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Gonadal Function after Busulfan Compared with Treosulfan in Children and Adolescents Undergoing Allogeneic Hematopoietic Stem Cell Transplant

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A B S T R A C T

Gonadal impairment is an important late effect with a significant impact on quality of life of transplanted patients. The aim of this study was to compare gonadal function after busulfan (Bu) or treosulfan (Treo) conditioning regimens in pre- and postpubertal children. This retrospective, multicenter study included children transplanted in pediatric European Society for Blood and Marrow Transplantation (EBMT) centers between 1992 and 2012 who did not receive gonadotoxic chemoradiotherapy before the transplant. We evaluated 137 patients transplanted in 25 pediatric EBMT centers. Median age at transplant was 11.04 years (range, 5 to 18); 89 patients were boys and 48 girls. Eighty-nine patients were prepubertal at transplant and 48 postpubertal. One hundred eighteen children received Bu and 19 Treo. A higher proportion of girls treated with Treo in the prepubertal stage reached spontaneous puberty compared with those treated with Bu ($P = .02$). Spontaneous menarche was more frequent after Treo than after Bu ($P < .001$). Postpubertal boys and girls treated with Treo had significantly lower luteinizing hormone levels ($P = .03$ and $P = .04$, respectively) compared with the Bu group. Frequency of gonadal damage associated with Treo was significantly lower than that observed after Bu. These results need to be confirmed in a larger population.

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INTRODUCTION

Gonadal impairment represents an important late effect occurring after hematopoietic stem cell transplantation (HSCT), affecting the quality of life of young transplanted patients. The proportion and severity of gonadal late impairment depends on type and cumulative dosage of gonadotoxic chemotherapeutic agents, type of HSCT, age at HSCT, and additional use of total body irradiation (TBI) for conditioning or radiotherapy (cranial and pelvic radiotherapy) administered before HSCT [1]. Gonadal complications are recognized as a consequence of myeloablative conditioning regimens, in particular based on TBI or busulfan (Bu), and include hypergonadotropic hypogonadism and infertility. Radiation and/or alkylating agents induce irreversible ovarian failure in older girls because of the rapid decline in the number of follicles, whereas in younger girls a recovery of ovarian function may be observed [2-6]. In boys the spermatogenesis is more sensitive to radiation, and also low doses of radiotherapy may cause azoospermia [3].

The preparative regimen including Bu is associated with damage to the testicular function as reported by Sanders et al. [10] in 83% of patients. Although gonadal toxicity related to TBI has been frequently described [7-9], there are few reported studies on gonadal toxicity of Bu, in particular regarding prepubertal children. Sanders et al. [10] and Thibaud et al. [5] concluded that Bu induced a complete ovarian failure with extremely rare spontaneous recovery. Moreover, Sanders et al. [10] demonstrated that Bu administered at myeloablative doses (≥8 mg/kg) induced damage to testicular function and was associated with increased levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

Treosulfan (Treo; L-treitol-1,4-bis-methanesulfonate) is a bifunctional alkylating agent with myeloablative and immunosuppressive effects. It is administered as a conditioning agent before HSCT in patients with malignant and nonmalignant diseases, such as primary immune deficiencies, metabolic disorders, hemoglobinopathies, histiocytic disorders, and autoimmune diseases [11-15]. Its limited extramedullary toxicity and the less frequent occurrence of graft-versus-host disease is reported in a retrospective European Society for Blood and Marrow Transplantation (EBMT) study including children who received Treo as preparative regimen [10].

To the best of our knowledge, no published studies have compared gonadal damage induced by Bu versus Treo in pre- and postpubertal children. This prompted us to perform a retrospective, multicenter study involving patients treated in pediatric EBMT centers who received Bu or Treo as part of their preparative regimen for HSCT.

METHODS

This is a retrospective, multicenter, joint study conducted through the collaboration between the Pediatric Disease Working Party and the Transplant Complications Working Party of the EBMT society including pediatric patients treated with Bu or Treo at myeloablative doses (≥8 mg/kg and 36 to 42 g/m^2, respectively) between January 1992 and December 2012 and who did not receive chemotherapy and/or radiotherapy before HSCT. The underlying diseases included in the study that typically did not required gonadotoxic chemo- and/or radiotherapy before HSCT were primary immunodeficiency, Shwachman-Diamond syndrome, hemophagocytic lymphohistiocytosis, chronic myeloid leukemia, and juvenile myelomonocytic leukemia. Patient eligibility criteria also included age at HSCT between 5 and 20 years, follow-up ≥ 2 years, and being alive at the last follow-up. Patients who received chemo- and/or radiotherapy before HSCT, those affected by diseases causing iron overload such as hemoglobinopathies and primary diseases including gonadal failure, and patients who underwent pelvic surgery were excluded from the study.

Puberty was defined as the development of secondary sexual characteristics associated with skeletal and somatic growth; the time of onset of puberty was different in girls (normally from 8 to 13 years) and boys (from 9 to 4 years). In this study patients were stratified into 2 groups according to pubertal stage at HSCT, namely prepubertal and postpubertal patients. To simplify the analysis, we decided to include in the prepubertal group children aged between 5 and 14 years and in the postpubertal group those aged over 14 years. Spontaneous puberty (SP) was defined as the development of secondary sexual characteristic without hormonal replacement therapy (HRT).

The study was based on data from the EBMT database "Promise" and from those obtained through a questionnaire sent to all participating pediatric EBMT centers. The questionnaire contained questions about pubertal development and measurement of hormone levels at different time points of pubertal development, in particular for girls, the levels of LH, FSH, and 17β-estradiol before HSCT and at the last follow-up and the inception of HRT administered for gonadal failure, and for boys, the levels of LH, FSH, and testosterone before HSCT and at the last follow-up. Hormone levels at the last follow-up were measured only in the postpubertal group. The evaluation of anti-Müllerian hormone and inhibin B as markers of gonadal function in girls and boys [16] was not recorded for the inhomogeneous determination of these markers in participating EBMT centers.

The primary endpoints of this study were to evaluate gonadal function after Bu versus Treo and to analyze the differences in the onset of SP and spontaneous menarche (SM) between patients treated with Bu versus Treo during prepubertal and postpubertal phases. Secondary endpoints were to analyze gonadotropin levels in these 2 groups of patients, number of patients on HRT, and results of sperm analysis.

All patients or legal guardians gave their informed consent to the use of their data in retrospective studies. The study was approved by the Institutional Review Board of the Pediatric Disease Working Party and by the Transplant Complication Working Party.

Statistical Analysis

Continuous data were summarized using median, interquartile range (IQR), minimum, and maximum values. All categorical/qualitative data were presented using absolute counts and percentage.

Patient-, disease-, and transplant-related characteristics of the 2 groups (Bu and Treo) were compared by using the chi-square or Fisher exact test for categorical variables and Wilcoxon test for continuous variables. Analysis was conducted in the 2 groups according to pubertal status at the time of HSCT. For measurement of hormone serum levels at last follow-up, only postpubertal patients were considered, and comparisons were stratified by gender. Two-sided $P < .05$ was considered statistically significant. Because of the limited number of patients, multivariate analysis was not conducted. Statistical analyses were performed with SPSS 24.0 (SPSS Inc., Chicago, IL) and R 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of 137 patients included in this study, all were conditioned with a myeloablative dose of Bu or Treo administered in 25 pediatric EBMT centers between 1992 and 2012. The mean age at HSCT was 11.04 years (range, 5.02 to 18.55; IQR, 7.61 to 15.01). Of 137 patients, 89 (65%) were boys and 48 (35%) were girls. At the time of HSCT, 89 children (65%) were prepubertal (median age, 8.88 years; range, 5.02 to 14.3; IQR, 6.58 to 10.74) and 48 (35%) were postpubertal (median age, 15.83 years; range, 12.31 to 18.55; IQR, 14.97 to 17.11). The characteristics of these 137 patients are shown in Table 1. The difference in the number of patients who received Bu versus Treo from 1992 to 2007 and from 2008 to 2012 was statistically significant: patients 63 (53.39%) were treated with Bu versus 3 (15.79%) with Treo in the first period, and 55 (46.61%) were treated with Bu versus 16 (84.21%) with Treo in the second period ($P = .0023$).

Among the 89 prepubertal patients (62 boys and 27 girls), 77 (86.5%) received Bu (56 boys and 21 girls) and 12 Treo (13.5%; 6 boys). Median age at the last follow-up of the prepubertal patients was 17.03 years (range, 8.23 to 29.97; IQR, 14.49 to 19.66).

Among the 48 patients transplanted during the postpubertal period, 41 received Bu (85.5%; 21 boys and 20 girls) and 7 Treo (14.5%; 5 girls and 2 boys). Median age at the last follow-up in postpubertal patients was 23.9 years (range, 17.7 to 33.9; IQR, 21.9 to 25.9) in the Bu group and 23.9 years (range, 20.5 to 26.1; IQR, 21.6 to 24.7) in the Treo group ($P = .65$).

Data about SP were available for 85 of 89 prepubertal patients (58 boys and 27 girls) (Table 2). SP occurred in 64 of 85 prepubertal patients (75.3%; 50 boys [86.2%] and 14 girls [51.9%]). Considering the type of conditioning regimen, SP was achieved in 45 (80.3%) of 53 prepubertal boys who received Bu versus 5 treated with Treo (100%; $P = 1$) and in 8 (38.1%) of 21 prepubertal girls who received Bu versus 6 treated with Treo (100%; $P = .02$); the median age at SP in girls was 13 years (range, 11.5 to 16.9; IQR, 12 to 14.7).

Data about SM were available for 25 of 27 prepubertal girls (Table 3). SM occurred in 7 of 25 prepubertal girls (28%) and in 14 of 21 postpubertal girls (66.6%). Two of 20 prepubertal girls who received Bu had SM (10%) versus 5 of 5 patients treated with Treo (100%; $P < .001$). In the postpubertal group, 11 (64.7%) of 17 girls who received Bu had SM versus 3 (75%) of 4 who were treated with Treo ($P = .70$).

Table 1
Characteristics of the 137 Eligible Patients

	Population (N = 137)	Busulfan (n = 118)	Treosulfan (n = 19)	*P*
Puberty at HSCT				
No	89 (64.96)	77 (65.25)	12 (63.16)	.1957
Yes	48 (35.04)	41 (34.75)	7 (36.84)	
Median age at HSCT, yr (range) [IQR]	11.04 (5.02-18.55) [7.61-15.01]	10.7 (5-18.6) [7.6-15.1]	12.2 (6.1-18.4) [8.4-14.3]	.6161
Median age at diagnosis, yr (range) [IQR]	8.11 (0-18.04) [4.05-13.14]	7.7 (0-17.5) [4-13.1]	10.8 (1.3-18) [5.4-13.1]	.3486
Median interval between diagnosis and HSCT, mo (range) [IQR]	10.85 (.3-207.15) [5.57-54.62]	11.8 (.3-207.1) [5.5-56.8]	8.9 (.3-129.3) [5.7-42.7]	.6074
<12 mo	71 (51.82)	59 (50)	12 (63.16)	.2867
≥12 mo	66 (48.18)	59 (50)	7 (36.84)	
Median years from HSCT to last follow-up (range) [IQR]	7.79 (2.07-22.69) [6.2-10.51]	7.8 (2.1-22.7) [6.3-11.2]	7 (3-9.1) [5.9-8.2]	.1286
Median age at last follow-up, yr (range) [IQR]	19.39 (8.23-33.86) [16.1-23.53]	19.2 (8.2-33.9) [16.2-23.6]	19.8 (12-26.1) [14.9-21.7]	.4057
Median year of HSCT (range) [IQR]				
1992-2007	66 (48.18)	63 (53.39)	3 (15.79)	.0023
2008-2012	71 (51.82)	55 (46.61)	16 (84.21)	
Gender				
Male	89 (64.96)	80 (67.8)	9 (47.37)	.0832
Female	48 (35.04)	38 (32.2)	10 (52.63)	
Diagnosis				
CML	41 (29.93)	38 (32.2)	3 (15.79)	
MDS	28 (20.44)	23 (19.49)	5 (26.32)	
Shwachman-Diamond syndrome	1 (.73)	1 (.85)	0 (0)	
CI	60 (43.7)	49 (41.52)	11 (57.89)	
HLH	7 (5.11)	7 (5.93)	0 (0)	
Source of stem cells				
BM	95 (69.34)	82 (69.49)	13 (68.42)	.5224
PB	34 (24.82)	30 (25.42)	4 (21.05)	
CB	8 (5.84)	6 (5.08)	2 (10.53)	
CR associated				
Cy (CED: 4000-6600 mg/m^2)	120 (87.5)	101 (85.5)	0	
Flu	32 (23.3)	17 (14)	15 (78.9)	
L-PAM	1 (7.2)	0	1 (5.2)	

Values are n (%) unless otherwise defined. CML indicates chronic myeloid leukemia; MDS, myelodysplastic syndrome; CI, congenital immunodeficiency; HLH, hemophagocytic lymphohystiocytosis; BM, bone marrow; PB, peripheral blood; CB, cord blood; Cy, cyclophosphamide; Flu, fludarabine; L-PAM, melphalan.

Table 2
Spontaneous puberty achievement in pre-pubertal children

No. of Patients (%)	Bu n (%)	Treo n (%)	*P*
85 prepubertal patients*	74	11	
64 Spontaneous puberty (75.3%)	53 (71.6%)	11 (100%)	.06
58 prepubertal boys	53	5	
50 Spontaneous puberty (86.2%)	45 (84.9%)	5 (100%)	1
27 pre pubertal girls	21	6	
14 Spontaneous puberty (51.8%)	8 (38.0%)	6 (100%)	.02

Bu= Busulfan; Treo=Treosulfan.
* In 4 of 89 prepubertal children, data were missing.

Table 3
SM in Prepubertal and Postpubertal Girls

No. of Girls	Bu *	Treo	*P*
25 prepubertal	20	5	
7 SM (28%)	2 (10)	5 (100)	.001
21 postpubertal	17	4	
14 SM (66.6%)	11 (64.7)	3 (75)	.70

Values are n or n (%).
* In 2 of 27 prepubertal girls, data were missing.

One hundred twelve patients were postpubertal at the last follow-up, and the results of hormone levels were available in 81 of these patients (72.3%) (Table 4). LH levels were significantly lower in both boys ($P = .03$) and girls ($P = .04$) who received Treo compared with those treated with Bu. Moreover, in girls, FSH levels were also statistically lower ($P = .02$) versus girls treated with Bu.

HRT was administered in 14 (51.8%) of 27 prepubertal girls and 13 (70%) of 21 postpubertal girls. Median age at HRT was 15.3 years (range, 12.8 to 18.5; IQR, 13.7 to 16.6). In 27 girls (62.7%), HRT was administered until the last follow-up.

Seventy-seven boys were postpubertal at the last follow-up, and data about sperm analysis were available for only 8 (17%). The results of sperm analysis were oligoazoospermia in 5 patients, whereas in 3 the test result was missing.

DISCUSSION

This study is the first multicenter study comparing the effect of Bu and Treo on gonadal function. In the literature, although some studies have reported on gonadal damage secondary to Bu [17-21], data about gonadal damage in prepubertal children receiving Bu are lacking and studies on gonadal damage secondary to Treo are very few [22,23]. Studies on the gonadotoxic effects of the conditioning regimen on pubertal development and fertility often have the weakness of not adequately considering the additional gonadotoxic effect of chemotherapy and radiation administered before HSCT. Therefore, we chose to limit our study to patients affected by diseases that did not require chemo- and/or radiotherapy before HSCT.

Previous studies [2,3,24] reported a very high rate of gonadal dysfunction in patients treated with myeloablative conditioning regimen, more frequent hypergonadotropic hypogonadism in older patients, higher gonadotropin levels in females than in males, and major incidence of ovarian insufficiency with more advanced pubertal stage at HSCT. An important study by Sanders et al. [10] reported that only 1 of 73 females (1.3%) treated with Bu/cyclophosphamide had ovarian function recovery. More recently, Vatanen et al. [24] evaluated a group of 92 pubertal female survivors confirming that preserved ovarian function is more frequent in patients conditioned with cyclophosphamide alone in comparison with females prepared with either a TBI-containing (29%) or Bu-based (25%) regimen. Bresters et al. [2] demonstrated that almost half of the girls who were prepubertal or postpubertal at HSCT required hormonal induction of puberty and described the association of Bu with ovarian insufficiency in patients conditioned with chemotherapy alone. Panasiuk et al. [20] reported that girls treated with melphalan combined with fludarabine entered puberty spontaneously and required HRT to a lesser extent compared with girls receiving Bu/cyclophosphamide.

To the best of our knowledge, gonadal function in patients who received Treo has not been reported yet. An Italian study

Table 4
Post-Transplant Hormone Levels According to Conditioning Regimen

	Bu	Treo	*P*
All patients (n = 81)	66	15	
Age, yr	18.5 (11.4-32.6)	15.2 (12.6-24.4)	.04
LH, IU/L	6.6 (.1-73.2)	6.1 (.4-16)	.31
FSH, IU/L	9.8 (.2-141.1)	6.5 (2.5-32)	.08
Boys (n = 53)	47	6 18.6 (13.2-24.4)	
Age, yr	19 (11.4-30.5)	2.8 (.4-16)	.96
LH, IU/L	5.6 (.2-41.9)	5.5 (2.6-32)	.03
FSH, IU/L	8.1 (.6-108.7)	11.4 (.2-18)	.7
Testosterone, IU/L	13.5 (01-220.7)		.51
Girls (n = 28)	19 16.8 (12.8-32.6)	9	
Age, yr	31.7 (.1-73.2)	14.6 (12.6-20.4)	.10
LH, IU/L	43.9 (.2-141.1)	8.1 (1.9-15)	.04
LH before HRT, IU/L	31.7 (1.4-119) [20.1-46.1]	7.4 (.2-60) [4.1-9]	.018
FSH, IU/L	36.7 (10.7-301)	7.1(2.5-15)	.02
FSH before HRT, IU/L	78.5 (4-200) [47.7-104.5]	6.6 (2.5-92) [4.7-8.3]	.0025
17β-Estradiol, IU/L	36.7 (10.7-301)	119.4 (18.4-279)	.35
17β-Estradiol before HRT, IU/L	12 (.2-94) [10-24.8]	60.3 (5-351) [10.8-119]	.16

Values are median (range); values in brackets are IQR.

on Treo-based conditioning regimen in children with sickle cell disease [15] reported an incidence of gonadal failure after Treo of 25%; however, the number of patients included in the study was too low to draw any conclusion. Recently, an experimental study [23] conducted in mice indicated that, unlike Bu, Treo has a differential gonadal toxicity profile, manifested as mild testicular toxicity contrasted with severe ovarian toxicity in both postpubertal and prepubertal mice, due to decrease of the number of primordial ovarian follicles.

In our study the number of patients treated with Bu was higher than those treated with Treo, because Treo is a relatively new treatment option adopted over the last 10 years. The discrepancy between patient sample size and the retrospective nature of the study represents a major limitation, and the low number of patients in the Treo group precludes any adjustment for potential confounding factors. Moreover, in this cohort of patients, most received Bu associated with cyclophosphamide, and the cyclophosphamide equivalent dose (CED) was between 4000 and 6600 mg/m^2, whereas those treated with Treo receiving fludarabine.

It is recognized that alkylating agents may interfere with fertility differently in males and females [25]. In particular, in males impaired spermatogenesis is described in patients who received CED $>$ 4000 mg/m^2 [26], whereas in females the exposure to CED $\geq$ 8000 mg/m^2 was associated with premature ovarian failure [27]. In our study the CED was include between 4000 and 6600 mg/m^2. This observation could suggest that in our study CED did not influenced the gonadal function in girls treated with Bu or Treo because the dose of CED was not high, whereas in boys CED could have interfered on the fertility also in our patients.

Regarding female patients, we observed that girls who received Treo during the prepubertal period more often reached SP compared with those treated with Bu ($P = .02$). This observation suggests that Treo has less impact on pubertal development. Also, SM occurred more frequently in girls treated with Treo compared with those treated with Bu ($P < .001$), which suggests that, contrary to experimental data, Treo seems to be less toxic to ovaries than Bu. Moreover, girls treated with Treo had statistically significant lower levels of LH, FSH, and 17β-estradiol compared with girls conditioned with Bu.

In boys, there was no statistically significant difference between the Treo and Bu groups in the development of SP, but the low number of patients did not allow a conclusion but only to report these results. Moreover, boys had statistically significant lower levels of LH in the Treo group versus the Bu group, whereas the difference in FSH and testosterone levels between the 2 groups was not significant.

In our study we compared gonadal function and its recovery after Bu versus Treo considering the periods between 1992 and 2007 and between 2008 and 2012. However, comparing the 2 conditioning regimes and selecting only patients transplanted between 2007 and 2012, the results were consistent with those of the entire population (data not shown). These data imply that Treo is less gonadotoxic than Bu in girls, whereas in boys its effect may be similar; however, further prospective studies are needed to confirm these results.

In conclusion, despite some limitations to our study, we can conclude that SP was more frequent in children treated with Treo compared with those conditioned with Bu. This result is particularly evident in girls in whom the occurrence of SM was more frequent after Treo, suggesting a greater ovarian reserve and a higher opportunity of being fertile. In boys, the levels of FSH were comparable in the 2 groups, suggesting similar effects of both agents on spermatogenesis. Further prospective studies that include measurements of anti-Müllerian hormone and inhibin B and conducted in larger patient population are needed to better evaluate the impact of these 2 different regimens on fertility.

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REFERENCES

1. Tichelli A, Rovó A, Passweg J, et al. Late Effects Working Party of the European Group for Blood and Marrow Transplantation. Late complications after hematopoietic stem cell transplantation. *Expert Rev Hematol*. 2009;2:583–601.
2. Bresters D, Emons JA, Nuri N, et al. Ovarian insufficiency and pubertal development after hematopoietic stem cell transplantation in childhood. *Pediatr Blood Cancer*. 2014;61:2048–2053.
3. Dvorak CC, Gracia CR, Sanders JE, et al. NCI, NHLBI/PBMTC first international conference on late effects after pediatric hematopoietic cell transplantation: endocrine challenges-thyroid dysfunction, growth impairment, bone health, & reproductive risks. *Biol Blood Marrow Transplant*. 2011;17:1725–1738.
4. Tauchmanovà L, Selleri C, Rosa GD, et al. High prevalence of endocrine dysfunction in long-term survivors after allogeneic bone marrow transplantation for hematologic diseases. *Cancer*. 2002;95:1076–1084.
5. Thibaud E, Rodriguez-Macias K, Trivin C, et al. Ovarian function after bone marrow transplantation during childhood. *Bone Marrow Transplant*. 1998;21:287–290.
6. Cohen A, Békássy AN, Gaiero A, et al. EBMT Paediatric and Late Effects Working Parties. Endocrinological late complications after hematopoietic SCT in children. *Bone Marrow Transplant*. 2008;41(Suppl 2):S43–S48.
7. Schneider RA, Schultze J, Jensen JM, et al. Long-term outcome after static intensity-modulated total body radiotherapy using compensators stratified by pediatric and adult cohorts. *Int J Radiat Oncol Biol Phys*. 2008;70:194–202.
8. Bresters D, Lawitschka A, Cugno C, et al. Incidence and severity of crucial late effects after allogeneic HSCT for malignancy under the age of 3 years: TBI is what really matters. *Bone Marrow Transplant*. 2016;51:1482–1489.
9. Faraci M, Barra S, Cohen A, et al. Very late nonfatal consequences of fractionated TBI in children undergoing bone marrow transplant. *Int J Radiat Oncol Biol Phys*. 2005;63:1568–1575.
10. Sanders JE, Hawley J, Levy W, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulphan or total-body irradiation and bone marrow transplantation. *Blood*. 1996;87:3045–3052.
11. Slatter MA, Boztug H, Pötschger U, et al. EBMT Inborn Errors and Paediatric Diseases Working Parties. Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases. *Bone Marrow Transplant*. 2015;50:1536–1541.
12. Lehmberg K, Albert MH, Beier R, et al. Treosulfan-based conditioning regimen for children and adolescents with hemophagocytic lymphohistiocytosis. *Haematologica*. 2014;99:180–184.
13. Slatter MA, Rao K, Abd Hamid IJ, et al. Treosulfan and fludarabine conditioning for hematopoietic stem cell transplantation in children with primary immunodeficiency: UK experience. *Biol Blood Marrow Transplant*. 2018;24:529–536.
14. Greystoke B, Bonanomi S, Carr TF, et al. Treosulfan-containing regimens achieve high rates of engraftment associated with low transplant morbidity and mortality in children with non-malignant disease and significant co-morbidities. *Br J Haematol*. 2008;142:257–262.
15. Marzollo A, Calore E, Tumino M, et al. Treosulfan-based conditioning regimen in sibling and alternative donor hematopoietic stem cell transplantation for children with sickle cell disease. *J Med J Hematol Infect Dis*. 2017;9.
16. Laporte S, Couto-Silva AC, Trabado S, et al. Inhibin B and anti-Müllerian hormone as markers of gonadal function after hematopoietic cell transplantation during childhood. *BMC Pediatr*. 2011;11:20.
17. Bakker B, Oostdijk W, Bresters D, et al. Disturbances of growth and endocrine function after busulphan-based conditioning for haematopoietic stem cell transplantation during infancy and childhood. *Bone Marrow Transplant*. 2004;33:1049–1056.
18. Grigg AP, McLachlan R, Zaja J, et al. Reproductive status in long-term bone marrow transplant survivors receiving busulfan-cyclophosphamide (120 mg/kg). *Bone Marrow Transplant*. 2000;26:1089–1095.
19. Teinturier C, Hartmann O, Valteau-Couanet D, et al. Ovarian function after autologous bone marrow transplantation in childhood: high-dose busulphanis a major cause of ovarian failure. *Bone Marrow Transplant*. 1998;22:989–994.
20. Panasiuk A, Nussey S, Veys P, et al. Gonadal function and fertility after stem cell transplantation in childhood: comparison of a reduced intensity

conditioning regimen containing melphalan with a myeloablative regimen containing busulfan. *Br J Haematol*. 2015;170:719–726.
21. Afify Z, Shaw PJ, Clavano-Harding A, Cowell CT. Growth and endocrine function in children with acute myeloid leukaemia after bone marrow transplantation using busulfan/cyclophosphamide. *Bone Marrow Transplant*. 2000;25:1087–1092.
22. Brachet C, Heinrichs C, Tenoutasse S, et al. Children with sickle cell disease: growth and gonadal function after hematopoietic stem cell transplantation. *J Pediatr Hematol Oncol*. 2007;29:445–450.
23. Levi M, Stemmer SM, Stein J, et al. Treosulfan induces distinctive gonadal toxicity compared with busulfan. *Oncotarget*. 2018;9:19317–19327.
24. Vatanen A, Wilhelmsson M, Borgström B, et al. Ovarian function after allogeneic hematopoietic stem cell transplantation in childhood and adolescence. *Eur J Endocrinol*. 2013;170:211–218.
25. Ginsberg JP. New advances in fertility preservation for pediatric cancer patients. *Curr Opin Pediatr*. 2011;23:9–13.
26. Green DM, Liu W, Kutteh WH, et al. Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. *Lancet Oncol*. 2014;15:1215–1223.
27. Chemaitilly W, Li Z, Krasin MJ, et al. Premature ovarian insufficiency in childhood cancer survivors: a report from the St. Jude Lifetime cohort. *J Clin Endocrinol Metab*. 2017;102:2242–2250.