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## Hematopoietic stem cell transplantation for acquired aplastic anemia

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### Abstract

**Purpose of review**—There has been steady improvement in outcomes with allogeneic bone marrow transplantation (BMT) for severe aplastic anemia (SAA), due to progress in optimization of the conditioning regimens, donor hematopoietic cell source and supportive care. Here we review recently published data that highlight the improvements and current issues in the treatment of SAA.

**Recent findings**—Approximately one-third of AA patients treated with immune suppression therapy (IST) have acquired mutations in myeloid cancer candidate genes. Because of the greater probability for eventual failure of IST, human leukocyte antigen (HLA)-matched sibling donor BMT is the first-line of treatment for SAA. HLA-matched unrelated donor (URD) BMT is generally recommended for patients who have failed IST. However, in younger patients for whom a 10/10-HLA-allele matched URD can be rapidly identified, there is a strong rationale to proceed with URD BMT as first-line therapy. HLA-haploidentical BMT using post-transplant cyclophosphamide (PT-CY) conditioning regimens, is now a reasonable second-line treatment for patients who failed IST.

**Summary**—Improved outcomes have led to an increased first-line role of BMT for treatment of SAA. The optimal cell source from an HLA-matched donor is bone marrow. Additional studies are needed to determine the optimal conditioning regimen for HLA-haploidentical donors.

### Keywords

Anti-thymocyte globulin; allogeneic bone marrow transplantation; severe aplastic anemia

### Introduction

Aplastic anemia (AA) is a rare and potentially life threatening disorder that is caused by immune-mediated destruction of hematopoietic stem and progenitor cells (HSC). Allogeneic bone marrow transplantation (BMT) is well established as curative treatment. However,

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patients may also have a response to immune suppressive therapy (IST). Although prospective randomized clinical trials have not been performed, studies have consistently shown a survival benefit for patients who underwent human leukocyte antigen (HLA)-identical sibling BMT(1–6)\*. New insights into the genetic alterations that occur with bone marrow failure and the inferior outcome with rabbit anti-thymocyte globulin (ATG) as IST, support the rationale for patients to proceed to allogeneic BMT as first-line treatment. With reduced toxicity conditioning regimens and optimization of the donor graft, the long term outcome of HLA-matched unrelated donor (URD) BMT has significantly improved. The challenge for hematologists is to determine when to treat patients with SAA with IST or HLA-matched URD BMT or HLA-haploidentical hematopoietic stem cell transplantation (HSCT). Recent data support the decision to proceed to URD BMT as first-line therapy for younger patients if an HLA-matched donor can be rapidly identified.

### **Etiology of Aplastic Anemia and Immune Suppressive Therapy (IST)**

The value of immunosuppressive agents for the treatment of SAA was first described by Mathé *et al.* in 1970(7). Subsequently, cyclosporine (CSP) added to ATG achieved better responses(8). In a randomized study conducted at the NIH, horse ATG was shown to be superior to rabbit ATG as first-line IST for SAA(9). Unfortunately, horse ATG is not available in most countries outside the United States. Follow-up reports confirmed that rabbit ATG+CSP was significantly less effective than horse ATG+CSP as initial IST for AA(10–12) The responsiveness of AA to IST is evidence for the immune-mediated pathophysiology of the disease(13).

### **Clonal Hematopoiesis and Somatic Mutations in Acquired Aplastic Anemia**

For many years it has been recognized that a subset of patients with AA treated with IST has clonal hematopoiesis and some eventually develop cytogenetic abnormalities that lead to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML)(14–16). With the development of high-throughput sequencing, the molecular basis of clonal hematopoiesis in AA has been comprehensively assessed(17)\*. Yoshizato *et al.* detected genetic abnormalities in ~50% of 439 patients at 6 months after IST(18)\*\*. One-third of these patients had acquired somatic mutations in myeloid cancer candidate genes. With the exception of PIGA mutations and uniparental disomy of the 6p arm (6pUPD), the most frequent mutations were in genes mutated in MDS/AML including DNMT3A, ASXL1 and BCOR/BCORL1. These mutations appeared to have distinct patterns of evolution over time with distinct impact on clinical outcomes. AA patients with BCOR/ BCORL1 or PIGA mutations had a better response to IST and better long term survival with IST while AA patients with ASXL1, DNMT3A, TP53, RUNX1, JAK2, JAK3, and CSMD1 mutations had a significantly inferior response to IST and worse overall survival(18)\*\*.

Kulasekararaj *et al.* found acquired somatic mutations in one-fifth of AA patients and most involved ASXL1, DNMT3A and BCOR. Patients with somatic mutations had longer disease duration, shorter telomere lengths and a 40% risk of subsequent transformation to MDS(19)\*. In a similar study, accelerated telomere attrition was seen in SAA patients with somatic myeloid cancer gene mutations that progressed to MDS or AML with

monosomy-7(20). Another study found mutations in ASXL1 were associated with progression to MDS(21). Using whole exome sequencing, one group reported somatic mutations in 73% of SAA patients, involving 51 genes, including PIGA, 6pUPD, and in signaling pathways STAT5B and CAMK2G(22)\*.

In summary, deep sequencing for commonly mutated MDS/AML genes may be useful to determine if patients with poor-prognosis somatic mutations should be referred for allogeneic BMT either prior to failure of IST or to prevent the subsequent progression to MDS/AML. Although patients with SAA who evolve to MDS/AML can be successfully treated with BMT, outcomes are inferior to BMT early after the diagnosis of SAA(23, 24).

## Cytogenetic abnormalities after treatment with TPO-mimetics

Eltrombopag, an oral thrombopoietin (TPO) receptor agonist, has been evaluated in refractory SAA patients who remained pancytopenic after IST. TPO receptor is present on HSCs and eltrombopag stimulates proliferation of residual HSCs. In a follow-up report of 43 SAA patients, the overall response rate to eltrombopag was 40% at 4 months. A small proportion of patients were able to discontinue eltrombopag with normal blood counts. However, 19% developed cytogenetic abnormalities including -7, a poor prognosis finding(25). These results are similar to the observation that G-CSF treatment concurrent with IST increased the risk of development of -7 abnormalities and progression to MDS/AML(26–28).

While IST may be effective in ameliorating the pancytopenia of AA, the development of somatic mutations results in significant risk for evolution to MDS/AML. The new molecular findings of a relatively high incidence of acquired mutations in myeloid cancer genes support the rationale to proceed to allogeneic BMT as first-line treatment, if a suitable donor can be identified, since BMT offers the greatest potential for definitive curative therapy of SAA.

## HLA-identical sibling donor BMT

Although there had been a steady and impressive improvement in survival after HLA-identical sibling donor BMT, when several transplant centers began using G-CSF mobilized peripheral blood stem cells (PBSC) as the graft source, outcomes actually worsened(29) owing to an increased incidence of chronic graft-versus-host disease (GVHD)(30) and associated mortality. Bone marrow is the only acceptable HSC source for patients with SAA. Additional details regarding the optimal conditioning regimen, cell dose and post-transplant immunosuppression were recently reviewed(31).

Reduction in the incidence of late chronic GVHD is achieved when the infused donor bone marrow cell dose (total nucleated cells corrected for peripheral white blood cell count) is limited to  $2.5 \times 10^8/\text{kg}$ (32). This is most effective when infused after cyclophosphamide (CY)-200 mg/kg and horse ATG, followed by GVHD prophylaxis with CSP and methotrexate (MTX). Using this regimen for HLA-identical sibling donors, the risk of chronic GVHD is less than 10%, and the overall survival is outstanding, particularly for younger patients where 100% survival has been reported(33).

In summary, a very effective conditioning regimen is the Seattle regimen of CY-200/ horse ATG/ HLA-identical sibling BMT and CSP/MTX. Tacrolimus appears to be approximately equivalent to CSP to prevent GVHD(34). However, for older patients, the development of fludarabine (FLU)-based conditioning regimens with reduced CY dose has achieved good survival outcomes(35, 36).

## Unrelated donor BMT

The outcomes after URD BMT for SAA have steadily improved over the past 3 decades. Bacigalupo *et al.* compared the outcome of HLA-matched URD ( $n=508$ ) to sibling ( $n=940$ ) HSCT in European registries between 2005–2009. The incidences of acute and chronic GVHD were greater for URD recipients than HLA-identical recipients, 25% and 26% vs. 13% and 14%, respectively. Although the overall survival after URD was less than after HLA-identical sibling transplantation, the difference was not significant. The strongest negative predictor of survival was the use of PBSC, followed by the interval of diagnosis to transplant of 180 days, patient age > 20 years, no ATG in the conditioning regimen, and positive donor/recipient cytomegalovirus status(37)\*.

A prognostic risk score was developed by adding each negative predictor identified in multivariate analysis (stem cell source, interval diagnosis to transplant, age, ATG use, CMV status). This identified low-, intermediate- and high-risk groups. With a median follow-up of 3 years, survivals were 83%, 77% and 64%, respectively(37).

In summary, PBSC should not be used as HSC source with HLA-matched URD transplantation. To reduce the incidence of acute and chronic GVHD, ATG should be given with the conditioning regimen. When following these guidelines, including high-resolution 10/10-HLA-allele level matching, URD BMT for SAA results in excellent overall survival, especially for age <20 years(37, 38)\*\*.

Previously, the standard conditioning regimen for URD BMT was CY-200 mg/kg, horse ATG 90 mg/kg and 2 Gray (Gy) total body irradiation (TBI). However, this regimen is associated with increased risk of transplant related mortality particularly in patients >age 20 years(39).

An important step in improving the outcome of URD BMT has been to reduce toxicity of the conditioning regimen by reducing the dose of CY and increasing the dose of FLU. A multicenter trial(38) determined the optimal conditioning regimen to include: horse/ rabbit ATG 30 mg/kg/day or 3 mg/kg/day, respectively, on days -4 to -2, CY 50 mg/kg on day -4, FLU 30 mg/m<sup>2</sup>/day on days -5 to -2, and 2 Gy TBI on day -1. Bone marrow was used from either 8/8-HLA-matched or 1-HLA-antigen mismatched URDs. The study compared CY-0, -50, -100 and -150 mg/kg. The incidence of graft failure following CY-0 was unacceptably high and organ toxicity following CY-150 was unacceptably high. Most patients received either CY-50 ( $n=38$ ) or CY-100 mg/kg ( $n=41$ ). However, the distribution of HLA-mismatches in the 2 groups was not balanced: 18% and 34% of patients in the CY-50 and CY-100 group, respectively, received 7/8 HLA-matched bone marrow. The incidence of graft failure at 1 year was 11.7% and 14.6% in the CY-50 and -100 groups, respectively. Of the 4

CY-50 patients with graft failure, 3 survived, 2 after second transplant. Of the 6 CY-100 patients with secondary graft failure, all died, despite second transplants. The use of HLA-mismatched grafts contributed to the increased incidence of graft failure. The incidence of acute GVHD was 23.7% and 26.8% and chronic GVHD was 22.5% and 31.7% in the CY-50 and -100 groups, respectively. With a median follow-up of 17 and 24 months, the 1-year overall survival was 97.4% and 80.5% in the CY-50 and -100 cohorts, respectively. With longer follow-up, there were 3 deaths (8%) in the CY-50 group: 1 from graft failure and 2 from chronic GVHD. There were 10 deaths (24%) in the CY-100 group: 6 from graft failure, 2 from acute/ chronic GVHD, 1 from sepsis(38)\*\*.

In summary, the combination of ATG, CY 50 mg/kg, FLU 120 mg/m<sup>2</sup> and 2 Gy TBI followed with a 10/10 HLA-allele matched URD BMT appears to be the optimal regimen and graft source. The optimal regimen for 1-HLA-antigen mismatched URD BMT is not yet defined.

Marsh *et al.* showed that alemtuzumab (median dose 0.96 mg/kg) given in place of rabbit ATG during the conditioning regimen, combined with FLU 30 mg/m<sup>2</sup>/day × 4 days and CY 300 mg/m<sup>2</sup>/day × 4 days achieved improved overall survival following URD BMT. Fifty-five patients, median age 18 years, received HLA-matched URD transplants (19 PBSC and 36 BM) after alemtuzumab-FLU-CY conditioning. Graft failure was observed in 9%; acute and chronic GVHD incidences were 29% and 11%, respectively, and 5-year overall survival was 88%. Bone marrow graft recipients had improved overall survival compared to PBSC recipients. This study is significant because the alemtuzumab-FLU-CY conditioning regimen has eliminated the use of low dose TBI for URD BMT(36).

Both ATG-CY50-FLU-2 Gy TBI and alemtuzumab-CY-FLU are reasonable conditioning regimens for HLA-matched URD BMT. However, the limited availability, high cost, and infectious complications associated with alemtuzumab may limit its broader use in URD-BMT for SAA.

Dufour *et al.* reported on the outcome of children with acquired SAA treated with (a) URD HSCT as first-line treatment (*n*=29, no IST prior to the transplant), and compared the outcome to the following historical matched control groups (b) HLA-identical sibling donor BMT (*n*=87), (c) IST with horse ATG and CSP (*n*=58) and (d) second-line therapy with URD transplant after IST failure (*n*=24). The 2-year overall survival and event-free survival in each group were as follows: (a) first-line URD HSCT: 96% and 92%, (b) HLA-matched sibling donor BMT: 91% and 87%, (c) first-line IST: 94% and 40%, (d) URD HSCT after IST failure: 74% and 74%, respectively (40)\*. Others also support the recommendation to proceed with HLA-matched URD BMT as first-line therapy for children with SAA(41, 42).

These data suggest that first-line treatment with an HLA-matched URD BMT should be strongly considered for children with acquired SAA who lack an HLA-identical sibling donor. Given the improvement in outcomes with the FLU-based conditioning regimens with low dose TBI, CY and ATG or alemtuzumab(36, 38), and given the increased risk of failure and late complications after IST, strong consideration should also be made for young adults with SAA to proceed with definitive BMT if a 10/10 HLA-matched URD is readily

identified. Although URD BMT can be successfully performed after IST failure or after evolution to MDS/AML, overall survival appears to be reduced when URD BMT is used as second-line treatment.

## HLA-haploidentical family donor BMT

The successful application of post-transplant CY (PT-CY) conditioning for HLA-haploidentical donors has recently been reported for SAA patients. Clay *et al.* described 8 patients, 4 with refractory SAA following IST and 4 with primary graft failure after URD or cord blood transplantation(43)\*\*. The conditioning regimen was FLU 30 mg/m<sup>2</sup>/day days -6 to -2, CY 14.5 mg/kg/day (days -6 and -5) and 2 Gy TBI on day -1. HLA-haploidentical, unmanipulated, G-CSF-mobilized PBSC were infused on day 0, followed by PT-CY 50 mg/kg/day on days +3 and +4. Starting on day +5, post-transplant GVHD prophylaxis included tacrolimus for 12 months and mycophenolate mofetil (MMF) until day +35. Six patients had sustained donor engraftment, 2 patients failed to engraft and subsequently died and one patient who engrafted subsequently died of multiple infectious complications. The 2 patients who failed to engraft had persistent, multiple anti-donor HLA-antibodies. Five of the 8 patients survived 3–40 months after transplant, 1 had successfully treated grade II skin GVHD(43)\*\*. Other investigators have reported similar results with this regimen, but patients received 2 to 6 Gy TBI(44).

The use of PT-CY to achieve in vivo T-cell-depletion has simplified the clinical use of HLA-haploidentical grafts and has achieved encouraging early results. It remains to be determined if bone marrow or PBSC is the preferred graft source from HLA-haploidentical donors. Currently, HLA-haploidentical grafts should be used as second- or third-line therapy for SAA once IST has failed and if an HLA-matched URD is unavailable.

## Conclusion

The overall survival after HSCT for SAA has improved significantly over the past few years, particularly for HLA-matched URD BMT. Survival outcomes from URD BMT are now approaching the success seen with HLA-identical sibling BMT. Recent studies with molecular sequencing of bone marrow blood cells from SAA patients treated with IST have found a large proportion of patients with clonal hematopoiesis and acquired mutations in myeloid leukemia genes. These mutations appeared early after IST and increased in frequency over time. Some of these acquired mutations confer a very high risk of progression to MDS/AML.

Given the high incidence of treatment failure with IST, the risk of evolution to MDS/AML after IST, and the improved outcomes with URD BMT, it is now reasonable to consider that younger patients with SAA should proceed to URD BMT as first-line treatment if a 10/10 HLA-matched donor is rapidly identified. With increased availability of URDs and rapid, high-resolution HLA-typing, an urgent URD search may be completed within several weeks. FLU-based conditioning regimens appear to reduce the risk of transplant related toxicity for URD BMT, especially in older patients. As with HLA-identical sibling transplantation, bone marrow is the only appropriate stem cell source from URDs for first-time HSCT for SAA.

With the introduction of PT-CY conditioning regimens, HLA-haploidentical HSCT appears to be a good second- or third-line treatment alternative for patients with refractory SAA who do not have an HLA-matched donor, particularly if patients lack anti-donor HLA-antibodies. The data from small studies are encouraging, but larger multicenter studies with optimized conditioning regimens and determination of the optimal stem cell source for HLA-haploidentical HSCT are needed.

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## References and Recommended Reading

Papers of particular interest, published within the annual period of review have been highlighted as:

\* Of special interest

\*\* Of outstanding interest

1. Dufour C, Pillon M, Socie G, Rovo A, Carraro E, Bacigalupo A, et al. Outcome of aplastic anaemia in children. A study by the severe aplastic anaemia and paediatric disease working parties of the European group blood and bone marrow transplant. *Br J Haematol.* 2015; 169(4):565–573. [PubMed: 25683884] \* This manuscript presents the most recent EBMT registry pediatric SAA data. HLA-identical sibling BMT remains the first choice of treatment for SAA.
2. Dufour C, Pillon M, Passweg J, Socie G, Bacigalupo A, Franceschetto G, et al. Outcome of aplastic anemia in adolescence: a survey of the Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation. *Haematologica.* 2014; 99(10):1574–1581. [PubMed: 25085353]
3. Yoshida N, Kobayashi R, Yabe H, Kosaka Y, Yagasaki H, Watanabe K, et al. First-line treatment for severe aplastic anemia in children: bone marrow transplantation from a matched family donor versus immunosuppressive therapy. *Haematologica.* 2014; 99(12):1784–1791. [PubMed: 25193958]
4. Doney K, Leisenring W, Storb R, Appelbaum FR. Primary treatment of acquired aplastic anemia: outcomes with bone marrow transplantation and immunosuppressive therapy. Seattle Bone Marrow Transplant Team. *Annals of internal medicine.* 1997; 126(2):107–115. [PubMed: 9005744]
5. Kojima S, Horibe K, Inaba J, Yoshimi A, Takahashi Y, Kudo K, et al. Long-term outcome of acquired aplastic anaemia in children: comparison between immunosuppressive therapy and bone marrow transplantation. *Br J Haematol.* 2000; 111(1):321–328. [PubMed: 11091219]
6. Locasciulli A, Oneto R, Bacigalupo A, Socie G, Korthof E, Bekassy A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica.* 2007; 92(1):11–18. [PubMed: 17229630]
7. Mathe G, Amiel JL, Schwarzenberg L, Choay J, Trolard P, Schneider M, et al. Bone marrow graft in man after conditioning by antilymphocytic serum. *British medical journal.* 1970; 2(5702):131–136. [PubMed: 4909449]
8. Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmeier H. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. *Blood.* 2003; 101(4):1236–1242. [PubMed: 12393680]
9. Scheinberg P, Nunez O, Weinstein B, Scheinberg P, Biancotto A, Wu CO, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med.* 2011; 365(5):430–438. [PubMed: 21812672]

10. Marsh JC, Bacigalupo A, Schrezenmeier H, Tichelli A, Risitano AM, Passweg JR, et al. Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party. *Blood*. 2012; 119(23):5391–5396. [PubMed: 22544699]
11. Takahashi Y, Muramatsu H, Sakata N, Hyakuna N, Hamamoto K, Kobayashi R, et al. Rabbit antithymocyte globulin and cyclosporine as first-line therapy for children with acquired aplastic anemia. *Blood*. 2013; 121(5):862–863. [PubMed: 23372154]
12. Yoshimi A, Niemeyer CM, Fuhrer MM, Strahm B. Comparison of the efficacy of rabbit and horse antithymocyte globulin for the treatment of severe aplastic anemia in children. *Blood*. 2013; 121(5):860–861. [PubMed: 23372153]
13. Young NS. Current concepts in the pathophysiology and treatment of aplastic anemia. *Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program*. 2013; 2013:76–81.
14. Socie G, Henry-Amar M, Bacigalupo A, Hows J, Tichelli A, Ljungman P, et al. Malignant tumors occurring after treatment of aplastic anemia. *European Bone Marrow Transplantation-Severe Aplastic Anaemia Working Party*. *N Engl J Med*. 1993; 329(16):1152–1157. [PubMed: 8377778]
15. Ohara A, Kojima S, Hamajima N, Tsuchida M, Imashuku S, Ohta S, et al. Myelodysplastic syndrome and acute myelogenous leukemia as a late clonal complication in children with acquired aplastic anemia. *Blood*. 1997; 90(3):1009–1013. [PubMed: 9242530]
16. Tichelli A, Gratwohl A, Wursch A, Nissen C, Speck B. Late haematological complications in severe aplastic anaemia. *Br J Haematol*. 1988; 69(3):413–418. [PubMed: 3044440]
17. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. *Blood*. 2016 e-pub online: April 29, 2016. \* Concise yet comprehensive review article of recent molecular data evaluating clonal hematopoiesis and acquired mutations in SAA.
18. Yoshizato T, Dumitriu B, Hosokawa K, Makishima H, Yoshida K, Townsley D, et al. Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia. *N Engl J Med*. 2015; 373(1):35–47. [PubMed: 26132940] \*\* The most comprehensive analysis to date involving a large cohort of SAA patients revealed acquired mutations in approximately 50%. This study revealed a high frequency of myeloid cancer gene mutations in SAA patients treated with IST.
19. Kulasekararaj AG, Jiang J, Smith AE, Mohamedali AM, Mian S, Gandhi S, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. *Blood*. 2014; 124(17):2698–2704. [PubMed: 25139356] \* One of the initial studies of SAA patients treated with IST that used molecular DNA analysis to reveal acquired somatic mutations. Mutations were found in a limited number of genes associated with myeloid cancers and these mutations predicted evolution to MDS.
20. Dumitriu B, Feng X, Townsley DM, Ueda Y, Yoshizato T, Calado RT, et al. Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia. *Blood*. 2015; 125(4):706–709. [PubMed: 25406353]
21. Huang J, Ge M, Lu S, Shi J, Li X, Zhang J, et al. Mutations of ASXL1 and TET2 in aplastic anemia. *Haematologica*. 2015; 100(5):e172–e175. [PubMed: 25596262]
22. Babushok DV, Perdignes N, Perin JC, Olson TS, Ye W, Roth JJ, et al. Emergence of clonal hematopoiesis in the majority of patients with acquired aplastic anemia. *Cancer Genet*. 2015; 208(4):115–128. [PubMed: 25800665] \* Very comprehensive DNA sequence analysis of the coding region of the genome of bone marrow cells in a small cohort of mostly children with SAA.
23. Kim SY, Le Rademacher J, Antin JH, Anderlini P, Ayas M, Battiwalla M, et al. Myelodysplastic syndrome evolving from aplastic anemia treated with immunosuppressive therapy: efficacy of hematopoietic stem cell transplantation. *Haematologica*. 2014; 99(12):1868–1875. [PubMed: 25107891]
24. Yoshimi A, Strahm B, Baumann I, Furlan I, Schwarz S, Teigler-Schlegel A, et al. Hematopoietic stem cell transplantation in children and young adults with secondary myelodysplastic syndrome and acute myelogenous leukemia after aplastic anemia. *Biol Blood Marrow Transplant*. 2014; 20(3):425–429. [PubMed: 24316460]
25. Desmond R, Townsley DM, Dumitriu B, Olnes MJ, Scheinberg P, Bevans M, et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. *Blood*. 2014; 123(12):1818–1825. [PubMed: 24345753]



26. Kojima S, Ohara A, Tsuchida M, Kudoh T, Hanada R, Okimoto Y, et al. Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukemia after immunosuppressive therapy in children. *Blood*. 2002; 100(3):786–790. [PubMed: 12130487]
27. Socie G, Mary JY, Schrezenmeier H, Marsh J, Bacigalupo A, Locasciulli A, et al. Granulocyte-stimulating factor and severe aplastic anemia: a survey by the European Group for Blood and Marrow Transplantation (EBMT). *Blood*. 2007; 109(7):2794–2796. [PubMed: 17110459]
28. Hama A, Takahashi Y, Muramatsu H, Ito M, Narita A, Kosaka Y, et al. Comparison of long-term outcomes between children with aplastic anemia and refractory cytopenia of childhood who received immunosuppressive therapy with antithymocyte globulin and cyclosporine. *Haematologica*. 2015; 100(11):1426–1433. [PubMed: 26273061]
29. Bacigalupo A, Socie G, Schrezenmeier H, Tichelli A, Locasciulli A, Fuehrer M, et al. Bone marrow versus peripheral blood as the stem cell source for sibling transplants in acquired aplastic anemia: survival advantage for bone marrow in all age groups. *Haematologica*. 2012; 97(8):1142–1148. [PubMed: 22315497]
30. Schrezenmeier H, Passweg JR, Marsh JC, Bacigalupo A, Bredeson CN, Bullorsky E, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. *Blood*. 2007; 110(4):1397–1400. [PubMed: 17475907]
31. Georges, GE., Storb, R. Hematopoietic Cell Transplantation for Aplastic Anemia. In: Forman, SJ, Negrin, RS, Antin, JH., Appelbaum, FR., editors. *Thomas' hematopoietic cell transplantation: stem cell transplantation*. Vol. 1. Chichester, UK: Wiley Blackwell; 2016. p. 517-536.
32. Kahl C, Leisenring W, Deeg HJ, Chauncey TR, Flowers ME, Martin PJ, et al. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long-term follow-up. *Br J Haematol*. 2005; 130(5):747–751. [PubMed: 16115132]
33. Burroughs LM, Woolfrey AE, Storer BE, Deeg HJ, Flowers ME, Martin PJ, et al. Success of allogeneic marrow transplantation for children with severe aplastic anaemia. *Br J Haematol*. 2012; 158(1):120–128. [PubMed: 22533862]
34. Inamoto Y, Flowers ME, Wang T, Urbano-Ispizua A, Hemmer MT, Cutler CS, et al. Tacrolimus versus Cyclosporine after Hematopoietic Cell Transplantation for Acquired Aplastic Anemia. *Biol Blood Marrow Transplant*. 2015; 21(10):1776–1782. [PubMed: 26033280]
35. Maury S, Bacigalupo A, Anderlini P, Aljurf M, Marsh J, Socie G, et al. Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a comparison with conventional conditioning regimen. *Haematologica*. 2009; 94(9):1312–1315. [PubMed: 19734425]
36. Marsh JC, Pearce RM, Koh MB, Lim Z, Pagliuca A, Mufti GJ, et al. Retrospective study of alemtuzumab vs ATG-based conditioning without irradiation for unrelated and matched sibling donor transplants in acquired severe aplastic anemia: a study from the British Society for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2014; 49(1):42–48. [PubMed: 23912664]
37. Bacigalupo A, Socie G, Hamladji RM, Aljurf M, Maschan A, Kyrz-Krzemien S, et al. Current outcome of HLA identical sibling versus unrelated donor transplants in severe aplastic anemia: an EBMT analysis. *Haematologica*. 2015; 100(5):696–702. [PubMed: 25616576] \* This manuscript presents an analysis of recent European SAA registry data. Outcomes after HLA-matched URD BMT are now almost equal to HLA-identical sibling BMT for SAA.
38. Anderlini P, Wu J, Gersten I, Ewell M, Tolar J, Antin JH, et al. Cyclophosphamide conditioning in patients with severe aplastic anaemia given unrelated marrow transplantation: a phase 1–2 dose de-escalation study. *The Lancet Haematology*. 2015; 2(9):e367–e375. [PubMed: 26685770] \*\* Multicenter clinical trial in SAA patients evaluated different doses of CY conditioning prior to URD-BMT. The study found a CY dose that was less toxic, achieved reliable engraftment with improved long-term survival.
39. Deeg HJ, O'Donnell M, Tolar J, Agarwal R, Harris RE, Feig SA, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood*. 2006; 108(5):1485–1491. [PubMed: 16684959]

40. Dufour C, Veys P, Carraro E, Bhatnagar N, Pillon M, Wynn R, et al. Similar outcome of upfront-unrelated and matched sibling stem cell transplantation in idiopathic paediatric aplastic anaemia. A study on behalf of the UK Paediatric BMT Working Party, Paediatric Diseases Working Party and Severe Aplastic Anaemia Working Party of EBMT. *Br J Haematol.* 2015; 171(4):585–594. [PubMed: 26223288] \* This is the first study to report on first-line treatment of SAA with HLA-matched URD BMT, with excellent survival outcome in younger patients.
41. Mackarel J, Iatan M, Kumar L, Storey L, O'Marcaigh A, Smith O. In support of upfront stem cell transplantation as first-line therapy for paediatric patients with idiopathic severe aplastic anaemia who lack a sibling donor. *Br J Haematol.* 2016
42. Mortensen BK, Jacobsen N, Heilmann C, Sengelov H. Allogeneic hematopoietic cell transplantation for severe aplastic anemia: similar long-term overall survival after transplantation with related donors compared to unrelated donors. *Bone Marrow Transplant.* 2016; 51(2):288–290. [PubMed: 26437067]
43. Clay J, Kulasekararaj AG, Potter V, Grimaldi F, McLornan D, Raj K, et al. Nonmyeloablative peripheral blood haploidentical stem cell transplantation for refractory severe aplastic anemia. *Biol Blood Marrow Transplant.* 2014; 20(11):1711–1716. [PubMed: 25016195] \*\* This the first report of a small cohort of SAA patients receiving PT-CY conditioning with HLA-haploidentical PBSC transplantation. As a result of this study, a multi-center clinical trial of HLA-haploidentical HSCT with PT-CY for SAA has been initiated.
44. Esteves I, Bonfim C, Pasquini R, Funke V, Pereira NF, Rocha V, et al. Haploidentical BMT and post-transplant Cy for severe aplastic anemia: a multicenter retrospective study. *Bone Marrow Transplant.* 2015; 50(5):685–689. [PubMed: 25730184]

### Key Points

- Patients with severe aplastic anemia (SAA) treated with immune suppressive therapy (IST) acquire somatic mutations in myeloid cancer candidate genes at high frequency.
- HLA-identical sibling bone marrow transplantation (BMT) remains first-line treatment of SAA. Outcomes with HLA-identical sibling BMT are consistently superior to IST.
- Bone marrow is the only acceptable stem cell source for first-time HLA-identical sibling and HLA-matched unrelated donor (URD) hematopoietic stem cell transplantation (HSCT) for SAA.
- Due to improved outcomes with conditioning regimens consisting of fludarabine (FLU)/ low-dose cyclophosphamide (CY)/ low-dose total body irradiation (TBI), HLA-matched URD BMT can now be considered as first-line treatment for SAA in younger patients.
- HLA-haploidentical family member HSCT with the post-transplant cyclophosphamide (PT-CY) conditioning regimen has encouraging results and is considered second- or third-line treatment after failure of IST if no HLA-matched donor is available.