Impact of graft source and composition on outcomes after allogeneic stem cell transplantation

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Since many graft-related factors may affect outcomes after allogeneic stem cell transplantation, graft selection is one of the crucial steps of transplant preparation. Optimal graft selection may offer the best chance of successful transplantation. Here, we reviewed the impact of graft-related factors on post transplant outcomes in light of new data that may help to refine the strategy for graft and graft source selection.

(Belg J Hematol 2015;6(4):162-8)

Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) offers potential curative treatment for a wide range of haematological disorders.[1,2] Practically, the challenges of alloHSCT are multiple. The primary aim is the control of the underlying haematological disease. However, the long-term success of alloHSCT also relies on the complete recovery of hematopoietic functions through sustained engraftment, limitation of transplant-related toxicity and morbidity (specifically graft-versus-host disease (GVHD)) and reconstitution of a fully efficient adaptive immune system to ensure long-term defences against infections and secondary malignancies. Several pre transplant factors may condition the long-term success of alloHSCT, including patient-, disease- and transplant-related factors. Graft-related factors may also considerably impact post transplant outcomes, such as graft source and graft composition. Hence, graft selection is a crucial step of the transplant procedure preparation, to allow the best chance of successful alloHSCT.

Graft and graft source selection for alloHSCT

Current common criteria for graft source selection are summarised in *Figure 1*. Pioneering alloHSCT studies in the late 1960's led to the crucial discovery that donor/recipient genetic disparities at human leukocyte antigens (HLA) were the most important risk factors for the development of graft rejection and lethal GVHD after alloHSCT.[3] Currently, donor/recipient HLA-matching status is the primary criterium for donor selection. The best donor is considered to be a HLA genotypically matched related donor (MRD). Unfortunately, only 30% of patients who require alloHSCT have a suitable MRD. For those who have not, a search for a HLA-matched unrelated donor (MUD) is undertaken on international registries. For MUD selection, the National Marrow Donor Program currently recommends HLA-A,-B,-C,-DRB1 high resolution DNA typing.[4] This enables identification of 8/8 HLA-matched donor/recipient pairs. However, many centres also further recommend HLA-DQB1 typing for MUD selection, which is then referred

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Conflict of interest: The authors have nothing to disclose and indicate no potential conflict of interest.

Keywords: Allogeneic stem cell transplantation, donor, graft composition, graft source, post transplant outcomes.

Acknowledgments: This work was supported by grants from F.R.S-FNRS Télévie, Leon Fredericq Fund and Anti-Cancer Centre at the University of Liège.

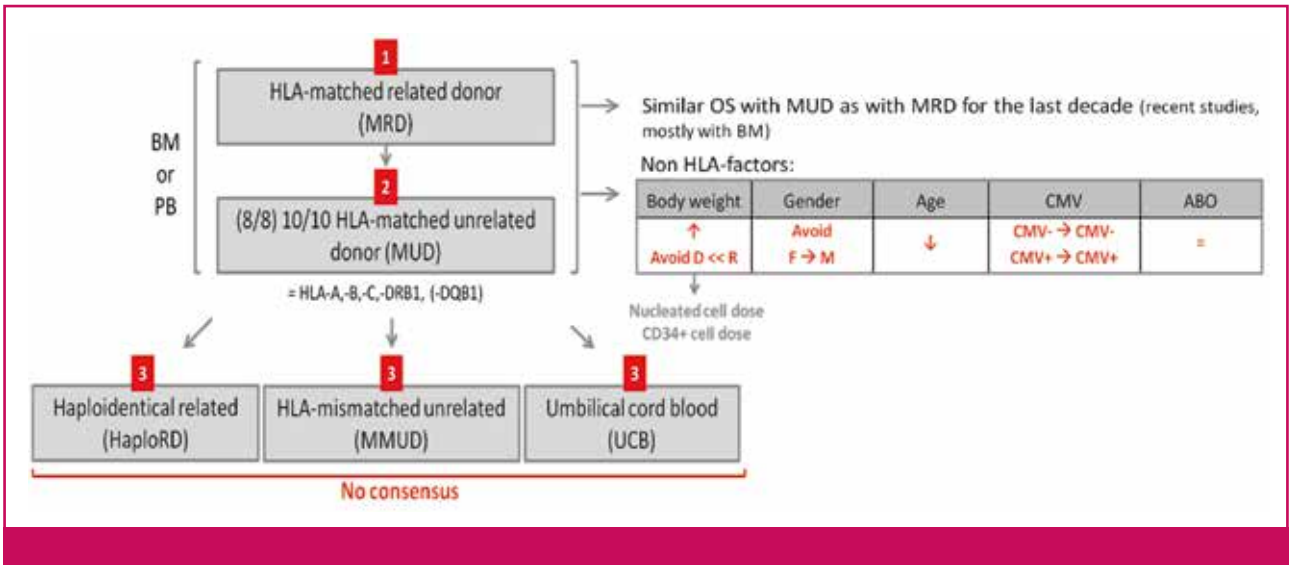

Figure 1. Algorithm for graft source selection for alloHSCT.
BM: bone marrow; CMV: cytomegalovirus; D: donor; F: female gender; HLA: human leukocyte antigens; M: male gender; OS: overall survival; PB: peripheral blood stem cells; R: recipient.

to as a 10/10 HLA-MUD. When several MRD or MUD are identified, non-HLA factors may secondarily be considered for donor selection (*Figure 1*). Some studies have reported a positive association between increasing graft's CD34+ cell dose and survival after alloHSCT.[5,6] Since the number of stem cells collected within the graft depends on the donor's body weight, selection of the largest donor may guarantee the highest chances for successful alloHSCT. On the other hand, transplantation from a donor whose body weight is highly inferior to the recipient's weight may result in a high risk of graft failure and must be avoided whenever possible.[7] Other factors that may be considered for donor selection are gender, age, cytomegalovirus (CMV) serostatus and ABO blood group. Graft sources may include bone marrow (BM) or mobilised peripheral blood stem cells (PB). Choice between these graft sources mainly depends on the patient's age, type of disease, centre habits and donor's preference.[8]

When no suitable MRD or MUD is identified, three alternative graft sources may be considered for alloHSCT: HLA-mismatched unrelated donor (MMUD), haploidentical related donor (haploRD - who shares only one of the two HLA-haplotypes with the recipient) and umbilical cord blood (UCB) (*Figure 1*).[9,10] UCB grafts have some specific characteristics when compared to PB or BM grafts. They contain lower absolute counts of stem cells and T-cells. This results in higher risk of graft failure after UCB-alloHSCT.[7] Hence, the total nucleated cell dose is considered the primary criteria for UCB unit selection for alloHSCT. Alternatively, UCB grafts have specific immunological properties such as higher proportions of naïve T-cells, regulatory T-cells and immune cells with some degree of functional immaturity, that enable reduced stringency of HLA-matching requirements for UCB unit selection.[11] There is no consensus about what is the best choice among MMUD, haploRD and UCB. Each of them has advantages and limitations (*Table 1*). A common concern after alloHSCT with alternative grafts is the high incidence of infection-related morbidity and mortality.[12-14] Surprisingly, few studies are aimed at directly comparing infection risks according to the source of alternative grafts. Hence, such data may be important to select the optimal alternative graft source.

Graft- and donor-related predictive factors of survival after PB-alloHSCT with MRD or MUD

Over the past decade, PB has progressively overtaken BM as a source of stem cells for alloHSCT.[15] In a first study, we focused on PB-alloHSCT from HLA-matched donors (MRD or MUD).[16] By performing the retrospective analysis of 442 patients with haematological malignancies, we assessed pre transplant predictors of long-term survival after alloHSCT. We observed a similar 5-year overall survival (OS) after PB-alloHSCT with MUD and MRD (46% and 45%, respectively; p=.49). This was in accordance with large previous studies, mostly with BM as graft source.[17,18] Among other graft-

Table 1. Advantages and disadvantages of alternative stem cell sources.

	HaploRD	MMUD	UCB
Donor availability	Nearly 100%	20-80%, depending on ethnicity	Nearly 100%
Time to find donor	<4 Weeks	8-10 Weeks	<4 Weeks
Cell dose	Targeted to recipient weight	Targeted to recipient weight	Fixed, depending on what is available in the UCB unit
Product quality	Low variability	Low variability	High variability
Cost	Low donor acquisition costs	US $20,000-35,000	US $20,000-$40,000 per UCB unit
Additional cell therapy (DLI)	Yes, readily available	Yes, but may be lengthy wait	No
PMN engraftment	Fast (15-20 days)	Fast (15-20 days)	Slow (20-28 days)
Graft failure risk	High, if TCD	Moderate	High
Relapse risk	High, if TCD	Moderate	Moderate
GVHD risk	Low, if post transplant Cy High, if no TCD	High	Moderate
Infection risk	High	High	High

Cy: cyclophosphamide; DLI: donor lymphocyte infusion; GVHD: graft-versus-host disease; HaploRD: haploidentical related donor; MMUD: HLA-mismatched unrelated donor; PMN: polymorphonuclear cells; TCD: T-cell depletion (*ex vivo* or *in vivo*); UCB: umbilical cord blood.

and donor-related factors, graft composition ($CD34^+$ stem cells and $CD3^+$ T-cell doses) and donor age were significantly associated with survival after PB-alloHSCT in our study.

Specifically, a lower risk of mortality was observed after transplant with a $CD34^+$ cell dose ≥$4.5x10^6$/kg of recipient's weight (HR: 0.56; p=.002) and CD3+ cell dose ≥$3x10^8$/kg of recipient's weight (HR: 0.61; p=.01). Favourable outcomes with infusion of larger $CD34^+$ cell numbers have been largely reported in the literature.[5,6] To the contrary, reports on the impact of graft $CD3^+$ cell dose on outcomes after alloHSCT with unmanipulated PB are scarcer. Interestingly, a higher $CD3^+$ graft cell dose was not associated with incremental risks of chronic GVHD in our cohort.

Donor age also impacted long-term survival after PB-alloHSCT in our study. Lower survival, a higher rate of relapse and a lower rate of chronic GVHD were observed in patients transplanted with older (≥60 years) MRD, as compared with patients transplanted with younger (<60 years) MRD or with MUD (≤60 years, by definition). These results raised the question of potential effects of donor age on alloimmune reactivity of the graft.[19] In fact, there is accumulating evidence that ageing is associated with a decline in immune (and specifically T-cell) functions (a concept designated as 'immune-senescence').[20] Our study further questioned what should be the best donor choice between an old MRD and a younger MUD? Few studies have specifically addressed this question and results remain controversial.[21-23] In our study, we reported a higher risk of late mortality (HR at five years: 4.41; p=.006) and treatment failure (HR at five years: 6.33; p=.009) with MRD ≥60 as compared to MUD. However, these results have to be validated in larger studies.

Immune reconstitution and infection burden after UCB- and MMUD-alloHSCT

In the second part of the study, we compared post transplant outcomes after alloHSCT to MMUD (n=36) and UCB (n=30), with a specific focus on immune reconstitution and late (>3 months) infection burden.[24] Previous studies have reported higher rates of early (<100 days) infections after UCB-alloHSCT as compared to alloHSCT from other stem cell sources.[13,25,26] Whether UCB-alloHSCT also predisposes patients to

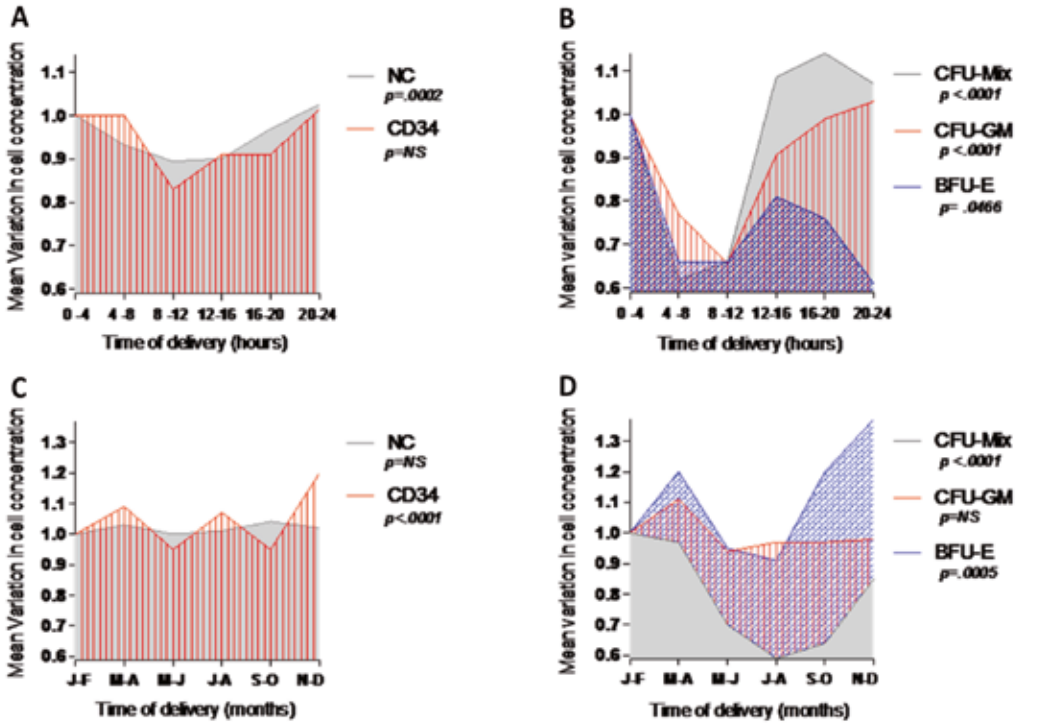

Figure 2. Mean variation in cell concentrations according to time of day and month of delivery. Circadian (A-B) and circannual (C-D) variations of nucleated cells (NC), CD34+ hematopoietic stem cells (CD34), and hematopoietic progenitor cells (Myeloid Colony-Forming Units, CFU-GM; Burst Forming Units Erythroid, BFU-) and multilineage Colony-Forming Units, CFUMix) are shown. Variations are presented with the 0:00–4:00 period of daytime (A-B) and January-February (C-D) as the reference groups. J-F: January-February; M-A: March-April; M-J: May-June; J-A: July-August; S-O: September-October; N-D: November-December.

higher risk of infections in the later post transplant (>100 days) period is less well known. In our study, the 18-month cumulative incidence of late infections was high after UCB-alloHSCT but similar to that after MMUD-alloHSCT (57% versus 72%, respectively, p=.34). The rate of infection per twelve patient-month was roughly two in both groups (2.3 and 2.2 after UCB- and MMUD-alloHSCT, respectively, p=.88). The 4-year overall survival was also similar (62% versus 60% after UCB- and MMUD-alloHSCT, respectively, p=.96). We further assessed circulating immune cell phenotype in the peripheral blood of patients during the first year after transplant. We observed that the kinetics of immune recovery was different according to the type of alternative graft source, with delayed recovery of T-cells (specifically $CD8^+$ and naive T-cells) but faster recovery of natural killer cells after UCB- as compared to MMUD-alloHSCT. Finally, we assessed predictive factors of late infections after alternative alloHSCT. Graft source (MMUD versus UCB) did not impact late infection risks. Immunological variables solely predicted infections. Hence, low $CD4^+$ (specifically central memory) T-cell counts and high $CD8^+$ (specifically effector memory and late effector memory) T-cell counts at three months were linked to increased risks of late infections (HR: 0.45 and 1.59 for $CD4^+$ T-cell and $CD8^+$ T-cell counts [as log cells/μL] respectively; p=.0001 and p=.014), with CD4+ T-cell counts mostly associated with bacterial and CD8+ T-cell counts with viral infections. This was in accordance with the results of previous studies with various graft sources.[27-29] Hence, phenotypic analysis of circulating lymphoid cells at three months after alloHSCT with alternative graft sources should help to evaluate late infection risks and to adjust infection prophylaxes.

Factors affecting UCB composition

Several studies have demonstrated that UCB unit composition is an important factor that may predict outcomes after UCB-alloHSCT, with higher doses of transplanted

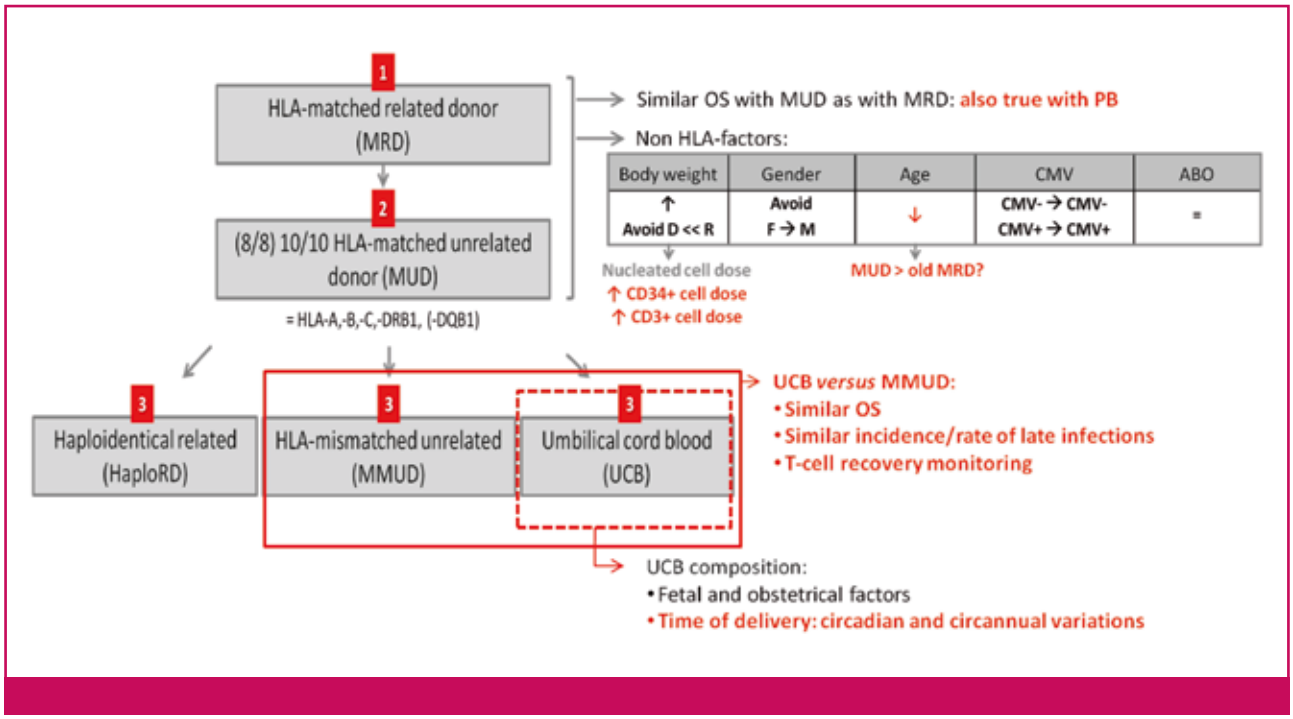

Figure 3. Algorithm for graft source selection for alloHSCT: new insights from our studies. Contributions from our studies are highlighted in red. CMV: cytomegalovirus; D: donor; F: female gender; HLA: human leukocyte antigens; M: male gender; OS: overall survival; PB: peripheral blood stem cells; R: recipient.

nucleated cells and hematopoietic stem and progenitor cells being associated with faster engraftment and better overall survival.[30-32] In a third study involving three Belgian centres, we analysed factors potentially affecting UCB cell composition (n=1127 UCB units).[33] In accordance with several previous publications, gestational age, birth weight and baby's gender influenced the concentration of nucleated and hematopoietic progenitor cells in UCB units.[34-36] Reassuringly, we did not observe any negative influence of obstetrical techniques, such as epidural anaesthesia, pharmacologically induced labour and use of oxytocin. Epidural anaesthesia and use of oxytocin were associated with even higher concentrations of hematopoietic progenitors. Interestingly, we also observed significant fluctuations in cord blood composition according to time of day and month of delivery (*Figure 2*). The lowest concentrations of nucleated cells and progenitor cells were observed in cord blood units collected from infants born during the morning and summer months. Previous studies have suggested circadian and circannual oscillations in haematopoiesis and in hematopoietic stem and progenitor cell traffic from the bone marrow to the peripheral blood in adult individuals.[37,38] Our findings suggest that such physiological rhythms may not be restricted to postnatal life. The study may also have practical implications for strategies for banking and selection of UCB units, by suggesting that time of delivery should help to target UCB units with the highest hematopoietic potential.

Conclusion

Our results confirm that graft source and composition significantly impact outcomes after alloHSCT. This work may also bring some new data that may help to refine the strategy for graft selection (*Figure 3*).

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Key messages for clinical practice

(Contributions from our studies are highlighted in italic)
How to select the optimal graft source for alloHSCT?

1. **The best donor choice is a MRD.**

2. **When no MRD is available, the second choice is a (8/8) 10/10 HLA-MUD. Reassuringly, several groups have reported similar long-term survival after MUD- and MRD-alloHSCT, mostly with BM.** *In the first study, we observed that this was also true with PB as the graft source.*

3. **Non-HLA factors may affect outcomes after alloHSCT. When several MRD or MUD are identified, it is classically recommended to avoid a female donor for a male recipient and to select the larger, younger, CMV- and ABO-matched donor.** *In the first study, we observed that a $CD34^+$ cell dose $\geq 4.5x10^6$/kg and a CD3+ cell dose $\geq 3x10^8$/kg were associated with better survival after PB-alloHSCT. We also observed a negative impact of increasing donor age on survival after PB-alloHSCT. The study further suggested survival benefit of alloHSCT with young MUD as compared with older MRD (age $\geq$ 60 years). However, this has to be confirmed in larger studies.*

4. **When no MRD or MUD is identified, three alternative graft sources may be considered for alloHSCT: haploRD, MMUD or UCB. There is currently no consensus about what is the best choice among them.** *In the second study, we compared MMUD- and UCB-alloHSCT and did not observe any difference in survival nor in the incidence/rate of late infections. However, infections were frequent after alloHSCT with these alternative graft sources. We observed that close monitoring of $CD4^+$ and $CD8^+$ T-cell recovery might help anticipating late infection risks in that setting.*

5. **One of the main limiting factors of successful UCB-alloHSCT is the infused nucleated, stem and progenitor cell doses.** *In the third study, we observed that, besides obstetrical and foetal factors, time of day and time of year of delivery also significantly affected UCB cell composition.*

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