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Outcomes of allogeneic transplantation for haemoglobin Bart's hydrops fetalis syndrome in Hong Kong

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Keywords:	hematopoietic stem cell transplantation, alpha-thalassemia, Hong Kong
Abstract:	Background Hemoglobin Bart's hydrops fetalis syndrome (BHFS) was once considered a fatal condition universally. Medical advances over past three decades have resulted in increasing numbers of BHFS survivors. This retrospective review summarized local territory-wide experience and outcomes of BHFS patients who received allogeneic hematopoietic stem cell transplantation (HSCT) in Hong Kong. Methods All BHFS patients who underwent allogeneic HSCT in Hong Kong, either in one of the two former pediatric transplant centers (Queen Mary Hospital and Prince of Wales Hospital) on or before 2019 or in the single territory-wide pediatric transplant center (Hong Kong Children's Hospital) since 2019, from 1 January 1996 till 31 December 2020 were included. Basic demographic data, perinatal history, transplant details, long-term outcomes and morbidities were reviewed. Results Total five allogeneic HSCT were performed in two males and three

	females at a median age of 22 months, which include one 8/8 matched-sibling bone marrow transplant, one 5/6 matched-sibling cord blood transplant with HLA-DR antigenic mismatch, two 12/12 matched-unrelated peripheral blood stem cell transplant (PBSCT) and one haploidentical PBSCT with TCRαβ/CD45RA depletion from maternal donor. Neutrophil and platelet engrafted (>20 x 109/L) at a median of 15 and 22 days respectively. All achieved near full donor chimerism at 1 month. All patients survived and remained transfusion-independent without significant morbidities with median follow up duration of 10 years. Conclusion To conclude, local data demonstrated favorable outcome of allogeneic HSCT for BHFS patients, but sample number is small. Nondirective approach in counselling and international collaboration is recommended.

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Abbreviations

BHFS	Hemoglobin Bart's hydrops fetalis syndrome
BM	Bone marrow
CB	Cord blood
GVHD	Graft-versus-host disease
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem cell transplantation
IUT	Intrauterine transfusion
MSD	Matched sibling donor
MUD	Matched unrelated donor
PBSCT	Peripheral blood stem cell
PCR	Polymerase chain reaction
SEA	Southeast Asian
SOS	Sinusoidal obstruction syndrome
TDT	Transfusion-dependent thalassemia

MANUSCRIPT

ABSTRACT

Background

Hemoglobin Bart's hydrops fetalis syndrome (BHFS) was once considered a fatal condition universally. Medical advances over past three decades have resulted in increasing numbers of BHFS survivors. This retrospective review summarized local territory-wide experience and outcomes of BHFS patients who received allogeneic hematopoietic stem cell transplantation (HSCT) in Hong Kong.

Methods

All BHFS patients who underwent allogeneic HSCT in Hong Kong, either in one of the two former pediatric transplant centers (Queen Mary Hospital and Prince of Wales Hospital) on or before 2019 or in the single territory-wide pediatric transplant center (Hong Kong Children's Hospital) since 2019, from 1 January 1996 till 31 December 2020 were included. Basic demographic data, perinatal history, transplant details, long-term outcomes and morbidities were reviewed.

Results

Total five allogeneic HSCT were performed in two males and three females at a median age of 22 months, which include one 8/8 matched-sibling bone marrow transplant, one 5/6 matched-sibling cord blood transplant with HLA-DR antigenic mismatch, two 12/12 matched-unrelated peripheral blood

stem cell transplant (PBSCT) and one haploidentical PBSCT with TCRαβ/CD45RA depletion from maternal donor. Neutrophil and platelet engrafted (>20 x 10^9/L) at a median of 15 and 22 days respectively. All achieved near full donor chimerism at 1 month. All patients survived and remained transfusion-independent without significant morbidities with median follow up duration of 10 years.

Conclusion

To conclude, local data demonstrated favorable outcome of allogeneic HSCT for BHFS patients, but sample number is small. Nondirective approach in counselling and international collaboration is recommended.

Keywords (MeSH terms 2020): alpha-thalassemia, hydrops fetalis, hematopoietic stem cell transplantation, peripheral blood stem cell transplantation, Hong Kong

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MAIN BODY TEXT

INTRODUCTION

Hemoglobin Bart's hydrops fetalis syndrome (BHFS), also known as homozygous α^0-thalassaemia major or homozygous α-thalassemia 1, was first described in 1960 (1, 2). It was once considered a fatal condition universally, and fetuses died in utero, were stillborn or died during the early neonatal period (3). When prenatal screening and diagnosis for thalassemia first started in Hong Kong in 1983, BHFS was advocated as an indication for termination of pregnancy (4). Improvements in intrauterine interventions and perinatal intensive care over past three decades have resulted in increasing numbers of BHFS survivors, both internationally (5) and locally (6). Successful hematopoietic stem cell transplantation (HSCT) offers a cure for this disease (7-9), albeit at the expense of possible significant morbidities or even mortality. This retrospective review summarized local territory-wide experience and outcomes of BHFS patients who received HSCT in HK.

METHODS

Patient recruitment

All BHFS patients who underwent allogeneic HSCT in Hong Kong, either in one of the two former pediatric transplant centers (Queen Mary Hospital and Prince of Wales Hospital) on or before 2019 or in the single territory-wide pediatric transplant center (Hong Kong Children's Hospital) since 2019, from 1 January 1996 till 31 December 2020 were included, in which three cases had been described separately in two previous publications (6, 7). Basic demographic data, perinatal history, transplant

details and outcomes, long-term outcomes and morbidities were reviewed.

Joint antenatal counselling by obstetrician and pediatric hematologists

For pregnant women bearing fetus with diagnosed BHFS and received antenatal care in public hospital, joint antenatal counselling by obstetricians and pediatric hematologists would be offered to couples or families. Need of intrauterine transfusions, neonatal intensive care, associated comorbidities would be discussed (details could be referred to **Appendix 1** of previous publication (6)). For those opted for continuation of pregnancy, couples would be counselled on different treatment strategies for BHFS survivors including regular transfusion and chelation versus HSCT with advantages and drawbacks of each option explained.

Counselling for HSCT

Different options of donor (matched sibling donor MSD, matched unrelated donor MUD, or haploidentical donor) and stem cell sources (bone marrow BM, cord blood CB, peripheral blood stem cells PBSC) were addressed and decided based on the high-resolution molecular typing for both HLA class I and class II loci (HLA-A, B, C, DRB1, DQB1) and following stringent criteria of compatibility with recipient. Information sheet were also provided upon counselling with written consent obtained. For patients without MSD or MUD, the option of HSCT from haploidentical donor was offered as individual case consideration under research protocol.

Procedures and evaluations prior to HSCT

Donors and recipients underwent donor and recipient workup respectively to ensure they were in optimal health condition to undergo HSCT. Investigations include blood and urine tests, chest X-ray and electrocardiogram. Regular transfusions were given to BHFS patients at 3-6 weekly interval pre-transplant to maintain pre- and post-transfusion hemoglobin level of 10 gm/dL and 14 gm/dL respectively. Iron overloading status in recipients would be assessed by serum ferritin, echocardiogram and magnetic resonance imaging (MRI) T2* of heart, liver, pancreas and pituitary glands. Oral deferasirox would be initiated when serum ferritin >1000 ng/ml (2200 pmol/L). Urine beta-2 microglobulin would be monitored while patients were put on deferasirox. For patient intolerant to deferasirox, oral deferiprone would be used instead. Combined use of oral chelators with intravenous deferoxamine would be considered for patients with suboptimal chelation with single agent. Autologous BM of BHFS patients would be harvested and cryopreserved before HSCT, which would be infused as rescue for marrow aplasia in case of non-engraftment. Double lumen central venous catheter would be inserted before transplant, preferably in the same session of general anesthesia for autologous BM harvest. Elected donor would undergo peripheral blood stem cell mobilization and harvest.

Conditioning, stem cell transplantation and supportive care

Patients were nursed in a high-efficiency particulate air-filtered room in the HSCT unit. Same transplant protocol on conditioning and supportive medications was generally employed for all patients with same

donor type and stem cell source, with modifications on drug selection and doses based on individual circumstances and clinical judgment with reference to updated medical literature.

Definition and endpoints

Myeloid engraftment was defined as the first of 3 consecutive days with ANC >0.5×10^9/L. Platelet engraftment was defined as the first of 7 consecutive days with a platelet count >20×10^9/L without platelet transfusion. Acute and chronic graft-versus-host diseases (GVHD) were diagnosed and graded according to the established criteria (10). Chimerism of donor/recipient DNA in peripheral blood was analyzed using either fluorescent in situ hybridization of X and Y chromosomes or short tandem repeat PCR depending on the technology available at the time of transplant.

Ethical approval

This retrospective study complied with the Declaration of Helsinki and approval was obtained from the Institutional Review Board of the University of Hong Kong / Hospital Authority Hong Kong West Cluster Clinical Research Ethics Committee (HKUCTR-2148).

RESULTS

Basic demographics, congenital malformations and neonatal outcomes (Table 1)

Total five patients were identified, of whom two males and three females. Median duration of follow up was 10 years. All were Chinese and were homozygous for Southeast Asian (SEA) α-thalassemia

deletion. Three out of five were diagnosed antenatally by amniocentesis, chorionic villi sampling or cordocentesis during concomitant intrauterine transfusion (IUT). All three couples were counselled antenatally by both obstetricians and pediatric hematologists, and they all decided to continue the pregnancy. IUT were performed in all 3 patients, ranging from two to four times in antenatal period. The remaining two without antenatal care was diagnosed postnatally by hemoglobin pattern at birth. Four out of five patients presented with different degrees of congenital malformations including urogenital anomalies **(Cases 4 and 5)**, secundum atrial septal defect **(Case 3)** and dental deformation with malocclusion **(Case 2)**. Two out of five were delivered prematurely **(Cases 2 and 5)**. All five patients required intubation and mechanical ventilation in intensive care unit during the neonatal period.

Transplant details and outcomes (Table 2)

Concerning HSCT, one patient underwent BM transplant from 8/8 MSD, one underwent CB transplant from 5/6 MSD with HLA-DR antigen mismatch, two underwent PBSCT from 12/12 MUD while one underwent haploidentical PBSCT with TCRαβ/CD45RA depletion from maternal donor. Median age of HSCT was 22 months. Neutrophil and platelet engrafted (>20 x 10^9/L) at a median of 15 and 22 days respectively. All achieved near full donor chimerism at 1 month and remained transfusion-independent. Four patients **(Cases 2 to 5)** experienced grade II acute GVHD of skin which resolved readily with topical and systemic steroid. Two patients **(Cases 3 and 5)** experienced grade II to III gut acute GHVD which resolved with systemic steroid and/or ruxolitinib. Iron overloading post-HSCT was encountered in three patients **(Cases 1, 3 and 4)** with median ferritin level of 8000 pmol/L, which fell to below 1000 pmol/L

spontaneously **(Case 1)**, by venesection **(Case 4)** or chelation **(Case 3)**.

Long-term growth, puberty and neurocognitive outcomes (Table 3)

Amongst all three post-pubertal patients **(Cases 1 to 3)**, all had short stature despite normal puberty. Two had primary gonadal failure but not requiring hormonal replacement therapy **(Cases 2 and 3)**. Three out of five patients encountered mild degree of developmental delay **(Cases 1, 2 and 4)** which improved with training and support. One patient **(Case 3)** had mild spastic diplegia due to fetal hypoxia.

Discussion

Paradigm shift in management of BHFS in HK over past 2 decades

The SEA deletion is present at an allele frequency of 4% to 8% in Southern China and HK (11). Since 1983, prenatal screening had been practiced in HK using a maternal mean corpuscular volume of ≤80 fL as cut-off and prenatal diagnosis using chorionic villus sampling, amniocentesis, and cordocentesis. Termination of pregnancy had been advocated in view of grave prognosis to BHFS fetuses. With improvements in intrauterine interventions and perinatal intensive care over past three decades, increasing numbers of BHFS survivors had been reported globally and locally. Currently largest cohort reported worldwide was from the international registry including 69 long-term BHFS survivors (5). There were total 10 BHFS survivors in Hong Kong over past 25 years from 1 January 1996 till 31 December 2020, which had been described in two previous publications (6, 7). Reasons for continuation of pregnancy include religious reason, personal preference or failure in antenatal diagnosis (6). All families were being counselled on different treatment strategies including regular

transfusion and chelation versus HSCT with advantages and drawbacks of each option explained.

Challenges in HSCT counselling for BHFS patients

Unlike leukemias or other malignancies, BHFS is a non-malignant disease and there is no imminent urgency for transplantation. Regular transfusion and chelation are reasonable alternative treatment options. Patients with transfusion-dependent thalassemias (TDT) such as BHFS can achieve reasonable life expectancy with the availability of safe blood product, improved compliance with oral chelators as compared to subcutaneous deferoxime as well as MRI T2* in assessing iron-overload related organ toxicities in addition to serum ferritin. Family may not opt for undertaking an allogeneic hematopoietic stem cell transplantation which may be associated with around 5-10% transplant-related mortality, also transplant-related complications such as acute and chronic GVHD and conditioning-related organ toxicities. In counselling parents and caregivers contemplating HSCT versus regular transfusion and chelation, indirective approach with non-judgmental attitude and cautious optimism is imperative in guiding parents making important decision. Provision of adequate and up-to-date information facilitate disease acceptance and alignment of management and expectations (6). As in our locality, five out of ten were transplanted after counselling, one was waitlisted and scheduled for HSCT in near future while four refused for the aforementioned reasons.

For those families finally accepted for HSCT, their main consideration was that successful HSCT allows BHFS patients to enjoy better quality of life as compared to those treated conventionally (12) as lifelong regular transfusion and chelation is cumbersome, difficult to adhere, costly and not without side effects

similar to other types of TDT (13-15). Moreover, many patients still have inadequate iron removal despite chelation and develop various complications or die prematurely (16-18). Stable supply of safe blood products may also be a concern in times of pandemic such as COVID-19. In addition, although HSCT for BHFS is not as imminent as hemic malignancies, it should still be done as soon as possible before development of iron overload and iron-related tissue damage. In our locality, we tend to perform allogenic HSCT for TDT patients before 8 years of age to minimize risk of subsequent development of endocrinopathies especially compromise on growth, puberty and fertility (19). Concerning transplant-related mortalities and morbidities, availability of graft manipulation such as TCRαβ/CD45RA depletion lowers risk of GVHD and allow extension of donor choice to haploidentical parents, making allogeneic HSCT feasible even for patients without MSD or MUD. The risk of transplant-related deaths also much decreased with the improvement in conditioning regimen, infection control and supportive care. Families would accept the option of HSCT when they perceive the benefits outweigh the possible risks. From hospital administrative point of view, one-time cost for an allogenic HSCT was less than long-term cost of regular transfusion and chelation for a single patient.

Extension of donor and stem cell types

For our first patient **(Case 1)**, 8/8 MSD BMT was performed with 5-year-old elder brother as donor. For our second patient **(Case 2)**, CBT was performed using 5/6-matched MSD with DR-antigen mismatch as her parents gave birth to younger brother who was one year younger than our patient who demonstrated hypochromic microcytic blood picture but normal hemoglobin pattern. Allogeneic HSCT

using BM or CB from MSD is a routinely offered treatment option to TDT patients as recommend by the European Blood and Marrow Transplantation Inborn Error Working Party and the Pediatric Diseases Working Party (20), and HSCT is recommended to be done as soon as possible before development of iron overload and iron-related tissue damage.

For our third and fourth cases **(Cases 3 and 4)**, both were transplanted using 12/12 MUD PBSC as MSD could not be identified, and the proposal for parents produce a subsequent sibling serving as a 'savior baby' raised ethical concern (21). With the advancements in molecular technology, extended typing of HLA could be performed and thus allowing extension of donor type from MSD to MUD. At the time of HSCT, MUD had been increasing employed to treat TDT patients with similar survival and reasonably low rate of severe complications such as GVHD (22-24). Li from China reported 3-year OS and transfusion-free survival of 92.3% and 90.4% respectively for MUD HSCT in 84 TDT patients (22)

As for the most recent case being transplanted **(Case 5)**, as no MSD or MUD could be identified and parents keen for transplantation after counselling, haploidentical donor had been sought for. As patient demonstrated no mismatches in killer immunoglobulin-like receptors (KIR) with both parents while mother is homozygous at both HLA-A and HLA-B alleles, mother was chosen as donor. Two doses of weekly intravenous rituximab 375mg/m^2/dose were given on D-13 and D-6 in view of low level of donor-specific antibody against HLA-DRB1*09 with mean fluorescence intensity of 5246. This is the first reported case in literature of successful transplantation of BHFS patients with haploidentical PBSCT employing TCRαβ/CD45RA depletion method in achieving transfusion-independency, which is novel to

the reported cohort of 18 out of 69 BHFS patients receiving allogeneic HSCT worldwide registered in the international registry (5).

Long-term outcomes and morbidities

Congenital malformations are often encountered in BHFS patients, the most common being genitourinary and musculoskeletal defects attributable by in-utero fetal anemia affecting organogenesis but are usually amenable to surgical correction. It is worth noting that all male babies in local BFHS cohort displayed hypospadias (6), including the two male BFHS patients who had been transplanted. International BHFS registry addressed concerns on long term growth and developmental outcome as forty percent demonstrated growth retardation and twenty percent were noted to have developmental delay in their cohort while hyper-transfusion regime was proposed to be associated with better neurological outcome (5). Another overseas case report also echoed with the findings (25). For the three out of five cases **(Cases 3 to 5)** reported here with IUT given, one **(Case 3)** had mild spastic diplegia with bilateral tight Achilles' tendons but not affecting locomotion. She had normal intelligence and attend normal mainstream secondary school. For **Case 4** with mild speech delay and learning difficulty especially with reading and writing, it was presumably accounted by under stimulation during pre-school age, which readily improved with training by speech and occupational therapists. The remaining child **(Case 5)** had normal development so far.

Strengths and limitations of this study

BHFS was once considered a fatal condition and was advocated for termination of pregnancy. International BHFS registry reported the world largest cohort of 69 long-term survivors in 2017, of which 18 received allogeneic HSCT (5). This paper described a local case series of 5 BHFS patients who underwent allogeneic HSCT, of which 2 had not be reported before. Local cohort also demonstrated the evolution of practice in the field of allogeneic HSCT for transfusion-dependent thalassemias over past two decades in Hong Kong in terms of donor choice, stem cell types, graft manipulation, and modifications in conditioning regimen. Our latest successful transplanted case is a haploidentical peripheral stem cell transplantation employing TCRαβ/CD45RA depletion method in achieving transfusion-independency, which had not been reported in literature before in BHFS setting. Favorable outcome had been demonstrated in terms of 100% thalassemia-free survival, 0% transplant-related mortality and reasonably acceptable morbidity. Although sample size is small, this paper addresses a very rare condition for which limited scientific evidence can be collected, and information provided by this paper is useful for future handling of such patients in the field of HSCT.

Future prospect in management of BHFS patients

BHFS is no longer destined to be fatal, and termination of pregnancy should not be the only option offered to parents. Detailed antenatal counselling of parents involving obstetricians, neonatologists, hematologists and stem cell transplant physicians with non-judgmental attitude and cautious optimism are imperative. Formulation of standard treatment protocol in regional, national and/or international level is recommended to facilitate streamlined multidisciplinary management of BHFS patients. New

advances such as gene therapy (26) and fetal HSCT (27) may revolutionize the management of TDT including BHFS in the future.

Conclusion

To conclude, local data demonstrated favorable outcome of allogeneic HSCT for BHFS patients, but sample number is small. International collaboration is recommended to further consolidate experience, facilitate research and improve outcome.

Disclosure

All authors have disclosed no conflicts of interest.

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Table 1. Basic demographics and perinatal history of the five BHFS patients who received allogeneic transplantation in Hong Kong over past 25 years (1996-2020)

Patient number	1	2	3	4	5
Ethnicity	Chinese	Chinese	Chinese	Chinese	Chinese
Gender	Female	Female	Female	Male	Male
Genetics	Homozygous SEA deletion	Homozygous SEA deletion	Homozygous SEA deletion	Homozygous SEA deletion	Homozygous SEA deletion
Diagnosis	No antenatal care	No antenatal care, postnatal diagnosis by hemoglobin pattern at birth	Antenatal diagnosis by amniocentesis	Antenatal diagnosis by chorionic villi sampling and cordocentesis	Antenatal diagnosis by cordocentesis
Antenatal intervention	Not applicable	Not applicable	IUT 2 times, DVET once	IUT 4 times, SVET once	IUT 3 times, SVET once
Mode of delivery	Emergency Caesarean for fetal distress	SVD	Emergency Caesarean section for failed induction	SVD	SVD
Birth weight (kilograms)	1.92 (edematous) 1.2 (3rd centile, SGA)	1.00 (>97th centile, LGA)	2.09 (<3rd centile, SGA)	2.51 (3rd centile, SGA)	1.88 (50th centile, AGA)
Gestation at delivery (weeks)	35	24	37	38	32
Apgar score (1 / 5 minutes)	0 (1), 1 (5)	2 (1), 7 (5)	5 (1), 8(5)	7(1), 8(5)	4(1), 7 (5)
Neonatal complications	Resuscitation at birth and ventilator therapy	Prematurity (24 weeks), PPHN, PDA	PPHN, PDA	Congenital pneumonia, right side pneumothorax	Prematurity (32 weeks), RDS, IVH
Congenital malformations	No	Dental deformation, anodontia, malocclusion	Small secundum ASD	Proximal penile hypospadia, urethroplasty and release of chordee done at 14 months old	Penoscrotal hypospadia and left undescended testes, surgery at 15 months old
Transfusion interval	4 weekly	4 weekly	5 weekly	4-6 weekly	4-5 weekly

AGA: appropriate for gestational age; ASD: atrial septal defect; DV: double volume; ET: exchange transfusion; IUT: intra-uterine transfusion; IVH: intraventricular hemorrhage; LGA: large for gestational age; PDA: patent ductus arteriosus; PPHN: persistent pulmonary hypertension of newborn; RDS: respiratory distress syndrome; SEA: southeast Asian; SGA: small for gestational age; SV: single volume; SVD: spontaneous vaginal delivery.

Table 2. Transplant details and outcomes of the five BHFS patients who received allogeneic transplantation in Hong Kong over past 25 years (1996-2020)

Patient number	**1**	**2**	**3**	**4**	**5**
HSCT center	Prince of Wales Hospital	Queen Mary Hospital	Queen Mary Hospital	Queen Mary Hospital	HK Children's Hospital
Donor	MSD	MSD	MUD	MUD	Haploidentical mother
Stem cell source	BM	CB	PBSC	PBSC	PBSC
HLA matching	8/8 matched	5/6 (DR Ag mismatched)	12/12 matched (HKBMDR)	12/12 matched (CMDP)	Haploidentical
Graft manipulation	Unmanipulated	Unmanipulated	Unmanipulated	Unmanipulated	TCRαβ/CD45RA depletion
Cell dose	TNC 18.6 x 10^8/kg GM-CFU 6.19 x 105/kg CD34 41.4 x 10^6/kg	TNC 4.8 x 10^7/kg	CD34 5.9 x 10^6/kg	CD34 12.17 x 10^6/kg	CD34 7.7 x 10^6/kg CD45RO 1 x 10^6/kg
Conditioning	Bu/CPM	Bu/CPM	Bu/CPM	HU/AZA/CPM/Bu/TT/Flu	HU/AZA/CPM/TT/Flu/Treo
GVHD prophylaxis	CSA, MTX, horse ATG	CSA, MTX, horse ATG	CSA, MTX, horse ATG	CSA, MTX, MMF, rabbit ATG	Rabbit ATG
Age at transplant	21 months	20 months	22 months	28 months	60 months
Neutrophil engraftment	D+17	D+26	D+15	D+11	D+13
Platelet engraftment	Not available	D+38 (platelet >20) D+56 (platelet >50)	D+17 (platelet >20) D+25 (platelet >50)	D+10 (platelet >20) D+12 (platelet >50)	D+27 (platelet >20)
Donor chimerism	99% at 1 month (by XY FISH)	100% at 1 month (by XY FISH)	100% at 1 month (by XY FISH)	98% at 1 month (by STR PCR)	99% at 1 month (by STR PCR)
Transfusion dependency	No need regular transfusion	No need regular transfusion	No need regular transfusion	No need regular transfusion	No need regular transfusion
Transplant complications	Nil	Grade 2 skin aGVHD resolved with systemic steroid, HHV-6 viremia resolved with foscarnet	Grade 2 aGVHD of skin and grade 3 aGVHD of gut resolved with systemic steroid, HHV-7 viremia spontaneously resolved	Grade 2 skin aGVHD resoled with systemic steroid, EBV and HHV-6 viremia resolved spontaneously. RV/EV	Klebsiella bacteremia cleared with antibiotics. Grade 2 skin aGVHD resolved by topical steroid, grade 2 gut

				URTI. MRCNS line sepsis.	aGVHD resolved with steroid and ruxolitinib

Ag: antigen; aGVHD: acute graft-versus-host disease; ATG: antithymocyte globulin; AZA: azathioprine; BM: bone marrow; Bu: busulphan; CB: cord blood; CMDP: China marrow donor program; CPM: cyclophosphamide; CSA: cyclosporine; EBV: Epstein-Barr virus; EV: enterovirus; FISH: fluorescent in situ hybridization; Flu: fludarabine; GM-CFU: granulocyte-macrophage colony-forming units; HK: Hong Kong; HKBMDR: Hong Kong bone marrow donor registry; HU: hydroxyurea; MMF: mycophenolate mofetil; MRCNS: methicillin-resistant coagulase-negative staphylococcus; MSD: matched sibling donor; MTX: methotrexate; MUD: matched unrelated donor; PBSC: peripheral blood stem cell; PCR: polymerase chain reaction; RV: rhinovirus; STR: short tandem repeat; TT: thiotepa; Treo: treosulphan; URTI: upper respiratory tract infection

Table 3. Long-term outcomes and morbidities of the five BHFS patients who received allogeneic transplantation in Hong Kong over past 25 years (1996-2020)

Patient number	**1**	**2**	**3**	**4**	**5**
Follow up period	25 years	23 years	10 years	3 years	3 months
Growth, puberty and endocrine problems	Short stature (3rd centile)	Primary gonadal failure not requiring HRT. Menarche at age 12 with regular menses. Hypertriglyceridemia and hyperuricemia	Short stature (BH just below 3rd centile with MPH on 10th centile) and obesity. Menarche at age 9 with normal menses Evolving gonadal failure	Pre-pubertal	Pre-pubertal
Neurology and development	Mild intellectual disability	Mild intellectual disability (IQ 80-89), graduated from mainstream secondary school with stable employment	Normal secondary school student in mainstream school. Bilateral tight Achilles tendons not affecting locomotion	Mild speech delay improved after speech therapy, preschooler with parental concern on reading and writing	Normal development up to date (5 years old)
Teeth and bones	Unremarkable	Anodontia, malocclusion	Unremarkable	Unremarkable	Unremarkable
Iron overloading post-HSCT	Severe biopsy-proven hepatic hemosiderosis but no fibrosis due to multiple blood transfusions, ferritin 3916 → 1408 pmol/L post-HSCT 20 months	No concern on cardiac / hepatic / pancreatic / pituitary iron overloading	Hyperferritinemia highest 8016 pmol/L with mild hepatic iron overload (MRI T2* 6.2ms), normalized with 6-month course of deferasirox	Hyperferritinemia highest 8133 pmol/L post HSCT, on 8-weekly venesection till ferritin dropped to <1000 pmol/L	Not applicable

BH: body height; HRT: hormonal replacement therapy; HSCT: hematopoietic stem cell transplantation; IQ: intelligence quotient; MPH: mid-parental height