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Research Article

A Single Center Experience with Haploidentical Stem Cell Transplantation for Pediatric Patients with Advanced-Stage Malignant Solid Tumors

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Abstract

Introduction: Natural killer (NK) cells are key players of the innate immune system. In haploidentical stem cell transplantation (haplo-SCT) allo-reactive NK cells mediate potent antileukemia effects and preclinical models show that paediatric malignant solid tumours (PMST) might be susceptible to NK-cell mediated cytotoxicity.

Methods: Haplo-SCT with ex vivo graft manipulation (CD3/CD19- and αβ/CD19-depletion) was offered as compassionate therapy to 11 patients with primary metastatic or metastatic relapse of Ewing's sarcoma (n = 6), osteosarcoma (n=3) and high-risk neuroblastoma (n=2). Donors were selected according to the receptor-ligand mismatch model.

Results: There were no treatment-related deaths. Graft failure, viral infection complications and grade II-IV acute and moderate chronic graft versus host disease were frequent. Six patients are alive in remission after a median follow up of 27 months [9-126] since haplo-SCT, and five patients relapsed and died of disease. All 6 survivors underwent HSCT in remission, including

three patients undergoing haplo-SCT in second remission after metastatic relapse of Ewing's sarcoma. Median survival after primary diagnosis of cancer was significantly higher in the group of patients undergoing haplo-SCT when compared with a historical control group of patients (46 versus 27 months, p = 0.0177).

Conclusions: Haplo-SCT increased overall survival in a cohort of advanced PMST. Remission status before transplantation might condition outcome.

Keywords: Haploidentical Stem Cell Transplantation; High-Risk Solid Tumours; Alloreactive NK Cells; NK-Cell Mediated Cytotoxicity

Abbreviations: ADV: Adenovirus; BUMEL: Busulphan Plus Melphalan; BKV: BK Virus; CMV: Cytomegalovirus; COJEC: Vincristine, Carboplatin, Cisplatin, Cyclophosphamide, Etoposide; CR1: First Complete Remission; CR2: Second Complete Remission; ES: Ewing's Sarcoma; GF: Graft Failure; GVHD: Graft Versus Host Disease; HHV6: Human Herpes Virus 6; HR: High-Risk; Haplo-SCT: Haploidentical Stem Cell Transplantation; MAT: Megatherapy And Autologous Stem Cell Rescue; MDIC: Methotrexate, Doxorubicin, Ifosfamide And Cisplatin; mIBG: Iodine-131 Metaiobenzylguanidine; NK: Natural Killer; PMST: Pediatric Malignant Solid Tumours; OS: Overall Survival; PD: Progressive Disease; PR: Partial Remission; TVD: Topotecan Plus Vincristine And Doxorubicin; VIDE: Vincristine, Ifosfamide, Doxorubicin And Etoposide

Introduction

Prognosis of primary metastatic and metastatic relapse of high-risk (HR) paediatric malignant solid tumours (PMST) is poor. Chemotherapy, radiotherapy and surgery are insufficient for the management of these patient and alternative strategies such as targeted therapy and immunotherapy are needed [1-10]. Although these patients are well candidates for early phase clinical trials, surgical removal of lung or other metastases is often pursued to confirm diagnosis or even achieve a new remission which may prolong survival, particularly in patients with osteosarcoma [5,11,12]. However, the absence of measurable disease after surgery often precludes their inclusion in such trials. In addition, the absence of ongoing paediatric early trials or available slots reduces treatment opportunities for these children. Natural killer (NK) cells are key players of the innate immune system [13]. In haploidentical stem cell transplantation (haplo-SCT), allo-reactive NK cells mediate potent antileukemia effects and preclinical models show that PMST might be also susceptible to NK-cell mediated cytotoxicity [3,14-16]. There are anecdotal reports supporting the role of haplo-SCT in paediatric patients with advanced stage malignant solid tumours [17-22]. Here we present our single centre experience with such approach in 11 children.

Methods

This was a retrospective single centre study of children with primary metastatic and metastatic relapse of HR PMST who underwent haplo-SCT under a compassionate use program. Written informed consent was obtained from parents and all eleven patients were transplanted at our institution. For donor selection, the receptor-ligand mismatch model was the preferred method applied [23]. Donors were mobilized using granulocyte colony-stimulating factor. Conditioning regimen consisted of a combination of methylprednisolone, fludarabine, busulfan and thiotepa in 10 patients, one patient diagnosed with relapsed HR neuroblastoma received therapeutic iodine-131 metaiobenzylguanidine (mIBG) early before conditioning and received melphalan instead of busulphan. Ex vivo graft manipulation included CD3+/CD19+ depletion in 2 patients transplanted before 2013, and αβ+/CD19+ depletion in the remaining 9 patients. Primary graft failure was diagnosed when a patient did not achieve an absolute neutrophils count > 500/µl by day +28. Decline of neutrophil counts bellow 500/µl after previous engraftment was recorded as secondary graft failure. Chimerism was periodically evaluated by PCR analysis in both total mononuclear cells and CD3+ cell subset in peripheral blood. Graft versus host disease (GVHD) prophylaxis consisted of a combination of cyclosporine and methotrexate in 5 patients and mycophenolate in one. Five patients with CD3αβ+ graft cell contents below 25 x 10^3/kg received no pharmacological prophylaxis. Standard criteria were applied for the definition and grading of acute and chronic GVHD [24,25]. Serum immunoglobulin levels as well as absolute number of CD3+, CD3+CD4+, CD3+CD8+, CD3-CD56+ and CD20+ cells were evaluated every 30 days after engraftment. Overall survival (OS) was defined as the time form primary diagnosis to death from any cause or last contact and the probability of OS was calculated according to the Kaplan-Meier method. The log-rank test was used to compare the difference in OS with and historical control group of 24 patients with relapsed Ewing`s sarcoma (n=8), osteosarcoma (n=10) and HR neuroblastoma (n=6).

Results

From November 2010 to May 2021 eleven patients with PMST underwent haplo-SCT. Median ages at primary diagnosis and at haplo-SCT were 13 years [1-15] and 14 years [3-17], respectively. Clinical characteristics of these patients are described in Table 1. Three patients were diagnosed with localized (limb) Ewing's sarcoma (ES) and receive a combination of vincristine, ifosfamide, doxorubicin and etoposide (VIDE), followed by surgical resection of the primary tumour before metastatic relapse

as solitary lung metastasis in two (13 and 27 months after primary diagnosis), and as multiple skull metastases in one (36 months after diagnosis). Treatment after relapse and before haplo-SCT included surgical resection of lung metastasis or radiotherapy for skull metastasis, followed by a combination of topotecan plus cyclophosphamide in two, and high-dose ifosfamide followed by myeloablative therapy (MAT) with busulfan plus melphalan (BUMEL) and autologous stem cell rescue in one. Three patients were diagnosed with primary metastatic (lung metastasis in two and multiple bone in one) pelvic ES and received VIDE followed by MAT (BUMEL) and radiotherapy to achieve remission before haplo-SCT; one progressed during VIDE and received radiotherapy, a combination of topotecan plus cyclophosphamide followed by MAT (carboplatin, etoposide plus melphalan) and achieved a partial remission (PR) before haplo-SCT. One patient with localized osteosarcoma received a combination of methotrexate, doxorubicin, ifosfamide and cisplatin (MDIC) followed by surgical resection of primary tumour, then locally relapsed 22 months after primary diagnosis and had local and metastatic (lung) progression during second line chemotherapy with ifofamide plus etoposide, and achieved a second remission (CR2) after surgical removal of primary tumour and lung metastases. Two patients with primary metastatic (multiple lung nodules) osteosarcoma underwent upfront surgical removal of lung metastasis followed by MDIC chemotherapy and local surgery before haplo-SCT.

Two patients with MYCN-amplified HR neuroblastoma received neoadjuvant chemotherapy with vincristine, carboplatin, cisplatin, cyclophosphamide and etoposide (COJEC) followed by a combination of topotecan plus vincristine and doxorubicin (TVD) and MAT (BUMEL), surgical removal of primary tumour and local radiotherapy. One progressed during COJEC chemotherapy and received additional chemotherapy with cyclophosphamide plus topotecan before MAT and then therapeutic mIBG and isotretinoine. Both neuroblastoma patients underwent haplo-SCT with active progressive disease (PD). In total, four patients with primarily metastatic disease received haplo-SCT in first CR (CR1), four in CR2 after metastatic relapse, one in PR, and two with PD. For seven patients who relapsed/progressed before haplo-SCT, median time from primary diagnosis to relapse/progression was 13 months [3-36]. Median time from primary diagnosis to haplo-SCT was 19 months [9-50]. One patient was transplanted from her haploidentical brother and the remaining ten from one progenitor. NK alloreactivity was recorded in four patients (2DL3 and 3DL1 missing ligand in two patients each). Details on graft, NK alloreactivity and infused cells are shown in Table 2. Median number of infused CD34+ and NK cells were 8.93 x 10^6/kg [3.26-25.21] and 21.99 x 10^6/kg [2.26-74.37], respectively. Median number of infused CD20+ was 31.22 x 10^3/k [0-1,913]. For nine patients who received an αβ+ depleted graft, the median number of infused αβ+ and γδ+ T-cells was 8.95 x 10^3/kg [0-355] and 9.04 x 10^6/kg [0.8-21.06], respectively. Primary graft failure (GF) occurred in 3 patients, two of whom were rescued with a second transplantation from the same haploidentical donor and from an HLA compatible sibling donor, respectively; one patient rejected 2 subsequent haplo-SCT procedures from the same donor and was finally rescued with a fourth CD45RA ex vivo depleted graft from an alternative haploidentical donor (sister) after conditioning with fludarabine, therosulfan and 2 Gy total body irradiation. Secondary GF occurred in one patient who was rescued with a second transplantation from an alternative HLA compatible sister. Median number of infused CD34+ in these four patients was 6.7 x 10^6/kg [3.36-12] (Table 2 and 3). Non-fatal grade 2-4 acute GVHD was diagnosed in 4 patients after the first haplo-SCT, including one grade 4, and in 2 additional patients after subsequent haplo-SCT. Three patients developed moderate chronic GVHD successfully managed with steroids, extracorporeal photoaphreresis and ruxolitinib. As expected, NK populations recovered rapidly after haplo-SCT followed by CD8+, CD4+ and CD20+ subsets (Table 4) (20,26,27). Non-fatal viral infection complications were frequent (Table 2). Cytomegalovirus (CMV) reactivation without disease occurred in 5 patients. BK virus (BKV) associated haemorrhagic cystitis was diagnosed in 5 patients, one of whom developed BK virus nephropathy after prolonged high plasma titters of BKV. One patient developed Adenovirus (ADV)-related colitis, one was diagnosed with visceral disseminated Varicella Zoster virus infection, and one developed transient human Herpesvirus 6 (HHV6) encephalitis after his fourth haplo-SCT with CD45RA+ depletion. There were no treatment-related deaths. Five patients relapsed 2 to 8 months after HSCT and died of disease. Six patients are alive in remission after a median follow up of 27 months since haplo-SCT [14-126]. Median OS after primary diagnosis of cancer was significantly higher in the group of patients undergoing haplo-SCT when compared with a historical control group of 24 patients with primary metastatic o metastatic relapse of PMST (46 versus 27 months, $p = 0.0177$) after a median follow up of 35 [12-148] and 26 [8-86] months, respectively (Table 1 and 2; figure 1 and 2).

Patient	Diagnosis	Site of primary tumor	Stage at diagnosis	Site of M	Age at diagnosis	First-line treatment	Relapse or progression before haploSCT (site)	Time from diagnosis to relapse or progression (m)	Treatment after relapse or progression before haploSCT	Time from relapse or progression to haploSCT (m)	Status at haploSCT	Relapse or progression after haploSCT (m)	Current Status	Survival since haploSCT (m)	Survival since diagnosis (m)
#1	ES	Femur	L	NA	13 y	StFLC + L Sx + RT	M (lung)*	13	M Sx + CT (Topo-Cyclo)	9	CR2	No	Alive CR2	126	148
#2	ES	Femur	L	NA	11 y	StFLC + L Sx	M (lung)*	27	M Sx + CT (Topo-Cyclo)	6	CR2	No	AliveCR2	103	136
#3	ES	Humerus	L	NA	13 y	StFLC + L Sx	M (bone)	36	RT + CT (hdIFO) + MAT BUMEL	14	CR2	No	Alive CR2	25	74
#4	ES	Pelvis	M	Lung	15 y	StFLC + MAT BUMEL + RT	No	NA	NA	NA	CR1	No	Alive CR1	22	35
#5	ES	Pelvis	M	Bone	13 y	StFLC + MAT BUMEL + RT	No	NA	NA	NA	CR1	No	Alive CR1	14	26
#6	ES	Pelvis	M	Lung	13 y	StFLC	M (lung, bone)	4	RT + CT (Topo-Cyclo) + MAT CARBO-VP-MEL	5	PR	Yes (2)	DOD	3	12
#7	OS	Humerus	L	NA	10 y	StFLC + L Sx	L	23	CT (Ifo-VP) + L Sx + M Sx	5	CR2	Yes (3)	DOD	7	35
#8	OS	Femur	M	Lung	5 y	M Sx + StFLC + L Sx	No	NA	NA	NA	CR1	Yes (8)	DOD	30	46
#9	OS	Tibia	M	Lung	13 y	M Sx + StFLC + L Sx	No	NA	NA	NA	CR1	No	Alive CR1	29	45
#10	NB	Adrenal	M	Bone,BM	20 m	StFLC + MAT BUMEL+ L Sx + RT	M (bone)	12	CT (Irino-TMZ) + thMIBG + CT (Irino-TMZ)	7	PD	Yes (5)	DOD	5	24
#11	NB	Adrenal	M	Bone,BM	5 y	StFLC	M (bone)	3	CT (TVD, Topo-Cyclo) + MAT BUMEL+ thMIBG (x3) + CT (Topo) + RA	20	PD	Yes (7)	DOD	12	35

BM, bone marrow; BUMEL, busulfan-melfalan; CARBO-VP-MEL, carboplatin-etoposide-melfalan; CR1, first complete remission; CR2, second complete remission; CT, chemotherapy; Cyclo, cyclophosphamide; DOD, dead of disease; ES, Ewing sarcoma; FLU-BU-TT, fludarabine-busulfan-thiotepa; hdIFO, high-dose ifosfamide; Ifo, ifosfamide; Irino, irinotecan; L, localized/local; M, metastatic/metastasis; m, months; MAT, myeloablative therapy; NA, not applicable; NB, neuroblastoma; OS, osteosarcoma; PD, progressive disease; PR, partial remission; RA, retinoic acid; RT, radiotherapy; StFLC, standard first-line chemotherapy; Sx, surgery; TMZ, temozolomide; thMIBG, therapeutic metaiodobenzylguanidine; Topo, topotecan; TVD, topotecan-vincristine-doxorubicine; VP, etoposide; y, years; RA, 13-cis retinoic acid (isotretinoin).
* Solitary nodule

Table 1: Clinical characteristics of 11 patients with solid tumours who underwent haploidentical stem cell transplantation (haplo-SCT).

Patient	Graft		NK alloreactivity:	Cell infusión						GvHD prophylaxis	Engraftment		Diagnosis of GvHD		Infections			
	Donor	Depletion	Receptor-ligand mismatch	$CD34^+$ ($x10^6$/kg)	$CD3^+$ ($x10^3$/kg)	$\alpha\beta^+CD3^+$ ($x10^3$/kg)	$\gamma\delta^+CD3^+$ ($x10^6$/kg)	NK cells ($x10^6$/kg)	$CD20^+$ ($x10^3$/kg)		Graft rejection	Best chimerism (d)	Acute (grade)	Chronic (stage)	Bacterial infections (CTCAE grade)	CMV status (donor/receptor)	CMV reactivation	Other viral infections (CTCAE grade)
#1	Father	CD3+/CD19+	2DL3	3.29	31.22	NA	NA	6,46	31.22	Csa + MTX	Secondary	100% (d+34)	No	No	*E. coli* sepsis (3)	+ / –	Yes	No
#2	Mother	αβ+/CD19+	2DL3	8.66	12160	36,6	12.02	11.43	101.71	Csa + MTX	No	100% (d+30)	Yes (4)	Yes (mod)	*C. difficile* colitis (3)	+ / +	Yes	BKV cystitis (3)
#3	Father	αβ+/CD19+	None	5.59	2050	0	0.87	4.41	90	No	No	100% (d+30)	Yes (3)	Yes (mod)	No	– / –	Yes	BKV cystitis (3); BKV nephritis (3); HHV6 viremia (2)
#4	Father	αβ+/CD19+	None	12	11140	0.56	9.04	26.09	2.79	No	Primary	NA	No*	Yes (mod)	*P. aeruginosa* bacteremia (2)	– / +	Yes	HHV6 viremia (2)
#5	Brother	αβ+/CD19+	None	11.33	14150	0	14.15	74.37	0	No	No	100% (d+15)	No	No	No	– / –	No	HHV6 viremia (2); EBV viremia (2)
#6	Mother	αβ+/CD19+	3DL1	5.88	6800	355.04	6.09	34.49	177.5	Csa + MTX	No	100% (d+30)	Yes (2)	No	*E. faecium* bacteremia (2)	+ / –	No	BKV cystitis (2)
#7	Mother	αβ+/CD19+	None	8.93	1250	14.72	0.8	24.28	1913.08	Csa + MTX	No	100% (d+15)	No	No	No	+ / –	No	BKV cystitis (1)
#8	Mother	αβ+/CD19+	None	18.5	25540	8.95	21.06	46.68	17.89	No	No	100% (d+30)	No	No	No	– / –	No	VZV esophagitis (3); VZV gastritis (3)
#9	Father	αβ+/CD19+	3DL1	3.26	2000	3.04	1.64	2.26	16.94	No	Primary	NA	No*	No	No	+ / +	Yes	BKV cystitis (3); ADV colitis (3); HHV6 encephalitis (3)
#10	Father	αβ+/CD19+	None	25.21	19060	224.68	17.15	21.99	922.37	Csa + MTX	No	100% (d+20)	Yes (2)	No	*S. ovis* bacteremia (2)	+ / +	No	No
#11	Mother	CD3+/CD19+	None	10.11	127.94	NA	NA	19.06	0	MF	Primary	NA	No	No	*E. coli* bacteremia (2)	– / –	No	BKV cystitis (2); EBV viremia (2)

ADV, adenovirus; BKV, BK virus; Csa, cyclosporine; d, day; CMV, cytomegalovirus; CTCAE, common terminology criteria for adverse events; EBV, Epstein-Barr virus; GvHD, graft-versus-host disease; HHV6, human herpesvirus 6; MF, micofenolate; mod, moderate; MTX, methotrexate; NK, natural killer cells; VZV, varicella-zoster virus.
* Patients #4 and #9 developed acute grade 2 GvHD after second and fourth haploSCT, respectively.

Table 2: Details of graft, NK alloreactivity, infused cells and complications of 11 patients with solid tumours who underwent haploidentical stem cell transplantation (haploSCT).

Patient	1st haploSCT	2nd allograft											3rd allograft										4th allograft										
	Graft failure	Donor	Conditioning	*Ex vivo* manipulation	Cell infusion								Outcome	Donor	Conditioning	*Ex vivo* manipulation	Cell infusion						Outcome	Donor	Conditioning	*Ex vivo* manipulation	Cell infusion						Outcome
					$CD34^+$ ($x10^6$/kg)	$CD3^+$ ($x10^6$/kg)	$CD3^+45RA^+$ ($x10^3$/kg)	$CD3^+45RO^+$ ($x10^6$/kg)	$\alpha\beta^+CD3^+$ ($x10^3$/kg)	$\gamma\delta^+CD3^+$ ($x10^6$/kg)	NK cells ($x10^3$/kg)	$CD20^+$ ($x10^3$/kg)					$CD34^+$ ($x10^6$/kg)	$CD3^+$ ($x10^6$/kg)	$CD3^+CD4^+$ ($x10^6$/kg)	$CD3^+CD8^+$ ($x10^6$/kg)	NK cells ($x10^6$/kg)	$CD20^+$ ($x10^6$/kg)					$CD34^+$ ($x10^6$/kg)	$CD3^+$ ($x10^3$/kg)	$CD3^+45RA^+$ ($x10^3$/kg)	$CD3^+45RO^+$ ($x10^6$/kg)	NK cells ($x10^6$/kg)	$CD20^+$ ($x10^6$/kg)	
#1	Secondary	HLAc sister	Flu + TMG + TBI (2 Gy)	No	3.04	0.7	NA	NA	NA	NA	uk	uk	Successful																				
#4	Primary	Same haploSCT	Flu + Mel + TMG	CD45RA+/ CD19+	0.154	0.313	0	49,719	NA	NA	48,108	1,443	Successful																				
#9	Primary	Same haploSCT	Flu + Mel + TMG	CD3αβ+/ CD19+	1.60	0.12	NA	NA	13.28	0	20	0	Primary GF	Same haploSCT	No	No (cyclo-post)	3.06	92.3	56.96	32.50	29.79	42.22	Primary GF	Alternative haploSCT (sister)	Flu + Threos + TBI (2 Gy)	CD45RA+/ CD19+	11.1	11.81	49.68	11.76	0.01	0.13	Successful
#11	Primary	HLAc sister	Flu + Cy + ATG	No	2.64	0.002	NA	NA	NA	NA	uk	0.73	Successful																				

ATG, anti-thymocyte globulin; Cy, cyclophosphamide; Flu, fludarabine; GF, graft failure; HLAc, HLA-compatible; Mel, melphalan; NA, not applicable; TBI, total body irradiation; Threos, threosulfan; TMG, thymoglobulin; uk, unknown

Table 3: Management and outcome of subsequent allografts in 4 patients who developed graft failure after haploidentical stem cell transplantations (haplo-SCT).

	1 month post-SCT				3 month post-SCT			6 month post-SCT			12 month post-SCT		
Lymphocyte subsets	N	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
CD3+ T lymphocytes (%)	10	57.57	14.86	99.21	50.59	14.17	88.89	46.71375	29.21	88.99	65.995	51.24	92.7
CD3+ T lymphocytes (Cels/µl)	10	211.197	6.73	934.3	283.113	80	988.19	504.5	154	1093.8	797.445	187	2014
CD3+CD4+ (%)	10	24.623	3.47	45.07	19.272	7.78	33.7	18.22875	10.62	25.89	20.501667	9.75	30.12
CD3+CD4+ (Cels/µl)	10	115.964	2.4	639.81	109.187	31	277.36	193.5125	75	325	213.94833	71	336.14
CD3+CD8+ (%)	10	27.862	2.39	72.4	31.885	1.53	66.82	34	20	61.5	41.245	16.27	67.4
CD3+CD8+ (Cels/µl)	10	74.245	4	228	148.594	9	674.49	266.95	62	755.97	548.76667	94	1708
CD16+CD56+ NK cells (%)	10	37.006	0.46	81.78	39.6735	3.99	82.1	39.8675	8.16	65.6	19.558333	6.01	40.24
CD16+CD56+ NK cells (Cels/µl)	10	174.298	0.05	441.81	211.273	21.61	546	482.05375	100.32	1312	207.76167	36.08	435
CD19+ B lymphocytes (%)	10	1.1768	0	4.65	6.737	0	28.2	10.97	0.2	42.35	11.086667	0.81	18.95
CD19+ B lymphocytes (Cels/µl)	10	5.535	0	27	34.44	0	162	131.5275	1	385	181.19	3	565
Serum Immunoglobulins													
IgG	11	583.27273	188	1430	690.03636	22.4	1490	783.88889	353	1390	621.16667	238	1160
IgA	11	53.267273	5.04	136	50.187273	2.84	152	70.6625	17.6	196	32.8	12	63.1
IgM	11	45.360909	2.14	289	65.867273	1.08	236	103.3875	17.2	187	40.776667	2.26	85.7

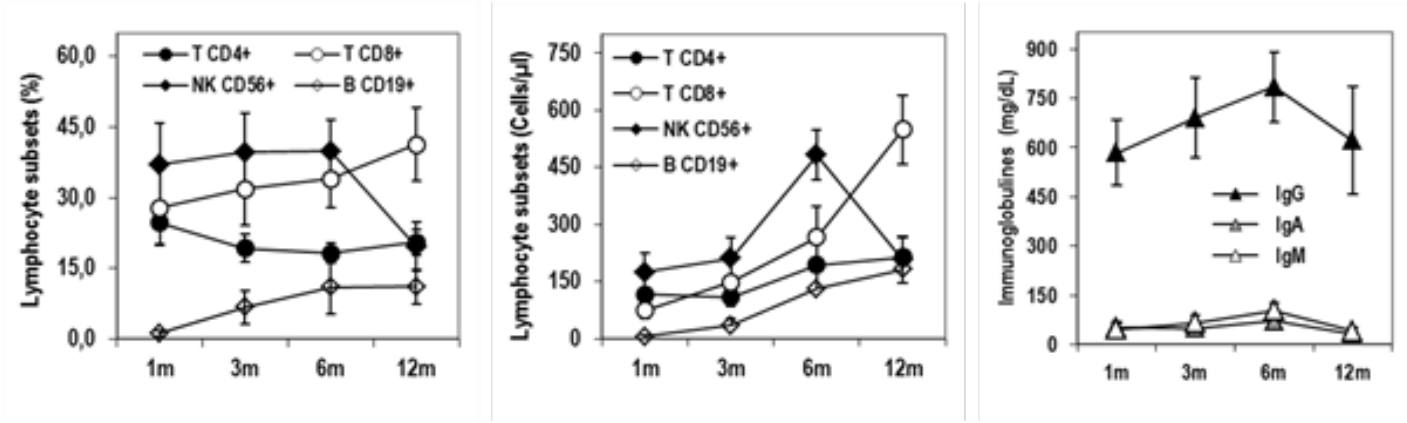

Table 4: Lymphocyte subsets and serum immunoglobulin immunorecovery after haploidentical stem cell transplantation (SCT).

Patient	Diagnosis	Site of primary tumor	Stage at diagnosis	Site of metastases	Age at diagnosis (y)	First-line treatment	First relapse or progression (site)	Time from diagnosis to first relapse or	Second-line treatment	Status	Survival after first relapse (m)	Survival since diagnosis (m)
#12	ES	Femur	L	NA	14	StFLC + L Sx	M (lung)	10	CT (Topo-Cyclo-Carbo-Imatinib) + RT	DOD	9	19
#13	ES	Femur	M	Lung	10	StFLC + L Sx + MAT_{BUMEL} + RT	M (bone)	11	CT (Topo-Cyclo)	DOD	4	15
#14	ES	Fibula	L	NA	8	StFLC + L Sx	L + M (lung)	20	CT (Topo-Cyclo) + MAT BUMEL + RT	DOD	21	42
#15	ES	Not available	L	Not available	11	Not available	Not available	16	Not available	DOD	16	32
#16	ES	Pelvis	M	Bone, lung	9	StFLC + MAT_{BUMEL} + RT	L + M (bone, lung)	16	CT (Topo-TMZ-Dasatinib)	DOD	10	26
#17	ES	Pelvis	L	NA	9	StFLC + MAT_{BUMEL} + RT	M (not available)	15	Not available	DOD	10	25

#18	ES	Vertebra	M	Lung	5	StFLC + $MAT_{CARBO-TT}$	L + M (bone, lung, mediastinum)	10	No	DOD	3	13
#19	ES	Multicentric (bone)	M	Lung, soft T	9	StFLC	M (bone, lung, soft T)	5	CT (Topo-Cyclo) + RT	DOD	3	8
#20	OS	Femur	L	NA	8	StFLC + L Sx	L + M (bone, soft T)	20	CT (Gem-Tax-beva)	DOD	11	32
#21	OS	Femur	M	Lung	15	StFLC + L Sx + M Sx	No	NA	NA	Alive	NA	7
#22	OS	Femur	L	NA	12	StFLC + L Sx	M (lung)	49	M Sx + CT (Gem-Tax-beva)	DOD	37	86
#23	OS	Fibula	L	NA	9	StFLC + L Sx	M (lung)	18	M Sx	Alive	19	37
#24	OS	Femur	L	NA	14	StFLC + L Sx	M (lung)	9	CT (Gem-Tax)	DOD	4	13
#25	OS	Femur	M	Lung	15	StFLC + L Sx	M (lung)	4	Early clinical trial *	Alive	5	9
#26	OS	Tibia	L	NA	7	StFLC + L Sx	M (lung)	12	M Sx + CT (Gem-Tax-beva)	DOD	29	41
#27	OS	Tibia	M	Bone	10	StFLC + L Sx	M (lung)	20	Early clinical trial *	DOD	16	36
#28	OS	Femur	L	NA	6	StFLC + L Sx	L + M (LN)	18	CT (Gem-Tax-beva)	Alive	53	61
#29	OS	Femur	M	Lung	3	L Sx + StFLC + M Sx + $MAT_{CARBO-VP-TT}$	M	12	CT (Cyclo-Dasatinib)	DOD	2	14

#30	NB	Adrenal	M	Bone, BM	4	StFLC + L Sx + MAT_{BUMEL} + RT	M (bone)	12	RT + CT (CADO)	DOD	7	19
#31	NB	Adrenal	M	Bone, LN	2	StFLC + L Sx + $MAT_{CARBO-VP-MEL}$ + RT + RA	M (bone)	9	CT (TVD) + RT	DOD	4	14
#32	NB	Adrenal	M	Bone, BM	3	StFLC + L Sx + MAT_{BUMEL} + RT + aGD2-IL2	M (bone)	38	CT (TOTEM + CAV)	DOD	25	64
#33	NB	Abdominal	M	Bone, BM	6	StFLC + L Sx + MAT_{BUMEL} + RT + RA	L + M (bone)	17	CT (TVD) + thMIBG + $MAT_{CARBO-VP-MEL}$	DOD	28	45
#34	NB	Abdominal	M	Bone, BM	4	StFLC + MAT_{BUMEL} + L Sx + RT	M (bone)	8	CT (TVD)	DOD	6	13
#35	NB	Not available	M	Bone, BM	5	Not available	Not available	19	Not available	DOD	8	27

aGD2-IL2, antiGD2-interleukin 2; Beva: bevacizumab; BM: bone marrow; BUMEL: busulfan-melfalan; CADO: Cyclophosphamide-Doxorubicine-Vincristine; CARBO-VP-MEL: carboplatin-etoposide-melfalan; Carbo: carboplatin; CARBO-TT: carboplatin-thiotepa; CARBO-VP-TT: carboplatin-etoposide-thiotepa; CAV: Cyclophosphamide-doxorubicine-vincristine; CR1: first complete remission; CR2: second complete remission; CT: chemotherapy; Cyclo: cyclophosphamide; DOD: dead of disease; ES, Ewing sarcoma; FLU-BU-TT: fludarabine-busulfan-thiotepa ; Gem: gemcitabine; hdIFO: high-dose ifosfamide; Ifo: ifosfamide; Irino-TMZ: irinotecan-temozolomide; L: localized/local; LN: lymph nodes; M: metastatic/metastasis; m: months; MAT: myeloablative therapy; NA, not applicable; NB: neuroblastoma; OS: osteosarcoma; PD; progressive disease; PR: partial remission; RT: radiotherapy; soft T: soft tissues; StFLC: standard first-line chemotherapy; Sx: surgery; Tax: docetaxel; thMIBG: therapeutic metaiodobenzylguanidine; TMZ: temozolomide; Topo: topotecan; TOTEM: topotecan-temozolomide; TVD: topotecan-vincristine-doxorubicine; VP: etoposide; y: years; RA: 13-cis retinoic acid (isotretinoin).

*Early clinical trial containing oral receptor tyrosine kinase inhibitor lenvatinib

Table 5: Clinical characteristics of 24 patients with primary metastatic or relapsed Ewing sarcoma (ES), osteosarcoma (OS), or neuroblastoma (NB).

Discussion

NK cells are part of the innate immune system capable to eliminate tumour cells and their role in cancer immune-surveillance is well established [28,29]. There is evidence from preclinical studies that NK cells may mediate antitumor activity against osteosarcoma lung metastases, ES cells were particularly sensitive in vitro to activated and expanded NK cell-mediated cytotoxicity and the administration of expanded NK cells was able to reduce the development of lung metastases of ES in murine models [3,14,16]. Expression of ligands for activating NK-cell receptors in combination with down-regulation of HLA class I molecules on ES tumour cells are probably associated to this particular sensitivity [13,16,20]. NK cells are considered the main effector of tumour cell killing in paediatric patients who receive anti-GD2 monoclonal antibodies [4]. Allogeneic NK cells may scape the inhibition of autologous NK cells provided by self-HLA signals [15,28]. The use of allogeneic NK cells yielded promising results in children with neuroblastoma and the administration of isolated or pre-activated and/or expanded autologous and allogeneic NK cell infusions is currently under clinical evaluation in haematological malignancies and paediatric solid tumours such as neuroblastoma, ES, osteosarcoma, rhabdomyosarcoma and central nervous system tumours [4,7,28]. Haplo-SCT represents an approach to exploit the ability of NK cells to kill MHC class I defective tumour cells, and has been successfully applied for the treatment of high risk leukaemia [29,30]. In the paediatric setting, many groups adopt the platform of ex vivo T-cell depletion in order to prevent severe GVHD after haplo-SCT [30,31]. Selective depletion of specific cell subpopulations within the graft such as αβ+ T cells allows the administration of large amounts of γδ+ T cells and NK cells, which might protect the recipient from leukaemia relapse and severe infection, and this approach was reported to be effective in patients with haematological malignancies [30-33]. By contrast, the eventual role and benefit of haplo-SCT for the management of PMST has not been explored in depth [26,27].

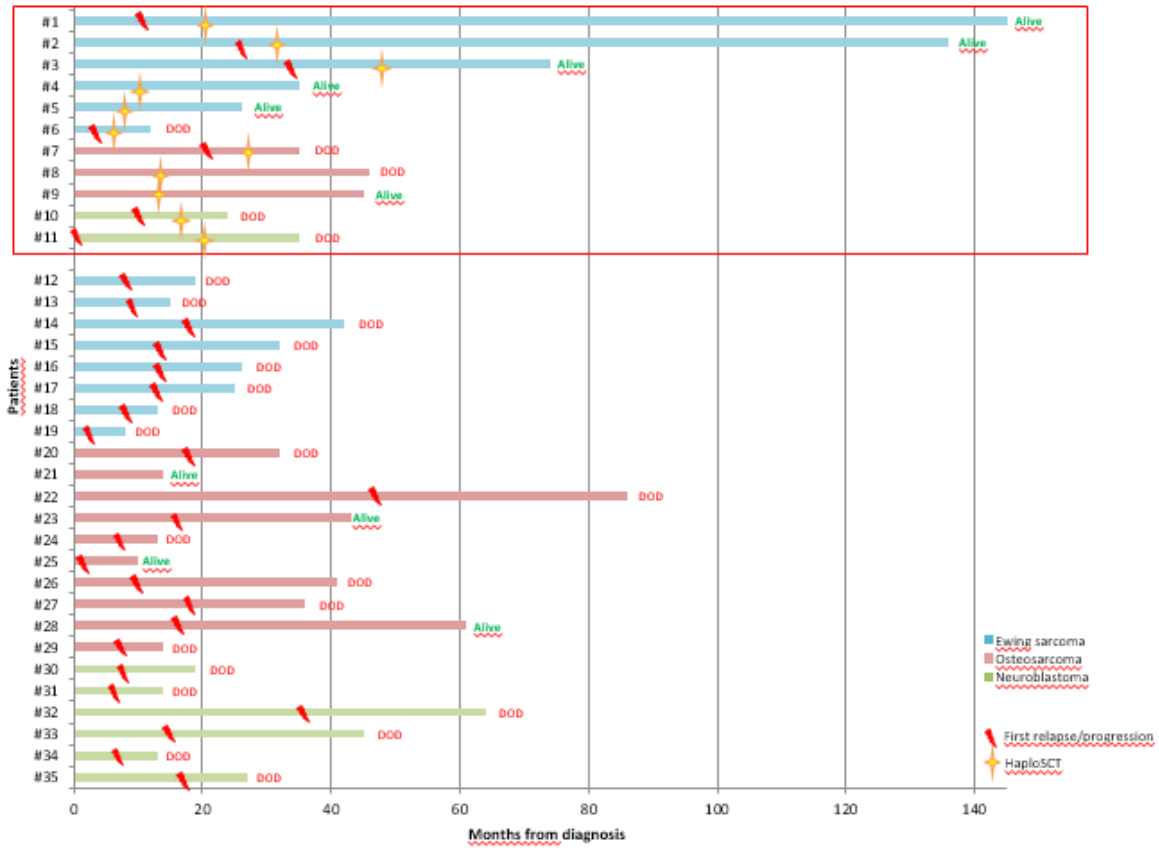

Figure 1: Outcome of 11 patients with high-risk solid tumors who underwent haploidentical stem cell transplantation (surrounded by a red square). The outcome of a cohort of 24 patients with similar characteristics is also shown. HaploSCT, haploidentical stem cell transplantation; DOD, dead of disease.

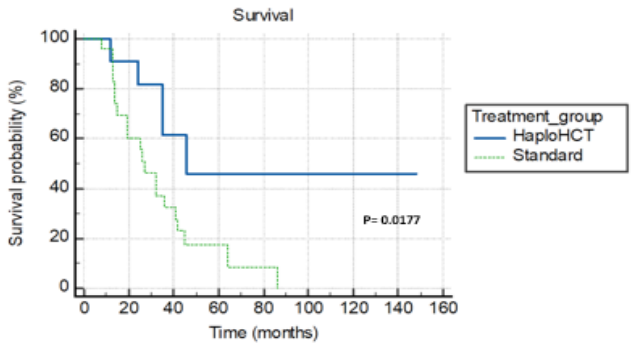

Figure 2: Kaplan-Meier survival curves of overall survival analysis in a cohort of 11 patients with high-risk solid tumors who underwent haploidentical stem cell transplantation (haploSCT) compared with a cohort of 24 patients with similar characteristics who did not receive haploSCT.

Supported by a few previously reported encouraging clinical experiences, we offered this approach as compassionate treatment to 11 patients [17-22]. Different criteria for haploidentical donor selection have been described in order to improve NK cell alloreactivity, including progenitor sex (mother versus father), the KIR (receptor)-ligand mismatch model, presence of KIR B/x genotype, donor B-haplotype content score, KIR2DS1/HLA-C1+ donor for HLA-C2+ recipient, circulating numbers of NK and γδ+ T cells in donor peripheral blood, expression of NKp46, and presence of NKG2C [23,30,32]. When available, we preferred haploidentical donors with NK alloreactivity according to the receptor-ligand mismatch model [23]. Only 1 out of 4 of our patients with an NK allo-reactive donor rapidly progressed and died of disease after haplo-SCT. This patient underwent transplantation in PR. One of these long-term survivors after metastatic relapse of ES rejected the haploindentical allo-reactive graft and was rescued with an allogeneic transplantation from an HLA compatible sibling without NK alloreactivity suggesting that, even in the absence of a successful engraftment, allo-reactive NK cells within the initial graft might exert an antitumor activity by the elimination of circulating tumour cells [28]. Three additional patients survive in remission 8, 18 and 19 months after haplo-SCT from a non-allo-reactive donor. However, apart from haematological malignancies, donor selection according to the KIR-ligand mismatch model or other KIR/HLA genotype approaches did not prove to be useful in previous studies exploring NK cell therapies, and NK-cell response to cancer cells is not entirely dependent upon down-regulation of MHC molecules but can also be triggered by overexpression of activating ligands such as MICA, MICB, and UL16-bindings proteins (ULBPs) [7,14,19,28]. We are currently prospectively studying the expression of ligands on tumour cells from PMST for both inhibitory and activating NK cell receptors.

In this study, all 6 survivors underwent haplo-SCT in remission (CR1 or CR2), while all three patients undergoing haplo-SCT in PR or PD progressed and died 3, 5, and 12 months after transplantation. Of note, three patients undergoing haplo-SCT in CR2 after metastatic relapse of ES are alive in remission up to 126 months after HSCT. Thus, disease status before haplo-SCT seems to be an important determinant of response and outcome. In fact, it is the effector-to-target ratio which plays an important role for success in NK cell therapy strategies and it is determined by disease status before haplo-SCT and the number of infused cells [7,14,19,27,28,34]. In our series, all patients receive ≥ 1 x 10^6/kg NK cells within the graft, which has been proposed as an adequate number in previous NK cell infusion studies for neuroblastoma [7]. We also found a rapid NK cell population reconstitution after engraftment, and this early expansion of functional NK cell after transplantation is suggested to be an effective mechanism for systemic tumour control and preventing metastatic relapse [19,20,27]. Therefore, haplo-SCT, rather than promote or induce remission in patients with residual disease might serve as a mean to prevent systemic relapses in those undergoing transplantation in remission [26]. In fact, although tumour permeability to NK cell infiltration differs among different tumour types, infiltrating NK cells were generally found within the stroma and not in direct contact with the tumour cells. Moreover, tumour microenvironment interacts and may suppress the immune response by different mechanisms including down-regulation of expression and function of natural cytotoxicity receptors, shedding of soluble forms of NKG2D ligands, and modulation of chemokine-receptor repertoire and inhibitory checkpoint expression on NK cells [13,29,33]. In addition, the high numbers of γδ+ T cells within the grafts might have contributed to prevent fatal infections and non-relapse mortality [30,31].

We had a high incidence of GF but, fortunately, all four patients rejecting the primary graft could be rescued with subsequent transplants from the same or alternative donors. Obviously, this translated into prolonged hospitalization and a high rate of viral reactivation and infection complications. Asymptomatic CMV reactivation, and ADV-associated disease are frequent in children undergoing haplo-SCT [26,27,31].

Interestingly, five of our six survivors had CMV reactivation in contrast with no single case of reactivation among the 5 patients who progressed/relapsed and died after haplo-SCT. In patients with leukaemia undergoing αβ/B cell-depleted haplo-SCT, NK-cell responses were reported to be partly dependent upon exposure to CMV, CMV reactivation/infection played an important role in the NK cell maturation process and CMV-induced NK cells were able to persist over one year after transplantation which was hypothesized to prevent relapse [28,32]. BKV-haemorrhagic cystitis and HHV6 viremia were also frequent; one patient

developed grade 3 BKV-nephropathy after prolonged viremia and one had a reversible episode of HHV6-induced encephalitis after a fourth haplo-SCT with CD45RA+ depletion. One additional patient developed visceral disseminated varicella-zoster virus infection. By contrast with previous reports, we had a high incidence of grade II-IV aGVHD and moderate cGVHD (6 and 3 out of 11 patients, respectively) but all affected patients were successfully managed with immunomodulation treatment [26,30,31]. In this study, haplo-SCT as consolidation therapy for HR PMST seems to improve overall survival when compared with an historical control group of patients with primary metastatic and metastatic relapse of PMST. Outcome of three patients with ES undergoing haplo-SCT in CR2 after systemic relapse is particularly intriguing. However, advanced ES was defined by other groups as multifocal disease (≥ 2 bone metastases) or bone marrow involvement at primary diagnosis or relapse occurring ≤ 24 months after diagnosis, and these criteria were met only in two out of 5 survivors with ES in our series [34]. Thus, one can indisputably argue that these patients might well have been cured without haplo-SCT. To conclude, haplo-SCT from an NK-cell allo-reactive donor represents a realistic and attractive approach for the management of HR PMST. Given the associated risk of potentially severe acute and late complications, candidates should be carefully selected. Remission status at transplantation seems to play an important role in outcome.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as potential conflict of interest.

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