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Advances in *Ex Vivo* T Cell Depletion – Where Do We Stand?

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Abstract

Graft-versus-host (GVHD) is an important cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). As donor T cells are recognized as key drivers of GVHD, some approaches to prevent GVHD have focused on T cell depletion of the allograft. In this review we summarize methods and outcomes of *ex vivo* T cell depleted (TCD) HCT with a focus on CD34+ selection. This platform is efficacious in preventing acute and chronic GVHD across a wide range of hematologic malignancies, and with the exception of chronic myeloid leukemia, is not associated with adverse relapse or survival outcomes compared to conventional GVHD prophylaxis platforms. In retrospective comparisons recipients of CD34+ selected HCT have higher rates of GVHD-free relapse-free survival (GRFS) than conventional HCT counterparts. Although CD34+ selected allografts require myeloablative and antithymocyte-globulin based conditioning to support engraftment, abrogation of calcineurin inhibitors and methotrexate in this approach reduces its toxicity such that it can be considered in select older and more comorbid patients who could benefit from ablative HCT. A trial comparing GVHD prophylaxis regimens (BMT CTN 1301, NCT 02345850) has completed accrual and will be the first to compare CD34+ selected HCT with conventional HCT in a randomized prospective setting. Its findings have potential to establish CD34+ selected HCT as a new standard-of-care platform for GVHD prevention.

Keywords

Hematopoietic stem cell transplantation; T cell depletion; CD34+selection; graft-versus-host disease

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Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

Conflicts of interest

Dr Perales reports personal fees from Abbvie, Merck, Incyte, Novartis, Takeda, and Nektar Therapeutics. He serves on the DSMB for Servier and Medigene, and the scientific advisory boards of MolMed and NexImmune. He has also received research support (clinical trials) from Incyte and Miltenyi Biotec. Dr. Bryant declares no conflicts of interest.

Introduction

Despite decades of experience with allogeneic hematopoietic cell transplantation (HCT), graft-versus-host disease (GVHD) remains an important challenge, leading to potentially devastating post-HCT morbidity and an estimated 14-19 % of all non-relapse mortality (NRM).¹ The most commonly used regimens to prevent GVHD were developed more than three decades ago, and generally involve the combination of a calcineurin inhibitor (CNI) and methotrexate. Although these regimens have improved control of acute GVHD (aGVHD), aGVHD rates with their use remain high in both HLA-matched related donor (MRD; Grade II-IV aGVHD 35-50%) and matched unrelated donor (MUD; Gr II-IV aGVHD 40-70%) HCT.^{2,3} These regimens have also failed to demonstrate clear efficacy in decreasing rates of chronic GVHD (cGVHD).

The recognition that donor T cells are key drivers of the GVHD process has led to widespread exploration of the potential GVHD-mitigating effects of allograft T cell depletion (TCD). Methods used for T cell Depletion are numerous and include both *in vivo* and *ex vivo* depletion techniques.

In vivo T cell depletion

Clinical exploration of *in vivo* T cell depletion has primarily involved peri-transplant infusion of anti-T cell antibodies, mostly with antithymocyte globulin (ATG) in various formulations or with the anti-CD52 antibody alemtuzumab, and most often in the matched related and matched unrelated donor settings. Outcomes using *in vivo* TCD have been reported over two decades in many prospective randomized and non-randomized settings, including two recent large multicenter randomized controlled trials (RCTs), and were compared systematically in a recent review and meta-analysis.⁴⁻⁶

While not a method of T cell depletion, post-transplant cyclophosphamide has gained increasing prominence as T cell-directed GVHD prevention strategy, primarily in the setting of haploidentical and mismatched donor HCT. Its application in the post transplant setting has been postulated to selectively destroy alloreactive T cells and increase populations of GVHD-moderating T regulatory cells, thereby decreasing GVHD rates in the short and long term.⁷ With GVHD-specific efficacy demonstrated in mismatched donors, this strategy is being increasingly explored in the matched donor setting, and represents one arm in the large multicenter study (BMT CTN 1301, NCT 02345850) comparing two strategies of calcineurin-inhibitor free prophylaxis (*ex vivo* CD34+ selection or post transplant cyclophosphamide) to a conventional calcineurin-inhibitor based strategy.

A more comprehensive review of *in vivo* TCD and of post-transplant cyclophosphamide falls beyond the scope of this discussion. We direct you to key references in the bibliography.⁴⁻⁸

Methods of *ex vivo* T cell depletion

Techniques used for *ex vivo* T cell depletion have varied over time. Efficacy and extent of T cell removal can vary based on technique used, cell subtypes depleted, stem cell source,

degree of donor-recipient HLA matching, and use and type of post-transplant immune suppression. These factors are important considerations in interpreting and comparing outcomes in *ex vivo* TCD HCT.

Ex vivo T cell depletion can be achieved by either positive or negative selection of CD34+ cells. Negative selection methods include a) physical approaches such as counterflow elution, or soybean lectin agglutination followed by sheep red blood cell rosette depletion (E-rosetting); and b) immunologic approaches that employ anti T cell monoclonal antibodies (e.g. anti-CD6) conjugated to immunomagnetic beads that are used as targets for separation.^{9–12} When compared to positive selection techniques, grafts depleted by physical or immunologic negative selection retain a larger number of T cells and other effector lymphocytes such as NK cells.¹³

Over time, positive selection of CD34+ cells has become the predominant *ex vivo* T cell depletion technique in clinical use. Earlier positive selection techniques employed the ISOLEX 300i magnetic cell selection system (Baxter; Deerfield, Illinois) followed by E-rosetting.^{14,15} Current studies use the more efficient CliniMACS CD34 Reagent System (Miltenyi Biotec; Bergisch Gladbach, Germany), which uses super-paramagnetic particle conjugated antibodies.^{16–18} The CliniMACS system is able to achieve a 5-log T cell depletion while retaining a median viability of 98%, and in direct comparison with ISOLEX 300i the CliniMACS system yielded a product with higher CD34+ purity (90 vs 78%, $p=0.004$), and lower median T cell content (0.06 vs 0.44%; $p = 0.003$).¹⁹ The CliniMACS CD34 Reagent System was approved by the FDA for use for GVHD prevention in patients with acute myeloid leukemia (AML) in first remission receiving a transplant from an HLA-identical sibling donor in January 2014, and remains the only FDA approved to CD34+ selection system for use in HCT. The CliniMACS system can also be used to selectively deplete CD3+ T cells, CD19+ B cells, and T cell receptor $\alpha\beta$ positive (TCR $\alpha\beta$ +) T cells.^{20,21}

***Ex Vivo* CD3+/CD19+ and $\alpha\beta$ T Cell Depletion**

Combined CD3/CD19 depletion aims to simultaneously deplete allografts of CD3+ T cells responsible for GVHD, and of CD19+ B Cells that may result in donor-derived EBV post-transplant lymphoproliferative disease (PTLD). This technique has been explored almost exclusively in the setting of haploidentical donor HCT and most often in pediatric populations.²² The largest series employing this strategy in adults included 29 and 61 haploidentical HCT recipients and reported grade II-IV acute GVHD of 46-48%, and with longer follow-up, chronic GVHD rates of 18% and 2-year NRM of 44%.^{23,24} The efficacy of this GVHD strategy compared to others used in the haploidentical setting (ie. post-transplant cyclophosphamide), and its wider applicability in adults and in matched donor HCT setting are as yet unknown and may be the focus of future prospective studies.

Selective depletion of $\alpha\beta$ -TCR T cells has been explored with the intent to reduce the $\alpha\beta$ T cell-mediated adaptive immune response that drives GVHD while preserving the $\gamma\delta$ T cell-mediated innate immune response that provides infectious protection. Earlier studies used anti- $\alpha\beta$ antibodies to bind and target $\alpha\beta$ T cells for complement-mediated lysis. A large

multicenter study comparing *ex vivo* T cell depletion using $\alpha\beta$ -depletion as described or global T cell depletion through counterflow centrifugal elution, against a standard cyclosporine/methotrexate-based strategy noted that TCD was associated with more rapid neutrophil recovery, reduced grade III-IV toxicity, and less grade III-IV acute GVHD (18 v 37%), but higher rates of CMV infection (28 v 17%), lower 3 year disease free survival (27 v 34%) and significantly higher relapse rates in CML patients (20 vs 7%).²⁵

More recent studies have employed CliniMACS-based selective immunomagnetic $\alpha\beta$ depletion, as this method provides profound and selective depletion and reproducible results.²⁶ Reports to date have been largely pediatric-focused and almost exclusively in haploidentical HCT, and have demonstrated similar findings to those using other $\alpha\beta$ selection methods.²⁷ One recent retrospective review of 41 pediatric recipients of haploidentical HCT for acute leukemia, MDS or non-malignant disease using combined immunomagnetic $\alpha\beta$ TCR and CD19+ depletion noted rates of acute grade II and III-IV GVHD of 10 and 15% respectively, and also reported more rapid and robust recovery of CD56+ natural killer, CD3+ T, and CD3+4+ T helper early after HCT (days 14, 30, and 30 respectively) when compared to CD34+ selected historical controls.²⁸ In one the largest reported adult series of 34 acute leukemic adult haploidentical HCT recipients, grade III/IV and chronic GVHD rates were low at 5.6 and 6.1% respectively, immune reconstitution of CD3+ T cells and subsets appeared to be more rapid than in reports using CD3+/CD19+ depletion or CD34+ selection, but relapse-free survival was also low (33% at 2 years) and relapse-driven deaths were high at 56%.²⁹ Further exploration $\alpha\beta$ T cell depletion and its potentially advantageous immune recovery is being prospectively examined in trials involving pediatric and adult recipients of matched, mismatched, or haploidentical HCT in both malignant and non-malignant disease (EudraCT 2011-005562-38, NCT03615144). For a detailed discussion of $\alpha\beta$ TCD we direct the reader to comprehensive reviews on the subject.²⁷

CD34+ selection does not impair engraftment when using ablative ATG-containing conditioning regimens

It has been postulated that donor T cells present in the allograft may diminish graft rejection by eliminating residual components of the host immune system present at time of allograft infusion. This concept was supported by the early clinical experience with CD34+ selected allografts reporting high rates of graft failure.³⁰⁻³² This barrier was overcome through the use of ablative TBI- or chemotherapy-based conditioning, and by increasing the immunodepleting potential of the conditioning regimen with incorporation of ATG. With these measures, engraftment in CD34+ selected HCT matches those seen in conventional HCT. The requirement of an ablative conditioning platform currently restricts CD34+ selected HCT to younger and non-comorbid patients.^{33,34} Avoidance of CNI and methotrexate with this approach reduces toxicity compared to conventional ablative HCT, however, and may open this approach to patients in whom toxicity risks of conventional myeloablation would otherwise be prohibitive (see below). With the goal of making CD34+ selected HCT safe for a wider patient population, exploration of techniques to reduce the

conditioning intensity required for this approach are active areas of investigation, as outlined at the end of this review.

CD34+ selection decreases rates of acute and chronic GVHD

CD34+ selected HCT has been consistently associated with decreased rates of acute and/or chronic GVHD across a variety of hematologic malignancies. Single center studies are summarized in Table 1,^{14,15,17,34–37} and studies comparing outcomes of CD34+ selected HCT with conventional HCT are summarized in Table 2.^{17,38–42} From these studies two common themes emerge:

- a** The use of CD34+ selection is consistently associated with low rates of acute and chronic GVHD and significantly and often markedly lower than those in unmodified HCT
- b** GVHD experienced by CD34+ selected HCT recipients is most commonly low grade or limited, with exceedingly rare incidence of severe acute (grade III-IV) or extensive chronic GVHD

CD34+ selected HCT does not adversely affect relapse or survival in the most common HCT indications

Depleting donor allografts of T cells has raised concerns of decreasing T cell mediated contribution to graft-versus-leukemia (GVL) effect, and a resultant increased risk of relapse. In clinical practice, this increased relapse risk was demonstrated in a retrospective study of chronic myelogenous leukemia (CML) patients in which 47 patients who received *ex vivo* TCD HCT had a 2.5 fold increased risk of relapse compared to 40 patients received conventional non-TCD HCT (3 year relapse incidence 62 vs 24%; $p < 0.001$).⁴³ Twenty patients who relapsed subsequently received donor lymphocyte infusion (DLI), and 17 (85%) achieved a complete remission (CR), ultimately resulting in similar overall survival (OS) in both cohorts. These observations support the well recognized importance of GVL in CML, and suggest that donor T cells in the allograft or in post-HCT infusions are important GVL mediators. Although DLI salvaged remission in most relapsed patients in this cohort, T-cell depleted allografts are not routinely recommended for CML patients undergoing HCT.

In contrast, CD34+ selection has not been associated with an increased relapse risk in other hematologic malignancies, including some types of non-Hodgkin lymphoma (NHL), and in direct comparative studies in AML,^{38,39,41} acute lymphoblastic leukemia (ALL),⁴⁰ and myelodysplastic syndrome (MDS),^{38,42} in which no differences were observed in relapse rates, relapse-free survival (RFS) or in OS between recipients of CD34+ selected and conventional HCT (Table 2). The majority of these studies included myeloablative regimens in both arms. One cross-institutional study compared AML and MDs patients receiving either CD34+ selected HCT or a reduced intensity conditioned (RIC) unmodified HCT in older patients however.³⁸ This study reported no differences in RFS or OS between cohorts, but a significant decreased relapse incidence in the CD34+ selected group (3-year 19 vs 33%, $p = 0.001$), which may speak to the relapse-preventive qualities of myeloablative conditioning. Low rates of relapse and prolonged 10-year RFS and OS (43% and 50%) were

also reported in a mixed population of high-risk NHL patients undergoing CD34+ selected HCT, (Table 2)³⁷ though it should be noted that most patients with NHL currently undergo reduced intensity HCT with unmodified grafts. The use of CD34+ selected HCT should be considered in carefully selected NHL patients.

Overall these observations may suggest that, in selected hematologic malignancies, a) GVL is not a primary determinant of disease control; b) T cells contribute less to GVL; c) the decreased T cell dose and slower immune reconstitution observed in CD34+ selected HCT does not compromise the T cell contribution to GVL, and/or d) that ablative conditioning chemotherapy contributes more to relapse prevention than does GVL. This absence of a deleterious effect on disease control in CD34+ selected HCT results in similar RFS and OS in these patients compared to recipients of unmodified grafts. Furthermore, a recent landmark analysis in 276 patients with AML, ALL or MDS who underwent CD34-selected HCT reported 5-year RFS and OS of 73% and 76%, respectively in patients alive and without evidence relapse at one year post HCT.⁴⁴

Taken together, these reports suggest that relapse, relapse-free survival, and OS rates in HCT are not adversely impacted by T cell depletion of allografts in AML, ALL, and MDS. Increased rates of relapse seen in CML patients undergoing CD34+ selected HCT are explained by the important contribution of GVL to CML disease control.

CD34+ selected HCT is associated with increased rates of GVHD-free survival

In recent years the composite outcome of GVHD-free relapse-free survival (GRFS) has rapidly gained acceptance in the research and clinical HCT realms, in part because it represents the ideal HCT outcome: a patient that is alive, free of disease, and without debilitating morbidity of GVHD.⁴⁵ Though this newer composite outcome is not reported in all comparisons of conventional and CD34+ selected HCT, a common conclusion is consistently reported in these studies: recipients of T cell depleted HCT do not have different likelihood of relapse, RFS, or OS compared to conventional HCT recipients, but are more likely to be free of acute and chronic GVHD.^{39–42} Where reported, CD34+ selected HCT is consistently associated with higher GRFS than unmodified HCT:

- AML: In 44 CD34+ selected and 84 unmodified HCT patients, 2-year GVHD-free survival (GFS) was reported at 42 vs 19% ($p=0.006$).⁴¹
- MDS: In 60 CD34+ selected and 121 unmodified HCT patients, 3-year chronic-GRFS (CRFS) was 60 vs 19% ($p<0.001$).⁴²
- AML/MDS: In 204 ablative CD34+ selected HCT and 152 RIC unmodified HCT patients, 3-year CRFS was 51 vs 7% ($P<0.001$).³⁸

While these studies illustrate a consistent message, they are limited by their retrospective and cross-institutional comparative designs. These limitations are currently being addressed in an ongoing three-arm multicenter randomized controlled trial in patients < 65 with AML, ALL or MDS and an 8/8 HLA MRD or MUD comparing conventional GVHD HCT prophylaxis (methotrexate + CNI) with CD34+ selected HCT and with post-transplant

cyclophosphamide (BMT CTN 1301, NCT 02345850), with CRFS as the primary endpoint. As of June 2018, the trial has completed accrual. Its results are eagerly anticipated and have the potential to alter HCT standard of care.

Decreased toxicity with CD34+ selected HCT permits use of ablative HCT in older and more comorbid patients

The standard GVHD prophylaxis regimen of CNIs and methotrexate contributes substantially to HCT toxicity. Methotrexate can be hepatotoxic, nephrotoxic, and not uncommonly causes mucositis and related complications. Calcineurin inhibitors pose serious risk of renal toxicity after HCT and can cause hypertension, electrolyte disturbances, dyslipidemia, glucose intolerance, tremor, posterior reversible leukoencephalopathy syndrome (PRES), and thrombotic microangiopathy. The prevention and management of these complications requires careful and frequent monitoring of serum CNI levels, drug-drug interactions, fluid balance, renal function, blood pressure monitoring, and electrolyte monitoring and replacement.

The added potential toxicities associated with standard GVHD prophylaxis can be prohibitive to HCT in some patients, particularly elderly patients, those with renal dysfunction, and patients with a high pre-transplant comorbidity burden. Approaches to such patients typically involve either forgoing HCT altogether in favor of palliative or of lower risk non-curative options, or reducing HCT conditioning intensity to decrease risks of serious toxicity or transplant-related mortality (TRM). While the latter approach may reduce toxicity associated with the HCT, a recent multicenter trial in which AML and MDS patients were randomized to high versus reduced intensity HCT was stopped early due to a clear signal of increased mortality in the reduced intensity arm, the majority of which was driven by relapse. RFS was worse in AML and MDS patients receiving reduced intensity regimens (18 month RFS AML 45 vs 65%, $p=0.003$; MDS 56 vs 78%, $p=0.07$).²¹ Premature study cessation resulted in an underpowered MDS analysis, however, and a subsequent similar European multicenter trial reported similar relapse and survival outcomes in RIC and MAC-based HCT for patients with MDS and secondary AML.⁴⁶ Although a robust comparison of RIC or MAC-based HCT falls beyond the scope of this review, consensus remains that an ideal HCT platform should strike a balance between disease-controlling myeloablation and the potential for serious toxicity.

With CD34+ selected HCT, the elimination of methotrexate and CNI from the transplant regimen substantially reduces transplant related toxicity, and has the potential to open myeloablative HCT to elderly or comorbid patients who otherwise would have been denied HCT or offered a reduced intensity approach. This safety and efficacy of ablative CD34+ selected HCT in elderly and/or comorbid patients is supported by the following observations

- A 2018 retrospective comparative analysis of 200 patients undergoing CD34+ HCT CD34+ for hematologic malignancies in which patients ≥ 60 had acceptable 1 year NRM (23%) and OS (70%) that were not significantly different than 120 patients age < 60 (NRM 13%, $p=0.38$; OS 78%, $P=0.07$).⁴⁷

- A 2017 non-comparative analysis reporting low NRM (10%) and acceptable long term RFS and OS (4-year 44% and 41%, respectively) in a cohort of 214 elderly (>55) and comorbid (50% HCT-CI ≥ 3) patients undergoing CD34+ selected HCT.⁴⁸
- A 2017 report validating the hematopoietic stem cell transplant comorbidity index (HCTCI) in CD34+ selected HCT, in which rates of NRM in patients with moderately low HCT-CI (score 1–2) and with prohibitively high HCT-CI (≥ 7) were lower than would have been expected in myeloablative unmodified HCT, for which this score was originally validated.^{49,50}
- A 2018 report referenced above and detailed in Table 2 comparing ablative CD34+ selected (n= 204) to unmodified RIC HCT (n=152) for patients with AML and MDS in which NRM (1-year 18 vs 11%, p = 0.11), RFS (1-year 68 vs 61%, p=0.13), and OS (1-year 74 vs 68%, p=0.28) were similar between CD34+ selected and RIC cohorts, respectively.³⁸

Taken together these observations suggest that, by abrogating additional conditioning toxicity attributed to CN1 and methotrexate, an ablative CD34+ selected HCT platform can be used in some elderly and comorbid patients, and allow such patients both the curative potential of HCT and the potential added efficacy of ablative conditioning.

Immune recovery is delayed in CD34+selected HCT

The speed and quality of immune recovery after HCT can vary according to conditioning regimen, composition of the allograft, and patient thymic activity, which decreases with age.^{51,52} The use of T cell depleted allografts results in delayed immune recovery, whether measured by absolute number of CD4+ and CD8+ T cells and T cell subsets, CD4+/CD8+ ratio, T cell diversity indicators such as T cell Receptor (TCR) repertoire diversity, or thymic output measures such as TCR excision circles (TRECs).^{52–55} Although immune recovery in CD34+ selected HCT begins to approach that of conventional grafts by 12–18 months, the initial delay has been associated with increased rates of opportunistic infections, the majority of which involve viruses such as cytomegalovirus (CMV), Epstein Barr Virus (EBV), adenovirus, and human herpes virus-6 (HHV-6).^{52–56}

Of these viruses, CMV is the most common and potentially lethal. Though CMV viremia is common in CMV seropositive CD34+ selected HCT recipients, a 2016 study of 213 recipients of patients undergoing CD34+ selected alloHSCT for AML, MDS, CML or myeloproliferative disorders reported that with effective pre-emptive antiviral therapy, CMV seropositive patients experience low rates of conversion to CMV disease (11%) and no difference in 1 year OS compared to CMV seronegative counterparts.⁵⁷ Historical rates of CMV infection seen in CD34+ HCT may also decline with the use of letermovir, an antiviral agent that was recently FDA approved for use as post-HCT CMV prophylaxis.⁵⁸

In studies comparing CD34+ selected and conventional HCT in AML, ALL, and MDS, recipients of CD34+ selected HCT had higher rates of infectious-related mortality compared to conventional HCT recipients (10–17% vs 3–7%).^{39,40,42} In none of these comparisons was any difference RFS or OS observed however, suggesting that the increase rate of

infectious deaths observed in CD34+ selected HCT recipients was offset by the comparatively higher rates of GVHD observed in conventional HCT recipients. Notably GVHD itself may impair immune recovery either directly through thymic suppression, or indirectly through the immune suppressing therapies required for its treatment. Strategies to improve immune reconstitution post CD34+ selected HCT remain an important area of unmet need and are a key focus of active clinical investigation.

Advantages and drawbacks of CD34+ selected HCT and optimal patient selection

The decision to use a T cell depleted allograft over an unmodified one should be based on individual patient factors including but not limited to underlying disease, donor availability and degree of HLA match, and patient comorbidities. These patient-specific factors can provide context to weigh the potential advantages and drawbacks of a CD34+ selected HCT approach, which are summarized in Table 3.

In general, CD34+ selected HCT should be considered in all myeloablative conditioning candidates who fall into the following categories:

- Patients with AML, MDS, ALL, and high grade NHL are ideal candidates given the similar rates of relapse, RFS, and OS seen in these diseases compared to unmodified grafts
- Patients at high risk of GVHD, such as recipients of mismatched related or unrelated donor allografts
- Patients with renal insufficiency in whom the risk of long term CNI use is prohibitive
- Elderly or comorbid patients with diseases (AML, MDS) that would benefit from myeloablative HCT in whom risks of NRM with conventional ablative HCT are prohibitive. Although CD34+ selected ablative conditioning may be better tolerated due to omission of CNI and methotrexate, risks and benefits should nonetheless be carefully weighed in all patients proceeding to HCT, with Particular caution in older adults and patient with multiple comorbidities.
- Patients in whom the close monitoring of post-HCT CNI and its associated adverse effects are prohibitively challenging

Future directions

CD34+ selected HCT is a promising HCT platform that is both efficacious in treating hematologic diseases and in preventing GVHD. Future research is aimed primarily at expanding its applications, preventing complications, and improving post-HCT immune reconstitution.

Expanding Applications

As summarized above, CD34+ selected HCT requires a conditioning regimen that is ablative to permit engraftment of the T cell depleted stem cell product. This stipulation generally excludes patients unable to tolerate ablative regimens. Additionally, although CD34+ selection has not been associated with increased rates of relapse outside of CML, clinicians are often hesitant to use T cell depleted allografts in disease at high risk of relapse where GVL effect may play a more important role. Studies aimed at expanding applications of the CD34+ selected platform are aimed both at decreasing conditioning toxicity and at preventing relapse in high risk disease:

- In one pilot study (NCT03531736) patients will undergo TCR- $\alpha\beta$ T cell depleted RIC (fludarabine/total body irradiation) HCT supported by high dose ATG, post transplant cyclophosphamide and rituximab. It is hypothesized that removal of TCR- $\alpha\beta$ and preservation of TCR-gamma-delta T cells and NK cells may contribute to improved engraftment and disease control, and that high dose ATG and post transplant cyclophosphamide will provide sufficient host immune suppression to prevent allograft rejection and obviate the need for myeloablative conditioning.
- In additional ongoing and planned studies, patients with high risk malignancies undergoing CD34+ selected HCT will be supported by relapse prevention strategies that include post HCT maintenance azacytidine in high risk MDS and AML (NCT01995578), and checkpoint blockade in refractory AML (planned) and relapsed refractory multiple myeloma (planned).

Improving Immune Reconstitution and Preventing Complications

- Interleukin-7 has an important role in T cell development and survival, and in murine HCT models has been shown to improve immune recovery. In a phase I clinical trial, administration of escalating weekly doses of IL-7 to CD34+ selected HCT recipients was associated with increased levels of functional T cells and of TCR diversity, without increases in GVHD, compared to historical institutional controls.⁵⁹ At time of writing, this intervention is being planned for investigation in the phase II and III setting.
- Sex steroids also play a critical role in immune regulation, and their presence is associated with decline in thymic size and immune function over time. Administration of Lupron, a sex steroid ablative agent, has been associated with enhanced T cell reconstitution and function after HCT,⁶⁰ and is part of an ongoing clinical trial investigating its effects on post CD34+ selected HCT immune reconstitution (NCT01746849).
- CD34+ selection results in efficient and global deterioration of both TCR- $\gamma\delta$ and TCR- $\alpha\beta$ T cells. In murine HCT models TCR- $\gamma\delta$ T cells have demonstrated important roles in pathogen responses, and in pediatric HCT the use $\gamma\delta$ replete TCD allografts was associated with decreased infection rates and improved event free survival.²⁷ The aforementioned open TCR- $\alpha\beta$ T cell depleted RIC HCT pilot study (NCT03531736) and $\alpha\beta$ TCD study in adults with non malignant

diseases (NCT03615144) will explore these possibilities and potentially provide groundwork for exploration of this TCD method on a larger scale.

Conclusions

T cell depleted HCT is a transplant platform that offers relapse-free and overall survival that matches those seen in conventional HCT, but with comparatively lower rates of acute and chronic GVHD, and improved CRFS. Through its omission of CNi and methotrexate based GVHD prophylaxis, this approach may also provide a means to offer ablative HCT to elderly or comorbid patients in whom the risks of traditional myeloablative HCT may be prohibitively high.

While all the current data is subject to potential bias due to its retrospective nature, an ongoing three-arm multicenter prospective randomized controlled trial is comparing conventional GVHD HCT prophylaxis with CD34+ selected HCT and with post-transplant cyclophosphamide (BMT CTN 1301, NCT 02345850). The results of this trial have potential to establish CD34+ selected HCT as a new standard-of-care platform for GVHD prevention in HCT.

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Clinical implications

CD34+ selected HCT is a platform that can be considered in patients AML, ALL, MDS, and NHL who are candidates for myeloablative conditioning in that this platform is associated with decreased rates of acute and chronic GVHD and does not adversely impact relapse or survival, ultimately resulting in higher GRFS than conventional HCT. The reduction in toxicity from the abrogation of calcineurin inhibitors and methotrexate in this regimen reduce its toxicity such that it can be considered in select older and more comorbid patients who may benefit from ablative HCT.

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Table 1

Single-center studies of CD34+ selected HCT in hematologic malignancies

	N	Disease	Cond	Regimen	Donor	CD34+ Selection Method	Graft Source	GVHD	RFS and OS
Papadopoulos 1998 ³⁴	39	AML	TBI Thio CY		MRD	SBA-sRBC	BM	aGVHD Gr I 5% Gr II-IV 0% Extensive cGVHD 1/35 evaluable patients	CR1 4y RFS 77% CR2 4y RFS 50%
Jakubowski 2007 ¹⁴	52	AML ALL CML MDS NHL TPLL	TBI Thio Flu		MRD	ISOLEX-sRBC	PBSC	aGVHD Gr II 8% Gr III-IV 0% cGVHD Any 9%	3y RFS 61% 3y OS 62%
Castro-Malaspina 2008 ³⁵	49	MDS MDS→AML	TBI-based BU-based		MRD	SBA-sRBC (BM) ISOLEX-sRBC (PBSC)	BM PBSC	aGVHD Gr I-III 7% Gr IV 0% cGVHD Any 2%	All 3y RFS 37% 3y OS 31% CR or < RCMD pre- HCT 3y RFS 50% 3y OS 54%
Perales 2010 ³⁷	61	NHL	TBI Thio CY TBI Thio Flu		MRD MUD MMRD MMUD	SBA-sRBC (BM) ISOLEX-sRBC (PBSC)	BM PBSC	aGVHD Gr II-III 18% Gr IV 0% cGVHD Any 12%	10y RFS 43% 10y OS 50%
Jakubowski 2011 ¹⁵	35	AML ALL CML MDS TPLL	TBI Thio Flu ATG		MUD	SBA-sRBC (BM) ISOLEX-sRBC (PBSC)	BM PBSC	aGVHD Gr I-III 9% Gr IV 0% cGVHD Any 29%	4y RFS 57% 4y OS 59%
Goldberg 2013 ³⁶	56	ALL	TBI Thio CY TBI Thio Flu		MRD MUD MMRD MMUD	SBA-sRBC (BM) ISOLEX-sRBC (PBSC)	BM PBSC	aGVHD Gr II-III 20% Gr IV 0% cGVHD Any 15% Extensive 5%	2y RFS 38% 5y RFS 38% 2y OS 39% 5y OS 38%
Tamari 2015 ¹⁷	102	MDS	BU Mel Flu TBI Thio CY/Flu		MRD MUD MMRD MMUD	ISOLEX-sRBC CliniMACS	PBSC	aGVHD Gr II-IV 10% cGVHD Any 4%	2y RFS 52% 5y RFS 48% 2y OS 57% 5y OS 49%

Abbreviations used - aGVHD: acute GVHD; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; ATG: antithymocyte globulin; BM: Bone marrow; BU: busulfan; cGVHD: chronic GVHD; CML: chronic myeloid leukemia; CR1, CR2: complete remission 1, 2; CY: cyclophosphamide; Flu: fludarabine; ISOLEX-sRBC: ISOLEX 300i magnetic cell selection followed by sheep RBC-rosette depletion MDS: myelodysplastic syndrome; MRD: Matched related donor; MUD: Matched unrelated donor; MMRD: Mismatched related donor; MMUD: Mismatched unrelated donor; NHL: non Hodgkin lymphoma; OS: overall survival PBSC: Peripheral blood stem cell; RCMD: refractory cytopenia with multilineage dysplasia; RFS: relapse free survival; SBA-sRBC: Soybean lectin agglutination (SBA) followed by sheep red blood cell (sRBC)-rosette depletion; TBI: Total body irradiation; TCD: T cell depletion; Thio: thiopeta; TPLL: T cell prolymphocytic leukemia

Table 2

Comparison of GVHD, relapse, and survival outcomes in CD34 selected and unmodified HCT

	Populations	Disease	Donor	CD34+ Selection Method	Graft Source	Gr II-IV aGVHD (TCD vs IST)	Any cGVHD (TCD vs IST)	Relapse Survival (TCD vs IST)
Pasquini 2012 ⁴¹	TCD graft BMTCTN 0303 n= 44 Unmod +IST BMT CTN 0101 n= 84	AML	MRD	CliniMACS	PBSC	23 vs 39% p=0.07	19 vs 50% p<0.001	2y RFS 54 vs 55% 2y OS 65 vs 59%
Bayraktar 2013 ³⁹	TCD graft MSKCC n= 115 Unmod +IST MDACC n= 181	AML	MRD MUD MMRD MMUD	SBA-sRBC (BM) ISOLEX-sRBC or CliniMACS (PBSC)	BM PBSC	5 vs 18% p=0.005	13 vs 53% p<0.001	3y RFS 58 vs 60% 3y OS 57 vs 66%
Hobbs 2015 ⁴⁰	TCD graft MSKCC n= 52 Unmod +IST MDACC n= 115	ALL	MRD MUD MMRD MMUD	SBA-sRBC (BM) ISOLEX-sRBC or CliniMACS (PBSC)	BM PBSC	17 vs 43% p=0.001	14 vs 33% p=0.006	3y RFS 43 vs 36% 3y OS 43 vs 43%
Tamari 2018 ⁴²	TCD graft MSKCC n= 60 Unmod +IST n= 121 (MDACC)	MDS	MRD MUD MMRD MMUD	ISOLEX- sRBC CliniMACS	BM PBSC	Low Risk 13 vs 41% p=0.015 High Risk 16 vs 22% p=0.752	Low Risk 5 vs 48% p<0.001 High Risk 0 vs 24% p=0.013	3y RFS Low Risk 60 vs 53% High Risk* 32 vs 11% 3y OS Low Risk 59 vs 54% High Risk 36 vs 15%
Barba 2018 ³⁸	TCD graft MAC cdx MSKCC n = 204 Unmod +IST RIC cdx Spanish cohort N = 152	AML MDS	MRD MUD	ISOLEX-sRBC CliniMACS	PBSC	18 vs 46% p<0.001	6 vs 55% p<0.001	3y RFS 55 vs 50% 3y OS 58 vs 56%

Abbreviations used - aGVHD: acute GVHD; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BM Bone marrow; BMTCTN: Bone Marrow Transplant Clinical Trials Network; cGVHD: chronic GVHD; ISOLEX-sRBC: ISOLEX 300i magnetic cell selection followed by sheep RBC-rosette depletion; IST: immune suppression therapy; MDACC: MD Anderson Cancer Center; MDS: myelodysplastic syndrome; MRD: Matched related donor; MUD: Matched unrelated donor; MMRD: Mismatched related donor; MMUD: Mismatched unrelated donor; MSKCC: Memorial Sloan Kettering Cancer Center; OS: overall survival PBSC: Peripheral blood stem cell; RFS: relapse free survival; SBA-sRBC: Soybean lectin agglutination (SBA) followed by sheep red blood cell (sRBC)-rosette depletion; TBI: Total body irradiation; TCD: T cell depletion; Unmod: unmodified allograft.

Table 3

Advantages and Drawbacks of CD34+ Selected HCT

	Advantage	Neutral	Drawback
Engraftment		Engraftment comparable to unmodified HCT provided conditioning is myeloablative and uses ATG	Rates of graft failure increased in absence of conditioning that is myeloablative and includes ATG
GVHD Prophylaxis Toxicity	Absence of methotrexate reduces mucositis Absence of CNi reduces renal toxicity Less toxic GVHD prophylaxis makes ablative conditioning more tolerable and increases safety of ablative conditioning in elderly and/or comorbid patients		
GVHD	Decreased rates of acute and chronic GVHD		
Relapse		Relapse rate and RFS comparable to unmodified HCT in ALL, AML, MDS, NHL	Relapse rates increased and RFS decreased compared to unmodified HCT in CML
Immune recovery			Delay in immune recovery may result in higher rates of infectious complications and infectious-related mortality
Overall Survival		OS comparable to unmodified HCT in ALL, AML, MDS, NHL, and in CML (if DLI available)	
Post HCT Immune Suppression	No need for immune suppression after HCT a) simplifies HCT management and follow-up b) abrogates need to manage CNi-related toxicities		
Post HCT Quality of Life	Potential QoL benefits related to a) decreased likelihood of CNi-related toxicities b) decreased rates of chronic GVHD		

Abbreviations used - aGVHD: acute GVHD; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; ATG: antithymocyte globulin; BM: Bone marrow; BU: busulfan; cGVHD: chronic GVHD; CML: chronic myeloid leukemia; DLI: donor lymphocyte infusion; MDS: myelodysplastic syndrome; NHL: non Hodgkin lymphoma; OS: overall survival; QoL: quality of life; RFS: relapse free survival