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# Biology of Blood and Marrow Transplantation

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Pediatric

## Relapse of Aplastic Anemia with Majority Donor Chimerism (Donor-Type Aplasia) Occurring Late after Bone Marrow Transplantation



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### A B S T R A C T

There have been sporadic reports of the development of delayed disease recurrence after bone marrow transplantation for severe aplastic anemia despite sustained majority or full donor chimerism. This is termed “donor-type aplasia” (DTA). We describe the management and outcome of 11 pediatric patients from 8 institutions in Europe, the United States, and the Middle East who developed DTA at a mean of 35 months post-transplant. These patients were initially transplanted at a mean age of 10.0 years (range, 5.8 to 16.0 years), 9 from matched sibling donors and 2 from matched unrelated donors. Attempts to treat DTA with varying combinations of additional immunosuppression (including intravenous immunoglobulin, donor lymphocyte infusions, stem cell boosts, and other therapies) failed. Ten patients have received a conditioned second transplant, 9 from the same donor and 1 from a new matched unrelated donor. Aplasia has resolved in the remaining patient in response to ongoing eltrombopag therapy. All patients were alive at a mean of 92 months (range, 26 to 195) after a second transplant; 6 are in complete remission, but 4 suffered from second/recurrent DTA at 16 to 129 months after retransplant and required further transplant therapy.

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### INTRODUCTION

The treatment of choice for pediatric severe aplastic anemia (SAA) is currently an HLA matched sibling donor (MSD) transplant, with 2- to 5-year overall survival of 82% to 92% [1–4]. Historically, immunosuppressive therapy (IST) was given to those who lacked an MSD, but matched unrelated donor (MUD) transplant is now considered an alternative upfront treatment if a donor can be found quickly [2]. This change partly reflects excellent results from alternative donor transplants but also a lack of consistent long-term remission for those treated with IST alone [5–7].

Post-transplant chimerism testing is widely used to monitor engraftment and identify primary or secondary graft failure [8]. Intuitively, full (100%) donor chimerism is the outcome most likely to be associated with minimal risk of long-term myelodysplasia or relapse of aplastic anemia [9]. However, it has been shown that stable mixed chimerism may be superior for survival in aplastic anemia because of the low risk of graft-versus-host disease (GVHD) [10,11].

The European Society for Blood and Marrow Transplantation Severe Aplastic Anaemia (EBMT-SAA) Working Party data show that graft failure after hematopoietic stem cell transplant (HSCT) for SAA remains the third most common cause of death after infections and GVHD, with an incidence of 4% to 18% [12]. Graft failure after transplant can be either primary (where sustained engraftment never occurs and the patient remains pancytopenic, with chimerism of recipient origin) or secondary

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(engraftment occurs but is followed by progressive autologous reconstitution). Secondary graft failure produces 1 of 2 outcomes, either return of marrow aplasia and pancytopenia or retention of normal or acceptable blood counts because of immune suppression inherent in the transplant procedure allowing autologous hemopoietic reconstitution [13]. Autologous reconstitution, with recovery of peripheral blood counts accompanied by 100% recipient alleles in chimerism testing, has been reported in 4.2% of patients [14].

Following variable periods of normal blood counts after donor cell engraftment, some patients develop pancytopenia despite sustained high level mixed or complete donor chimerism, implying that SAA has occurred in donor cells. The term “donor-type aplasia” (DTA) has been introduced to describe this phenomenon [15]. DTA has been reported as late as 10 years after MSD transplant [16]. It is rare, having only been reported in single or small series case reports and conference abstracts, predominantly from Asia [15,17–19]. We report a series of 11 patients who developed DTA after transplant for SAA from Europe, the Middle East, and the United States and focus on management and outcome.

## METHODS

Patients were identified from the presentation of several cases at the 2014 EBMT Annual Meeting and subsequent extensive discussions with clinicians from the EBMT-SAA Working Party. Patients were included if younger than age 18 years at HSCT.

Neutrophil and platelet engraftment were defined as the first of 3 consecutive days with neutrophils exceeding  $.5 \times 10^9/L$  and platelets exceeding  $50 \times 10^9/L$ . Donor chimerism of >95% was categorized as full donor chimerism. DTA was defined as bone marrow aplasia in the presence of majority or full donor chimerism. Complete remission was defined as achieving normal blood counts without the need for transfusions, hematopoietic growth factors, or immunosuppression and partial remission as independence from transfusions without normalization of blood counts [20]. Medical charts were reviewed and data collected for diagnosis of SAA, pretransplant IST (if any), donor graft, conditioning therapy, engraftment details, transplant complications, aplasia recurrence and its treatment, and chimerism testing results.

## RESULTS

### Diagnosis

Eleven children (7 boys and 4 girls; Table 1) from 8 institutions in Europe, the United States, and the Middle East were identified as having developed DTA. They were transplanted between 2001 and 2014. Eight were of White ethnicity, 1 African American, and 2 Asian. Patients were initially diagnosed with SAA (5 patients) or very SAA (6 patients).

All initial diagnoses of SAA were believed to be idiopathic except unique patient number (UPN)1, where aplasia appeared to be secondary to parvovirus infection (detected by PCR at 28,000 copies/mL in blood at presentation). Cytogenetic analysis was normal in 10 cases and unavailable in 1. Fanconi anemia was excluded by the finding of normal chromosome fragility in all cases. Dyskeratosis congenita was excluded on the grounds of a lack of characteristic clinical features and/or genetic testing. A paroxysmal nocturnal hemoglobinuria clone was not detected in any patient.

All patients received RBC and platelet transfusions after diagnosis, but exact numbers could not be obtained because their pretransplant care occurred in multiple institutions. Transfusion refractoriness to platelet transfusions occurred in 1 patient (UPN2) in whom human platelet antigens-1b and -2b and HLA class I antibodies were detected and HLA antibodies to B44, B45, B57, and B58 were detected in another patient (UPN3).

**Table 1**  
Patient and First Transplant-Related Characteristics (n=11)

| Characteristic  | Value             |
|---|-------------------|
| <b>Ethnicity</b>  |                   |
| White   | 8                 |
| African American  | 1                 |
| Asian   | 2                 |
| <b>M:F</b>  | 7:4               |
| <b>Age at first transplant, yr</b>                        |                   |
| Mean (range)  | 10 (5.8–16.0)     |
| <b>Conditioning</b>                                       |                   |
| CY + ATG  | 5                 |
| CY + ALEM   | 3                 |
| CY + ALEM + FLU   | 2                 |
| CY + ALEM + TBI   | 1                 |
| <b>GvHD prophylaxis</b>                                   |                   |
| CSA and MTX   | 7                 |
| CSA   | 4                 |
| <b>Graft Type</b>   |                   |
| Bone marrow   | 11                |
| <b>Donor-recipient HLA match</b>                          |                   |
| MSD   | 9                 |
| MUD   | 2                 |
| <b>Donor-recipient sex match</b>                          |                   |
| Male donor / male recipient                               | 4                 |
| Male donor / female recipient                             | 2                 |
| Female donor / female recipient                           | 2                 |
| Female donor / male recipient                             | 3                 |
| <b>Donor-recipient CMV status</b>                         |                   |
| - / -   | 7                 |
| - / +   | 0                 |
| + / +   | 4                 |
| + / -   | 0                 |
| <b>Donor Age, yr</b>                                      |                   |
| Mean (range)  | 14 (4–41)         |
| <b>ABO Incompatibility</b>                                |                   |
| Major   | 5                 |
| Minor   | 1                 |
| Major + minor   | 0                 |
| None  | 5                 |
| <b>CD34+ cell dose, <math>\times 10^6</math> cells/kg</b> |                   |
| Mean (range)  | 5.54 (2.64–16.34) |
| Unknown   | 2                 |
| <b>TNC dose, <math>\times 10^8</math> cells/kg</b>        |                   |
| Mean (range)  | 4.41 (2.10–8.40)  |
| Unknown   | 2                 |

CY indicates cyclophosphamide, ATG, anti-thymocyte globulin; ALEM, Alemtuzumab; FLU, fludarabine; TBI, total body irradiation; GvHD, graft versus host disease; CSA, ciclosporin; MTX, methotrexate; HLA, human leucocyte antigen, CMV, cytomegalovirus; TNC, total nucleated cell.

### First Transplant

All transplants used bone marrow as the stem cell source. Nine patients received transplants from a MSD (matching to 10 antigen level in 8 patients and 6 antigens in 1 patient). The remaining 2 patients underwent transplantation from 10/10 MUDs; in UPN1 after failure to respond to IST and in UPN3 as an upfront procedure due to rapid availability of a 10/10 MUD in accordance with UK and EBMT-SAA working party guidance [2,3]. Transplants were performed at a mean age of 10 years 0 months (range, 5.7 to 16.0) and mean of 4 months (range, 1 week to 25 months) from diagnosis, respectively. Donors and recipients were sex matched in 6 cases. All cases were donor-recipient cytomegalovirus (CMV) status matched. ABO incompatibility was present in 6 patients (5 major and 1 minor).

Conditioning for MSD HSCT comprised cyclophosphamide at a cumulative dose of 200 mg/kg over 4 days plus antithymocyte globulin (ATG) in 5 cases (rabbit ATG in 4, horse in 1), alemtuzumab (3 cases) or alemtuzumab and 4 Gy total body irradiation in 1 case. GVHD prophylaxis comprised cyclosporin (CSA) and methotrexate in 7 patients and CSA alone in the

remaining 2 MSD transplants. The intention was to use immune suppression for a minimum of 9 to 12 months in all cases [21].

Conditioning in the 2 MUD transplants comprised cyclophosphamide 120 mg/kg, fludarabine 150 mg/m<sup>2</sup>, and alemtuzumab .9 mg/kg total dose; CSA alone was used as GVHD prophylaxis. UPN3 developed renal impairment secondary to CSA administration and was switched to mycophenolate mofetil after 2 months. IST was continued for a minimum of the intended 12 months in 4 patients but was stopped early in 7 patients (at a mean 4.5 months; range, 6 weeks to 8 months).

Neutrophil engraftment occurred at a mean of 23 days post-transplant (range, 15 to 38) and platelet engraftment at 44 days (range, 13 to 135) (data from 10 patients). UPN1 experienced ongoing thrombocytopenia with platelet counts fluctuating from 6 to 65 × 10<sup>9</sup>/L in the first 3 months post-transplant with otherwise satisfactory engraftment, receiving 5 platelet transfusions. CSA was stopped 3 months post-transplant with little improvement in platelets. One month later an unconditioned CD34<sup>+</sup> stem cell boost (2.5 × 10<sup>6</sup>/kg) was administered after which the patient became transfusion independent.

UPN4 (CMV -/-) developed primary CMV infection 5 weeks post-transplant. Ganciclovir was administered, but the patient remained persistently CMV-PCR positive (although without signs of active disease) and immunosuppression was therefore withdrawn at 2 months. The patient also fluctuated between having negative and low level Epstein-Barr virus (EBV) infection during this time, eventually becoming PCR negative for both CMV and EBV 6 months after transplant. UPN6 developed EBV reactivation at a level of 150,000 copies/mL 6 weeks after transplant and was successfully treated with doses of weekly rituximab. EBV transiently reactivated 7 months later but at a lower level of below 40,000 copies/mL, which did not require further treatment. Only 1 patient (UPN5) developed limited GVHD in the form of de novo chronic skin GVHD at 6 months post-transplant.

### Donor-Type Aplasia

DTA developed at a mean of 35 months post-transplant (median, 36 months; range, 8 to 93) (Table 2). Bone marrow biopsies were reported in 9 patients, and all showed hypocellular bone marrow. Four patients had full donor chimerism (>95%) and the remaining 7 patients mixed chimerism from 61% to 94% at development of DTA.

At the time of initial diagnosis of DTA, 4 of 11 patients (36.4%) were diagnosed with concomitant viral infections: 3 with parvovirus (2 with primary parvovirus and 1 with parvovirus reactivation) and 1 with EBV (Table 2). One further patient (UPN5) had *Aspergillus* pneumonia at the time of DTA diagnosis. UPN11 had autoimmune hemolytic anemia with direct antiglobulin test 2+, alongside anti-HLA antibodies and donor-specific antibody at the time of DTA.

Two patients proceeded straight to a second HSCT after development of DTA. The remaining 9 patients were initially treated using the following approaches, either alone or in combination: CD34<sup>+</sup> cell infusion (4 patients), CSA (n = 3), intravenous immunoglobulin (n = 3), ATG (n = 2), steroids (n = 2), donor lymphocyte infusion (n = 1), plasmapheresis (n = 1), rituximab (n = 1), sirolimus (n = 1), and eltrombopag (n = 1). Full details are given in Table 2.

Only the single patient (UPN3) treated with eltrombopag became transfusion independent, with blood counts gradually improving over 15 months from starting therapy to a normal hemoglobin, neutrophils > 1 × 10<sup>9</sup>/L, and platelets 90 to

110 × 10<sup>9</sup>/L. This patient remains well and has not been retransplanted; donor chimerism remained stable at 99% (CD3 98%) at 6 and 18 months after commencing eltrombopag.

### Second Transplant

A second HSCT was performed using the same donor in 9 cases and a new 10/10 antigen matched MUD in 1 (UPN7). Second transplants were carried out at a mean of 6 months (range, 2 to 13) after development of DTA. The average duration of peripheral cytopenia in each cell line between DTA and second transplant (data from 8/10 patients who underwent a second transplant) was 4.1 months of anemia, 5.2 months of thrombocytopenia, and 4.4 months of neutropenia. Second transplant characteristics are detailed in Table 3.

### Follow-Up

All patients were alive at the latest follow-up at a mean of 92 months (range, 26 to 195) after receiving their second transplant. The patient who remains under observation after additional IST and treatment with eltrombopag (UPN3) is alive at 62 months after the first transplant in partial remission.

Complications are listed in Table 2. Seven patients were in complete remission. However, 2 had significant *Aspergillus* infections (UPN4 and UPN8) and 3 had CMV reactivations (UPN4, UPN10, and UPN11). No patient developed signs of myelodysplasia during follow-up. One patient (UPN1) had mild anemia (Hb 117) and impaired renal function at 86 months post-transplant, considered to be secondary to severe atypical hemolytic uremic syndrome that developed as a complication of retransplantation.

Four patients (UPN4 to 6 and UPN11) developed a second episode of DTA, of whom 2 received a third transplant. UPN5 developed low platelets and anemia with a hypocellular bone marrow despite 100% donor chimerism 20 months after MSD retransplantation and underwent a third transplant procedure 14 months later using a 10/10 MUD. Despite engraftment on day +20, the patient suffered from liver and pulmonary toxicity and had poor graft function with RBC and platelet transfusion dependence at the time of writing. UPN6 required a third transplant after a second episode of DTA after a herpes zoster infection at 16 months after the second transplant. GVHD prophylaxis was stopped 6 months after the second transplant because of a persistent reactivation of parvovirus. She received a 5/6 unrelated cord transplant with CD34-selected haplo-identical stem cell support and is in complete remission at 83 months after the third transplant.

The 2 other patients who developed recurrent DTA (UPN4 and UPN11) did so at 129 and 16 months, respectively, after the second transplant (donor chimerism 95% [CD3 91%] and 93% to 97%, respectively; all viral PCRs were negative). UPN4, who had completely normal blood counts for over 10 years after MSD retransplantation, is currently requiring significant blood product support, and a MUD transplant is currently planned. UPN11 received a 2-month course of eltrombopag but remained severely thrombocytopenic and RBC transfusion and granulocyte colony-stimulating factor dependent. A peripheral blood stem cell boost caused an increase in platelets by 200 to 300 × 10<sup>9</sup>/L. At day +100 after the boost, the patient developed chronic GVHD of the mouth.

### DISCUSSION

DTA was first reported in 2 patients with “late graft rejection”; surprisingly, 1 of these occurred at 10 years after MSD transplant and in the context of 76% to 99% donor chimerism [16]. Retransplantation from the same donor was successful,

**Table 2**

Characteristics of Patients at the Time of DTA, Donor Source, and Complications after Replantation

| Patient No.<br>(Gender, Age at<br>First Transplant) | Initial<br>Transplant<br>Donor | Timing of DTA<br>(months post-<br>transplant) | Infection at<br>the Time of DTA | Whole Blood<br>Chimerism at the<br>Time of DTA (CD3<br>Chimerism Stated<br>Where Available) | Treatment Given for<br>DTA Before Second<br>HSCT   | Donor for Second<br>Transplant    | Complications  | Complete/ Partial<br>Remission   |
|---|--------------------------------|---|---------------------------------|---|--|-----------------------------------|--|--|
| UPN1 M, 9y 4m                                       | MUD                            | 12  | Parvovirus reactivation         | 93% (80%)   | CD34 <sup>+</sup> cell infusion  | Same                              | Atypical HUS; residual<br>anemia (Hb 117 g/L),<br>and elevated creatinine                                    | CR WBC (100%; CD3<br>100%)   |
| UPN2 M, 6y 2m                                       | MSD                            | 22  | Suspected viral infection       | 92% (83%)   | None   | Same                              | Eczema   | CR   |
| UPN3 F, 11y 1m                                      | MUD                            | 8   | None                            | 100%  | 2x CD34 <sup>+</sup> cell infusion,<br>trial of IVIG/ sirolimus/<br>rituximab, eltrombopag | No second transplant<br>performed | N/A  | PR; platelets 80-100,<br>neutrophils >1 × 10 <sup>9</sup> /L               |
| UPN4 F, 7y 3m                                       | MSD                            | 18  | Primary parvovirus              | 79% (71%)   | CD34 <sup>+</sup> cell infusion  | Same                              | CMV and EBV reactiva-<br>tion, <i>Aspergillus</i> , DTA<br>recurrence  | Awaiting MUD<br>transplant   |
| UPN5 M, 16y   | MSD                            | 93  | <i>Aspergillus</i> pneumonia    | 100%  | CD34 <sup>+</sup> cell infusion, ATG   | Same                              | Squamous cell carci-<br>noma of lip, DTA<br>recurrence   | Third transplant (10/10<br>MUD); RBC and platelet<br>transfusion dependent |
| UPN6 F, 5y 8m                                       | MSD                            | 44  | Primary parvovirus              | 91% (72%)   | 2x IVIG  | Same                              | DTA recurrence after<br>herpes zoster infection  | Third transplant (hap-<br>loidentical and 5/6<br>unrelated cord); CR       |
| UPN7 M, 10y 7m                                      | MSD                            | 40  | None                            | 96% (90%)   | None   | MUD                               | Grade II gut GVHD, renal<br>impairment, AVN femo-<br>ral condyle   | CR (although initial<br>thrombocytopenia, Rx<br>eltrombopag)               |
| UPN8 M, 15y 8m                                      | MSD                            | 24  | None                            | 100%  | CSA  | Same                              | <i>Aspergillus</i> infection<br>(pulmonary and renal)  | CR   |
| UPN9 M, 7y  | MSD                            | 36  | None                            | 61%   | DLI x 3, CSA and ATG   | Same                              | None reported  | CR   |
| UPN10 M, 10y 11m                                    | MSD                            | 41  | EBV (29,000 copies/mL)          | 83%   | Steroids   | Same                              | <i>Clostridium difficile</i> coli-<br>tis, CMV reactivation  | CR   |
| UPN11 F, 10y 10m                                    | MSD                            | 46  | None                            | 92%-94%   | Plasmapheresis; IVIG,<br>steroids and CSA  | Same                              | CMV reactivation, ade-<br>novirus gastroenteritis,<br>internal jugular vein<br>thrombosis, DTA<br>recurrence | Eltrombopag and PBSC<br>boost; developed<br>chronic GVHD                   |

HUS indicates hemolytic uremic syndrome; CR, complete remission; PR, partial remission; IVIG, intravenous immunoglobulin; AVN, avascular necrosis; DLI, donor lymphocyte infusion; PBSC, peripheral blood stem cell.

**Table 3**  
Characteristics of Second Transplant

| Characteristic                     | Value | Characteristic   | Value            |
|------------------------------------|-------|--|------------------|
| Graft type                         |       | GVHD prophylaxis                                       |                  |
| BM                                 | 6     | CSA  | 4                |
| PBSC                               | 4     | CSA + MMF  | 2                |
|                                    |       | MTX + tacrolimus                                       | 1                |
| Conditioning                       |       | CSA + MTX  | 1                |
| CY + FLU + ALEM                    | 2     | CSA + tacrolimus                                       | 1                |
| CY + ATG                           | 2     | None (ALEM added to graft)                             | 1                |
| CY + FLU + ATG                     | 1     |  |                  |
| CY + FLU + ATG + rituximab         | 1     | CD34 <sup>+</sup> cell dose, ×10 <sup>6</sup> cells/kg |                  |
| CY + FLU + ATG + TBI               | 1     | Mean (range)   | 5.08 (2.60–8.48) |
| ALEM + FLU + treosulfan + thiotepa | 1     |  |                  |
| FLU + busulfan + ALEM + ATG        | 1     |  |                  |
| FLU + ATG + rituximab              | 1     |  |                  |

BM indicates bone marrow; MMF, mycophenolate mofetil; PBSC, peripheral blood stem cells; CY, cyclophosphamide; FLU, fludarabine; ALEM, Alemtuzumab; ATG, anti-thymocyte globulin; TBI, total body irradiation; GvHD, graft versus host disease; CSA, ciclosporin; MTX, methotrexate.

and normal counts were reported at 8 and 16 years after the second transplant, respectively.

A subsequent study of 3 patients who had received MSD transplants reported development of DTA at 10 to 24 months post-transplant with myeloid donor chimerism from 26% to 82% [17]. All were retransplanted from their original donor, and their blood counts remained normal at 7 to 22 months post-transplant at publication. However, subsequently 1 of these patients had 2 further episodes of DTA after repeat transplants from the original MSD. Problems only resolved after a fourth transplant using a MUD; this patient remained well at 5 years follow-up from last transplant (Shakila Khan, personal communication).

Several studies from Japan have looked at the incidence and risk factors for DTA [15,18]. The Japanese registry data from 1980 to 2010 identified DTA in 5.7% of 660 patients younger than age 20 years after either MSD or MUD transplants [15]. Statistically significant correlations were reported between DTA and use of fludarabine for conditioning ( $P < .0001$ ), low infused total nucleated cell dose ( $\leq 3 \times 10^8$ /kg,  $P = .008$ ), and use of IST ( $P = .04$ ) pretransplant.

South Korean data have shown a considerably higher incidence of DTA at 26.2% (11/42 patients) [19]. Similar to the Japanese studies, statistically significant associations were seen with a low infused cell dose ( $P = .002$ ) and prior use of IST ( $P = .003$ ). An association was also seen if the patient had received >40 transfusions before transplant ( $P = .008$ ). Stem cell rescue was used in 4 of 11 cases of DTA, with only 2 of these treatments providing long-term restoration of graft function.

In the current series of 11 patients, the first episode of DTA appeared at a mean of 35 months post-transplant, with the latest at 93 months; 9 cases (82%) followed MSD transplants. We could not, however, confirm any correlations seen in the Japanese and South Korean studies. Only 1 patient had received prior IST, 2 received fludarabine in their conditioning regimen, and only 3 patients had a reported cell dose  $\leq 3 \times 10^8$  cells/kg (mean,  $5.54 \times 10^8$  cells/kg). Furthermore, our patients were not highly transfused because mean time from diagnosis to transplantation was only 4 months.

The true incidence of DTA in Europe and the United States is unknown. Cesaro et al. [22] reported that 14% of pediatric and adult EBMT SAA patients receiving allogeneic bone marrow or peripheral blood stem cell transplants between 1998 and 2009 experienced primary or secondary graft failure and that 38% of these patients underwent a second HSCT. However, no specific data on how many patients experienced DTA or the

chimerism analysis at the time of secondary graft failure were obtained (Simone Cesaro, personal communication).

Although graft failure appears to be conceptually well understood [13], the cause of DTA is not. It is noteworthy that there were no cases of DTA reported in a series of 45 fludarabine, cyclophosphamide, and ATG conditioned transplants in adults where all patients received GVHD prophylaxis for at least 6 months and 84% for over 1 year post-transplant despite mixed T cell chimerism [23]. By contrast, early withdrawal of GVHD prophylaxis had occurred in 7 patients in this series for a variety of reasons, including protracted primary CMV infection, persistent unexplained cytopenia, and low donor chimerism. The long interval between cessation of GVHD prophylaxis and onset of DTA in most patients argues against early withdrawal of immunosuppression as a cause. However, it is possible that the time course of stem cell destruction may be more protracted than believed; there is recurrence of the “malignant” marrow-destroying lymphoid clone, despite high levels of overall donor cell engraftment; or recurrence of SAA critically requires a “second hit” such as precipitating viral infection. If the latter were true, it may be relevant that 4 patients had viral infections identified at onset of DTA (2 primary parvovirus infections, 1 parvovirus reactivation, and 1 EBV).

It is believed that antigen-driven proliferation of pathogen-specific recipient T cells explains frequent persistence of recipient CD8 T cells even beyond 1 year post-transplant in fludarabine/cyclophosphamide/alemtuzumab transplants for SAA [23]. T cell depletion with ATG or alemtuzumab was used in all patients in our series. Mixed T cell chimerism was detected in all patients in whom it was assessed (3 in whom T cell chimerism was not assessed had 100% whole blood chimerism). A cross-reactive immune attack on stem cells by persisting recipient T cells could therefore potentially be the cause of DTA.

Of all treatments used initially for DTA (including CD34<sup>+</sup> stem cell infusion, donor lymphocyte infusion, intravenous immunoglobulin, or reintroduction of immunosuppression) only eltrombopag produced sustained remission (in the single patient treated for an adequate period). Ten patients proceeded to a formal second conditioned HSCT, 9 from their original donor (8 MSD and 1 MUD), and this was successful in 5 of these 9. It could be argued that retransplantation succeeds by driving cross-reactive T cells to extinction or below a critical threshold. However, it should be noted that in 4 of our patients and 1 of 3 previously reported by Rodriguez et al. [17], DTA recurred after a second HSCT using the original MSD. Two of these patients were eventually successfully transplanted from



an alternative unrelated donor; 1 will undergo a MUD transplant and the fourth will proceed to MUD transplant if a trial of eltrombopag and peripheral blood stem cell boosting does not produce sustained remission.

One patient avoided a second transplant after eltrombopag treatment (duration 20 months at publication) after unsuccessful attempts at a CD34<sup>+</sup> stem cell boost, intravenous immunoglobulin, and immunosuppression with rituximab and sirolimus. Eltrombopag has efficacy as upfront treatment in SAA, in the refractory or relapse setting, either as monotherapy or combined with immunosuppression [24–28]. However, cytogenetic clonal evolution has been reported in up to 19% of treated patients, mostly within 6 months of initiation, leaving the advisability of this approach unclear, especially in a pediatric population [25,28].

Although aplastic anemia is predominantly still regarded as idiopathic, not caused by somatic mutations, all but 2 of our cases occurred after MSD transplant. We therefore cannot exclude the possibility that genetic influences may play a role in DTA. If this were the case, retransplantation would be expected to be ineffective using the primary donor. Exome/whole genome sequencing or telomere analysis of the sibling pairs where DTA occurs may therefore prove to be an important area of future study.

In summary, recurrent immunosuppression or stem cell boosting after DTA occurring after transplant for aplastic anemia has not been shown to be effective in this series of patients. Treatment with eltrombopag was effective in the only patient treated for an adequate period, but long-term efficacy and risks are unknown. A second transplant from the same or a different donor appears to have a high chance of success, but substantial risk of recurrent DTA still persists and may occur more than a decade post-transplant, requiring a third transplant. Time may prove that the optimal treatment for recurrent DTA is retransplantation with an alternative donor.

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