Immune suppression for childhood acquired aplastic anemia and myelodysplastic syndrome: where next?

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The childhood bone marrow failure disorders are a heterogeneous group of diseases of which the commonest ones are acquired severe aplastic anemia (SAA) and myelodysplastic syndromes. SAA is characterized by the presence of pancytopenia with a hypocellular marrow in the absence of malignant infiltration or fibrosis. Myelodysplastic syndromes are characterized by ineffective hematopoiesis, dyplasia and cytopenias. The commonest subset of childhood myelodysplastic syndrome is refractory cytopenia of childhood (RCC) accounting for half of all cases of childhood myelodysplastic syndrome. The majority of cases of RCC are hypocellular and distinguishing between SAA and hypocellular RCC can be challenging. Most cases of hypoplastic RCC lack a cytogenetic abnormality and conversely some patients with aplastic anemia have an abnormal cytogenetic clone. Thus careful morphological assessment is required to distinguish between SAA and RCC. Future use of next-generation sequencing to detect acquired somatic mutations typical of myeloid disorders may help to distinguish between the two conditions.[1]

It is postulated that, in both hypoplastic myelodysplastic syndrome and SAA, autoimmunity and T-cell-mediated suppression of hematopoiesis contribute to the pathogenesis of these disorders. In both sets of disorders, there is a plethora of data demonstrating oligoclonal T-cell expansion, leading to over-expression of cytokines, such as interferon-γ and tumor necrosis factor-α, which suppress hematopoiesis.[2] In acquired SAA, there is absolute and functional deficiency of regulatory T cells.[3,4] The best evidence for an immune pathogenesis is provided by the response to immune suppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine. IST with ATG and cyclosporine is currently used as first-line treatment for children with SAA who do not have a matched sibling donor for bone-marrow transplantation. Historically, horse ATG was adopted as first line IST with rabbit ATG being used for relapse or refractory cases. Until 2007, there were two preparations of horse ATG; Lymphoglobulin (Genzyme) and ATGAM (Pfizer). Lymphoglobulin was withdrawn from the market in 2007 and because of the initial unavailability of ATGAM in most European countries, rabbit ATG (Thymoglobulin; Sanofi) was then adopted as first-line therapy. There have been numerous studies (Table 1) comparing the efficacy of rabbit ATG *versus* horse ATG. As described in this issue of the Journal, Jeong *et al.* performed a large retrospective comparison between Thymoglobulin and Lymphoglobulin in pediatric acquired SAA.[5] While there was no difference in overall response rate at 6 months, patients in the horse ATG cohort had a superior overall survival and lower relapse rate. Thus so far, none of the seven published studies comparing horse ATG and rabbit ATG has demonstrated superiority of rabbit ATG.[5-11] In five, rabbit ATG led to inferior overall survival or response rates[5-7,9,10] and in the other two[8,11] there was no significant difference between the two preparations.

Table 1. Summary of studies of immunosuppression comparing horse ATG/cyclosporine and rabbit ATG/cyclosporine in acquired SAA. There were two horse ATG preparations (Lymphoglobulin or ATGAM). The rabbit ATG preparation was Thymoglobulin.

Study	Age range (years)	ATG preparation	N.	OR (%)	Relapse rates (%)	Transplantation free survival (survival with hematologic response and thus with no need for transplant)	OS (%)
[a]NIH (6)	(2-77)	ATGAM Thymoglobulin	60 60	68* 37*	28 11	NA	96* at 3 years 76* at 3 years
[b]EBMT (7)	(17-75)	Lymphoglobulin Thymoglobulin	105 35	[d] 67 [d] 60	N/A	80* 64*	86* at 2 years 68* at 2 years
[b]Cleveland Clinic (8)	(3-80)	ATGAM Thymoglobulin	67 20	58 50	16 5	NA	64 at 5 years 65 at 5 years
[b]EWOG (9)	<18	Lymphoglobulin Thymoglobulin	96 32	65* 34*	N/A	73* 30*	92 at 3 years 92 at 3 years
[c]Brazil (10)	(1-66)	Lymphoglobulin Thymoglobulin	42 29	59.5* 34.5*	36 30	NA	78.4* at 2 years 55.4* at 2 years
[c]Korea, China, Japan (5)	<18	Lymphoglobulin Thymoglobulin	297 158	61 55	7* 15*	NA	92* at 10 years 87* at 10 years
[c]Korea (11)	(14-75)	Lymphoglobulin Thymoglobulin	20 46	45.7 49.1	22.7 27.3	NA	83.5 at 5 years 82.7 at 5 years

*[a]Randomized prospective study; [b]prospective study compared to historical control; [c]retrospective study; [d]best response. *Significant difference between horse ATG and rabbit ATG. (P<0.05). ATG: antithymocyte globulin; EBMT: European Society of Blood and Marrow Transplantation; EWOG: European Working Group of MDS and JMML in Childhood; N: number of patients, NA: not available; NIH: National Institutes of Health; OR: overall response rate; OS: overall survival.*

In a similar study published in this issue of the Journal, Yoshimi *et al.*[12] prospectively evaluated responses following IST with horse ATG (Lymphoglobulin) until 2007 and rabbit ATG (Thymoglobulin) after 2007 in a selected cohort of children with RCC. All children enrolled in this study were carefully selected to maximize response to IST and minimize disease progression. Only children with a diagnosis of hypocellular RCC and karyotypes other than monosomy 7/7q- or three or more chromosomal abnormalities, were potentially eligible. As in the SAA studies, horse ATG led to a superior overall response at 6 months (74% for horse ATG *versus* 53% for rabbit ATG; *P*=0.04). This translated into superior transplantation-free and failure-free survival in the horse ATG group. The overall message from these comparison studies is clear: horse ATG with cyclosporine is the IST of choice in SAA and low-risk hypocellular RCC.

Rabbit ATG should only be used when horse ATG is not available. Further studies comparing the two preparations will be of limited value, whereas future efforts should concentrate on explaining the reasons for these differences, determining the optimal dose of the ATG preparations and enhancing existing response rates to IST. The reasons for the superiority of horse ATG is unclear. Rabbit ATG is more immunosuppressive and leads to lower absolute numbers of $CD4^+$ regulatory T cells compared to horse ATG,[6] but these findings may not necessarily explain the differences between the two preparations.

How can we improve the response to immune suppressive therapy?

The aforementioned studies demonstrate that IST with horse ATG and cyclosporine is still not fully satisfactory. The overall response rate is between 50-70%, relapse rates vary between 10-30% and there is a clonal evolution rate of 10-20%, with a significant risk of developing monosomy 7 aftter IST.[1,13] Many of the responses are partial remissions and thus the ensuing subnormal blood counts may lead to restrictions to children's quality of life. Long-term outcomes following IST with horse ATG and cyclosporine demonstrate that around 50% of children will require a second-line treatment (retreatment with IST or a transplant from an unrelated donor).[9] Attempts to enhance remission rates seen with ATG and cyclosporine by adding sirolimus, mycophenolate mofetil or danazol have not improved responses rates.[14] One potential agent that is currently being investigated which might improve IST responses is eltrombopag. Eltrombopag is an oral thrombopoietin receptor agonist and was originally developed to stimulate platelet production in immune thrombocytopenia. However, patients who lack the thrombopoietin receptor, c-mpl, develop a form of congenital bone marrow failure, called congenital amegakaryocytic thrombocytopenia. This suggests that thrombopoietin is critical to hematopoietic stem cell development and differentiation.

In a pilot dose escalation study in 25 adults with SAA who were refractory to IST, 44% of patients had hematologic responses to eltrombopag.[15] In a subsequent update of this study, the National Institutes of Health group reported that in a total of 43 patients, 17 (40%) achieved responses at 3-4 months. Five patients were subsequently able to stop eltrombopag and have maintained stable blood counts despite stopping the drug at a median of 13 months off eltrombopag (range, 1-15 months).[16] One concern about the use of the use of eltrombopag is the potential for clonal evolution. Eight of 43 patients (19%) developed new and early cytogenetic abnormalities, most by 3 months (range, 3-13 months) and most often monosomy 7. At present, it is too early to know whether eltrombopag increases the risk of clonal evolution. Randomized studies are underway or planned to determine the role of eltrombopag in combination with horse ATG/cyclosporine in acquired SAA. These studies will answer whether this agent can safely increase the response rates to IST beyond what is currently achievable.

Time to consider upfront transplantation from a matched unrelated donor?

While there has been no improvement in overall or failure-free survival rates in the last two decades following IST,[17] results of matched unrelated donor hematopoietic stem cell transplantation have improved dramatically over this period. This has been due to improvements in supportive care, the development of high resolution HLA typing and less toxic conditioning regimens. Results of matched unrelated donor hematopoietic stem cell transplantation in childhood acquired SAA and RCC in children now approach those of matched sibling donor hematopoietic stem cell transplantation.[18]

With the excellent outcomes currently seen in unrelated donor hematopoietic stem cell transplantation in pediatric idiopathic SAA/RCC, it is unclear whether newly diagnosed children lacking a matched sibling donor should receive IST with horse ATG or proceed directly to transplantation from an unrelated donor. With the disappointing results seen following rabbit ATG, there has been an increasing move towards upfront matched unrelated donor hematopoietic stem cell transplantation in children. Compared to IST, transplantation offers a more complete restoration of hematopoiesis, lower relapse rates and better protection against secondary cancers.[19] The major potential drawbacks of upfront matched unrelated donor transplantation are: (i) difficulties in finding donors; (ii) the time from diagnosis to stem cell donation; (iii) graft-*versus*-host disease/graft rejection; (iv) donors opting to donate peripheral blood stem cells rather than bone marrow; and (v) treatment-related mortality.

In the study by Yoshimi *et al.*, the median time from diagnosis to administration of IST was 60 days while it was 134 days in those who underwent upfront matched unrelated donor hematopoietic stem cell transplantation.[12] While IST can, undoubtedly, always be started earlier than an upfront matched unrelated donor transplant, the time to cellular recovery is slow following IST (typically 3-6 months). In contrast, cellular recovery following matched unrelated donor transplantation is much quicker (normally a month). Thus, time to cellular recovery following IST or upfront matched unrelated donor hematopoietic stem cell transplantation is often similar if a matched unrelated donor can be found quickly. With the advent of FCC (fludarabine, cyclophosphamide and alemtuzumab) conditioning regimens for acquired SAA, the rates of extensive chronic graft-*versus*-host disease and graft rejection are now less than 5%.[18,20] Thus, the current EBMT SAA rec-

ommendations are that if a matched unrelated donor can be found quickly, then transplantation could be considered a potential first-line option in those children who lack a matched sibling donor. The decision to proceed with horse ATG-based IST or an upfront transplant from a matched unrelated donor will, therefore, depend on the preferences of patients and physicians and donor availability until further data become available.

Conclusion

Survival outcomes for low-risk RCC and acquired SAA in children following IST with horse ATG/cyclosporine are excellent. Future strategies will now need to focus more on quality of life and failure-free survival so that further improvements can be made. This can only be achieved with well-designed prospective clinical studies.

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Assessing the prognostic impact of immune cell infiltrates in follicular lymphoma

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Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma (NHL) subtype in the Western world. Even without specific therapy, many patients show prolonged survival, but over time a significant proportion of patients progress to aggressive, often therapy-refractory and ultimately fatal disease. Although clinical tools such as the FL International Prognostic Index,[1] based on simple clinical parameters, allow risk stratification, the clinical course of FL in an individual patient is unpredictable, and the search for better tissue-based prog-