  | 50% MA               | 46             | 59 (50–71)        | --                        | --                                   | 27%                  | 30%          | 38%          | 7%           | 33%          | 60%          |
|               |                           | Haplo PT-Cy          |                |                    |                           |                                      |                      |              |              |              |              |
|               |                           | MRD                  | 50             | 58 (50–75)        | --                        | --                                   | 27%                  | 30%          | 38%          | 7%           | 33%          | 60%          |
|               |                           | 8/8 URD              | 51             | 58 (50–72)        | --                        | --                                   | 39%                  | 39%          | 54%          | 16%          | 34%          | 52%          |
| Reference                  | Malignancy         | Conditioning Regimen | No of Patients | Median Age (range) | Median Time to ANC ≥500/µL | Median Time to Platelet >20 × 10^9/L | aGVHD (II-IV) CI (%) | cGVHD CI (%) | TRM | Relapse | DFS |
|---------------------------|--------------------|----------------------|----------------|-------------------|---------------------------|--------------------------------------|---------------------|--------------|-----|--------|-----|
| **Comparative studies with Haplo-HCT** |                    |                      |                |                   |                           |                                      |                     |              |     |        |     |
| Raiola 2014               | Hematologic malignancies | 232 acute leukemia   | 105            | 40 (18–64)       | 23 days                  | 40 days                | 19%                  | 23%          | 35% | 30%    | 33% |
|                           |                    |                      |                |                   |                           |                                      |                     |              |     |        |     |
| Ciurea 2014 (ASH)         | 2174 AML           | MA-Haplo PT-Cy       | 104            | 21–70             |                           | D30 CI                  |                       | HR           | HR   |        |     |
|                           |                    | MA-8/8 URD           | 1245           |                   |                           |                         |                       | HR           | HR   |        |     |
| Luo 2014                  | Hematologic malignancies | 126 AML             |                |                   |                           | D90 CI                  |                       | HR           | HR   |        |     |
| Ruggeri 2015              | AML                | Haplo (32% PT-Cy)    | 360            |                   |                           |                         |                       | HR           | HR   |        |     |
|                           |                    | UCB (49%)            | 558            |                   |                           |                         |                       | HR           | HR   |        |     |
|                           |                    | Cy/Flu/TBI           |                |                   |                           |                         |                       | HR           | HR   |        |     |
11. Unmodified Haploidentical Graft with Intensive Immune Suppression

Another approach to minimizing the rate of GVHD after haploidentical transplantation is the use of intensive immunosuppression [109–111]. This strategy was first studied by Huang and colleagues, in which 250 patients with acute leukemia received a G-CSF-primed, unmanipulated, haploidentical, peripheral blood or bone marrow graft [111]. Myeloablative conditioning consisted of Bu/Cy/cytarabine/semustine and rabbit ATG, and the immunosuppression consisted of CSA, MMF, and methotrexate (MTX). Neutrophil engraftment was achieved in all except one patient, and the rates of grade II-IV acute GVHD, grade III-IV acute GVHD, and extensive chronic GVHD were 46%, 13%, and 23%, respectively. At three years, TRM and the relapse rate were higher for high-risk AML patients at 29% and 49%, respectively. In their most updated report Luo and colleagues compared their haplo-HCT experience with MRD and matched URD and identified higher TRM and lower relapse rate associated with haplo-HCT as compared to MRD graft; however, five-year LFS was similar in all three groups [112]. Most recently, the same group from China compared the TBI/Cy/simustine/ATG conditioning regimen with the Bu/Cy/simustine/cytarabine/ATG regimen in 115 patients with acute leukemia and reported similar clinical outcomes except for a higher rate of organ toxicities with the Bu-based regimen [113]. Another group from China reported their experience using T-cell-replete haplo-HCT with myeloablative conditioning consisting of Bu/Cy/cytarabine/lamustine and low-dose (10 mg/kg) ATG and immunosuppression consisting of CSA, MMF, and MTX [112]. They compared this haplo-HCT platform \((n = 99)\) to MSD \((n = 90)\) and URD \((n = 116)\) allografts and identified comparable long-term DFS in all three groups. While there was no difference in other clinical outcomes between haplo-HCT and URD grafts, haplo-HCT recipients had a higher incidence of TRM and acute GVHD, but a lower relapse rate, than MRD recipients. In addition, a G-CSF-priming conditioning regimen in T-cell-replete haplo-HCT with intensive immunosuppression resulted in a lower relapse rate and superior LFS and OS as compared to non-G-CSF priming in a Southwest China multicenter randomized controlled study [114]. A similar strategy of intensive immunosuppression after T-cell-replete haplo-HCT has been evaluated in 80 patients with high-risk hematologic malignancies [115]. GVHD prophylaxis consisted of five drugs (ATG, CSA, MMF, MTX and basiliximab), and most patients (80%) received myeloablative conditioning consisting mainly of Thio/Bu/Flu. This therapeutic approach produced acceptable hematopoietic engraftment and low rates of acute and chronic GVHD, leading to a three-year LFS rate of 30% for high-risk patients. A calcineurin inhibitor-free, sirolimus-based immunosuppressive platform in combination with ATG, MMF, and rituximab was another modality that was used after trosulfan/Flu conditioning and T-cell-replete haplo-HCT; this approach demonstrated rapid T-cell immune reconstitution and promoted in vivo expansion of Tregs [116,117]. On the basis of these clinical investigations, it is reasonable to view T-cell-replete haplo-HCT in combination with intensive immunosuppression to be another promising haplo-HCT platform.

12. The Way Forward

Alternative donor transplantation is now a reality that allows almost every patient who requires alloHCT to proceed with this potentially curative treatment modality. Mismatched URD represents yet
another alternative donor type, especially with our improved understanding of the HLA-system and HLA-matching, such as the identification of “permissive” mismatches [118]. Future advances in clinical care will require physicians to encourage their patients to participate in prospective clinical trials so that strategies to improve the clinical outcomes of HCT recipients using alternative donor types and platform options can be rigorously compared. One such clinical trial is the phase III, randomized, multicenter trial (BMT CTN protocol 1101) of RIC and double UCB HCT versus haplo-HCT with PT-Cy in adults with leukemia and lymphoma. This study will compare clinical outcomes as well as cost efficacy, quality of life, and immune reconstitution: important outcomes for defining the relative efficacy of the two donor types and helping to establish evidence-based standards of care.

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Author Contributions

Nelli Bejanyan, Housam Haddad and Claudio Brunstein wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Grewal, S.S.; Barker, J.N.; Davies, S.M.; Wagner, J.E. Unrelated donor hematopoietic cell transplantation: Marrow or umbilical cord blood? Blood 2003, 101, 4233–4244.
2. Confer, D.; Robinett, P. The US National Marrow Donor Program role in unrelated donor hematopoietic cell transplantation. Bone Marrow Transplant. 2008, 42 (Suppl. 1), S3–S5.
3. Ballen, K.K.; Spitzer, T.R.; Yeap, B.Y.; McAfee, S.; Dey, B.R.; Attar, E.; Haspel, R.; Kao, G.; Liney, D.; Alyea, E.; et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. Biol. Blood Marrow Transplant. 2007, 13, 82–89.
4. Brunstein, C.G.; Barker, J.N.; Weisdorf, D.J.; DeFor, T.E.; Miller, J.S.; Blazar, B.R.; McGlave, P.B.; Wagner, J.E. Umbilical cord blood transplantation after nonmyeloablative conditioning: Impact on transplantation outcomes in 110 adults with hematologic disease. Blood 2007, 110, 3064–3070.
5. Gladstone, D.E.; Zachary, A.A.; Fuchs, E.J.; Luznik, L.; Kasamon, Y.L.; King, K.E.; Brodsky, R.A.; Jones, R.J.; Leffell, M.S. Partially mismatched transplantation and human leukocyte antigen donor-specific antibodies. Biol. Blood Marrow Transplant. 2013, 19, 647–652.
6. Misawa, M.; Kai, S.; Okada, M.; Nakajima, T.; Nomura, K.; Wakae, T.; Toda, A.; Itoi, H.; Takatsuka, H.; Itsukuma, T. Reduced-intensity conditioning followed by unrelated umbilical cord blood transplantation for advanced hematologic malignancies: Rapid engraftment in bone marrow. Int. J. Hematol. 2006, 83, 74–79.
7. Brunstein, C.G.; Fuchs, E.J.; Carter, S.L.; Karanes, C.; Costa, L.J.; Wu, J.; Devine, S.M.; Wingard, J.R.; Aljitawi, O.S.; Cutler, C.S.; et al. Alternative donor transplantation after reduced intensity conditioning: Results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood* 2011, 118, 282–288.

8. Chang, Y.J.; Xu, L.P.; Liu, D.H.; Liu, K.Y.; Han, W.; Chen, Y.H.; Wang, Y.; Chen, H.; Wang, J.Z.; Zhang, X.H. Platelet engraftment in patients with hematologic malignancies following unmanipulated haploidentical blood and marrow transplantation: Effects of CD34+ cell dose and disease status. *Biol. Blood Marrow Transplant.* 2009, 15, 632–638.

9. Bashey, A.; Zhang, X.; Sizemore, C.A.; Manion, K.; Brown, S.; Holland, H.K.; Morris, L.E.; Solomon, S.R. T-cell-replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. *J. Clin. Oncol.* 2013, 31, 1310–1316.

10. Kasamon, Y.L.; Luznik, L.; Leffell, M.S.; Kowalski, J.; Tsai, H.L.; Bolanos-Meade, J.; Morris, L.E.; Crilley, P.A.; O’Donnell, P.V.; Rossiter, N.; et al. Nonmyeloablative HLA-haploidentical bone marrow transplantation with high-dose posttransplantation cyclophosphamide: Effect of HLA disparity on outcome. *Biol. Blood Marrow Transplant.* 2010, 16, 482–489.

11. Delaney, C.; Varnum-Finney, B.; Aoyama, K.; Brashem-Stein, C.; Bernstein, I.D. Dose-dependent effects of the Notch ligand Delta1 on *ex vivo* differentiation and *in vivo* marrow repopulating ability of cord blood cells. *Blood* 2005, 106, 2693–2699.

12. De Lima, M.; McNiece, I.; Robinson, S.N.; Munsell, M.; Eapen, M.; Horowitz, M.; Alousi, A.; Saliba, R.; McMannis, J.D.; Kaur, I. Cord-blood engraftment with *ex vivo* mesenchymal-cell coculture. *N. Engl. J. Med.* 2012, 367, 2305–2315.

13. Wagner, J.E.; Brunstein, C.G.; McKenna, D.; Sumstad, D.; Maahs, S.; Boitano, A.E.; Cooke, M.P.; Bleul, C.C. Safety and Exploratory Efficacy of *Ex Vivo* Expanded Umbilical Cord Blood (UCB) Hematopoietic Stem and Progenitor Cells (HSPC) Using Cytokines and Stem-Regenin 1 (SR1): Interim Results of a Phase 1/2 Dose Escalation Clinical Study. *Blood (ASH Annu. Meet. Abstr.)* 2013, 122, 698.

14. Frassoni, F.; Gualandi, F.; Podesta, M.; Raiola, A.M.; Ibatici, A.; Piaggio, G.; Sessarego, M.; Sessarego, N.; Gobbi, M.; Sacchi, N. Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: A phase I/II study. *Lancet Oncol.* 2008, 9, 831–839.

15. Frassoni, F.; Varaldo, R.; Gualandi, F.; Bacigalupo, A.; Sambuceti, G.; Sacchi, N.; Podestà, M. The intra-bone marrow injection of cord blood cells extends the possibility of transplantation to the majority of patients with malignant hematopoietic diseases. *Best Pract. Res. Clin. Haematol.* 2010, 23, 237–244.

16. Brunstein, C.G.; Barker, J.N.; Weisdorf, D.J.; Defor, T.E.; McKenna, D.; Chong, S.Y.; Miller, J.S.; McGlave, P.B.; Wagner, J.E. Intra-BM injection to enhance engraftment after myeloablative umbilical cord blood transplantation with two partially HLA-matched units. *Bone Marrow Transplant.* 2009, 43, 935–940.
17. Brunstein, C.G.; Miller, J.S.; Cao, Q.; McKenna, D.H.; Hippen, K.L.; Curtsinger, J.; Defor, T.; Levine, B.L.; June, C.H.; Rubinstein, P.; et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: Safety profile and detection kinetics. *Blood* 2011, 117, 1061–1070.

18. Locatelli, F.; Perotti, C.; Torretta, L.; Maccario, R.; Montagna, D.; Ravelli, A.; Giorgiani, G.; De Benedetti, F.; Giraldi, E.; Magnani, M.L.; et al. Mobilization and selection of peripheral blood hematopoietic progenitors in children with systemic sclerosis. *Haematologica* 1999, 84, 839–843.

19. Barker, J.N.; Weisdorf, D.J.; Wagner, J.E. Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. *N. Engl. J. Med.* 2001, 344, 1870–1871.

20. Rocha, V.; Cornish, J.; Sievers, E.L.; Filipovich, A.; Locatelli, F.; Peters, C.; Remberger, M.; Michel, G.; Arcese, W.; Dallorso, S.; et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood* 2001, 97, 2962–2971.

21. Wagner, J.E.; Barker, J.N.; DeFor, T.E.; Baker, K.S.; Blazar, B.R.; Eide, C.; Goldman, A.; Kersey, J.; Krivit, W.; MacMillan, M.L.; et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: Influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* 2002, 100, 1611–1618.

22. Michel, G.; Rocha, V.; Chevret, S.; Arcese, W.; Chan, K.W.; Filipovich, A.; Takahashi, T.A.; Vowels, M.; Ortega, J.; Bordigoni, P.; et al. Unrelated cord blood transplantation for childhood acute myeloid leukemia: A Eurocord Group analysis. *Blood* 2003, 102, 4290–4297.

23. Gluckman, E.; Rocha, V. Cord blood transplantation for children with acute leukaemia: A Eurocord registry analysis. *Blood Cells Mol. Dis.* 2004, 33, 271–273.

24. Escolar, M.L.; Poe, M.D.; Provenzale, J.M.; Richards, K.C.; Allison, J.; Wood, S.; Wenger, D.A.; Pietryga, D.; Wall, D.; Champagne, M.; et al. Transplantation of umbilical-cord blood in babies with infantile Krabbe’s disease. *N. Engl. J. Med.* 2005, 352, 2069–2081.

25. Laughlin, M.J.; Barker, J.; Bambach, B.; Koc, O.N.; Rizzieri, D.A.; Wagner, J.E.; Gerson, S.L.; Lazarus, H.M.; Cairo, M.; Stevens, C.E.; et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N. Engl. J. Med.* 2001, 344, 1815–1822.

26. Cornetta, K.; Laughlin, M.; Carter, S.; Wall, D.; Weinthal, J.; Delaney, C.; Wagner, J.; Sweetman, R.; McCarthy, P.; Chao, N. Umbilical cord blood transplantation in adults: Results of the prospective Cord Blood Transplantation (COBLT). *Biol. Blood Marrow Transplant.* 2005, 11, 149–160.

27. Takahashi, S.; Ooi, J.; Tomonari, A.; Konuma, T.; Tsukada, N.; Oiwa-Monna, M.; Fukuno, K.; Uchiyama, M.; Takasugi, K.; Iseki, T.; et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood* 2007, 109, 1322–1330.

28. Sanz, J.; Sanz, M.A.; Saavedra, S.; Lorenzo, I.; Montesinos, P.; Senent, L.; Planelles, D.; Larrea, L.; Martin, G.; Palau, J.; et al. Cord blood transplantation from unrelated donors in adults with high-risk acute myeloid leukemia. *Biol. Blood Marrow Transplant.* 2010, 16, 86–94.
29. Sanz, J.; Boluda, J.C.; Martin, C.; Gonzalez, M.; Ferra, C.; Serrano, D.; de Heredia, C.D.; Barrenetxea, C.; Martinez, A.M.; Solano, C.; et al. Single-unit umbilical cord blood transplantation from unrelated donors in patients with hematological malignancy using busulfan, thiotepa, fludarabine and ATG as myeloablative conditioning regimen. *Bone Marrow Transplant.* 2012, 47, 1287–1293.

30. Sanz, J.; Jaramillo, F.J.; Planelles, D.; Montesinos, P.; Lorenzo, I.; Moscardo, F.; Martin, G.; López, F.; Martínez, J.; Jarque, I.; et al. Impact on outcomes of human leukocyte antigen matching by allele-level typing in adults with acute myeloid leukemia undergoing umbilical cord blood transplantation. *Biol. Blood Marrow Transplant.* 2014, 20, 106–110.

31. Barker, J.N.; Weisdorf, D.J.; DeFor, T.E.; Blazar, B.R.; McGlave, P.B.; Miller, J.S.; Verfaillie, C.M.; Wagner, J.E. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* 2005, 105, 1343–1347.

32. Horwitz, M.E.; Morris, A.; Gasparetto, C.; Sullivan, K.; Long, G.; Chute, J.; Verfaillie, C.M.; Wagner, J.E. Myeloablative intravenous busulfan/fludarabine conditioning does not facilitate reliable engraftment of dual umbilical cord blood grafts in adult recipients. *Biol. Blood Marrow Transplant.* 2008, 14, 591–594.

33. Bradstock, K.; Hertzberg, M.; Kerridge, I.; Svennilson, J.; George, B.; McGurgan, M.; Huang, G.; Antonenas, V.; Gottlieb, D. Single versus double unrelated umbilical cord blood units for allogeneic transplantation in adults with advanced haematological malignancies: A retrospective comparison of outcomes. *Intern. Med. J.* 2009, 39, 744–751.

34. Kanda, J.; Rizzieri, D.A.; Gasparetto, C.; Long, G.D.; Chute, J.P.; Sullivan, K.M.; Morris, A.; Smith, C.A.; Hogge, D.E.; Nitta, J.; et al. Adult dual umbilical cord blood transplantation using myeloablative total body irradiation (1350 cGy) and fludarabine conditioning. *Biol. Blood Marrow Transplant.* 2011, 17, 867–874.

35. Kai, S.; Wake, A.; Okada, M.; Kurata, M.; Atsuta, Y.; Ishikawa, J.; Nakamae, H.; Aotsuka, N.; Kasai, M.; Misawa, M.; et al. Double-unit cord blood transplantation after myeloablative conditioning for patients with hematologic malignancies: A multicenter phase II study in Japan. *Biol. Blood Marrow Transplant.* 2013, 19, 812–819.

36. Hulegardh, E.; Nilsson, C.; Lazarevic, V.; Garelius, H.; Antunovic, P.; Rangert Derolf, A.; Möllgård, L.; Uggla, B.; Wennström, L.; Wahlin, A.; et al. Characterization and prognostic features of secondary acute myeloid leukemia in a population-based setting: A report from the Swedish Acute Leukemia Registry. *Am. J. Hematol.* 2015, 90, 208–214.

37. Shin, S.H.; Yahng, S.A.; Yoon, J.H.; Lee, S.E.; Cho, B.S.; Eom, K.S.; Lee, S.; Min, C.K.; Kim, H.J.; Cho, S.G.; et al. Survival benefits with transplantation in secondary AML evolving from myelodysplastic syndrome with hypomethylating treatment failure. *Bone Marrow Transplant.* 2013, 48, 678–683.

38. Barker, J.N.; Weisdorf, D.J.; DeFor, T.E.; Blazar, B.R.; Miller, J.S.; Wagner, J.E. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood* 2003, 102, 1915–1919.
39. Brunstein, C.G.; Eapen, M.; Ahn, K.W.; Appelbaum, F.R.; Ballen, K.K.; Champlin, R.E.; Cutler, C.; Kan, F.; Laughlin, M.J.; Soiffer, R.J.; et al. Reduced-intensity conditioning transplantation in acute leukemia: The effect of source of unrelated donor stem cells on outcomes. *Blood* **2012**, *119*, 5591–5598.

40. Rocha, V.; Crotta, A.; Ruggeri, A.; Purtil, D.; Boudjedir, K.; Herr, A.L.; Ionescu, I.; Gluckman, E.; Eurocord Registry. Double cord blood transplantation: Extending the use of unrelated umbilical cord blood cells for patients with hematological diseases. *Best Pract. Res. Clin. Haematol.* **2010**, *23*, 223–229.

41. Robin, M.; Ruggeri, A.; Labopin, M.; Niederwieser, D.; Tabrizi, R.; Sanz, G.; Bourhis, J.H.; van Biezen, A.; Koenecke, C.; Blaise, D.; et al. Comparison of Unrelated Cord Blood and Peripheral Blood Stem Cell Transplantation in Adults with Myelodysplastic Syndrome after Reduced-Intensity Conditioning Regimen: A Collaborative Study from Eurocord (Cord blood Committee of Cellular Therapy & Immunobiology Working Party of EBMT) and Chronic Malignancies Working Party. *Biol. Blood Marrow Transplant.* **2015**, *21*, 489–495.

42. Scaradavou, A.; Brunstein, C.G.; Eapen, M.; Le-Rademacher, J.; Barker, J.N.; Chao, N.; Cutler, C.; Delaney, C.; Kan, F.; Isola, L.; et al. Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. *Blood* **2013**, *121*, 752–758.

43. Kindwall-Keller, T.L.; Hegerfeldt, Y.; Meyerson, H.J.; Margevicius, S.; Fu, P.; van Héeckeren, W.; Lazarus, H.M.; Cooper, B.W.; Gerson, S.L.; Barr, P.; et al. Prospective study of one- vs. two-unit umbilical cord blood transplantation following reduced intensity conditioning in adults with hematological malignancies. *Bone Marrow Transplant.* **2012**, *47*, 924–933.

44. Newell, L.F.; Milano, F.; Gutman, J.A.; Riffkin, I.; Lopez, M.; Ziegler, D.; Nemecek, E.R.; Delaney, C. Treosulfan-Based Conditioning Is Sufficient to Promote Engraftment in Cord Blood Transplantation. *Biol. Blood Marrow Transplant.* **2011**, *17*, S227–S228.

45. Ponce, D.M.; Sauter, C.; Devlin, S.; Lubin, M.; Gonzales, A.M.; Kernan, N.A.; Scaradavou, A.; Giralt, S.; Goldberg, J.D.; Koehne, G.; et al. A novel reduced-intensity conditioning regimen induces a high incidence of sustained donor-derived neutrophil and platelet engraftment after double-unit cord blood transplantation. *Biol. Blood Marrow Transplant.* **2013**, *19*, 799–803.

46. Cutler, C.; Stevenson, K.; Kim, H.T.; Brown, J.; McDonough, S.; Herrera, M.; Reynolds, C.; Liney, D.; Kao, G.; Ho, V.; et al. Double umbilical cord blood transplantation with reduced intensity conditioning and sirolimus-based GVHD prophylaxis. *Bone Marrow Transplant.* **2011**, *46*, 659–667.

47. Rio, B.; Chevret, S.; Vigouroux, S.; Chevallier, P.; Furst, S.; Sirvent, A.; Bay, J.O.; Socie, G.; Ceballos, P.; Huynh, A.; et al. Reduced Intensity Conditioning Regimen Prior to Unrelated Cord Blood Transplantation in Patients with Acute Myeloid leukemia: Preliminary Analysis of a Prospective Phase II Multicentric Trial on Behalf of Societe Francaise De Greffe De Moelle Osseuse Et Therapie Cellulaire (SFGM-TC) and Eurocord. *Blood (ASH Annu. Meet. Abstr.)* **2010**, *116*, 911.
48. Wallet, H.L.; Sobh, M.; Morisset, S.; Robin, M.; Fegueux, N.; Furst, S.; Mohty, M.; Deconinck, E.; Fouillard, L.; Bordigoni, P.; et al. Double umbilical cord blood transplantation for hematological malignancies: A long-term analysis from the SFGM-TC registry. Exp. Hematol. 2013, 41, 924–933.

49. Rio, B.; Chevret, S.; Vigouroux, S.; Chevallier, P.; Furst, S.; Sirvent, A.; Bay, J.O.; Socié, G.; Ceballos, P.; Huynh, A.; et al. Decreased Nonrelapse Mortality after Unrelated Cord Blood Transplantation for Acute Myeloid Leukemia Using Reduced-Intensity Conditioning: A Prospective Phase II Multicenter Trial. Biol. Blood Marrow Transplant. 2015, 21, 445–453.

50. Robin, M.; Sanz, G.F.; Ionescu, I.; Rio, B.; Sirvent, A.; Renaud, M.; Carreras, E.; Milpied, N.; Mohty, M.; Beguin, Y.; et al. Unrelated cord blood transplantation in adults with myelodysplasia or secondary acute myeloblastic leukemia: A survey on behalf of Eurocord and CLWP of EBMT. Leukemia 2011, 25, 75–81.

51. Rocha, V.; Labopin, M.; Ruggeri, A.; Podestà, M.; Caballero, D.; Bonifazi, F.; Montserrat, R.; Gallamini, A.; Fagioli, F.; Socié, G.; et al. Unrelated Cord Blood Transplantation: Comparison After Single Unit Cord Blood Intrabone Injection and Double Unit Cord Blood Transplantation In Patients with Hematological Malignant Disorders. A Eurocord-EBMT Analysis. Blood (ASH Annu. Meet. Abstr.) 2010, 116, 223.

52. Verneris, M.R.; Brunstein, C.G.; Barker, J.; MacMillan, M.L.; DeFor, T.; McKenna, D.H.; Burke, M.J.; Blazar, B.R.; Miller, J.S.; McGlave, P.B.; et al. Relapse risk after umbilical cord blood transplantation: Enhanced graft-versus-leukemia effect in recipients of 2 units. Blood 2009, 114, 4293–4299.

53. Sanz, J.; Wagner, J.E.; Sanz, M.A.; DeFor, T.; Montesinos, P.; Bachanova, V.; Lorenzo, I.; Warlick, E.; Sanz, G.F.; Brunstein, C. Myeloablative cord blood transplantation in adults with acute leukemia: Comparison of two different transplant platforms. Biol. Blood Marrow Transplant. 2013, 19, 1725–1730.

54. Ruggeri, A.; Sanz, G.; Bittencourt, H.; Sanz, J.; Rambaldi, A.; Volt, F.; Yakoub-Agha, I.; Ribera, J.M.; Mannone, L.; Sierra, J.; Mohty, M.; et al. Comparison of outcomes after single or double cord blood transplantation in adults with acute leukemia using different types of myeloablative conditioning regimen, a retrospective study on behalf of Eurocord and the Acute Leukemia Working Party of EBMT. Leukemia 2014, 28, 779–786.

55. MacMillan, M.L.; Weisdorf, D.J.; Brunstein, C.G.; Cao, Q.; DeFor, T.E.; Verneris, M.R.; Blazar, B.R.; Wagner, J.E. Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: Analysis of risk factors. Blood 2009, 113, 2410–2415.

56. Wagner, J.E., Jr.; Eapen, M.; Carter, S.; Wang, Y.; Schultz, K.R.; Wall, D.A.; Bunin, N.; Delaney, C.; Haut, P.; Margolis, D.; et al. One-unit versus two-unit cord-blood transplantation for hematologic cancers. N. Engl. J. Med. 2014, 371, 1685–1694.

57. Eapen, M.; Rocha, V.; Sanz, G.; Scaradavou, A.; Zhang, M.J.; Arcese, W.; Sirvent, A.; Champlin, R.E.; Chao, N.; Gee, A.P.; et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: A retrospective analysis. Lancet Oncol. 2010, 11, 653–660.
58. Brunstein, C.G.; Gutman, J.A.; Weisdorf, D.J.; Woolfrey, A.E.; Defor, T.E.; Gooley, T.A.; Verneris, M.R.; Appelbaum, F.R.; Wagner, J.E.; Delaney, C. Allogeneic hematopoietic cell transplantation for hematologic malignancy: Relative risks and benefits of double umbilical cord blood. *Blood* 2010, 116, 4693–4699.

59. Laughlin, M.J.; Eapen, M.; Rubinstein, P.; Wagner, J.E.; Zhang, M.J.; Champlin, R.E.; Stevens, C.; Barker, J.N.; Gale, R.P.; Lazarus, H.M.; et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N. Engl. J. Med.* 2004, 351, 2265–2275.

60. Rocha, V.; Labopin, M.; Sanz, G.; Arcese, W.; Schwerdtfeger, R.; Bosi, A.; Jacobsen, N.; Ruutu, T.; de Lima, M.; Finke, J.; et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N. Engl. J. Med.* 2004, 351, 2276–2285.

61. Gutman, J.A.; Leisenring, W.; Appelbaum, F.R.; Woolfrey, A.E.; Delaney, C. Low relapse without excessive transplant-related mortality following myeloablative cord blood transplantation for acute leukemia in complete remission: A matched cohort analysis. *Biol. Blood Marrow Transplant.* 2009, 15, 1122–1129.

62. Majhail, N.S.; Brunstein, C.G.; Tomblyn, M.; Thomas, A.J.; Miller, J.S.; Arora, M.; Kaufman, D.S.; Burns, L.J.; Slungaard, A.; McGlave, P.B.; et al. Reduced-intensity allogeneic transplant in patients older than 55 years: Unrelated umbilical cord blood is safe and effective for patients without a matched related donor. *Biol. Blood Marrow Transplant.* 2008, 14, 282–289.

63. Majhail, N.S.; Brunstein, C.G.; Shanley, R.; Sandhu, K.; McClune, B.; Oran, B.; Warlick, E.D.; Wagner, J.E.; Weisdorf, D.J. Reduced-intensity hematopoietic cell transplantation in older patients with AML/MDS: Umbilical cord blood is a feasible option for patients without HLA-matched sibling donors. *Bone Marrow Transplant.* 2012, 47, 494–498.

64. Chen, Y.B.; Aldridge, J.; Kim, H.T.; Ballen, K.K.; Cutler, C.; Kao, G.; Liney, D.; Bourdeau, G.; Alyea, E.P.; Armand, P.; et al. Reduced-intensity conditioning stem cell transplantation: Comparison of double umbilical cord blood and unrelated donor grafts. *Biol. Blood Marrow Transplant.* 2012, 18, 805–812.

65. Le Bourgeois, A.; Mohr, C.; Guillaume, T.; Delaunay, J.; Malard, F.; Loirat, M.; Peterlin, P.; Blin, N.; Dubruille, V.; Mahe, B.; et al. Comparison of outcomes after two standards-of-care reduced-intensity conditioning regimens and two different graft sources for allogeneic stem cell transplantation in adults with hematologic diseases: A single-center analysis. *Biol. Blood Marrow Transplant.* 2013, 19, 934–939.

66. Weisdorf, D.; Eapen, M.; Ruggeri, A.; Zhang, M.J.; Zhong, X.; Brunstein, C.; Ustun, C.; Rocha, V.; Gluckman, E. Alternative donor transplantation for older patients with acute myeloid leukemia in first complete remission: A center for international blood and marrow transplant research-eurocord analysis. *Biol. Blood Marrow Transplant.* 2014, 20, 816–822.

67. Malard, F.; Milpied, N.; Blaise, D.; Chevallier, P.; Michallet, M.; Lioure, B.; Clément, L.; Hicheri, Y.; Cordonnier, C.; Huynh, A.; et al. Effect of graft source on unrelated donor hematopoietic stem cell transplantation in adults with acute myeloid leukaemia after reduced intensity or non-myeloablative conditioning: A study from the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. *Biol. Blood Marrow Transplant.* 2015, 21, 1059–1067.
68. Ponce, D.M.; Zheng, J.; Gonzales, A.M.; Lubin, M.; Heller, G.; Castro-Malaspina, H.; Giralt, S.; Hsu, K.; Jakubowski, A.A.; Jenq, R.R. Reduced late mortality risk contributes to similar survival after double-unit cord blood transplantation compared with related and unrelated donor hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.* 2011, 17, 1316–1326.

69. Atsuta, Y.; Suzuki, R.; Nagamura-Inoue, T.; Taniguchi, S.; Takahashi, S.; Kai, S.; Sakamaki, H.; Kouzai, Y.; Kasai, M.; Fukuda, T.; et al. Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood* 2009, 113, 1631–1638.

70. Appelbaum, F.R.; Asnassetti, C.; Antin, J.H.; Atkins, H.; Davies, S.; Devine, S.; Giralt, S.; Heslop, H.; Laport, G.; Lee, S.J.; et al. Blood and marrow transplant clinical trials network state of the Science Symposium 2014. *Biol. Blood Marrow Transplant.* 2015, 21, 202–224.

71. Powles, R.L.; Morgenstern, G.R.; Kay, H.E.; McElwain, T.J.; Clink, H.M.; Dady, P.J.; Barrett, A.; Jameson, B.; Depledge, M.H.; Watson, J.G.; et al. Mismatched family donors for bone-marrow transplantation as treatment for acute leukaemia. *Lancet* 1983, 1, 612–615.

72. Szydlo, R.; Goldman, J.M.; Klein, J.P.; Gale, R.P.; Ash, R.C.; Bach, F.H.; Bradley, B.A.; Casper, J.T.; Flomenberg, N.; Gajewski, J.L.; et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. *J. Clin. Oncol.* 1997, 15, 1767–1777.

73. Beatty, P.G.; Clift, R.A.; Mickelson, E.M.; Nisperos, B.B.; Flournoy, N.; Martin, P.J.; Sanders, J.E.; Stewart, P.; Buckner, C.D.; Storb, R. Marrow transplantation from related donors other than HLA-identical siblings. *N. Engl. J. Med.* 1985, 313, 765–771.

74. O’Reilly, R.J.; Keever, C.; Kernan, N.A.; Brochstein, J.; Collins, N.; Flomenberg, N.; Laver, J.; Emanuel, D.; Dupont, B.; Cunningham, I.; et al. Bone marrow transplantation from related donors other than HLA-nonidentical T cell depleted marrow transplants: A comparison of results in patients treated for leukemia and severe combined immunodeficiency disease. *Transplant. Proc.* 1987, 19 (6 Suppl. 7), 55–60.

75. Ash, R.C.; Horowitz, M.M.; Gale, R.P.; van Bekkum, D.W.; Casper, J.T.; Gordon-Smith, E.C.; Henslee, P.J.; Kolb, H.J.; Lowenberg, B.; Masaoka, T. Bone marrow transplantation from related donors other than HLA-identical siblings: Effect of T cell depletion. *Bone Marrow Transplant.* 1991, 7, 443–452.

76. Munchel, A.; Kesserwan, C.; Symons, H.J.; Luznik, L.; Kasamon, Y.L.; Jones, R.J.; Fuchs, E.J. Nonmyeloablative, HLA-haploidentical bone marrow transplantation with high dose, post-transplantation cyclophosphamide. *Pediatr. Rep.* 2011, 3 (Suppl. 2), e15.

77. Ciurea, S.O.; Zhang, M.J.; Bacigalupo, A.; Bashey, A.; Appelbaum, F.R.; Antin, J.H.; Chen, J.; Devine, S.M.; Fowler, D.H.; Nakamura, R.; et al. Survival after T-Cell Replete Haplo-Identical Related Donor Transplant Using Post-Transplant Cyclophosphamide Compared with Matched Unrelated Donor Transplant for Acute Myeloid Leukemia. *Blood (ASH Annu. Meet. Abstr.)* 2014, 124, 679.

78. Luznik, L.; Fuchs, E.J. High-dose, post-transplantation cyclophosphamide to promote graft-host tolerance after allogeneic hematopoietic stem cell transplantation. *Immunol. Res.* 2010, 47, 65–77.
79. Raiola, A.M.; Dominiello, A.; di Grazia, C.; Lamparelli, T.; Gualandi, F.; Ibatici, A.; Bregante, S.; Van Lint, M.T.; Varaldo, R.; Ghiso, A.; et al. Unmanipulated haploidentical transplants compared with other alternative donors and matched sibling grafts. Biol. Blood Marrow Transplant. 2014, 20, 1573–1579.

80. Perruccio, K.; Tosti, A.; Burchielli, E.; Topini, F.; Ruggeri, L.; Carotti, A.; Capanni, M.; Urbani, E.; Mancusi, A.; Aversa, F.; et al. Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation. Blood 2005, 106, 4397–4406.

81. Feuchtling, T.; Opherk, K.; Bethge, W.A.; Topp, M.S.; Schuster, F.R.; Weissinger, E.M.; Mohty, M.; Or, R.; Maschan, M.; Schumm, M.; et al. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. Blood 2010, 116, 4360–4367.

82. Comoli, P.; Basso, S.; Zecca, M.; Pagliara, D.; Baldanti, F.; Bernardo, M.E.; Barberi, W.; Moretta, A.; Labirio, M.; Paulli, M.; et al. Preemptive therapy of EBV-related lymphoproliferative disease after pediatric haploidentical stem cell transplantation. Am. J. Transplant. 2007, 7, 1648–1655.

83. Comoli, P.; Schilham, M.W.; Basso, S.; van Vreeswijk, T.; Bernardo, M.E.; Maccario, R.; van Tol, M.J.; Locatelli, F.; Veltrop-Duits, I.A. T-cell lines specific for peptides of adenovirus hexon protein and devoid of alloreactivity against recipient cells can be obtained from HLA-haploidentical donors. J. Immunother. 2008, 31, 529–536.

84. Leen, A.M.; Christin, A.; Myers, G.D.; Liu, H.; Cruz, C.R.; Hanley, P.J.; Kennedy-Nasser, A.A.; Leung, K.S.; Gee, A.P.; Krance, R.A.; et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. Blood 2009, 114, 4283–4292.

85. Ciceri, F.; Bonini, C.; Stanghellini, M.T.; Bondanza, A.; Traversari, C.; Salomoni, M.; Turchetto, L.; Colombi, S.; Bernardi, M.; Peccatori, J.; et al. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): A non-randomised phase I-II study. Lancet Oncol. 2009, 10, 489–500.

86. Vago, L.; Oliveira, G.; Bondanza, A.; Noviello, M.; Soldati, C.; Ghio, D.; Brigida, I.; Greco, R.; Lupo Stanghellini, M.T.; Peccatori, J.; et al. T-cell suicide gene therapy prompts thymic renewal in adults after hematopoietic stem cell transplantation. Blood 2012, 120, 1820–1830.

87. Di Stasi, A.; Tey, S.K.; Dotti, G.; Fujita, Y.; Kennedy-Nasser, A.; Martinez, C.; Straathof, K.; Liu, E.; Durett, A.G.; Grilley, B.; et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N. Engl. J. Med. 2011, 365, 1673–1683.

88. Nguyen, V.H.; Shashidhar, S.; Chang, D.S.; Ho, L.; Kambham, N.; Bachmann, M.; Brown, J.M.; Negrin, R.S. The impact of regulatory T cells on T-cell immunity following hematopoietic cell transplantation. Blood 2008, 111, 945–953.

89. Di Ianni, M.; Falzetti, F.; Carotti, A.; Terenzi, A.; Castellino, F.; Bonifacio, E.; Del Papa, B.; Zei, T.; Ostini, R.I.; Cecchini, D.; et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood 2011, 117, 3921–3928.
90. Martelli, M.F.; Di Ianni, M.; Ruggeri, L.; Falzetti, F.; Carotti, A.; Terenzi, A.; Pierini, A.; Massei, M.S.; Amico, L.; Urbani, E.; et al. HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. Blood 2014, 124, 638–644.

91. Mielke, S.; Nunes, R.; Rezvani, K.; Fellowes, V.S.; Venne, A.; Solomon, S.R.; Fan, Y.; Gostick, E.; Price, D.A.; Scotto, C.; et al. A clinical-scale selective allodepletion approach for the treatment of HLA-mismatched and matched donor-recipient pairs using expanded T lymphocytes as antigen-presenting cells and a TH9402-based photodepletion technique. Blood 2008, 111, 4392–4402.

92. Bastien, J.P.; Krosl, G.; Therien, C.; Rashkovan, M.; Scotto, C.; Cohen, S.; Allan, D.S.; Hogge, D.; Egeler, R.M.; Perreault, C.; et al. Photodepletion differentially affects CD4+ Tregs versus CD4+ effector T cells from patients with chronic graft-versus-host disease. Blood 2010, 116, 4859–4869.

93. Lafferty, K.J.; Cunningham, A.J. A new analysis of allogeneic interactions. Aust. J. Exp. Biol. Med. Sci. 1975, 53, 27–42.

94. Guinan, E.C.; Boussiotis, V.A.; Neuberg, D.; Brennan, L.L.; Hirano, N.; Nadler, L.M.; Fan, Y.; Gostick, E.; Price, D.A.; Scotto, C.; et al. Transplantation of anergic histoincompatible bone marrow allografts. N. Engl. J. Med. 1999, 340, 1704–1714.

95. Federmann, B.; Bornhauser, M.; Meisner, C.; Kordelas, L.; Beelen, D.W.; Stuhler, G.; Stelljes, M.; Schwerdtfeger, R.; Christopeit, M.; Behre, G.; et al. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: A phase II study. Haematologica 2012, 97, 1523–1531.

96. Godder, K.T.; Henslee-Downey, P.J.; Mehta, J.; Park, B.S.; Chiang, K.Y.; Abhyankar, S.; Lamb, L.S. Long term disease-free survival in acute leukemia patients recovering with increased gammadelta T cells after partially mismatched related donor bone marrow transplantation. Bone Marrow Transplant. 2007, 39, 751–757.

97. Federmann, B.; Hagele, M.; Pfeiffer, M.; Wirths, S.; Schumm, M.; Faul, C.; Vogel, W.; Handgretinger, R.; Kanz, L.; Bethge, W.A. Immune reconstitution after haploidentical hematopoietic cell transplantation: Impact of reduced intensity conditioning and CD3/CD19 depleted grafts. Leukemia 2011, 25, 121–129.

98. Locatelli, F.; Bauquet, A.; Palumbo, G.; Moretta, F.; Bertaina, A. Negative depletion of alpha/beta+ T cells and of CD19+ B lymphocytes: A novel frontier to optimize the effect of innate immunity in HLA-mismatched hematopoietic stem cell transplantation. Immunol. Lett. 2013, 155, 21–23.

99. Ciurea, S.O.; Mulanovich, V.; Saliba, R.M.; Bayraktar, U.D.; Jiang, Y.; Bassett, R.; Wang, S.A.; Konopleva, M.; Fernandez-Vina, M.; Montes, N.; et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. Biol. Blood Marrow Transplant. 2012, 18, 1835–1844.

100. Raiola, A.M.; Dominiello, A.; Ghiso, A.; Di Grazia, C.; Lamparelli, T.; Gualandi, F.; Bregante, S.; Van Lint, M.T.; Geroldi, S.; Luchetti, S.; et al. Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. Biol. Blood Marrow Transplant. 2013, 19, 117–122.
101. Solomon, S.R.; Sizemore, C.A.; Sanacore, M.; Zhang, X.; Brown, S.; Holland, H.K.; Morris, L.E.; Bashey, A. Haploidentical transplantation using T cell replete peripheral blood stem cells and myeloablative conditioning in patients with high-risk hematologic malignancies who lack conventional donors is well tolerated and produces excellent relapse-free survival: Results of a prospective phase II trial. *Biol. Blood Marrow Transplant. 2012, 18*, 1859–1866.

102. Chang, Y.J.; Xu, L.P.; Liu, D.H.; Liu, K.Y.; Han, W.; Chen, Y.H.; Wang, Y.; Chen, H.; Wang, J.Z.; Zhang, X.H.; *et al.* The impact of CD34+ cell dose on platelet engraftment in pediatric patients following unmanipulated haploidentical blood and marrow transplantation. *Pediatr. Blood Cancer 2009*, *53*, 1100–1106.

103. Ciceri, F.; Labopin, M.; Aversa, F.; Rowe, J.M.; Bunjes, D.; Lewalle, P.; Nagler, A.; Di Bartolomeo, P.; Lacerda, J.F.; Lupo Stanghellini, M.T.; *et al.* A survey of fully haploidentical hematopoietic stem cell transplantation in adults with high-risk acute leukemia: A risk factor analysis of outcomes for patients in remission at transplantation. *Blood 2008*, *112*, 3574–3581.

104. Aversa, F.; Terenzi, A.; Tabilio, A.; Falzetti, F.; Carotti, A.; Ballanti, S.; Falcinelli, F.; Velardi, A.; Ruggeri, L.; Aloisi, T.; *et al.* Full haplotype-mismatched hematopoietic stem-cell transplantation: A phase II study in patients with acute leukemia at high risk of relapse. *J. Clin. Oncol. 2005*, *23*, 3447–3454.

105. Luznik, L.; O’Donnell, P.V.; Symons, H.J.; Chen, A.R.; Leffell, M.S.; Zahurak, M.; Gooley, T.A.; Piantadosi, S.; Kaup, M.; Ambinder, R.F.; *et al.* HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol. Blood Marrow Transplant. 2008*, *14*, 641–650.

106. El-Cheikh, J.; Crocchiolo, R.; Furst, S.; Bramanti, S.; Sarina, B.; Granata, A.; Vai, A.; Lemarie, C.; Faucher, C.; Mohty, B.; *et al.* Unrelated cord blood compared with haploidentical grafts in patients with hematologic malignancies. *Cancer 2015*, *121*, 1809–1816.

107. Ruggeri, A.; Labopin, M.; Sanz, G.; Piemontese, S.; Arcese, W.; Bacigalupo, A.; Blaise, D.; Bosi, A.; Huang, H.; Karakasis, D.; *et al.* Comparison of outcomes after unrelated cord blood and unmanipulated haploidentical stem cell transplantation in adults with acute leukemia. *Leukemia 2015*, doi:10.1038/leu.2015.98.

108. Grosso, D.; Gaballa, S.; Alpdogan, O.; Carabasi, M.; Filicko-O’Hara, J.; Kasner, M.; Martinez-Outschoorn, U.; Wagner, J.L.; O’Hara, W.; Rudolph, S.; *et al.* A Two-Step Approach to Myeloablative Haploidentical Transplantation: Low Nonrelapse Mortality and High Survival Confirmed in Patients with Earlier Stage Disease. *Biol. Blood Marrow Transplant. 2015*, *21*, 646–652.

109. Huang, X.J.; Liu, D.H.; Liu, K.Y.; Xu, L.P.; Chen, H.; Han, W.; Chen, Y.H.; Wang, J.Z.; Gao, Z.Y.; Zhang, Y.C.; *et al.* Haploidentical hematopoietic stem cell transplantation without *in vitro* T-cell depletion for the treatment of hematological malignancies. *Bone Marrow Transplant. 2006*, *38*, 291–297.

110. Lu, D.P.; Dong, L.; Wu, T.; Huang, X.J.; Zhang, M.J.; Han, W.; Chen, H.; Liu, D.H.; Gao, Z.Y.; Chen, Y.H.; *et al.* Conditioning including antithymocyte globulin followed by unmanipulated HLA-mismatched/haploidentical blood and marrow transplantation can achieve comparable outcomes with HLA-identical sibling transplantation. *Blood 2006*, *107*, 3065–3073.
111. Huang, X.J.; Liu, D.H.; Liu, K.Y.; Xu, L.P.; Chen, H.; Han, W.; Chen, Y.H.; Zhang, X.H.; Lu, D.P. Treatment of acute leukemia with unmanipulated HLA-mismatched/haploidentical blood and bone marrow transplantation. *Biol. Blood Marrow Transplant.* 2009, 15, 257–265.

112. Luo, Y.; Xiao, H.; Lai, X.; Shi, J.; Tan, Y.; He, J.; Xie, W.; Zheng, W.; Zhu, Y.; Ye, X.; et al. T-cell-replete haploidentical HSCT with low-dose anti-T-lymphocyte globulin compared with matched sibling HSCT and unrelated HSCT. *Blood* 2014, 124, 2735–2743.

113. Fu, H.; Xu, L.; Liu, D.; Liu, K.; Zhang, X.; Chen, H.; Chen, Y.; Han, W.; Wang, Y.; Wang, J.; et al. Total body irradiation and cyclophosphamide plus antithymocyte globulin regimen is well tolerated and promotes stable engraftment as a preparative regimen before T cell-replete haploidentical transplantation for acute leukemia. *Biol. Blood Marrow Transplant.* 2014, 20, 1176–1182.

114. Gao, L.; Wen, Q.; Chen, X.; Liu, Y.; Zhang, C.; Gao, L.; Kong, P.; Zhang, Y.; Li, Y.; Liu, J.; et al. Effects of priming with recombinant human granulocyte colony-stimulating factor on conditioning regimen for high-risk acute myeloid leukemia patients undergoing human leukocyte antigen-haploidentical hematopoietic stem cell transplantation: A multicenter randomized controlled study in southwest China. *Biol. Blood Marrow Transplant.* 2014, 20, 1932–1939.

115. Di Bartolomeo, P.; Santarone, S.; De Angelis, G.; Picardi, A.; Cudillo, L.; Cerretti, R.; Adorno, G.; Angelini, S.; Andreani, M.; De Felice, L.; et al. Haploidentical, unmanipulated, G-CSF-primed bone marrow transplantation for patients with high-risk hematologic malignancies. *Blood* 2013, 121, 849–857.

116. Peccatori, J.; Forcina, A.; Clerici, D.; Crocchiolo, R.; Vago, L.; Stanghellini, M.T.; Noviello, M.; Messina, C.; Crotta, A.; Assanelli, A.; et al. Sirolimus-based graft-versus-host disease prophylaxis promotes the in vivo expansion of regulatory T cells and permits peripheral blood stem cell transplantation from haploidentical donors. *Leukemia* 2015, 29, 396–405.

117. Cicieri, F.; Bregni, M.; Peccatori, J. Innovative platforms for haploidentical stem cell transplantation: The role of unmanipulated donor graft. *J. Cancer* 2011, 2, 339–340.

118. Pidala, J.; Lee, S.J.; Ahn, K.W.; Spellman, S.; Wang, H.L.; Aljurf, M.; Askar, M.; Dehn, J.; Fernandez Viña, M.; Gratwohl, A.; et al. Nonpermissive HLA-DPB1 mismatch increases mortality after myeloablative unrelated allogeneic hematopoietic cell transplantation. *Blood* 2014, 124, 2596–2606.

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