Towards graft-*versus*-host disease-free alternative donor transplant platforms for patients with acquired aplastic anemia

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Abstract

Hematopoietic stem cell transplantation (HCT) is a well-established treatment option for acquired aplastic anemia. Historically, upfront HCT with HLA-matched sibling donors is used in young patients and immunosuppressive therapy (IST) is used for all others. Over time, innovations in the transplant platform have decreased unwanted complications such as graft-*versus*-host disease (GvHD), failure to engraft, and infections, thereby expanding the use of alternative donors. This has led to an evolution towards HCT and away from IST despite increased intensity of treatment due to improved event-free survival. The ideal conditioning regimen for HCT in aplastic anemia results in sustained engraftment, minimal toxicity, lack of GvHD, and is not limited by donor availability. Two transplant platforms have been refined to meet these needs. TCR $\alpha\beta^+$ T-cell depletion has excellent outcomes with minimal graft rejection and GvHD in the matched and mismatched unrelated donor setting. Haploidentical grafts, however, still require further optimization. Bone marrow grafts given with anti-thymocyte globulin and post-transplant cyclophosphamide have similarly excellent results with extremely low rates of GvHD. Peripheral mobilized grafts need further optimization in this setting. This review provides an overview of the current perspective on regimens that minimize GvHD in aplastic anemia and how they can be further refined so that all patients with aplastic anemia have curative therapy available to them.

Introduction

Acquired severe aplastic anemia (SAA) is an immune-mediated hematopoietic stem cell disorder that presents with a hypocellular marrow and pancytopenia.[1,2] In the historical therapeutic algorithm, most newly diagnosed patients are treated with immunosuppressive therapy (IST) unless they are young and have a suitable leukocyte antigen (HLA)-matched sibling donor.[3] Increasingly, paradigms for safe and effective allogeneic hematopoietic stem cell transplantation (alloHCT) are being explored to increase use of this therapeutic option. For HCT to become a more viable option for therapy, we need to ensure the toxicity of the conditioning regimen and the transplant are essentially isolated to on-target hematologic toxicity; graft immunologic effects must be minimized or eliminated since neither graf-*versus*-tumor nor graft-*versus*-host disease (GvHD) are desired.

Advances have been made in IST over the past 30 years through better understanding of clonal evolution and immunobiology of the disease.[4] Additionally, for adult patients with SAA, we now have the combination of eltrombopag with the historical IST backbone of equine anti-thymocyte globulin (ATG) and cyclosporine. The three-drug regimen decreases the time to hematologic response, but longer-term durability remains relatively unchanged.[5,6] Interestingly, a similar signal was not observed in pediatric patients treated with eltrombopag and IST.[7,8] The hematopoietic response after IST is approximately 70-80% and 5-year survival, 60-85%.[2,5,9,10] In the most recent clinical trial of eltrombopag combined with IST, 4-year survival was an excellent 92.5%.[11] Traditionally, failure-free survival for patients alive and in remission without clonal disease more than ten years after IST is <40% in longer term follow-up studies.[1,9,12-14] These long-term data are not yet available for

contemporary clinical trials of eltrombopag with IST, but they will be critical as we continue to attempt to adjudicate appropriate front-line therapy for each newly diagnosed patient. Newer precision-based targeted approaches to immune suppression therapy are also being developed and assessed based on preclinical models implicating the JAK/STAT pathways in disease pathogenesis.[15] Current clinical trials are investigating the upfront use of emapalumab (an anti-Interferon-γ monoclonal antibody) in pediatric patients with newly diagnosed aplastic anemia (*clinicaltrials.gov identifier 06430788*) and the use of ruxolitinib (JAK1/2 inhibitor) in the relapsed/refractory setting (*clinicaltrials.gov identifier 05998408*). However, the need to have multiple therapies available has spawned novel approaches to alloHCT to improve the event-free and overall survival for HCT in patients with SAA.[16,17]

Given the relatively unchanged treatment options for SAA, consensus recommendations for upfront therapy in North America have largely remained the same. This stems from overall survival (OS) rates of 70-75% for patients with SAA and an HLA-matched sibling donor (MSD), 60-65% for patients with SAA receiving IST, and 35-40% for alternative donor transplants for SAA in the 1980s.[18,19] Outcomes have steadily improved, and OS is currently >90% in young patients receiving MSD grafts or IST, largely secondary to improvements in supportive care.[20] Outcomes for alternative donor transplants, however, have also significantly improved due to refinements in supportive care as well as the novel transplant platforms described in this review. For newly diagnosed patients with SAA under 40 years of age who have an MSD, upfront HCT is the standard of care, with long-term survival rates approaching 90% in patients under 20 years of age and 76% for patients over 20 years of age.[2,21] Modern outcomes with MSD HCT for patients <40 years old with SAA have approached >95% and have led investigators to try minimizing toxicity by using fludarabine to reduce cyclophosphamide dosing from 200 mg/kg to 100-120 mg/kg.[22,23] In Europe, trials have shown modern matched-unrelated donor (MUD) HCT outcomes to be essentially equivalent to those of MSD HCT outcomes and have led to a paradigm change in how upfront SAA therapy is approached in Europe.[24-26] Similar randomized studies are currently being conducted in North America to evaluate upfront MUD HCT compared to upfront traditional IST in pediatric patients with SAA.[27] Transplant outcomes in older patients (>40 years of age) are less favorable due to reduced engraftment, infections and high rates of GvHD, as shown in recent studies.[28,29] A successful alloHCT from the best available donor minimizes the risk of relapse and secondary clonal disease. GvHD rates now can be quite low, even with alternative donors.[17,30,31] Although alloHCT for SAA still presents a high-risk of graft rejection, modern immune-ablative regimens continue to improve engraftment rates across donor types. This review will illustrate the strides we have made in the field leading to the question of whether alternative donor transplants should be considered earlier for patients with SAA (Figure 1).

Ex vivo T-cell depletion

Different modalities of *ex vivo* T-cell depletion have been used to broaden the available donor pool for alloHCT while maintaining acceptable rates of GvHD. Traditional methods of *ex vivo* T-cell depletion led to full removal of T cells from the donor graft either through a $CD3^+$ T-cell depletion or a $CD34^+$ HSC selection. These methods resulted in decreased GvHD when using alternative donors; however, there was an increased rate of non-engraftment and/or primary graft rejection. Thus, partial T-cell depletion (pTCD) approaches were developed to continue minimizing development of GvHD while increasing rates of engraftment.[17,32]

Initially, *ex vivo* T-cell depletion relied on sheep erythrocytes and soybean lectin, and progressed to the use of antibodies and complement.[33] Modern methods of *ex vivo* T-cell depletion utilize technologies such as the Miltenyi CliniMACS® device and provide the ability to engineer hematopoietic stem cell grafts more precisely. The high start-up costs associated with these technologies and appropriate laboratory set-up necessarily limit the number of centers at which they can be offered; however, these methodologies are critical to offer a platform for precise graft engineering. Continued refinement in cost structures and/or the ability to engineer grafts at central locations and ship to centers will increase access and equity of these platforms. Here, we will concentrate on modern techniques of *ex vivo* partial T-cell depletion in aplastic anemia.

A prior method of pTCD utilized an antibody-based T-cell depletion. In a single center study using T10B9 (a monoclonal anti-T cell antibody that spared γδT cells) for pTCD of MUD (N=9) and MMUD (N=19) with a myeloablative total body radiation (TBI) based-conditioning regimen, 28 pediatric patients with SAA were transplanted from 1986-1994. In this cohort, 3/28 patients had graft failure and there was a 54% OS at a median follow up of 33 months.[34] In a similar single-center study of pTCD of MUD (N=4) and MMUD (N=8), 12 pediatric patients (11/12 with SAA) were transplanted between 1992-2003 with a myeloablative TBI-based conditioning regimen. pTCD was accomplished with either T10B9 or OKT3 (a monoclonal anti-CD3 antibody) and the addition of complement. All patients engrafted, and 9/12 were alive 13-153 months post transplant.[32] These data showed promising results from the pTCD approach of MUD and MMUD, although additional optimization was needed. A modern method of pTCD incorporated the use of a CliniMACS® device (which allowed for more precise control and deeper depletion of targeted cell populations) to deplete $CD3^+$ T cells with a subsequent targeted addback of $1x10^5$ $CD3^+$ T cells/kg to the graft product prior to infusion. A single-center study investigated a cohort of 12 pediatric patients with non-malignant hematologic disorders treated from 2014-2017 on an expanded access protocol with

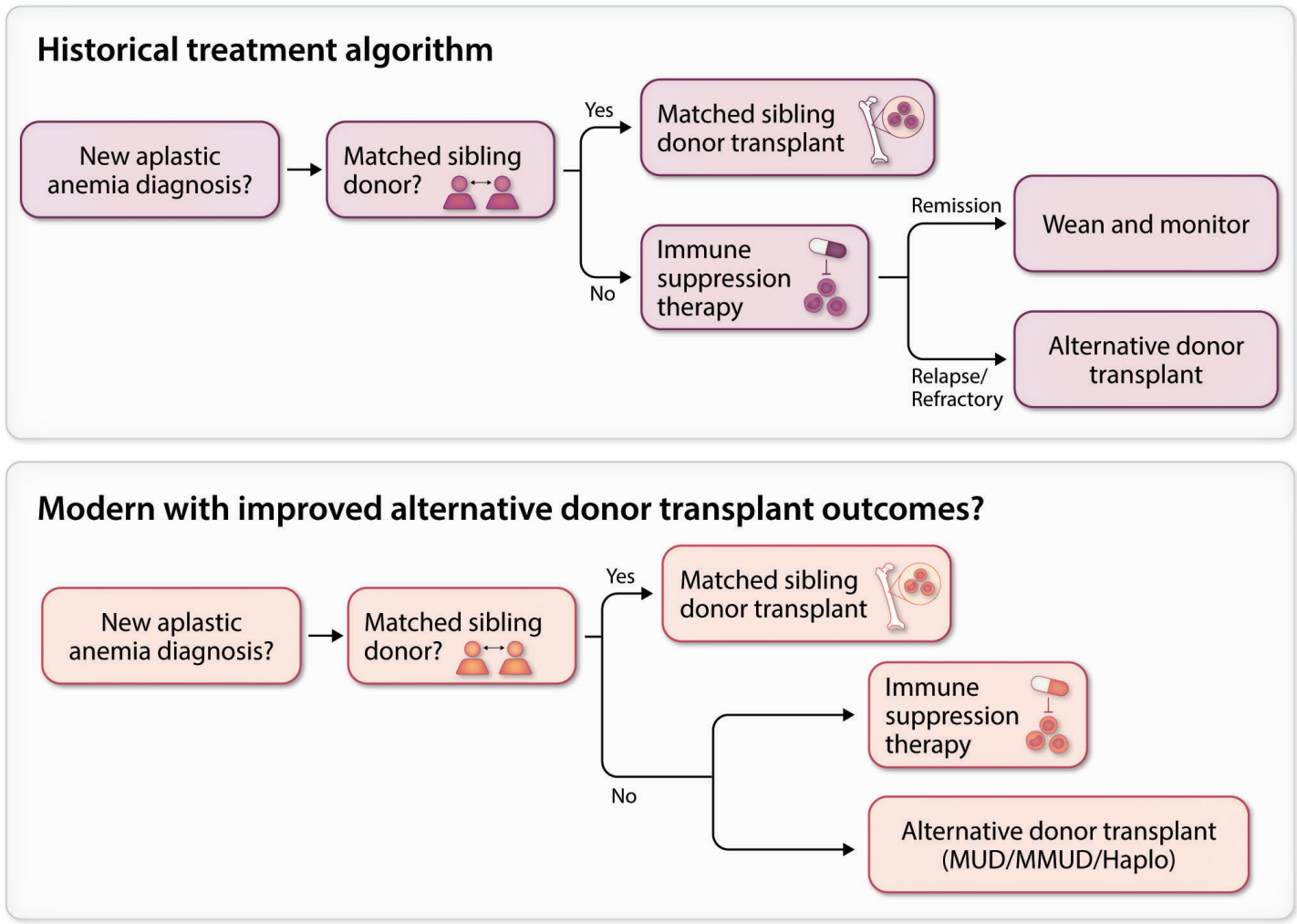

Figure 1. Historical treatment algorithm for severe aplastic anemia. The historical treatment algorithm for severe aplastic anemia (SAA) prioritized hematopoietic stem cell transplantation (HCT) for patients that had an available matched sibling donor and immunosuppressive therapy (IST) for others. Alternative donor HCT was previously reserved for patients that failed IST or for unique situations. We propose that improved outcomes in alternative donor HCT for SAA should allow for alternative donor HCT to be moved to upfront therapy alongside IST. Several clinical trials are currently exploring this question. Given these increased therapeutic options, biomarker-based and/or response-adapted therapy will be critical to personalize appropriate treatment decisions for upfront therapy (IST or alternative donor HCT) for each patient with newly diagnosed SAA.

matched and mismatched unrelated donor. Peripheral blood stem cells (PBSC) were mobilized using $CD3^+$ T-cell/$CD19^+$ depletion with targeted $CD3^+$ T-cell addback; 100% OS and disease-free survival (DFS) were reported with no graft rejections at a median follow up of two years. Seven of these patients had relapsed/refractory SAA and/or SAA with paroxysmal nocturnal hemoglobinuria (PNH). One patient developed acute grade II skin GvHD and 2 patients developed chronic limited eczema. Importantly, the majority of patients treated were of ethnicities under-represented on the donor registry, thus showing the utility of pTCD in helping achieve equity of care in HCT.[17] The added novelty of these approaches was the use of mismatched unrelated donors as opposed to haploidentical donors for *ex vivo* T-cell depletion. These data led to the development of a prospective clinical trial using $TCR\alpha\beta^+$ T-cell depletion of matched and mismatched unrelated donors and partially matched related donors as a more refined pTCD approach for pediatric patients with bone marrow failure syndromes (*clinicaltrials.gov identifier 03047746)*.

$TCR\alpha\beta^+$ T-cell depletion was developed as a new method for pTCD based on data implicating $TCR\alpha\beta^+$ T cells as mediators of GvHD. This method of pTCD relies on the full depletion of $TCR\alpha\beta^+$ T cells using the CliniMacs® device while leaving the $TCR\gamma\delta^+$ T cells in the graft product for increased anti-viral activity and improved engraftment. This method also simplifies the *ex vivo* graft manipulation as no additional addback step is required. The $\alpha\beta^+$ T-cell depletion platform has been used successfully in MSD, MUD, mismatched-unrelated donor and haploidentical donor transplants for pediatric malignant and non-malignant diseases.[35-39] In a single-center study, 26 pediatric patients were treated on a prospective trial investigating the efficacy and outcomes of $TCR\alpha\beta^+$ T-cell/$CD19^+$ depletion using matched (N=15) and mismatched (N=11) unrelated donor peripheral stem cell transplants for bone marrow failure syndromes. Twenty-two of the 26 patients had acquired SAA (11 upfront and 11 relapsed/refractory). Conditioning is highly immune-ablative and consisted of rATG, fludarabine, cyclophosphamide and TBI200 (300 cGy for patients with PNH clones >15% in the granulocyte compartment).[40] Graft rejection/GvHD prophylaxis consists of single-agent calcineurin inhibitor for six months. All 22 patients with SAA engrafted without graft rejections. Engraftment was

robust and rapid (neutrophil = 15 days and platelets = 15 days per CIBMTR criteria and in the absence of GCSF). There were no instances of acute GvHD and one patient developed chronic limited NIH skin score 1 GvHD. OS and EFS were 100% in the treatment-naïve cohort and 90% in the relapsed/refractory cohort at a median follow up of two years. Complications of transplant were minimal. Thus, TCR$\alpha\beta^+$ T-cell/CD19$^+$ depletion of unrelated donors with an immune ablative conditioning regimen represents an important method for expanding the available donor pool for sAA at centers that have this capability.

Interestingly, while the TCR$\alpha\beta^+$ T-cell depletion platform yielded excellent outcomes and minimal graft rejections for pediatric patients with SAA in the unrelated donor setting, the haploidentical donor setting still presents challenges. An advantage to using a haploidentical donor compared to an unrelated donor is typically a shorter time from diagnosis to transplant, which is important given the profound neutropenia that can be seen with SAA. In a series of cohorts of pediatric patients with non-malignant diseases, including SAA, Bertaina *et al*. and Merli *et al*. show excellent OS of 91.4% at a median follow up of 3.7 years. However, the graft rejection rate was 30.4% for the entire 70 patient cohort. When patients (N=28) at high risk of graft rejection including the 13 patients with SAA are excluded, these high-risk patients have a 55.5% graft rejection rate.[39,41] Thus, while many of these patients have a graft rejection, they are ultimately cured with a second (and sometimes third) graft. Hence, while the TCR$\alpha\beta^+$ T-cell depletion has promising results and excellent OS, particularly for matched and closely matched unrelated donors, further modifications are necessary in the haploidentical setting to minimize graft rejection events. The mechanisms for which haploidentical grafts need further optimization compared to mismatched unrelated donors in this platform also requires further elucidation.

High-dose anti-thymocyte globulin and the Beijing protocol

The transplant platform for haploidentical grafts in Beijing is based on 4 drugs for GvHD prophylaxis: mainly, high-dose rabbit ATG (10 mg/kg), conventional post-transplant MTX, CSA and MMF.[42] In a prospective, multicenter study of haplo-HSCT for SAA refractory to IST, Xu *et al*. analyzed the outcomes of 101 patients following a myeloablative conditioning with intravenous busulfan 6.4 mg/kg, CY 200 mg/kg; the graft source was combined G-CSF-stimulated bone marrow and PBSC. Compared with 48 patients grafted from MSD, recipients from haplo-HSCT had higher than grade II-IV acute GvHD (33.7 *vs*. 4.2%, P<0.001), more chronic GvHD (22.4 *vs*. 6.6%, P=0.014) at one year, but similar 3-year OS (89.0 *vs*. 91.0%, P=0.555) and failure-free survival (FFS) (86.8 *vs*. 80.3%, P=0.659). More recently, 183 SAA patients receiving haploidentical grafts were compared with 159 receiving MSD grafts;[43] again the conditioning regimen was myeloablative for the haploidentical grafts (BU CY) and conventional FLU CY for the MSD grafts. GvHD prophylaxis in the haploidentical group, was based on high-dose ATG, MTX, CS and MMF. The 9-year FFS was 88% (MSD) and 87% (haploidentical donor).[43] A small prospective study has now been reported with the addition of low-dose PTCy to the ATG-based GvHD prophylaxis, with encouraging results.[44]

Innovation of post-transplantation cyclophosphamide

Cyclophosphamide has had a role in the therapy for SAA (both transplant and non-transplant) for nearly five decades.[45,46] High-dose cyclophosphamide (HiCY) is highly immunosuppressive, but not myeloablative, allowing endogenous hematopoietic stem cells to reconstitute hematopoiesis — the major goal in any treatment paradigm for a bone marrow failure disorder such as acquired SAA. As such, HiCY is combined with ATG for one of the most commonly used HCT conditioning regimens for acquired SAA.[47] Historically, complete reconstitution of autologous hematopoiesis occurs in 10-15% of patients undergoing alloHCT for aplastic anemia.[48-50] Many of these patients have maintained long-term remissions despite autologous reconstitution. The EBMT reported that 10% of patients with SAA experience autologous reconstitution following HCT with this conditioning and a 10-year survival (84%) in those patients equivalent or better than in patients who engrafted (74%).[51]

Autologous recovery after HiCY and an allogeneic transplant suggests that the immunosuppressive effect of HiCY has allowed for hematologic recovery in those patients; as a consequence, HiCY has been tested for primary treatment of aplastic anemia, with some patients showing hematologic recovery.[52] However, the long-lasting cytopenias following HiCY and the risk of infections has discouraged most investigators from using HiCy for the treatment of aplastic anemia. Thus, the Johns Hopkins group incorporated past work in the malignant arena with cyclophosphamide in the post transplantation setting (PTCy) and began piloting this platform in non-malignant disorders.

Post-transplantation cyclophosphamide has allowed for transplantation of alloHCT from matched, mismatched, unrelated or haplo-identical donors in both malignant and non-malignant diseases.[53-57] Indeed, the administration of a properly timed, high dose of CY given after HCT inhibits both GvHD as well as graft rejection. Use of PTCy expands the donor pool, achieves engraftment in over 90% of patients, and maintains low rates of GvHD in both malignant and non-malignant diseases.[58-60] Relapse after HLA-haploidentical HCT and PTCy remains the leading cause of death for hematologic malignancies. The first successful use of PTCy for GvHD when using HCT to treat SAA was by DeZern *et al*. in an HLA matched setting, following a myeloablative busulfan and cyclophosphamide conditioning regimen.[61] Other centers have explored less intense conditioning with some success. Using a reduced intensity conditioning with PTCy and mobilized PBSC in 8 patients, Clay *et al*. report-

ed successful engraftment in 6 patients and low rates of acute GvHD and no chronic GvHD.[62] Esteves *et al*. also described haploidentical HCT using the Baltimore regimen in another small cohort with 16 patients, after failure of IST or graft failure following unrelated-donor or cord blood transplant. Graft source was bone marrow in 13 and PBSC in 3. The 1-year OS was 67.1% (95% CI: 36.5-86.4%).[63] These studies have brought PTCy into the mainstream for SAA GvHD prophylaxis.

Current data for lower rates of graft-*versus*-host disease

Arcuri *et al*. analyzed 87 pediatric and adult patients who underwent haplo-PTCy HCT for SAA between 2010 and 2019. Primary graft failure occurred in 15% of the patients, and secondary or poor graft function occurred in 5%. The incidences of grade II-IV acute GvHD was 14%, and that of chronic GvHD was 9%. Two-year overall survival and event-free survival (EFS) were 79% and 70%, respectively, again comparing favorably in refractory disease.[64] More recently, DeZern *et al*. reported on a single institutional prospective phase II trial of PTCy for relapsed/refractory disease.[30,65] Some treatment-naïve patients with SAA were also reported. The cumulative incidence of grade II-IV acute GvHD at day 100 was 11% and of chronic GvHD at two years was 8%. Similar results were seen in 10 patients with SAA who received the identical non-myeloablative regimen with PTCy but matched sibling or unrelated donor transplants. These single institution studies were further validated in the USA with a multi-center prospective trial. The recently completed Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 1502, was a prospective phase II clinical trial of haploidentical donor HCT with ATG and PTCy for relapsed or refractory SAA (*clinicaltrials.gov identifier: 02918292*).[31] Thirty-one patients with relapsed/refractory SAA were transplanted on this multi-center trial. The median age was 24.9 (range 2.1-70.3) years and median follow-up was 24.3 (range 11.2-40.3) months. Twenty-four patients were alive with engraftment at one year and one patient was alive with autologous recovery. The day-100 cumulative incidence of grade II-IV acute GvHD was 16% (95% CI: 6-31%.) The 1-year incidence of chronic GvHD was 26% (95% CI: 12-42.%). The 1-year OS was 81% (95% CI: 62-91%). Four patients had primary and one had secondary graft failure (GF).[31]

Increasing use of alternative donors

In the current era, the goals of SAA therapy continue to be focused on regimens which recover hematopoiesis with minimal toxicity. As modern outcomes for HCT continue to improve in SAA, efforts to expand the donor pool and provide equitable access to curative therapy have been increasing, especially given continued longer term concerns about traditional IST.[11,66,67] In pediatrics, there are ongoing trials in the treatment-naïve setting investigating upfront MUD BMT (*clinicaltrials.gov identifier: 05600426*), pTCD approaches (*clinicaltrials.gov identifier: 03047746*), and haplo-PTCy approaches (*clinicaltrials.gov identifier: 06517641*).

The use of haploidentical HCT in SAA is very practical, as a donor is usually available and this can shorten time from diagnosis to transplant. The use of upfront haploidentical HCT has already been reported in some series with and without PTCy. Xu *et al*. reported on outcomes of upfront HCT in 158 patients with SAA,[68] based on the Beijing GCSF/ATG platform.[42,68]

Graft failure

As in many non-malignant disorders, graft failure is a major issue for SAA patients and has been one of the main reasons for which alternative-donor HCT has not previously been used more frequently in the upfront setting.[69] The underlying mechanism of disease in SAA, involving auto-reactive T-cell destruction of HSC, makes it particularly high risk for graft failure.[70] The Beijing group has reported a very high rate of engraftment using a myeloablative BU_Cy conditioning regimen.[68] In the TCR$\alpha\beta^+$ T-cell depletion platform with haploidentical donors, graft failure has been reported in >50% of high-risk non-malignant disorders, including SAA.[39,41] This was not seen in the mismatched unrelated donor setting. Similarly in the original PTCy haploidentical cohort, 3 of the first 7 patients suffered a graft rejection necessitating an increase in TBI.[30] Thus, graft failure is still a risk in HCT for SAA, but current regimens with intensified immune ablation have been able to minimize these events.

Upfront use of a PTCy-based regimen with alternative (mostly haploidentical) donors was suggested by the BMT CTN State of the Science Symposium 2021 as the next needed innovation for the SAA field.[71] The Johns Hopkins group has already utilized ATG and PTCy in the upfront setting with very promising outcomes.[30] Notably, in the relapsed setting, engraftment had not been an issue. However, in the treatment-naïve setting, 3 of the first 7 patients treated with only 200 cGY TBI had primary or secondary graft failure. With augmentation to 400 cGy, no further graft loss was noted, suggesting this additional radiation is required for durability of the platform. Very low rates of acute and chronic GvHD were still seen.[30] One key feature of any chosen donor is the graft dose. The importance of cell dose to facilitate engraftment and survival has been demonstrated in several trials.[31,64] Unsurprisingly, severe infections and toxicities occurred at much higher rates in patients who had early or late graft rejection; thus, a high cell dose to maximize engraftment is a key to optimal outcomes. Going forward, it will be important to determine whether the ATG, fludarabine, cyclophosphamide and TBI with PTCy, mycophenolate mofetil and tacrolimus platform should become standard for all allografts in SAA (over ATG and CY conditioning with methotrexate and cyclosporin[72]) or just reserved for alternative donor HCT.

Donor specific antibody management

The issue of donor specific antibodies (DSA) can be quite complex in many patients, but especially in those with an

autoimmune disease such as SAA and when engraftment is so critical. In clinical trials, as reported above, most have not been eligible if the patient has documented DSA above a mean fluorescence intensity (MFI) of 1,000.[30,31] A recent report on haploidentical HCT following a myeloablative conditioning regimen did not find the presence of DSA to be predictive of graft failure.[73] However, desensitization has been pursued for some patients with SAA in order to use a haploidentical donor.[74] A recent retrospective analysis in 59 pediatric patients (≤21 years) who underwent haplo-PTCy HCT for non-malignant conditions (including 11 with SAA) showed engraftment was possible with bone marrow as the graft source using a fludarabine-based conditioning regimen.[75] Four patients received desensitization therapy with rituximab and plasmapheresis, whereas 7 patients were untreated. All patients with treated DSA achieved donor engraftment. In the multivariable analyses, untreated DSA were associated with lower neutrophil recovery, increased GF, and poor OS. This suggests that presence of DSA is an independent predictor of poor outcomes and DSA-positive donors should be avoided whenever possible. An important caveat is that these studies enrolled a limited number of subjects and were not specific to SAA. Thus, while the role of DSA is currently unknown in SAA, expanding the donor search for additional haploidentical related donor or unrelated donors to which the patient does not have DSA is prudent until additional data are generated.

Conclusions and future directions

Current results with TCR$\alpha\beta^+$ T-cell depletion, as well as PTCy and ATG platforms, have increased the utilization of alternative donor HCT for upfront therapy in SAA and have minimized the incidence of GvHD. This is particularly critical since while the probability of finding a matched unrelated donor is >70% for patients with European ancestry, those with African American, Hispanic, Middle Eastern or Asian ancestry have a <20% to <50% probability of finding a fully matched unrelated donor. By allowing a one antigen mismatch, the probability of finding an appropriate donor increases to at least 70% for all these populations.[25,76] The limitations of current published studies are the small numbers of patients treated in each study, as well as the limited follow-up time currently available. Prospective multi-center trials investigating efficacy and monitoring long-term effects of these platforms will be vital, and some of these data will be generated by the TransIT study (*clinicaltrials.gov identifier: 05600426*) and the BMT-CTN 2207 study (*clinicaltrials.gov identifier: 06517641*). In particular, the long-term effects of TBI will need to be assessed (particularly fertility and secondary malignancies) and the development of TBI-free regimens will be critical. Similarly, clinical investigations are currently ongoing to evaluate if PTCy dosing can be reduced in HCT for malignancy (*clinicaltrials.gov identifier: 04959175*) or a PBSC-based graft can be used in elderly patients (*clinicaltrials.gov identifier: 05436418*), and these results might inform future trial iterations in SAA. A retrospective study in India analyzed the addition of abatacept to a PTCy-based GvHD prophylaxis regimen in patients with SAA and showed decreased acute GvHD and a trend towards decreased chronic GvHD in patients that received abatacept, thus providing preliminary data for a future prospective study.[77] An additional study looking at decreasing MMF in the PTCy platform (*clinicaltrials.gov identifier: 03983850*) may also allow for optimizing the use of MMF in SAA.

Efforts aimed at shortening time to immune reconstitution, and decreasing transplant-related morbidity by using population-pharmacokinetic modeling of rATG have been used in several diseases and transplant platforms.[78-81] The use of pop-PK modeled rATG and other conditioning agents represents an additional variable that may be optimized across different HCT platforms for SAA. Further modifications to the TCR$\alpha\beta^+$ T-cell depleted platforms to modulate immune reconstitution and decrease viral reactivation are also underway (*clinicaltrials.gov identifier: 03810196*). Several groups are continuing to optimize haploidentical grafts in the TCR$\alpha\beta^+$ T-cell depletion platform to minimize complications of graft rejection. Lastly, studies comparing efficacy and complications of using bone marrow *versus* PBSC in the PTCy platform, as well as whether a maximum CD34$^+$ dose is to be recommended in both PTCy and TCR$\alpha\beta^+$ T-cell depletion platforms, should be defined.

Current studies are focused on improving alternative donor alloHCT and evaluating if standard IST or alternative donor alloHCT are better for all newly diagnosed patients with SAA. Ultimately, however, there will be a patient population for which IST is most appropriate, and a second population for which intensification of therapy with upfront HCT is best. As both IST and HCT continue to be refined and outcomes continue to improve, the major question that will need to be addressed will be how to appropriately identify which patient is right for either IST or HCT. So far, historical approaches at identifying predictive biomarkers for response have failed, and thus, further effort in this area will be critical so that patients receive the minimal required therapy for cure. Thus, while the field has made significant improvement in the treatment for SAA, there continues to be room for refinements.

There have been tremendous advances in the use of HCT for aplastic anemia, and outcomes are approaching those of matched sibling and matched unrelated donor transplants. These advances have allowed the potential donor pool to be widened, allowing for curative therapy to be delivered to patients that have traditionally been under-represented on the donor registry. Due to these improved outcomes, current studies are investigating the use of alternative donor transplant as upfront therapy in patients with SAA. Long-term monitoring of potential side effects, and identification of which patient population might be ideally suited for upfront BMT, will continue to

help refine and improve this powerful curative approach for patients with SAA.

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