

Review

Allogeneic Stem Cell Transplantation with CD34+ Cell Selection

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ABSTRACT

The success of allogeneic stem cell transplant is hampered by the development of acute and chronic graft-versus-host disease (GvHD) which has direct impact on treatment-related mortality and morbidity. As a result, T cell depletion through positive selection of CD34+ cells has emerged as a promising strategy to reduce acute and chronic GvHD in these patients.

In this review, we summarize the main characteristics of allogeneic stem cell transplant with CD34+ cell selection including risks of graft failure, GvHD, infection, organ toxicity, and long-term survival. Moreover, we highlight future strategies to improve the results of this platform and to consolidate its use in clinical practice.

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1. INTRODUCTION

Allogeneic stem cell transplant (alloSCT) is a curative therapy for a large number of malignant and nonmalignant hematological diseases. However, graft-versus-host disease (GvHD) is one of the main causes of treatment-related mortality (TRM) and morbidity, and may impair posttransplant quality of life [1]. The risk of grade II–IV acute GvHD is around 30%–50% in recipients of Human Leukocyte Antigen (HLA) identical siblings, while it varies between 40% and 70% in unrelated donor transplants [2]. Recently, multiple strategies have been used to optimize the management of GvHD, both in the prophylactic and treatment settings. Among them, the role of *ex vivo* T cell depletion (TCD) with positive selection of CD34+ cells in the graft has been investigated, based on the importance of alloreactive T cells for the development of GvHD [3–5]. In this review, we summarize the characteristics of this transplant platform (Table 1) including recent data that will contribute to optimize this modality of alloSCT.

2. METHODOLOGY

In the last decades, different TCD methodologies have been proposed, including *ex vivo* depletion techniques. The effectiveness of the latter in preventing GvHD varies with the technical procedure

Table 1 | CD34+ cell selection as compared to unmodified alloSCT.

Graft rejection	No higher incidence if myeloablative conditioning and ATG
GvHD	Decreased incidence of acute and chronic GvHD
Toxicity	Decreased renal toxicity Better tolerance in older age and in patients with comorbidities
Immune reconstitution	Delay in immune recovery of CD4+ cells
Infections	Inversion of CD4+/CD8+ ratio Increased incidence of opportunistic infections, especially viral
Relapse	Similar results in AML, MDS, ALL
Disease free survival	Similar results in AML, MDS, ALL
Overall survival	Similar results in AML, MDS, ALL
Quality of life	Fewer hospital admissions No immunosuppression post-SCT

Abbreviations: GvHD: Graft-versus-host disease; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; ALL: acute lymphoblastic leukemia; SCT: Stem cell transplantation.

used, the subtypes of depleted cells, the source of stem cells, and the use and type of posttransplant immunosuppression. Every selection technique generates a specific graft in terms of quality and quantity of depleted cells [6–8].

The *ex vivo* TCD of the graft can be carried out either by positive or negative selection [9]. The most frequently used techniques to achieve the TCD of the product are based on the positive selection of CD34+ cells through electromagnetic methods such as ISOLEX 300i (Baxter, Deerfield, IL) (nowadays disused) and, especially, the CliniMACS CD34 Reagent System (Miltenyi Biotec, Bergisch Gladbach, Germany), which allow an up to 5-log reduction of T cells in the final product [8,10,11].

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More recently, other TCD strategies have been developed including $\alpha\beta+$ TCR/CD19 depletion. This methodology may produce a better immune response against viral infections, with low risk of acute GvHD [12] by enhancing $\gamma\delta$ T cell reconstitution and lowering $\alpha\beta+$ TCR counts after transplantation. Noteworthy, most of the data on $\alpha\beta+$ TCR/CD19 depletion come from pediatric studies using haploidentical donors [13,14] and need further investigation in the setting of matched-related or unrelated donor transplants in adults.

3. CLINICAL IMPLICATIONS OF CD34 POSITIVE SELECTION

3.1. Engraftment

Initially, CD34+ cell selection showed a moderately increased risk of graft failure compared with other transplant modalities [15]. However, successive modifications in the initial platform, including the use of peripheral blood progenitors (as opposed to the initial use of bone marrow), technical innovations in the selection procedure and the use of total body irradiation (TBI), and anti-thymocyte globulin (ATG) in the conditioning, significantly decreased this risk [7,16–18]. Also, more immunosuppressive drugs such as fludarabine and thiotepa have been recently incorporated into the conditioning chemotherapy, allowing to perform alloSCT with CD34+ selection without the use of ATG or TBI, and achieving similar rates of engraftment failure to those of unmodified transplants [19–21].

3.2. Graft-Versus-Host Disease

The main goal of carrying out CD34+ cell selection is to decrease the risk of GvHD and, thus, the mortality and morbidity associated with this frequent complication. Numerous studies have shown the benefit of TCD transplants in terms of GvHD reduction. At the beginning of this decade, a multicenter phase II study conducted by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN 0303, NCT01119066) [5] including 44 patients with acute myeloid leukemia (AML) in first or second complete remission and receiving a CD34+ selected alloSCT showed an incidence of grade II–IV acute GvHD and chronic GvHD at 2 years of 22.7% and 6.8%, respectively. These incidences are significantly lower than those reported by using unmodified grafts for alloSCT in similar patient populations. Subsequent retrospective studies including patients with AML [19,22–24], acute lymphoblastic leukemia (ALL) [25,26], and myelodysplastic syndrome (MDS) [22,27] also demonstrated a lower incidence of acute and chronic GvHD compared to non-manipulated transplants, in the context of myeloablative conditioning. In the reduced-intensity conditioning setting, a retrospective study compared the use of CD34+ cell selection alloSCT with a non-manipulated reduced-intensity platform in patients with AML and MDS older than 50 years has been recently published. This report also observed a lower incidence of grade 2–4 acute GvHD (18% vs 46%) and chronic GvHD (6% vs 55%) in the TCD arm [28]. Recently, a study of more than 500 patients with acute leukemia comparing the ATG-based GvHD prophylaxis with CD34+ selected alloSCT showed lower incidence of acute and chronic GvHD in the latter group [29].

Studies focused on GvHD in the context of CD34+ cell selected transplants have not only confirmed these low incidences, but also

added additional observations: 1) Acute GvHD in this type of transplants is mostly cutaneous and upper gastro-intestinal. 2) Acute GvHD can be managed in the majority of patients with topical or limited oral absorption corticosteroids. Therefore, only a minority of patients with GvHD required systemic immunosuppressive treatment. 3) Reduction of GvHD is especially relevant in its chronic form (incidences <10% in successive studies [22]). This decrease in chronic GvHD could have an impact on posttransplant quality of life and on other variables such as length of hospitalization and use of nutritional support [4,30]. Unfortunately, at the present time, there are no comprehensive studies of quality of life focused on CD34+ selection alloSCT.

A prospective multicenter randomized phase III study (BMT CTN 1301, NCT02345850) is currently ongoing in AML and MDS patients who are randomized to receive one of three different strategies to prevent GvHD: CD34+ cell selection (using peripheral blood as stem cell source), posttransplant cyclophosphamide (using bone marrow) and a conventional GvHD prophylaxis with tacrolimus and methotrexate (also using bone marrow). The main objective of this study is chronic GvHD relapse-free survival, a composite endpoint that includes survival and, although indirectly, quality of life [31]. The accrual into this study finished in 2018 and results are expected shortly.

3.3. Immune Reconstitution and Infection

Immune reconstitution after transplantation depends on multiple factors, including the conditioning regimen, type of graft and thymic activity of the recipient [17,32]. Patients undergoing alloSCT with CD34+ selection have delayed recovery of CD4+ T-cells (absolute and naïve), longer time for the CD4+/CD8+ ratio inversion, as well as delayed mitogenic T-cell response. In addition, these patients have less functionality of the T-cell receptor (TCR) (thymic function marker) than those receiving unmodified grafts, although these differences disappear after 6–9 months of alloSCT [33,34]. Similarly, a slower recovery of the TCRs has been observed within the first year after alloSCT compared with unmodified grafts [35], whereas early recovery of T lymphocyte functionality has been associated with better survival in CD34+ selection alloSCT [36].

To overcome the caveat of delayed immune reconstitution, several approaches have been proposed. Among them, a phase 1 study (CYT107) carried out in patients with CD34+ selection alloSCT explored the role of interleukin-7 (IL-7) in the reconstitution and survival of T cells. The use of human recombinant IL-7 showed to be safe and, apparently effective in improving immune reconstitution after transplant, by increasing the number and functionality of CD8+ and CD4+ T cells, without increasing the risk of GvHD [37]. Another proposed strategy to enhance immune reconstitution is the use of hormonal ablation with Gonadotropin-releasing hormone (GnRH) agonists to favor thymopoiesis (NCT01746849) [38,39].

The aforementioned delay in the immune reconstitution is associated with an increased risk of opportunistic infections within the first 12 months after the procedure [40], especially viral infections, including cytomegalovirus (CMV) [41,42], Epstein-Barr virus (EBV), adenovirus (ADV), or Herpes virus 6 (HHV-6) [43–45]. In a study of 156 patients who received alloSCT with

CD34+ selection in one center, 85% developed at least one viremia in the first 100 days posttransplant [43].

CMV is the most frequent viral infection reported after CD34+ selected alloSCT, and has an early presentation. In a study including more than 200 patients [41], cumulative incidences of 85% and 62% were reported in CMV seropositive patients with seropositive or seronegative donors, respectively. Additionally, 5% of the patients developed organ CMV disease. In the risk factor analysis, the risk of CMV disease was associated with persistent positive viremia (more than 28 days). It is important to highlight that in this transplant platform, seronegative patients receiving a transplant from seropositive donors have a risk of transmission of CMV infection close to 0%, similarly to patients with a negative donor and recipient serology [41]. To prevent CMV disease and infection, prophylactic or anticipated antiviral treatments with drugs such as letermovir [46] or brincidofovir [47,48] have been studied in this platform.

Regarding EBV, the reported incidences of infection and EBV-related posttransplant lymphoproliferative disorder (PTLD) are 15% and 5%, respectively, with a median time of presentation at week 14 after transplant [43]. A study with 405 patients comparing *ex-vivo* TCD with T-cell repleted alloSCT showed a higher risk of PTLD in the TCD group (5% vs 1% [$p = 0.018$], respectively) [30].

For ADV, the risk of infection in CD34+ cell selection alloSCT is also higher than with the use of unmodified grafts [44]. In a study with 215 patients undergoing a CD34+ selection SCT conducted in a single center, 18 patients (8%) presented ADV viremia with a median appearance at 57 days [45]. One-third of these patients developed ADV organ disease.

Similarly, the risk of human HHV-6 infection is also higher than in alloSCT with unmodified grafts, reaching an incidence up to 17%, although only 10% of patients require targeted treatment [43].

Given the high incidence of these infections, alloSCT with TCD could be an ideal platform in which to expand the use of adaptive immunotherapy with specific T lymphocytes against viral infections. Both nonspecific (directed to common viral pathogens) and specific lymphocytes have been investigated in these patients with promising results [49,50].

3.4. Organ Toxicity

TRM is the most limiting factor for a successful alloSCT, and may prevent elderly or unfit patients to receive a potential curative therapy. TRM is mostly driven by GvHD and infections, as previously discussed. In addition to them, direct organ toxicities can also hamper the success of the transplant procedure.

Recently, the toxic effects of the CD34+ cell selection alloSCT platform were analyzed in a cohort of 200 patients within the first year after transplantation [51]. The most relevant toxicities in this period were infectious, metabolic, hematological, gastrointestinal, cardiac, and pulmonary complications. Noteworthy, renal complications were minimal and a low incidence of hepatic veno-occlusive disease was reported. Higher targeted busulfan levels, CMV seropositivity of the recipient, and a high comorbidity index (HCT-CI ≥ 3) were associated with an increased risk of death from any cause. Another study showed that fluid overload continues to be a cause of morbidity in CD34+ selection transplantation, despite the absence of

immunosuppressive drugs, especially in patients over 55 years old receiving chemotherapy-based conditioning regimens [52].

As for late complications, a recent *landmark analysis* on 276 patients who were alive and in complete remission after one year of CD34+ alloSCT disclosed that ferritin levels $>1,000$ ng/mL, a lymphocyte count $<0.5 \times 10^9/L$, and an albumin level of less than 4.0 g/dL were associated with an increased risk of toxicity after one year. The overall survival of patients after one year post-SCT was 77% at 4 years [53].

The most common GvHD prophylactic regimens include calcineurin inhibitors, methotrexate, or sirolimus, which contribute substantially to SCT toxicity. Methotrexate can be hepatotoxic, nephrotoxic, and, not uncommonly, causes mucositis and related complications. Calcineurin inhibitors pose serious risk of renal toxicity after SCT and can cause hypertension, electrolyte disturbances, dyslipidemia, glucose intolerance, tremor, posterior reversible leukoencephalopathy syndrome, and thrombotic microangiopathy (TMA). Sirolimus has been associated with a higher risk of Hepatic veno-occlusive disease (VOD), Thrombotic microangiopathy (TMA), and other endothelial-related complications [54,55].

The use of CD34+ selected HCT precludes the need for posttransplant GVHD prophylaxis, reducing transplant-related toxicity. It also has the potential to open myeloablative SCT to elderly or comorbid patients who would otherwise have been excluded from SCT or offered a RIC approach. Hence, two recent studies have shown a similar overall survival between patients older and younger than 55–60 years old [56,57]. In another recent study in patients older than 50 years, the CD34+ cell selection platform was not associated with an increased risk of TRM compared with an unmodified reduced intensity conditioning (RIC) approach [28]. Also, it has been confirmed that the HCT-CI [58] is useful to predict TRM in patients receiving CD34+ selected grafts, and can be helpful when deciding on the transplant indication for elderly or comorbid patients [59].

Finally, it has been demonstrated that the good performance status of patients after CD34+ selected alloSCT and the low incidence of GvHD facilitate the administration and tolerability of antineoplastic drugs after transplant, both prophylactically or as a treatment for disease relapse [60].

3.5. Relapse

The main objective of alloSCT in malignant diseases is to decrease the risk of relapse through the graft-*versus*-leukemia (GvL) effect. Since the latter is mostly mediated by T-cells, some concerns have been raised on the ability of CD34+ selected alloSCT to control disease relapse. The earlier reports using CD34+ selected grafts disclosed an increased risk of relapse in these patients [61]. However, those studies used old CD34+ cell selection methods, and posttransplant immunosuppression was routinely administered. Also, the main transplant indication in those studies was chronic myeloid leukemia (CML), which is a highly sensitive disease to the GvL effect. A retrospective analysis compared the results of two groups of patients with CML in chronic phase receiving CD34+ selection alloSCT ($n = 46$) and unmodified alloSCT ($n = 40$). The 3-year relapse incidence in the TCD group was 2.5 times higher than in the group without depletion. However, most of these patients were

rescued with donor lymphocytes infusions (DLI). Due to the high risk of relapse in this indication, the use of TCD alloSCT in CML is currently not recommended [61].

In contrast, several more contemporary studies have shown a similar risk of relapse in patients receiving CD34+ selected compared with unmodified grafts in several hematological malignancies, including AML [19,24], ALL [25,26], MDS [27], and in some subtypes of non-Hodgkin's lymphoma [62]. One of these reports focused on patients with AML in first complete remission evaluating the clinical outcome of 115 patients receiving CD34+ selected alloSCT at Memorial Sloan Kettering Cancer Center (MSKCC) and 181 patients treated with unmodified alloSCT at M.D. Anderson Cancer Center (MDACC) [19]. This study observed a similar risk of relapse at 1 year (17% vs 21%, $p = 0.4$) and at 3 years (18% vs 25%, $p = 0.9$) between the two groups. Other analyses in ALL [25]

and MDS [27] patients transplanted at MSKCC and MDACC with the same strategies also found a similar risk of relapse between the two groups. Finally, a recent study in patients older than 50 years with AML and MDS found a lower risk of relapse in those receiving CD34+ selected grafts compared with RIC-alloSCT with unmodified grafts [28]. This was probably because a higher intensity conditioning regimen could be administered in the CD34+ selected cohort, since these patients do not have the additional toxicity from posttransplant immunosuppression.

Donor lymphocyte infusions (DLI) have been used in this platform to stimulate the GvL effect, both as preemptive and as a therapeutic strategy. Bryant *et al.* [63] retrospectively reviewed a cohort of 58 CD34+ selected patients who received DLI to treat overt relapse ($n = 20$), minimal residual disease [MRD] ($n = 11$), or mixed chimerism ($n = 27$). Seventy-three percent of

Table 2 Comparative studies of CD34+ selection alloSCT versus unmodified alloSCT.

	Cohort	Donor	Disease	aGvHD Grade II-IV (TCD vs UMT)	cGvHD (TCD vs UMT)	DFS and OS (TCD vs UMT)
Pasquini <i>et al.</i> [64]	AlloTCD BMTCTN 0303 $n = 44$	Identical sibling	AML	23% vs 39% $P = 0.07$	19% vs 50% $p < 0.001$	2 years DFS 54% vs 55%
	UM+IS BMT CTN 0101 $n = 84$					2 years OS 65% vs 59%
Bayraktar <i>et al.</i> [19]	AlloTCD MSKCC $n = 115$	Identical sibling HLA 10/10 URD	AML	5% vs 18% $p = 0.005$	13% vs 53% $p < 0.001$	3 years DFS 58% vs 60%
	UM+IS MDACC $n = 181$	HLA 9/10 RD HLA 9/10 URD				3 years OS 57% vs 66%
Hobbs and Perales [26]	AlloTCD MSKCC $n = 52$	Identical sibling HLA 10/10 URD	AAL	17% vs 43% $p = 0.001$	14% vs 33% $p = 0.006$	3 years DFS 43% vs 36%
	UM+IS MDACC $n = 115$	HLA 9/10 RD HLA 9/10 URD				3 years OS 43% vs 43%
Tamari <i>et al.</i> [27]	AlloTCD MSKCC $n = 60$	Identical sibling HLA 10/10 URD	MSD	Low risk 13% vs 41% $p = 0.015$	Low risk 5% vs 48% $p < 0.001$	3 years DFS Low risk 60% vs 53% High risk 32% vs 11%
	UM+IS $n = 121$ (MDACC)	HLA 9/10 RD HLA 9/10 URD				High risk 16% vs 22% $P = 0.75$
Barba <i>et al.</i> [28]	AlloTCD MSKCC $n = 204$	Identical sibling HLA 10/10 URD	AML	18% vs 46% $p < 0.001$	6% vs 55% $p < 0.001$	3 years DFS 55% vs 50%
	UM+IS RIC. GETH $N = 152$		MSD			3 years OS 58% vs 56%

Abbreviations: AlloTCD: AlloSCT with T cell depletion; UM: Unmodified; IS: Immunosuppression; BMT CTN: Blood and Marrow Transplant Clinical Trials Network; MDACC: MD Anderson Cancer Center; MSKCC: Memorial Sloan Kettering Cancer Center; MAC: myeloablative conditioning; aGvHD: acute Graft versus Host Disease; cGvHD: chronic graft-versus-host disease; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; ALL: acute lymphoblastic leukemia; RD: related donor; URD: Unrelated donor; EFS: event free survival; OS: overall survival; RIC: reduced intensity conditioning; GETH, Grupo Español de Trasplante Hematopoyético.

patients with MRD achieved remission, *versus* 15% in those with overt relapse. DLI was associated with low rates of acute (3%) and chronic (7%) GvHD indicating that it could be an effective strategy to manage relapse, especially in patients with MRD+ disease.

3.6. Survival

Most of the comparative studies have shown a similar overall survival between CD34+ cell selection SCT and unmodified transplants in different diseases and conditioning intensities (Table 2). Again, the results of the prospective BMT CTN (NCT02345850) clinical trial are awaited and will help to clarify the role of CD34+ selection from a controlled, prospective, and multicentric perspective.

4. FUTURE DIRECTIONS

Probably, the main challenge of CD34+ selection alloSCT is to improve the management of infections. Exploring modifications to the conditioning regimen (e.g., by excluding or lowering the dose of ATG), modulating the anti-infectious prophylaxis with new antiviral drugs such as letermovir or maribavir, and extending the use of anti-infectious cell therapy with viral-specific T cells or with CAR-T cells can contribute to such improvement. Other pharmacologic strategies to enhance immune reconstitution as the use of IL-7 deserve further investigation.

Additional areas with unmet needs in the field of CD34+ selected alloSCT include the improvement in the selection of patients and the evaluation of patient-reported outcomes and quality of life.

5. CONCLUSIONS

The substantial scientific evidence published in the last decade has contributed to demonstrate that CD34+ selection alloSCT reduces the incidence and severity of acute and chronic GvHD without increasing the risk of disease relapse. At least similar survival has been reported between CD34+ selected alloSCT and unmodified transplants. Moreover, specific populations of patients, such as those with comorbidities (especially renal failure) or advanced age could also have a benefit in terms of survival. Infection-related complications (mostly viral) are the main challenge for this transplant platform. The results of the randomized phase III clinical trial comparing its use with other GvHD prophylaxis strategies (BMT CTN 1301, NCT02345850) will contribute decisively to define the place for this modality of transplantation in the near future.

CONFLICT OF INTERESTS

M.-A.P. declares have received honoraries from Abbvie, Incyte, Medigene, Merck, MolMed, Nektar Therapeutics, Novartis, Servier and Takeda, not related with the present article.

P.B. declares have received honoraries from Amgen, Celgene, Gilead, Incyte, Jazz Pharmaceuticals, MSD, Novartis, Pfizer and Roche, not related with the present article.

E.R. declares no conflicting interests.

AUTHORS' CONTRIBUTIONS

MAP and PB conceived the manuscript. All authors wrote and reviewed the manuscript.

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