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REVIEW

Conditioning regimens in allo-SCT for thalassemia major

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Allogeneic hematopoietic SCT remains the only treatment that can correct the hematological manifestations in patients with thalassemia major. Improving the clinical outcomes of high-risk, heavily transfused patients with liver fibrosis and inadequate iron chelation remains a challenge. Because of the relatively high probability of graft rejection and regimen-related toxicity in many patients receiving SCT for advanced thalassemia major, further development of new treatment regimens is warranted. This review addresses the reported clinical studies in patients with advanced thalassemia major and we have summarized our suggested conditioning approach to improve the outcome after SCT.

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INTRODUCTION

Allogeneic hematopoietic SCT remains the only curative option for patients with β-thalassemia major. The correction of this disorder by allo-SCT was first described by Thomas *et al.*[1] Subsequently, a myeloablative conditioning regimen of BU and CY was established for thalassemia major. Most of the high-dose regimens described have been utilized for patients with hematologic malignancies. This BU-based myeloablative therapy forms the basis for the currently used conditioning regimens in this condition.[2]

Alternative regimens to improve the clinical outcome, especially in high-risk patients, are evolving.[3] The development of new treatment regimens has, in general, been empirical with the evaluation of a variety of drugs and doses, and schedules.

RISK OF GRAFT REJECTION AND REGIMEN-RELATED TOXICITY (RRT) IN HIGH-RISK POPULATIONS

The current risk stratification of patients with β-thalassemia major undergoing myeloablative allo-SCT classifies them into three risk groups (Lucarelli or Pesaro Class I, II and III) based on liver size (>2 cm), presence of liver fibrosis and inadequate iron chelation.[4,5] Patients with none of the above risk factors are classified as Class I, those with one or two of these risk factors are Class II, whereas those who have all three adverse risk factors are classified as Class III. Patients in Classes I and II are considered to be at low risk and have an excellent long-term outcome following allo-SCT.[4,5] Class III patients on the other hand are considered to be at high-risk and have inferior outcomes following SCT. However, in a population with poor medical treatment before SCT, the above risk stratification is limited by the small number of patients who belong to Class I, the majority being in Class III.[6] In such a population, the current risk stratification strategy is also insensitive to the wide heterogeneity among patients in Class III. A major limitation of the existing risk stratification[4,5] is the inability to recognize a very high-risk subset of Class III in these populations. Increasingly, allo-SCT is being offered in many developing countries where this scenario of inadequate medical treatment before SCT is common.[6–8]

Before SCT, patients who were $\geqslant 7$ years old and had a liver size $\geqslant 5$ cm constituted what has been previously defined as a very high-risk subset of a conventional Class III group (Class III high-risk (HR)).[6] The adverse impact of age ($\geqslant 7$ years) and liver size ($>$ 2 cm) was further validated by an international collaborative analysis that was recently reported.[9] Class III and more specifically Class III HR subset have a high-risk of graft rejection and RRT, especially sinusoidal obstruction syndrome (SOS), leading to multi-organ failure and death. These complications are related to the high degree of allo-immunization and iron overload-related end organ damage in this cohort. The poor clinical outcome in this subset of older patients with very poor pre-transplant medical therapy, as reported previously, is not reflected in the western literature owing to the use of adequate blood transfusion and chelation support before transplant. However, when such a population is transplanted even in a developed country with expertise in such transplants the rejection rate is as high as 34%.[10]

CHALLENGES IN SELECTING THE CONDITIONING REGIMEN: REDUCING GRAFT REJECTION AND RRT

This has led to the evaluation of a number of novel conditioning regimens to improve the clinical outcome of Class III patients and more specifically in the Class III HR subset[4,5,11–14] (summarized in Table 1). The first approach to reduce the RRT among Class III patients involved reducing the cumulative dose of CY from 200 mg/kg to 160 mg/kg.[13] Although this approach significantly reduced the RRT-associated mortality, it was associated with an increase in graft rejection from 13 to 35%.[13] Subsequent attempts with reduced-intensity conditioning regimens to reduce RRT were also associated with an increased risk of graft rejection[15,16] and for the most part have been abandoned as an option in patients with transfusion-dependent hemoglobinopathies. The first reported successful attempt at improving clinical outcomes in Class III patients by modifying the conditioning regimen was reported by Sodani *et al.*[12] They used the same template of reducing the CY dosing to 160 mg/kg; however, based on their initial adverse

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Table 1. Major reported clinical studies that have attempted to improve the outcome of patients with Class III thalassemia major

	Year	*N*	*Median age (range) (years)*	*Proportion in Class III (%)*	*Proportion in Class III HR[a] (%)*	*Major defining feature of change in protocol*	*TRM (%)*	*Graft rejection (%)*	*EFS (%)*	*OS (%)*
Lucarelli *et al.*[13 b]	1996	115	11 (3–16)	100	NA	Reduction in cumulative CY from 200 mg/kg to 160 mg/kg	24	35	49	74
Sodani *et al.*[12]	2004	33	11 (5–16)	100	NA	Reduction in CY dose to ⩽160 mg/kg. Addition of azathioprine and fludarabine and intensification of immunosupression. Suppression of erythropoesis by hyeprtransfusion, chelation and hydroxyurea starting from day −45	6	6	85	93
Gaziev *et al.*[17]	2010	71	9 (1.6–27)	57.3	NA	I.v BU, dose adjustments with therapeutic drug monitoring	7	5	87	91
Chiesa *et al.*[10]	2010	53	8 (1–17)	47	NA	I.v BU, dose adjustments with therapeutic drug monitoring	4	15	79	96
Chiesa *et al.*[10 c]	2010	25	NA	100	NA	I.v BU, dose adjustments with therapeutic drug monitoring	4	34	66	96
Bernardo *et al.*[28]	2012	60	7 (1–37)	27[d]	NA	Treosulfan-based conditioning regimen	7	9	84	93
Choudhary *et al.*[29]	2013	28	9.6 (2–18)	75	39	Treosulfan-based conditioning regimen	21	7	71	79
Anurathapan *et al.* (*BBMT* in press)	2013	18	14 (10–18)	100	NA	Pre-conditioning immunosuppression therapy with fludarabine and dexamethasone; one or two courses one to two months prior to transplant. Conditioning regimen of fludarabine with i.v BU	5	0	89	89
Mathews *et al.*[25]	2013	50	11 (2–21)	100	48	Treosulfan-based conditioning regimen with PBSC graft in 74%	12	8	79	87
Mathews *et al.*[25 e]	2013[e]	24	12 (3–21)	100	100	Treosulfan-based conditioning regimen with PBSC graft in 74%	13	8	78	87

Abbreviations: HR = high-risk; NA = not applicable. [a]As defined previously.[6] [b]Only patients <17 years included in this table. [c]Subset of high-risk cases from the same paper. [d]Includes all adult cases as well (assumed to be Class III). [e]Subset of high-risk cases from the same paper.

experience with this approach, they augmented the immune suppression by adding fludarabine and azathioprine to the conditioning regimen. Additional elements starting from day −45 included intensive chelation and hyper-transfusion therapy along with hydroxyurea and growth factors. They achieved <10% graft rejection with a >85% EFS in a series of 33 consecutive Class III patients. There have been no subsequent series from other centers that have replicated these results. I.v. BU along with therapeutic dose monitoring and dose modifications was a promising strategy to reduce RRT and potentially reduce the risk of graft rejection as illustrated in the study by Gaziev *et al.*[17] However, as demonstrated in the study by Chiesa *et al.*[10] although this approach was effective in reducing RRT, it was not able to reduce the risk of graft rejection in patients with very high-risk thalassemia major. More recently, Anurathapan *et al*. reported a novel approach of administration of one or two courses of immune-suppressive therapy with a combination of fludarabine and dexamethsaone one to two months before the start of conditioning and followed this up with a reduced-toxicity myeloablative conditioning regimen consisting of fludarabine, i.v. BU and antithymocyte globulin with promising results in a small series of 18 patients with Class III HR thalassemia major (*BBMT*, in press). The caveat to interpretation of the above studies is that the proportion of Class III patients varies and the subset of patients who would fulfill the criteria for Class III HR is often not available. These variables, especially the proportion in Class III HR, would significantly impact the clinical outcome and make comparison across different studies difficult.

NOVEL CONDITIONING REGIMEN

Treosulfan (dihydroxybusulfan), in the recent past, has attracted a lot of attention as an agent to replace BU in view of its favorable toxicity profile.[18] Structurally it is similar to BU. Unlike BU, it is water soluble and easy to reconstitute and administer intravenously. It also has a linear pharmacokinetic profile with good systemic exposure and very low intra- and inter-individual pharmacokinetic variability.[19,20] In Phase I studies, even at cumulative doses of 56 g/m^2 (a dose not usually reached when used as an agent in conditioning regimens) there were no dose-limiting hepatic, renal, neurological or cardiac toxicities.[21] Hepatic SOS is a common problem with a conventional BU-based myeloablative regimen with an incidence ranging from 5 to 40%.[22] Use of a BU-based conditioning regimen was associated with an increased incidence of SOS on a multivariate analysis in a prospective study.[23] Similarly, the link between iron overload pre-transplant and SOS is well recognized.[24] Targeted BU levels and prophylaxis with defibrotide have significantly reduced this complication in patients with thalassemia major.[10,22] These interventions are either expensive or not available at the majority of centers carrying out SCT for thalassemia major in a developing country. In the absence of such interventions, the

cumulative incidence of SOS in the very high-risk subset (Class III HR) of patients has been reported to be as high as 78% and in 24% of such cases it leads to multi-organ failure and death.[25] Treosulfan was hence especially attractive in the context of an allo-SCT for high-risk β-thalassemia major because of its reported low hepatic toxicity profile and consistent pharmacokinetic profile, which are both significant problems with conventional BU in this population.[6,26,27] However, it is also important to recognize that the pharmacokinetic profile of treosulfan-based regimens, both in patients with thalassemia major and when it is combined with other high-dose chemotherapeutic agents as part of the conditioning regimen, have not been studied extensively.

Current data using treosulfan-based conditioning regimen

The first report on the use of treosulfan being used as part of the conditioning regimen for thalassemia was by Bernado *et al.*[14] in a small series of 20 patients, of which 45% were Class III and 18 were matched unrelated SCTs. Only two patients in this series developed transient liver enzyme elevation. The conditioning consisted in addition thiotepa and fludarabine and was very well tolerated and 17 cases had complete chimerism. Recently, the same group reported on their expanded experience with this reduced-toxicity myeloablative regimen.[28] In this expanded series of 60 cases with thalassemia major, the median age was 7 years, although only 7% of the 48 children were Class III and the remaining 12 were adults. Forty (67%) patients received an unrelated donor transplant. In 47 (79%) the stem cell source was the BM (18% umbilical cord blood and 3% PBSC). The regimen as previously reported was very well tolerated with a low ($<10\%$ graft failure) and a thalassemia-free survival of 84%.

A small series reported a comparable outcome between a similar treosulfan-based ($n=28$) and a historical BU-based regimen ($n=12$).[29] However, the median age in the BU group was 7 years versus 9.6 years in the treosulfan group and the treosulfan group had 75% Class III patient of whom 52.4% were Class III HR as defined previously. In the BU arm 58% were Class III and the number that fulfilled the criteria of Class III HR is not available. The age and risk group of the patients in the treosulfan arm that died because of RRT ($n=4$) and those that had a graft rejection ($n=2$) are not available. As reported previously the outcome of Class III HR can be significantly different from Class III as a whole.[6] Interpreting this data and comparing the two groups in the absence of this information must be done with caution, more so because of the small numbers in both groups. The inferior outcome in the treosulfan arm is likely to be related to the biology of Class III HR rather than the conditioning regimen.

High-risk population: balancing early versus late complications

In a larger series a clear advantage of a treosulfan-based regimen on the clinical outcome of Class III as a whole and the subset of Class III HR was recently reported.[25] A significant reduction in non-relapse mortality and RRT, especially SOS, was demonstrated in the Class III HR in comparison to the historical control arm that had used a conventional BU-based conditioning regimen.[25] However, in this very high-risk group there was a significantly increased risk of mixed chimerism that could be overcome with the use of a PBSC graft. The use of this regimen with a PBSC graft translated to a significantly superior OS and EFS in the Class III HR subset without a significant increased risk of GVHD.[25]

BM has been the preferred choice of stem cells to reduce the risk of GVHD in this nonmalignant condition, although the incidence of both acute and chronic GVHD in this predominantly pediatric population is low.[27,30] PBSC grafts, when used, have been reported to be associated with faster engraftment and lower requirement of blood product support in the peri-transplant period[25,31,32] and have also been associated with a low incidence of graft rejection.[25,33] However, the risk of chronic GVHD is increased.[25,31,32] Larger prospective studies are required to confirm the benefit of PBSC over BM in these high-risk patients.

SUGGESTED APPROACH

In summary, a treosulfan-based conditioning regimen is ideally suited for patients with thalassemia major including very high-risk patients. The low hepatic toxicity profile and the reliable pharmacokinetics obviating the need for drug dose monitoring make this as an attractive agent that can be used in the conditioning regimen. As SCT becomes more widely used in patients with thalassemia major, there will be a great need for controlled trials to evaluate the effectiveness of specific treatment regimens for specific groups of patients and cooperative study groups (for example, via global BMT-CTN) that can successfully perform the trials necessary to substantiate the effectiveness of a given treatment regimen.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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