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Diagnosis and Treatment of Aplastic Anemia

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Opinion Statement

Acquired aplastic anemia (AA) is a rare, life-threatening bone marrow failure (BMF) disorder that affects patients of all ages and is caused by lymphocyte destruction of early hematopoietic cells. Diagnosis of AA requires a comprehensive approach with prompt evaluation for inherited and secondary causes of bone marrow aplasia, while providing aggressive supportive care. The choice of frontline therapy is determined by a number of factors including AA severity, age of the patient, donor availability, and access to optimal therapies. For newly diagnosed severe aplastic anemia, bone marrow transplant should be pursued in all pediatric patients and in younger adult patients when a matched sibling donor is available. Frontline therapy in older adult patients and in all patients lacking a matched sibling donor involves immunosuppressive therapy (IST) with horse antithymocyte globulin and cyclosporine A. Recent improvements in upfront therapy include encouraging results with upfront closely matched unrelated donor transplants in younger patients and the emerging benefits of eltrombopag combined with initial IST, with randomized studies underway. In the refractory setting, several therapeutic options exist, with improving outcomes of matched unrelated donor and haploidentical bone marrow transplantation as well as the addition of eltrombopag to the non-transplant AA armamentarium. With the recent appreciation of frequent clonal hematopoiesis in AA patients and with the growing use of next-generation sequencing in the clinic, utmost caution should be exercised in interpreting the significance of somatic mutations in AA. Future longitudinal studies of large numbers of patients are needed to determine the prognostic significance of somatic mutations and to guide optimal surveillance and treatment approaches to prevent long term clonal complications.

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Compliance with Ethics Guidelines

Conflict of Interest

The authors have no conflicts of interests to disclose.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Introduction

Aplastic anemia (AA) is a rare, immune-mediated hematopoietic disorder associated with significant morbidity and mortality. In patients with suspected AA, rapid and accurate diagnosis and concomitant supportive care are critical. Historically, immunosuppressive therapy (IST) and bone marrow transplantation (BMT) in eligible patients have been the mainstay of AA treatment [1]. However, new frontline and salvage therapies are fundamentally changing how we approach therapy of AA, particularly in adult patients [2–4]. In pediatric patients, new transplant strategies and improvements in supportive care have led to greatly improved outcomes and increasing use of BMT in both upfront and refractory settings [5]. Furthermore, recent recognition of frequent clonal hematopoiesis in AA has changed our understanding of this immune-mediated blood disorder, reframing how we view somatic changes and a diagnosis of myelodysplastic syndrome (MDS) in patients with AA [6, 7]. Here, we present a comprehensive review of the diagnosis and treatment of AA, focusing on recent studies.

Clinical presentation and epidemiology

AA should be suspected in patients presenting with pancytopenia and a hypocellular bone marrow. Typical symptoms include fatigue and easy bruising or bleeding; infections may be present, but generally there is no long-standing illness. There is a well-recognized bimodal age distribution with one peak in mid to late childhood and another in the elderly [8]. The estimated annual incidence of AA is ~2 cases per million in Europe and North America, with a 2–3 fold higher incidence in East Asia [8]. In ~10% of patients, a history of non-viral hepatitis can precede the onset of AA [9]; an uncommon association with eosinophilic fasciitis has also been reported [10]. With rare exceptions, such as chloramphenicol, antiepileptics, and the emerging link to immunotherapies [8, 11], a causal relationship to medications or toxins can be difficult to establish.

Diagnosis and severity stratification

When AA is suspected, a comprehensive evaluation should be performed rapidly to exclude other mimicking conditions (Figure 1, Table 1). A baseline evaluation requires a full history and physical exam, a complete blood count with differential, a blood smear, a reticulocyte count, and a bone marrow aspirate with a core biopsy, with ancillary studies including cytogenetics and fluorescence in situ hybridization (FISH).

The search for alternative etiologies (Figure 1, Table 1) should focus on ascertainment of drug and toxin exposures, signs and symptoms suggestive of autoimmune or rheumatologic diseases, family and/or personal history suggestive of an inherited BMF disorder, infections, and nutritional deficiencies. Exclusion of inherited BMF is particularly relevant in children

and younger adults, where, at a minimum, an evaluation should include a detailed family history looking for lifelong cytopenias, congenital anomalies, cancers, and lung and liver pathology; in addition, patients should be screened for Fanconi anemia by testing the patient's lymphocytes for sensitivity to crosslinking agents and for Dyskeratosis congenita by measuring lymphocyte telomere lengths [12]. Lymphocyte telomere lengths may also be low in AA, particularly hepatitis-associated AA, requiring careful interpretation [13]. Additional causes of acquired BMF include autoimmune marrow aplasia due to a clonal T- or NK- large granular lymphocyte (LGL) expansion [14], which can be evaluated by T cell receptor rearrangement studies paired with lymphocyte flow cytometry. Morphologic and cytogenetic analyses are used to evaluate for hypoplastic MDS [15], although limited cellularity frequently precludes informative morphology assessment. Because of their association with acquired AA, detection of a paroxysmal nocturnal hemoglobinuria (PNH) clone (seen in up to 50% of AA patients) or copy number-neutral loss of heterozygosity of chromosome arm 6p (6p CN-LOH, seen in about 12% of AA patients) can be helpful in supporting the diagnosis of AA.

Once the diagnostic evaluation is complete, treatment is guided by the AA severity, established by the Camitta criteria (Figure 1) [16, 17]. For younger patients with severe aplastic anemia (SAA) or very severe aplastic anemia (VSAA), a transplant evaluation should be rapidly initiated. A referral to a tertiary center that specializes in the care of AA patients should be strongly considered.

Supportive Care

Throughout the diagnostic and treatment process, patients must be provided aggressive supportive care. Generally, restrictive transfusion targets (hemoglobin > 7 g/dL, platelets > 10,000 cells/ μ L) are preferred, especially in potential transplant candidates, given the risk of alloimmunization and transfusional iron overload [18]. Irradiated blood products should be used to prevent transfusion-associated graft-versus-host disease (GVHD). Because of the high mortality due to invasive mold infections, particularly *Aspergillus* species, antifungal prophylaxis with voriconazole or posaconazole should be used in patients with severe neutropenia (absolute neutrophil count < 500 cells/ μ L) [18]. *Pneumocystis jirovecii* pneumonia (PJP) prophylaxis should be used during the period of lymphopenia following ATG therapy, ideally selecting an alternative to trimethoprim-sulfamethoxazole because of its myelosuppressive effects. Antimicrobial prophylaxis with quinolone antibiotics in patients with VSAA can reduce the risk of gram-negative sepsis, but routine use of prophylactic antibiotics in patients with higher neutrophil counts is not advised in order to limit antibiotic resistance. Because granulocyte-colony-stimulating factor (G-CSF) does not improve overall survival when added to IST [19, 20], routine G-CSF use outside of episodes of febrile neutropenia remains controversial [21]. The benefits and risks of vaccines in AA also remain controversial due to the risk of immune activation, with some AA guidelines recommending against vaccinations outside of the post-transplant setting [21].

Transplant-based therapies for SAA/VSAA

Patient selection

In patients with SAA and VSAA eligible for transplant-based therapy, age remains the major factor predicting survival after matched sibling donor (MSD) allogeneic transplantation. A retrospective analysis from the Center for International Blood and Marrow Transplant Research (CIBMTR) of over 1,300 patients receiving MSD-BMT showed the adjusted 5-year overall survival (OS) of 53% in patients over the age of 40 years, as compared to 82% for patients under 20 years and 72% for patients aged 20–40 years [22]. The differences were primarily due to increased GVHD, infections, and delayed platelet recovery in the older cohort. In addition, these patients were more likely to have received prior IST, and/or to have additional comorbidities with poorer performance status and a longer interval between diagnosis and BMT [22]. Although outcomes in older patients transplanted with fludarabine-containing regimens have been more encouraging, these data are limited to retrospective analyses [23]. Thus, the current standard of care for patients older than 40 years is frontline IST, while BMT is the treatment of choice for children and young adults with SAA who have a MSD (Figure 1).

Donor choice

Historically, frontline transplantation for SAA in patients under 40 years of age has been largely limited to MSD transplants [24, 25]. However, a recent retrospective analysis of approximately 1,450 patients with AA transplanted between 2005 and 2009 showed no significant difference in OS between MSD and matched unrelated donor (MUD) transplant, although rates of acute and chronic GVHD were higher with MUD-BMT [26]. An analysis of 29 pediatric patients treated with Fludarabine/Cyclophosphamide/ATG (FCC) conditioning followed by unrelated donor transplantation showed similar overall and progression-free survival as compared to historical MSD-BMT controls, and superior outcomes compared to IST [5], suggesting that front-line therapy with MUD-BMT may be considered upfront in selected patients under age 20. Randomized trials are underway to compare outcomes of upfront MUD-BMT versus IST in pediatric patients without a matched sibling donor (Pediatric Blood and Marrow Transplant Consortium and the North American Pediatric Aplastic Anemia Consortium); studies exploring frontline MUD-BMT in adults under 40 are also ongoing (Blood and Marrow Transplant Clinical Trials Network). Pending these prospective studies, IST remains the standard upfront AA therapy in patients without MSD [26, 5].

The outcomes of mismatched or haploidentical donor transplantation in AA have also improved. In a prospective multicenter study of 101 AA patients receiving haploidentical transplants in China, 94% of patients achieved successful engraftment with 3-year overall and failure free survival of 89% and 86%, respectively [27]. A registry-based comparison of upfront haploidentical and MSD transplantation in 158 consecutive SAA patients in China have shown similar high rates of engraftment and OS, but significantly higher rates of grade III–IV acute GVHD (10% versus 1.5%) and chronic GVHD (31% versus 4.4%) for haploidentical transplants [28]. A more recent study of 16 patients receiving haploidentical or unrelated donor transplants with post-transplant cyclophosphamide showed encouraging

results with 100% engraftment and no instances of grade 3 or higher GVHD [29]. Novel approaches including co-infusion of mesenchymal stem cells [30] and selective T cell Receptor $\alpha\beta$ depletion [31] are being explored.

Graft source

Bone marrow grafts have been shown to produce superior OS compared to peripheral blood stem cell (PBSC) grafts in both pediatric [32] and adult [33] AA patients, due to lower rates of GVHD. More recent efforts to improve outcomes with PBSC showed encouraging results with partial T cell depletion [34]; larger randomized prospective studies are needed to confirm the efficacy and safety of this approach.

Conditioning Regimens

The standard conditioning regimen for MSD-BMT in younger patients is 200 mg/kg cyclophosphamide with antithymocyte globulin (ATG), with three-year survival rates of 92% [35]. However, subsequent studies in older transplant recipients (age > 30) did not show a survival benefit when compared to IST [36]. To reduce toxicity in older patients, newer regimens have incorporated fludarabine with lower-dose cyclophosphamide and with ATG (FCA) or alemtuzumab (FCC), with improved OS [37–39]. A CIBMTR analysis of 833 AA bone marrow transplants evaluated the role of ATG source on transplant outcomes, and demonstrated that rabbit ATG (Thymoglobulin, Sanofi, France) results in lower rates of acute and chronic GVHD for MSD transplants, improves survival, and lowers rates of acute GVHD for MUD transplants [40]. Conditioning for MUD and haploidentical transplants also includes 200 cGy total body irradiation [41].

Non-transplant therapy of AA

Immunosuppression

For patients older than 40 years with newly-diagnosed SAA/VSAA or younger patients without an MSD, immunosuppression with ATG and cyclosporine A (CsA) continues to be the recommended frontline therapy (Figure 1) [42], offering outcomes comparable to allogeneic BMT with reduced morbidity in older patients [43, 44]. Horse ATG is the recommended ATG source, based on a randomized-controlled trial of 120 patients showing a superior overall response (68% compared to 37%) and OS (96% compared to 76%) for horse ATG-based IST compared to rabbit ATG-based IST [45].

Role of Eltrombopag in Frontline Therapy

One of the most promising recent treatments for AA is the oral thrombopoietin (TPO) receptor agonist eltrombopag, previously approved for treatment of chronic idiopathic thrombocytopenic purpura [46]. Studies in mouse models showed that signaling via the TPO receptor c-mpl is necessary for hematopoietic stem cell maintenance and downstream expansion and differentiation [47, 48]. Interestingly, patients with congenital amegakaryocytic thrombocytopenia who have mutations in c-mpl can progress to aplastic anemia, suggesting that a deficiency in TPO signaling may play a role in AA pathogenesis [49]. Based on these studies, eltrombopag was initially tested as monotherapy in a Phase 2 study of 43 patients with relapsed/refractory SAA with persistent thrombocytopenia

following IST [2, 3]. The overall response rate after 3–4 months of therapy was 40% (17 of 43). Remarkably, one patient achieved a trilineage response, and 8 patients had neutrophil responses including 4 with severe neutropenia. With long-term treatment, 7 patients achieved trilineage responses. An early signal of increased clonal evolution in this high-risk population was also noted with 19% (8 of 43) patients developing cytogenetic abnormalities during follow-up [2, 3].

Based on these encouraging data, a phase 1/2 study evaluated whether the addition of eltrombopag to frontline IST can further improve patient outcomes [4]. 92 patients were assigned to receive daily eltrombopag in addition to horse ATG and CsA in three separate cohorts starting eltrombopag at either day 0 or day 14 and continuing for 3 or 6 months. Because of the concern for karyotypic evolution [2, 3], patients with cytogenetic abnormalities were excluded. The overall response rate at 6 months in the three cohorts ranged between 80–94%, as compared to 66% in the composite historical cohort of 102 patients [50, 45]. There was also an encouraging improvement in the rate of complete response, at 36% with eltrombopag as compared to a historical estimate of 10% with standard IST. During an initial phase of the study CsA was discontinued at 6 months, with a relapse rate of 32%–54%, leading to a protocol amendment to extend CsA duration to 2 years [4]. Encouragingly, the rates of chromosomal aberrations were similar to those in historical controls, ~8% at 2 years of follow-up [4]. Several prospective randomized trials combining eltrombopag therapy with IST are underway to confirm these findings and to better assess long-term efficacy and safety, particularly clonal evolution (Table 2). However, given these encouraging early results and low observed toxicity, we believe that in selected newly diagnosed SAA/VSAA patients without pre-existing karyotypic abnormalities, addition of six months of eltrombopag to upfront standard IST should be considered.

Cyclosporine Maintenance and Taper

Although there are no definitive data on the optimal duration of CsA, it is clear that early discontinuation leads to a high rate of early relapse. An 11-year follow-up of a randomized trial of horse ATG with or without CsA showed that CsA maintenance delays relapses. 26% of patients required CsA for greater than 6 months due to recurrent cytopenias on discontinuation [43]. Follow-up of two National Institutes of Health cohorts totaling 102 patients treated with standard IST for whom CsA taper was started at 6 months showed a cumulative relapse rate of 33% at 5 years of follow-up, with a median time to relapse of 2 years [51]. Compared to historical cohorts that discontinued CsA at 6 months, a tapering regimen delayed relapse by approximately 1 year [51]. The high relapse rates of 32%–54% in the recent study of eltrombopag added to upfront IST were also attributed to early CsA discontinuation, and were improved with extending CsA to a 2 year maintenance [4]. There are limited data on the rate of CsA taper, although an analysis of 33 pediatric AA patients suggested that a slower taper of <0.3mg/kg/month may lead to fewer relapses [52]. Putting these data together, our practice (Figure 1) is to continue full dose CsA, targeting therapeutic CsA trough of 200–300 mcg/L, for approximately 12 months after horse ATG therapy and until achievement of stable and maximally-improved blood counts, at which time we initiate a slow taper with no more than 10% dose reduction at a time over the course of approximately one year.

Treatment of nonsevere aplastic anemia (NSAA)

Unlike the fairly defined guidelines for frontline treatment of SAA/VSAA (Figure 1), the approach to nonsevere aplastic anemia (NSAA) is more nuanced. The natural history of patients with NSAA has been evaluated in several retrospective analyses, showing progression of cytopenias in ~20–67% of NSAA patients [53–56]. Interestingly, NSAA patients managed in more recent years (1997–2002) appear to have worse outcomes with a 56% 10-year survival as compared to 70% in NSAA patients treated between 1991–1996; this was associated with a significantly longer interval from diagnosis to treatment in the more recent cohort (52 versus 102 days) [57]. Expert AA guidelines recommend treating NSAA patients if they have transfusion dependence or neutropenia [21]. A prospective randomized study compared horse ATG and CsA to CsA monotherapy in 114 NSAA patients, showing significantly higher overall response (74% versus 46%) in the ATG and CsA arm [58]. Transfusion independence was achieved in 90% of ATG/CsA-treated patients compared to only 67% of patients receiving CsA alone; 5-year OS was equivalent in both groups [58]. The outcomes of a recent cohort of 95 Japanese NSAA patients treated with horse ATG and CsA were less encouraging, showing a lower 6-month response rate of 55% and a 10-year failure free survival of 44%, with the majority of patients needing second- and third-line therapies. The median time to initial treatment was 47 days [59].

More recently, eltrombopag has been proposed as a potential option for NSAA patients, with a number of ongoing studies studying the safety and efficacy of eltrombopag in combination with CsA in NSAA (Table 2) [60]. Given the excellent tolerability and efficacy of eltrombopag in the relapsed/refractory and first-line SAA/VSAA settings (Table 2) [2–4], we anticipate that eltrombopag-containing regimens would be similarly beneficial in NSAA and may allow for improved outcomes with lower toxicities in this population.

Several other treatments have been investigated in NSAA; however, to date, none have been demonstrated to be superior to standard IST. In 45 patients with NSAA treated with a recombinant humanized anti-IL2 receptor antibody daclizumab, 42% achieved a hematologic response at 3 months [61], although only 25% achieved transfusion independence at ~5 years of follow-up [62]. An antihelminthic agent levamisole, associated with immunomodulatory activity, was tested in combination with CsA in 118 Chinese patients with NSAA; the study found a nearly 100% overall response rate in 42 patients with newly-diagnosed NSAA and 87% overall response rate in chronic NSAA [63], suggesting that CsA combined with levamisole may be a promising therapy to be evaluated in future randomized studies.

Clonal evolution

The improvement in long-term survival of AA patients led to an increased appreciation of the long-term clonal sequelae of AA. ~15% of AA patients treated with IST go on to develop the late complications of MDS and acute myeloid leukemia (AML) [64, 65, 43]. Approximately 10% of AA patients (range 3–26%) develop cytogenetic changes during the course of their disease (reviewed in [7]), most commonly monosomy 7/del (7q) and trisomy 8, as well as del (13q), and trisomies of chromosomes 6, 15 and 21. In the context of AA,

monosomy 7 has been found to correlate with a poor prognosis, including a worse response to IST and increased progression to MDS, while del (13q) and trisomy 8 are associated with an improved response to IST and a better prognosis (reviewed in [6, 7]).

Newer techniques combining single nucleotide polymorphism arrays (SNP-A) and next-generation sequencing (NGS) have allowed to more precisely evaluate clonal hematopoiesis in AA. The majority of AA patients, including over 60% of children with AA, develop clonal genetic changes [66, 67]. Several recent reviews comprehensively addressed this topic [6, 7, 68]. The most common clonal abnormality in AA is the development of PNH clones, which can be detected by flow cytometry as cells lacking glycoprophosphatidyl-inositol-linked proteins due to a somatic mutation in the *PIGA* gene [69, 70], found in up to 50% of AA patients. The second most common is somatic loss of human leukocyte antigen (HLA) loci, that are detected as either regions of acquired 6p CN-LOH or as inactivating mutations in HLA class I genes in approximately 17% of AA patients [71–74]. Both PNH clones and HLA loss are hypothesized to occur due to immune escape, and, if present, can be helpful in corroborating a diagnosis of AA. The presence of even a subclinical PNH clone has been found to correlate with an improved response to IST [75–79, 70]. In contrast to PNH, the prognostic impact of somatic HLA loss is less clear [71, 72], with emerging data suggesting that HLA loss may be best viewed as a marker of a higher immune pathogenicity of a patient's inherited HLA alleles [74, 73].

Using targeted NGS of genes recurrently mutated in hematologic malignancies, several groups identified somatic mutations in MDS-associated genes in AA, detected in up to a third of adult AA patients [80–83, 66]. The most commonly mutated malignancy-associated genes in AA are *ASXL1*, *BCOR/BCORL1*, and *DNMT3A* [66, 81, 82]. The prognostic implications of somatic mutations in MDS-associated genes are not clear, due to the lack of prospective, long-term studies of large numbers of patients carrying these mutations. Nevertheless, available data suggest that mutations in *BCOR* and *BCORL1* may be predictive of an improved response to IST [66], and together with mutations in *PIGA*, comprise a group with “favorable” prognosis [66]. In the comprehensive study of clonal hematopoiesis in AA by Yoshizato and colleagues, no other genes were identified to have prognostic significance individually, perhaps due to the limited statistical power, although several genes (*DNMT3A*, *ASXL1*, *TP53*, *RUNX1*, and *CSMD1*) were associated with worse OS when analyzed in aggregate [66]. Putting together the available data, it is clear that the majority of pediatric and adult AA patients develop clonal hematopoiesis, with a large fraction of adult AA patients having detectable somatic mutations in genes that are frequently altered in aging and MDS. However, only ~15% of AA patients progress to the late complications of MDS and leukemia after 10 years of follow-up [64]. Thus, in the absence of prospective longitudinal studies evaluating the prognosis of AA patients carrying specific MDS-associated somatic mutations, we advise caution when factoring the presence of somatic mutations into therapeutic decisions in this patient population.

Salvage therapy for relapsed and refractory aplastic anemia

Despite overall improvement in AA outcomes, ~33–35% of patients who initially respond to IST will relapse during or after CsA taper, while another 35% will be refractory to frontline

IST [45, 1]. Most patients (~60–68%) who relapse following an initial response to IST can be salvaged with full-dose CsA monotherapy and/or a second course of IST with rabbit ATG and CsA [84, 52, 85, 42, 86] or transplant. Alternatives to ATG and CsA in relapsed disease include alemtuzumab, which, in a single-arm prospective study of 25 patients with relapsed AA, was shown to produce a hematologic response in 56% of patients, with 86% 3-year survival [87]. Because most patients will respond to a second round of IST [84], in adults, transplant therapies are usually reserved for relapsed patients who failed an attempt of salvage with a second course of immunosuppression, while excellent outcomes in children with salvage BMT after IST failure make MUD-BMT a reasonable second-line option [88].

Compared to relapsed AA patients who previously responded to IST, patients with primary refractory AA have worse outcomes, and only ~30% of refractory AA patients can be salvaged with rabbit ATG and CsA [84]. The failure-free survival in refractory pediatric patients treated with second-line IST can be as poor as 9.5%, as compared to >80% for salvage SCT [89]. Thus, refractory AA patients should be evaluated for salvage allogeneic transplant options, which may include HLA identical sibling, matched unrelated, or haploidentical bone marrow transplantation, depending on donor availability. Among non-transplant therapies, eltrombopag has hematologic response rates of ~40% in refractory AA, including some trilineage responses, and represents an important option, particularly for older adults or patients who are poor transplant candidates [2, 3].

There are several additional second- and third-line treatment options for refractory AA, of which danazol and alemtuzumab are more commonly used. In 48 patients with refractory AA randomized to receive alemtuzumab versus rabbit ATG with CsA, alemtuzumab was comparable to rabbit ATG arm, with a hematologic response of 37% and a 3-year survival of 83% [87]. Although androgen therapy has not been found to improve survival in combination with first-line IST [90, 91], a study of 16 refractory AA patients suggests that androgens may be helpful, particularly in female patients [92]. Cyclophosphamide in moderate to high doses also has efficacy in refractory AA [93–95], but significant toxicity with prolonged neutropenia and high rates of infectious have largely limited its use [96, 97].

Summary

AA is a rare, life-threatening, BMF syndrome that requires a systematic and timely approach to diagnosis and treatment. For a younger patient with a MSD, allogeneic BMT remains the standard frontline therapy, while other patients should receive frontline immunosuppression with horse ATG and CsA. Emerging data suggest that addition of eltrombopag to frontline IST can further improve outcomes and that outcomes following upfront MUD BMT may now be equivalent to MSD-BMT, at least in pediatric patients. Long-term prospective studies are underway to confirm the safety and efficacy of these approaches. As the outcomes of MUD and haploidentical transplantation improve and with emergence of eltrombopag as an effective agent in refractory AA, we expect that outcomes of patients with refractory AA will improve. Finally, with the recent findings of frequent clonal hematopoiesis in the majority of AA patients, utmost caution should be exercised in the interpretation of molecular changes which are common in this patient population and, in the absence of long-term prospective studies, do not have well-defined prognostic implications.

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Diagnosis

Treatment

Figure 1. Approach to diagnosis and treatment of acquired aplastic anemia

Initial screening evaluation of a patient with aplastic anemia is required to document pancytopenia with a hypocellular marrow, followed by testing to exclude alternative diagnoses. Aplastic anemia severity and outcomes of a transplant evaluation factor into determining an optimal treatment strategy. Patients with Severe or Very Severe Aplastic Anemia (SAA/VSAA) 40 years of age or younger with an HLA-matched sibling donor should undergo an evaluation for an allogeneic bone marrow transplant; older patients or patients without an HLA-identical sibling donor should be evaluated for frontline immunosuppressive therapy (IST) with horse ATG and CsA. **Based on recent data showing superior hematologic outcomes with the addition of eltrombopag [4], addition of 6 months of eltrombopag to standard IST in patients without pre-existing cytogenetic abnormalities can be considered. Cyclosporine A should be continued for ~12 months of therapy, followed by a slow taper to reduce relapse rates. Salvage therapies include alternative transplant modalities and a variety of nontransplant options. AA, aplastic anemia;

PNH, paroxysmal nocturnal hemoglobinuria; *6p CN-LOH*, copy number-neutral loss of heterozygosity of chromosome arm 6p; *alloSCT*, allogeneic stem cell transplant; *NSAA*, nonsevere aplastic anemia; *SAA*, severe aplastic anemia; *VSAA*, very severe aplastic anemia. *Cellularity criteria are determined on adequate bone marrow biopsy, and hypoplastic marrow can either be diagnosed on total cellularity or on bone marrow biopsy with <50 percent normal cellularity in which < 30 percent of the cells are hematopoietic. *HLA*, human leukocyte antigen; *alloBMT*, allogeneic bone marrow transplant; *IST*, immunosuppressive therapy; *hATG*, horse antithymocyte globulin; *CsA*, cyclosporine A; *CR*, complete response; *PR*, partial response; *Cy*, cyclophosphamide; *MRD*, matched related donor; *MUD*, matched unrelated donor; *haplo BMT*, haploidentical bone marrow transplant.

Table 1
Diagnostic evaluation of a patient with suspected acquired aplastic anemia

A variety of testing modalities in addition to a detailed personal/family history and exposure history are required both in the initial screening evaluation as well as the subsequent exclusion of alternative diagnoses.

Initial screening evaluation	
Peripheral blood	CBC with differential, peripheral blood smear, reticulocyte count Complete metabolic panel, LDH, haptoglobin, coagulation parameters
Bone marrow aspirate and biopsy with ancillary studies	Bone marrow aspirate and biopsy Metaphase cytogenetics and FISH panel for MDS-associated chromosomal abnormalities of chromosomes 5, 7, 8, 20 * Molecular studies
Exclusion of alternative diagnoses	
Infectious	HIV, Hepatitis B/C, parvovirus B19 PCR, EBV, CMV, bacterial, fungal (+/- mycobacterial testing)
Inherited bone marrow failure	Detailed family history focusing on cytopenias, congenital abnormalities, cancers and lung and liver pathology Chromosomal breakage testing for Fanconi anemia Lymphocyte telomere length for Dyskeratosis congenita Additional syndrome-specific testing if personal or family history are suggestive of specific disorders (IBMF, HLH)
Lymphoproliferative	Flow cytometry and T-cell receptor rearrangement testing for clonal LGL expansion
Medication or toxin-related	Detailed drug and occupational exposure history, with attention to excessive alcohol intake, antibiotics, prior cytotoxic chemotherapeutic agents, and immune-activating agents (e.g. interferon and checkpoint blockade inhibitors)
Nutritional	Vitamin B12, folate, copper, iron studies, ferritin
Rheumatologic	Antinuclear antibody, rheumatoid factor, erythrocyte sedimentation rate, C-reactive protein
Others (rare)	Exclude other rare etiologies of pancytopenia with a hypocellular marrow: e.g. graft-versus-host disease, HLH

CBC, complete blood count; LDH, lactate dehydrogenase; MPN, myeloproliferative neoplasm; FISH, fluorescence in-situ hybridization; MDS, myelodysplastic syndrome; HIV, human immunodeficiency virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; DEB, diepoxybutane; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; IBMF, inherited bone marrow failure; HLH, hemophagocytic lymphohistiocytosis; LGL, large granular lymphocyte.

* Although in current clinical practice molecular sequencing panels of somatic mutations in hematologic malignancy-associated genes are frequently included and can provide useful supporting information, in isolation, presence of somatic mutations in patients with AA should be interpreted with caution due to their high frequency in this patient population and uncertain prognostic significance (see text).

Table 2

Eltrombopag in the treatment of aplastic anemia.

	Type of study	Inclusion/Exclusion	Patients, n	Median age, yrs (range)	Treatment arms	Duration of Follow-up	Outcomes	Clonal Evolution
Townsend et al. (2017) [4]	Phase 1/2	<u>Inclusion:</u> Age >2 with previously untreated SAA <u>Exclusion:</u> FA, liver impairment, or abnormal cytogenetics	92	32 (3–82)	hATG/CsA + eltrombopag	Up to 24 months	Eltrombopag + hATG/CsA showed ORR of 80% and CR of 36%, improved from historical cohorts with ORR 66%, CR 10%	7/92 (7.6%) developed clonal abnormalities on cytogenetics
SOAR trial (pending) [98]	Phase 2	<u>Inclusion:</u> Age 6 with SAA <u>Exclusion:</u> Prior CSA, alemtuzumab, ATG, or TPO-R agonists; diagnosis of FA or abnormal cytogenetics	50 (target)	N/A	CsA +/- eltrombopag	Planned for up to 60 months	Pending trial; primary end point hematologic response at 6 months	Planned evaluation for clonal evolution to PNH, MDS, or acute leukemia
EBMT-SAA Working Party RACE trial (pending) [99]	Phase 3	<u>Inclusion:</u> Age >14 with SAA/VsAA <u>Exclusion:</u> Prior ATG; diagnosis of FA, DC, or MDS	200 (target)	N/A	hATG/CsA +/- eltrombopag	Planned for at least 2 years	Pending trial; primary end point complete response at 3 months	Planned evaluation for clonal evolution to PNH or clonal cytogenetics
Höschmann et al. EMMAA trial (pending) [60]	Phase 2/3	<u>Inclusion:</u> Age 2 with NSAA or multilineage BMF; PLT transfusion dependent <u>Exclusion:</u> ATG therapy in prior 6 months; diagnosis of FA	29 (target)	N/A	CsA +/- eltrombopag	Planned for 6 months after last dose eltrombopag	Pending trial; primary end point hematologic response at 6 months	Planned evaluation for clonal evolution to PNH or clonal cytogenetics
Olmes et al. (2012) Desmond et al. (2014) [2, 3]	Phase 2 with extension	<u>Inclusion:</u> Age >12 years with SAA refractory to 1 IST regimen; PLT <30K/uL <u>Exclusion:</u> Diagnosis of FA, familial marrow failure, or DC	43	44 (17–77)	Eltrombopag	Up to 4 years follow up	ORR at 3–4 months of 40% (17/43), including 1 patient with trilineage response, 9 patients with PLT transfusion independence, and 8 patients with neutrophil response	8/43 patients (18.6%) developed clonal abnormalities on cytogenetics

SAA, severe aplastic anemia; VsAA, very severe aplastic anemia; NSAA, nonsevere aplastic anemia; PLT, platelet; ORR, overall response rate; CR, complete response; FA, Fanconi anemia; DC, Dyskeratosis congenita; hATG, horse antithymocyte globulin; CsA, cyclosporine A; IST, immunosuppressive therapy; EMMT-SAA, European Society for Blood and Marrow Transplantation Severe Aplastic