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Allogeneic haematopoietic stem cell transplantation for mitochondrial neurogastrointestinal encephalomyopathy

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Haematopoietic stem cell transplantation has been proposed as treatment for mitochondrial neurogastrointestinal encephalomyopathy, a rare fatal autosomal recessive disease due to *TYMP* mutations that result in thymidine phosphorylase deficiency. We conducted a retrospective analysis of all known patients suffering from mitochondrial neurogastrointestinal encephalomyopathy who underwent allogeneic haematopoietic stem cell transplantation between 2005 and 2011. Twenty-four patients, 11 males and 13 females, median age 25 years (range 10–41 years) treated with haematopoietic stem cell transplantation from related ($n = 9$) or unrelated donors ($n = 15$) in 15 institutions worldwide were analysed for outcome and its associated factors. Overall, 9 of 24 patients (37.5%) were alive at last follow-up with a median follow-up of these surviving patients of 1430 days. Deaths were attributed to transplant in nine (including two after a second transplant due to graft failure), and to mitochondrial neurogastrointestinal encephalomyopathy in six patients. Thymidine phosphorylase activity rose from undetectable to normal levels (median 697 nmol/h/mg protein, range 262–1285) in all survivors. Seven patients (29%) who were engrafted and living more than 2 years after transplantation, showed improvement of body mass index, gastrointestinal manifestations, and peripheral neuropathy. Univariate statistical analysis demonstrated that survival was associated with two defined pre-transplant characteristics: human leukocyte antigen match (10/10 versus $<10/10$) and disease characteristics (liver disease, history of gastrointestinal pseudo-obstruction or both). Allogeneic haematopoietic stem cell transplantation can restore thymidine phosphorylase enzyme function in patients with mitochondrial neurogastrointestinal encephalomyopathy and improve clinical manifestations of mitochondrial neurogastrointestinal encephalomyopathy in the long term. Allogeneic haematopoietic stem cell transplantation should be considered for selected patients with an optimal donor.

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Abbreviations: HLA = human leukocyte antigen; HSCT = haematopoietic stem cell transplantation; MNGIE = mitochondrial neurogastrointestinal encephalomyopathy; TP = thymidine phosphorylase

Introduction

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive multisystem disorder due to *TYMP* mutations, which leads to premature death. *TYMP* mutations cause loss of thymidine phosphorylase (TP) activity (Hirano *et al.*, 1994; Nishino *et al.*, 1999), resulting in more than a 50-fold increase in levels of thymidine and deoxyuridine in plasma and tissues (Spinazzola *et al.*, 2002; Valentino *et al.*, 2007). As a consequence of this accumulation of nucleosides, intramitochondrial deoxynucleotide pools are unbalanced, which in turn impair mitochondrial DNA (mtDNA) replication leading to accumulation of site-specific point mutations, multiple deletions, and depletion of mtDNA (Hirano *et al.*, 1994; Papadimitriou *et al.*, 1998; Nishigaki *et al.*, 2003, 2004). As a result of the mtDNA instability, patients develop progressive mitochondrial oxidative phosphorylation (OXPHOS) dysfunction with gastrointestinal dysmotility, cachexia, peripheral neuropathy, skeletal myopathy, ophthalmoparesis and ptosis over the course of 2–4 decades (Papadimitriou *et al.*, 1998; Garone *et al.*, 2011). MRI of the brain reveals leukoencephalopathy; however, cognitive function is generally unimpaired. Diagnosis is usually made in the second or third decade of life although onset of first clinical signs is frequently described during childhood or adolescence. The disease is relentlessly progressive and causes death primarily due to cachexia and infections, with a median life expectancy of 37 years (Garone *et al.*, 2011).

To date, no widely available effective disease-modifying treatment is available for MNGIE. Attempts to reduce toxic nucleoside levels with dialysis failed because of rapid reaccumulation of thymidine (Spinazzola *et al.*, 2002; la Marca *et al.*, 2006; Yavuz *et al.*, 2007). Erythrocyte-encapsulated TP showed preliminary hints of efficacy in one patient but must be infused multiple times per year (Bax *et al.*, 2013). In a proof-of-concept trial, transfusions with platelets, which contain abundant TP, produced transient reduction of nucleoside levels (Lara *et al.*, 2006). This approach is not feasible as long-term therapy, but has stimulated the concept of enduring replacement of TP by

allogeneic haematopoietic stem cell transplantation (HSCT) as donor-derived leucocytes and platelets are rich in TP (Hirano *et al.*, 2006; Halter *et al.*, 2011). Because nucleosides diffuse between extra-and intracellular compartments, TP from normal donor cells can clear thymidine and deoxyuridine from plasma and presumably also from tissues of MNGIE patients. Biochemical success of this concept has been reported (Hirano *et al.*, 2006), however, clinical benefit has not yet been demonstrated.

Materials and methods

Study design

This retrospective study analysed data of all known MNGIE patients worldwide who started conditioning therapy for allogeneic HSCT, starting from the first patients who underwent HSCT in Haifa, Israel in July 2005 and in New York, USA in August 2005 (Hirano *et al.*, 2006). MNGIE is a very rare disease with only a few diagnostic laboratories performing metabolic and genetic analyses, hence virtually all patients with this disease have been brought to the attention of two of the co-authors (M.H., R.M.) who established a robust network for patient identification. Involved clinical teams were contacted and data were systematically collected by a questionnaire and personal contacts. Data collection included Karnofsky performance score, clinical general medical, neurological and ophthalmological exams; blood tests (complete blood counts, and routine blood chemistry); imaging studies (brain MRI, upper and lower gastrointestinal series, abdominal ultrasound); electrophysiological assessments (nerve conduction studies, electromyography); disease-specific biochemical studies (TP activity in buffy coat, thymidine and deoxyuridine plasma levels) and genetic testing (*TYMP* and mtDNA mutation screening). Data collection on HSCT included pre-transplant health status, conditioning, graft-versus-host disease (GvHD) prophylaxis and treatment, donor type, stem cell source, donor cell chimerism, post-transplant course and survival. The last follow-up evaluations were performed in June 2012, and the survival was last updated in December 2013.

Statistics

Survival was censored at the time of last follow-up (December 2013), death or time of second transplantation. Descriptive statistics were calculated for demographic, MNGIE-related and HSCT-related characteristics. The overall survival was estimated by the Kaplan-Meier method. Univariate analysis of the association between age, gender, body mass index, parenteral nutrition at the time of HSCT, history of intestinal pseudo-obstruction or perforation, liver disease (defined as transaminases more than twice the upper limit of normal or histological abnormality), donor type, human leukocyte antigen (HLA)-match, stem cell source, intensity of conditioning (intensive versus reduced intensity, classified according to published working definition) (Bacigalupo *et al.*, 2009) and overall survival was conducted. A *P*-value $\leqslant 0.05$ was considered significant. Due to the limited number of patients we were unable to complete multivariate analysis. Analyses were performed by IBM® SPSS® Statistics, Version 19 (SPSS, Inc. IBM copyright 1989, 2010).

Results

Patient population

A total of 26 patients (13 male, 13 female) with MNGIE were reported to have started conditioning treatment for HSCT (Fig. 1); this includes the seven patients who have already been reported (Hirano *et al.*, 2006; Bakker *et al.*, 2010; Filosto *et al.*, 2012; Sicurelli *et al.*, 2012; Finkenstedt *et al.*, 2013). Baseline clinical and genetic characteristics are presented in Table 1 and Supplementary Table 1.

Two of the initial 26 patients did not proceed to HSCT. One died before transplantation in septic shock with acute respiratory distress syndrome (ARDS) due to infection with *Acinetobacter baumanii* of unknown origin (Bakker *et al.*, 2010). The other developed acute cardiogenic shock after administration of alemtuzumab with subsequent cardiopulmonary failure. The patient recovered fully but died 5 years later due to disease progression. Both of these patients had a history of pseudo-obstruction and signs of liver disease. The first suffered also from two episodes of intestinal perforation. This report focuses on the 24 patients who underwent HSCT.

Median age at diagnosis of MNGIE was 21 years (range 9.5–34) but clinical onset was at a median age of 15.5 years (range 4–30). Diagnosis of MNGIE was confirmed in all patients by biochemical testing, identification of *TYMP* mutations or both (Table 1) (Hirano *et al.*, 2006; Schüpbach *et al.*, 2007; Poulton *et al.*, 2009; Bakker *et al.*, 2010). *TYMP* mutations resulted in drastically reduced TP activity ($n = 20$, median value below detection limit, range 0–28 nmol/h/mg protein, normal 634 ± 217) and detectable pyrimidine nucleosides in plasma ($n = 15$, median level of thymidine 9.6 µmol/l, range 4.1–96.2, median level of deoxyuridine 13.6 µmol/l, range 7.7–305.3; normal thymidine and deoxyuridine < 0.05). Blood lactate levels were elevated during fasting in 9 of 11 patients tested (median 3 mM, range 2.1–6.1; normal 0.5–2.2). Increased CSF protein levels (median 82 mg/dl, range 53–329; normal < 45) were observed in all eight patients who underwent lumbar puncture. Thymidine and deoxyuridine levels in CSF were measured in only two patients before transplantation. In both, levels of these nucleosides were similar to plasma levels, confirming previous findings that thymidine and deoxyuridine cross the blood–brain barrier (Thomas née Williams *et al.*, 1996). Brain MRI scans were performed in 19 patients and revealed symmetric leukoencephalopathy in all but one (95%). Common clinical manifestations included cachexia (100%), gastrointestinal dysmotility [gastrointestinal motility studies were abnormal in 11 of 14 patients tested (79%) with delayed gastric emptying, intestinal dysmotility or both], and ophthalmoparesis in 68%. Electromyography was performed in 14 patients

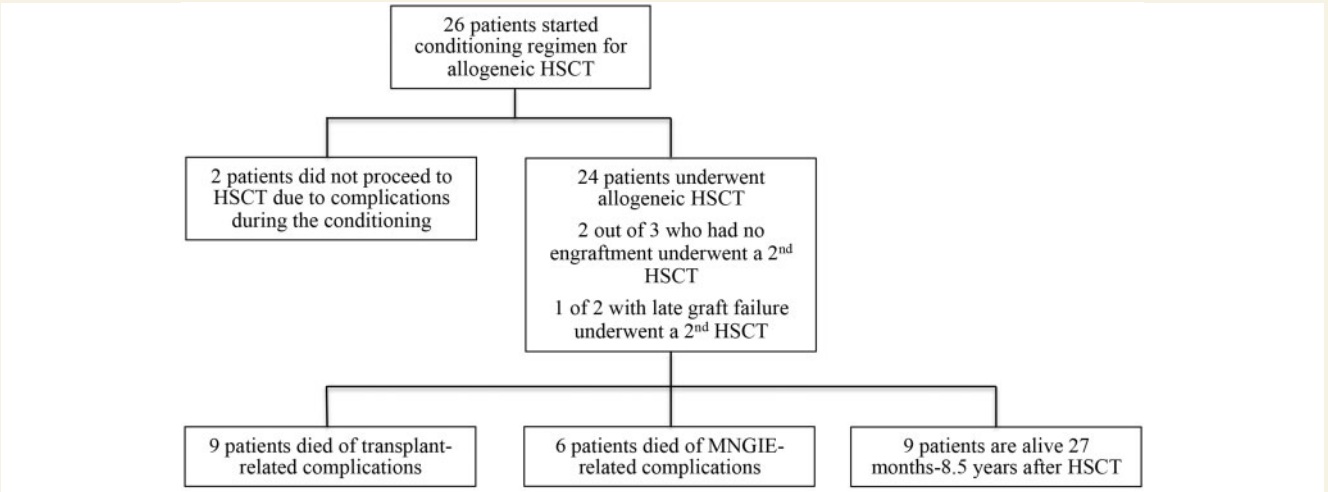

Figure 1 Number of patients treated and outcome.

and showed pathologic neurogenic, myopathic, or both patterns in nine (64%) and was normal in five patients. Nerve conduction studies were markedly abnormal in all 18 patients examined (100%) and revealed predominantly demyelinating sensorimotor peripheral neuropathy (Supplementary Table 1). In 10 patients, consanguinity of their parents was noted.

Pre-transplant characteristics

Median age at transplantation was 25 years (range 10–41). All patients were cachectic with a median body mass index of 13.5 kg/m^2 (range 10–19). Before HSCT, 12 patients were receiving parenteral nutrition. Median Karnofsky performance score was 70% (range 30–90) indicating the patients' frail status.

Twenty-four patients underwent a first HSCT. A variety of different regimens for conditioning and graft-versus-host disease prophylaxis were used in the first 12 patients with a preference for reduced intensity conditioning regimens (Table 2 and Supplementary Table 2). After a first consensus meeting in late 2008 (Halter *et al.*, 2011), a more homogenous transplant strategy has been followed. Fludarabin/busulfan ($n = 17$) in combination with anti-T cell antibodies ($n = 8$) was the most frequent conditioning regimen for first HSCT. Cyclophosphamide was used in eight patients; all but two before the consensus recommendations. More donors were unrelated ($n = 15$; 63%) than related ($n = 9$; 37%). Sixteen (67%) were either HLA-identical related donors ($n = 9$) or at least 10/10-HLA-matched unrelated donors ($n = 7$). Eight donors (33%) had at least one or more HLA-mismatches, including all five patients who underwent cord blood transplantation. Bone marrow was the preferred source of stem cells for first HSCT in half of the patients ($n = 13$, 54%), followed by peripheral blood stem cells ($n = 6$; 25%) and cord blood ($n = 5$; 21%).

Transplant outcome

Neutrophils (polymorphonuclear leucocytes; PMN) engrafted in 20/24 patients (83%) after a median of 16 days (range 8–26 days; in one patient PMN count was never $<500/\mu l$). One died before engraftment (21 days after transplant). Primary graft failure occurred in three patients, two of whom received a second HSCT with more intensive conditioning regimens 331 and 247 days after first transplants. Although both engrafted successfully, they died from transplant-related complications. The third patient died from progressive manifestations of MNGIE. In two other patients, late graft failure was observed 4 and 12 months after transplantation. One died from progressive disease, the other underwent a second HSCT from the same donor 473 days after first transplantation and is still alive with established full donor chimerism. All other patients had stable graft function after first transplantation until last follow-up.

Overall, nine patients were alive at last follow-up (37.5%) with a median follow-up of 1430 days (range 832–3084), all with stable engraftment (Fig. 2A). Fifteen patients have died. Seven patients died because of transplant-related causes at a median of 105 days (range 18–453: graft-versus-host disease in four, toxicity in two and infection in one). Two more patients died from transplant-related toxicity after second HSCT. In addition, six patients died because of MNGIE-related causes—mainly gastrointestinal complications—after a median of 198 days (84–1196 days). Pre-existing MNGIE-associated intestinal pathology likely contributed significantly to death during the first 6 months post-transplant in three of nine patients and was the main cause of death in four of six patients dying more than 6 months after first transplantation. By univariate analysis, HLA-match (10/10 versus $<10/10$) and the combination of indicators of disease burden (history of pseudo-obstruction, liver disease or both) were associated with overall survival (Fig. 2B and

Table 1 Pre-transplant clinical and genetic patient characteristics

Patient	sex	BMI (kg/m^2)	PFS	Age at HSCT (y)	*TYMP* mutations	Parenteral nutrition	History of pseudo-obstruction	Liver disease	External ophthalmoparesis	Ptosis	Reflexes
1	F	11.5	70	10	Homozygous c.1211insT	Absent	Absent	Absent	Absent	Mild	Absent
2	M	14.5	40	21	Homozygous c.854T>C	Present	Present	Present	Absent	Present	UL: absent LL: knee jerks weak, others absent
3	F	10	70	30	c.1160A>C and c.1382 ins-C	Present	Present	Absent	Vertical and horizontal	Present	Absent
4	M	11.1	70	33	Homozygous c.994_1011dup	Present	Present	Absent	n.a.	Present	UL: weak LL: absent
5	M	13.2	70	33	Homozygous c.1327_1346del	Absent	Absent	Present	Vertical and horizontal	Present	UL: present LL: absent
6	F	10.7	40	41	c.457G>A and c.866A>C	Present	Present	Absent	Vertical and horizontal	Present	Absent
7	M	15.5	90	25	Homozygous g.2182C>A	Absent	Absent	Absent	Vertical and horizontal	Absent	Normal
8	F	13.5	80	27	c.215-1G>C and c.866A>C	Absent	Present	Absent	Absent	Present	UL: normal LL: ankle jerk absent, others normal
9	M	15.3	90	26	Homozygous g.2182C>A	Absent	Absent	Absent	Vertical and horizontal	Absent	Normal
10	F	13.5	50	23	n.a.	Absent	Present	Absent	Vertical and horizontal	Present	Absent
11	M	13.2	40	23	c.1167T>C / c.1198_1203delGTGCTG	Present	Present	Absent	Vertical	Present	Absent
12	F	15.8	90	23	Homozygous c.1249 dupC	Absent	Absent	Absent	Absent	Present	Absent
13	F	12.3	30	21	Homozygous c.418-1G>T	Present	Present	Absent	Absent	Present	Absent
14	F	13.7	50	16	Homozygous c.1416delC	Absent	Present	Absent	Vertical	Present	Absent
15	M	18	70	34	Homozygous c.866A>C	Present	Absent	Present	Vertical and horizontal	Present	Absent
16	F	16.4	50	34	Homozygous c.1160-1G>A	Present	Absent	Absent	Horizontal	Present	UL: weak LL: ankle jerk absent, others weak
17	F	12	50	22	Homozygous c.1160-1G>A	Present	Present	Absent	Vertical and horizontal	Present	Absent
18	F	12	80	19	n.a.	Present	Absent	Present	Present, not further specified	Mild	Absent
19	F	13.6	40	25	homozygous c.1327_1346del	Absent	Absent	Absent	Vertical and horizontal	Present	Absent
20	M	14	90	14	c.215-14del13ins4/c.1159+2T>A	Absent	Absent	Absent	Absent	Absent	UL: absent LL: weak
21	F	11.2	80	17	Homozygous c.866A>C	Present	Present	Absent	Vertical and horizontal	mild	Weak
22	M	15	80	40	n.a.	Present	Present	Absent	n.a.	n.a.	n.a.
23	M	19	70	29	Homozygous c.1327_1346del	Absent	Absent	Present	Absent	Absent	Absent
24	M	12.9	70	25	c.689T>C and c.1112T>C	Absent	Absent	Absent	Horizontal	Present	Weak
f-1[a]	M	12	90	35	Homozygous c.433G>A	Present	Present	Present	Vertical and horizontal	Present	Normal
f-2[a]	M	15	40	38	Homozygous c.866A>C	Present	Present	Present	Present, not further specified	Present	Absent

UL = upper limbs, LL = lower limbs; BMI = body mass index; PFS = performance score; n.a. = not available.
[a]Patients who started conditioning but did not proceed to HSCT (see text).

Table 2 Transplant characteristics

Patient	Relationship D/R	Sex D/R	CMV D/R	HLA match	Conditioning regimen	GvHD prophlaxis	HSC source	NUC [$\times 10^8$/kg]	CD34+ cells [$\times 10^6$/kg]	Engraftment ANC $\geq$ 500/μl on day	plt $\geq$ 20 000/μl on day	Whole blood chimerism at last FU % donor cells
1	Rel	f/f	+/–	Ident	Flu/Bu/Cy/ATG-F	*In vitro* TCD	PBSC	n.d.	10.5	8	9	100%
2	URD	?/m	–/–	4/6	Flu/Cy/ATG -T	Tac/MMF	CB	0.47	n.d.	Primary non-engraftment with autologous reconstitution		n.a.
3	Rel	m/f	+/+	Ident	Flu/Cy/ATG-T	Sir/MMF	PBSC	8	8	11	Never below	100%
4	URD	m/m	+/+	Ident	Flu/Cy/ATG-T	CsA/MMF	PBSC	8.57	n.a	n.a.	n.a.	0% 4 month post-transplant (late graft failure)
										Engrafted	engrafted	
5.1	URD	m/m	–/+	4/6	Flu/Cy/ATG-T	Sir/MMF	CB1	0.231	0.13	Primary non-engraftment with autologous reconstitution		0%
		f/m	–/+	5/6			CB2	0.246	0.11			→ second HSCT 331 days later
5.2	URD (other)	m/m	+/+	9/10	Flu/Mel/TBI	CsA/Mtx	PBSC	8.90	5.04	12	n.a.	100%
6	Rel	f/f	–/–	Ident	Flu/Bu/MabC	CsA/Mtx	PBSC	n.d.	12.1	15	20	100%
7.1	URD	m/m	–/–	Ident	Flu/Bu/ATG-F	Sir/MMF	BM	3.58	2.31	26	Never below	48% (myeloid only, CD3 0%) → second HSCT 473 days later
7.2	URD (same)	m/m	–/–	Ident	Flu/Bu	CsA/Mtx	PBSC	n.d.	8.18	13	9	100%
8.1	URD	m/f	+/+	9/10	Flu/Cy/ATG-T	CsA/MMF	BM	1.81	1.7	Primary non-engraftment		0% → second HSCT 247 days later
8.2	URD (other)	f/f	–/+	Mismatch	Flu/Bu/ATG-T	CsA/MMF	CB	n.a.	n.a.	n.a.	n.a.	100%
9	URD	m/m	–/–	Ident	Flu/Bu/ATG-F	CsA/MMF	BM	5.91	7.1	23	Never below	100%
10	Rel	f/f	–/–	Ident	Flu/Bu/ATG-T	CsA/MMF	BM	5.8	nd	Never below	Never below	mixed
11	Rel	f/m	–/–	Ident	Flu/Bu/MabC	CsA	PBSC	7.47	12.8	12	Never below	73%
12	Rel	m/f	–/–	Ident	Flu/Bu	CsA/Mtx	BM	4.14	2.74	17	14	92%
13	URD	f/f	+/+	Ident	Flu/Bu	CsA/Mtx	BM	4.11	3.99	12	13	84% (d14)
14	URD	f/f	+/+	Ident	Flu/Bu	CsA/MMF	BM	3.4	4.01	19	19	86%
15	URD	f/m	+/–	11/12	Cy/Bu	CsA/Mtx	BM	2.69	n.a.	15	Not reached	n.d.
16	URD	f/f	–/+	9/10	Flu/Bu/ATG-T	CsA[a]/Mtx	BM	1.97	4.6	16	17	100%
17	Rel	m/f	+/+	Ident	Flu/Bu/ATG-T	CsA/Mtx	BM	7.4	13.3	17	14	100%
18	Rel	m/f	+/+	ident	Flu/Bu	CsA/Mtx	BM	8.5	7.2	15	13	100%
19	Rel	m/f	–/+	ident	Flu/Bu	Tac	PBSC	n.d.	5.34	13	15	100%
20	URD	m/m	+/+	Ident	Flu/Bu	CsA	BM	2.7	n.a.	16	17	100%
21	URD	f/f	–/+	4/6	Flu/Bu	CsA/Pdn	CB	0.49	0.07	24	Not reached	100%
22	URD	m/m	–/+	5/6	Flu/Bu	CsA/MMF/Pdn	CB1	0.54 (both)	0.11 (both)	Died before	Not reached	100% donor, mix of both units (d + 18)
		m/m	–/+	6/6			CB2					
23	URD	m/m	–/+	Mismatch	Cy/TBI/ATG	Tac/MMF	CB	n.d.	7.1	20	n.a.	n.a.
24	URD	f/m	+/–	10/10	Flu/Bu	CsA/Mtx	BM	6.75	n.a.	9	12	100%

Patients are identified by numbers, which is followed by a second number if the individual had more than one transplant, e.g. 5.1 and 5.2 indicates Patient 5 had two HSCTs.

ANC = absolute neutrophil count; ATG-F = ATG Fresenius, ATG-T = thymoglobulin; BM = bone marrow.; Bu = busulfan; CB = cord blood; CsA = cyclosporin A; Cy = cyclophosphamide; D/R = donor/recipient; f = female; Flu = fludarabine; HSC-source = source of haematopoietic stem cells; ident = HLA-identical donor (in unrelated donors 10/10 HLA-match if not otherwise indicated); m = male; MabC = alemtuzumab; MMF = mycophenolate mofetil; n.a. = not available; n.d. = not determined; NUC = nucleated cell count; PBSC = peripheral blood stem cells; plt = platelets; Rel = related donor; Tac = tacrolimus; TCD = T cell depletion; URD = unrelated donor.

[a]Later changed to MMF for possible cyclosporin A toxicity.

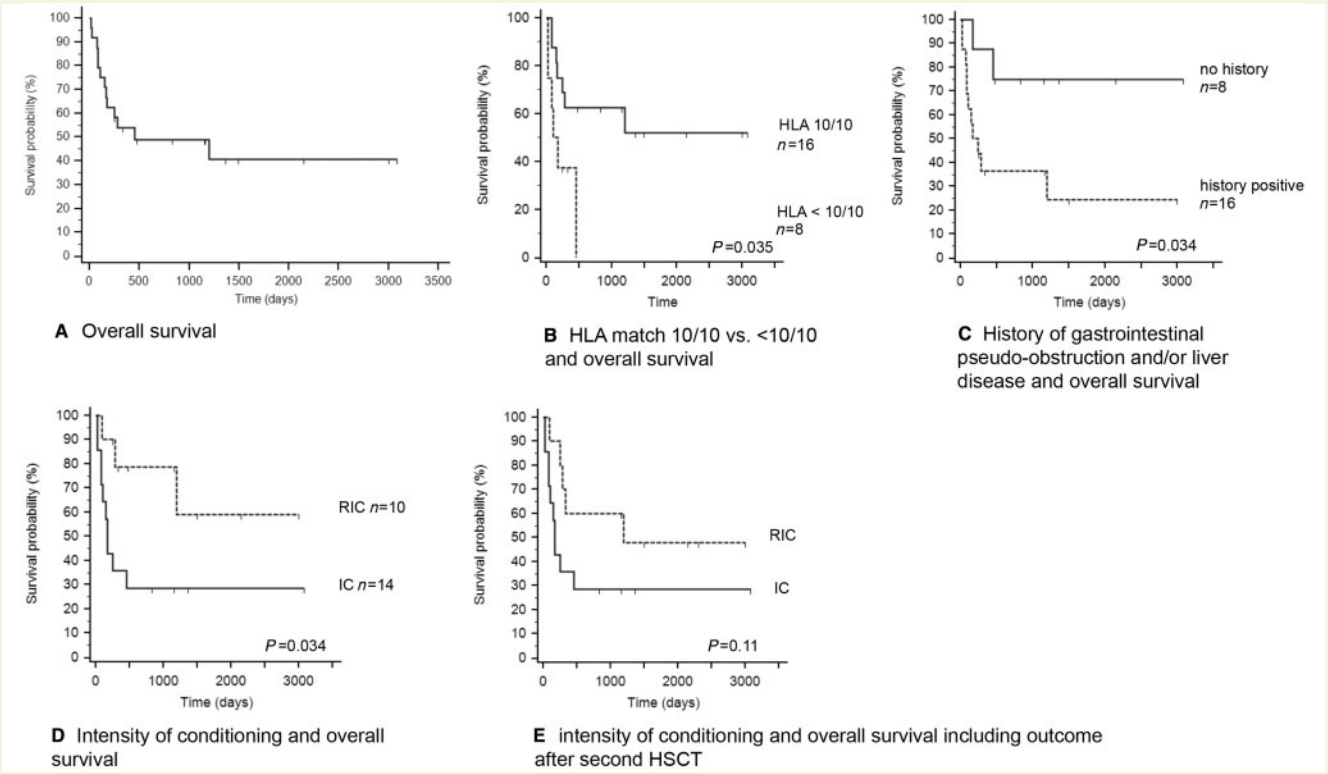

Figure 2 Overall survival. (**A**) Overall survival of the whole cohort with a median overall survival of 453 days. (**B–E**) Univariate analysis for factors associated with overall survival: HLA-match (**B**), history of gastrointestinal pseudo-obstruction and/or liver disease (**C**), intensity of conditioning regimen (**D**) and overall survival depending on intensity of conditioning regimen if outcome after second HSCT is included into survival analysis (**E**). RIC = reduced-intensity conditioning; IC = intensive, myeloablative conditioning.

C, and Supplementary Fig. 1). Although reduced intensity conditioning initially resulted in better survival (Fig. 2D) this advantage disappeared due to graft failure necessitating second HSCTs that were associated with a high transplant-related mortality (Fig. 2E).

Follow-up of MNGIE disease manifestations

After HSCT, buffy coat TP activity became detectable with engraftment and showed a rapid increase thereafter to normal levels, except in one patient with a donor who carried a *TYMP* mutation (median buffy coat TP activity 697 nmol/h/mg protein, range 262–1285; Fig. 3A). In parallel, normalization of plasma thymidine (median <0.05 μM/l, range 0–0.8) and deoxyuridine levels (median <0.05 μM/l, range 0–1.3) was observed, except in Patient 4 (0.8 μM/l) with ongoing late graft failure (Fig. 3B). Fasting plasma lactate levels were measured before and after transplantation in five patients with a follow-up of more than 2 years. Median lactate decreased from 2.3 mM (range 2.1–3.5) to 1.7 mM (range 1.5–1.9) after transplantation, suggesting improvement of mitochondrial respiratory chain function.

Slight clinically detectable improvements were observed as early as 6 months in some patients, but varied widely. Therefore, further analysis is focused on the seven patients that have survived for more than 2 years after first HSCT after a median follow-up of 53 months (range 27–85 months). Five patients have discontinued immunosuppressive treatment, and one is tapering immune-suppressants. Patient 6 died after more than 3 years post-transplantation. Long-term results of patients surviving at least 2 years after HSCT are shown in Fig. 3C–F.

Weight and gastrointestinal parameters

All patients were cachectic before transplantation. Patients lost weight during and in the initial period after HSCT. Despite high caloric food intake, patients experienced weight gain only after a year post-transplant. At last follow-up, median weight increase is 2.5 kg (range 0.7–18) (Fig. 3A). Diarrhoea resolved in five patients who also became free of abdominal pain. Diarrhoea improved in one patient and remained unchanged in another; in both, abdominal pain persisted. In three patients, parenteral nutrition was initiated after HSCT because of weight loss during post-transplant phase, but was later stopped in two; the third patient continues nocturnal parenteral nutrition to complement oral intake. Two further patients continue parenteral nutrition, which had already been initiated before transplantation; two never had parenteral nutrition.

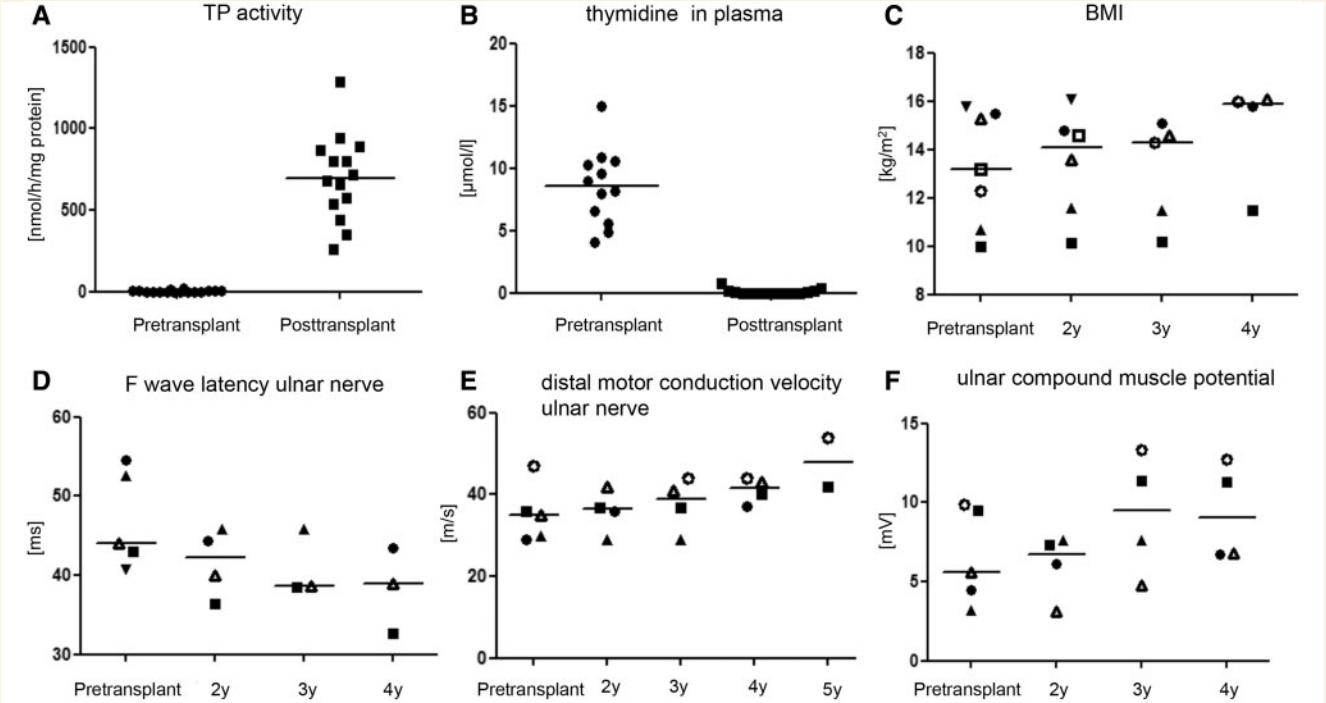

Figure 3 Metabolic and clinical MNGIE manifestations pre- and post-transplant. (**A**) TP activity in buffy coat before and after HSCT (paired *t*-test, two tailed: $P < 0.0001$). (**B**) Thymidine levels in plasma before and after HSCT ($P < 0.0001$). (**C–F**) Results of patients with follow-up >2 years. Shapes of symbols identify individual patients. Due to small patient numbers no statistical analysis was performed but a trend towards improvements of body mass index (BMI) and nerve conduction studies can be observed in long-term follow-up as exemplified by: (**C**) change of body mass index 2, 3, and 4 years after transplantation; (**D**) F-wave latency of ulnar nerve; (**E**) distal motor conduction velocity of ulnar nerve; and (**F**) ulnar compound muscle potential (mV).

Neurological findings

Peripheral neuropathy improved in affected patients with improved sensation (3/3) and restoration of tendon reflexes (4/5 with follow-up more than 3 years), starting as early as 6 months after transplantation and further improvements were observed in follow-up Years 2–4. One patient remains areflexic more than 5 years post-transplant. Three patients with glove and stocking-sensory deficits pre-transplant had partial improvement.

Five of the seven patients had ophthalmoparesis pre-transplant. This remained static for more than 1 year; however, eye movements slightly improved in the four individuals with more than 2 years follow-up.

Muscle atrophy was present in six of seven patients and persisted during long-term follow-up. After transient worsening of weakness following HSCT, muscle strength improved in all seven patients. Gait remained normal in four and improved in two. One patient remained unable to walk as prior to the HSCT. Cognition remained unaffected in all seven patients. Lumbar punctures were not performed after transplantation in any patient.

MRI of the brain

Leukoencephalopathy was present in all seven patients before HSCT. Although no formal volumetric analysis was performed, radiologists reported a slight increase of hyperintense lesions in three of them after 6 to 12 months but without clinical correlates. After a follow-up of more than 4 years, leukoencephalopathy improved in one patient and remained stable in five.

Electrophysiological studies

Improvement or at least stabilization of F-wave latencies and sensory and motor nerve conduction velocities was observed in all five patients assessed post-transplant. Results of measurements of the ulnar nerve are shown in Fig. 3D–F and Supplementary Table 3. These observations were confirmed by measurements of the median and peroneal nerve (data not shown). Compound muscle potentials also increased in all nerves measured in five patients assessed (Fig. 3F and Supplementary Table 3). Due to the small number of patients, no statistical analysis was performed but with increasing follow-up there seems to be a trend towards improvement of nerve conduction.

Discussion

In this retrospective study, we describe the experience of allogeneic HSCT in MNGIE patients. Despite the limitations of a heterogeneous cohort of patients transplanted under diverse protocols, this analysis produces several important conclusions. Firstly, we confirm in a large group of

patients that successful engraftment after allogeneic HSCT leads to a rapid normalization of TP enzyme activity and elimination of toxic metabolites but clinical improvement takes a much longer time and residual disease manifestations persist for years after HSCT. This is probably because the neurological and gastrointestinal features are due to multiple deletions, depletion, and point mutations of mtDNA that were acquired over many years of exposure to toxic levels of thymidine and deoxyuridine, causing progressive loss of mitochondrial function and cells with irreversible tissue destruction. Only after normalization of intra-mitochondrial deoxynucleotide pools, further damage to mtDNA may be prevented and improvements of the mtDNA depletion (but not deletions or point mutations) are likely to occur. Direct assessment of mtDNA in affected tissues was not performed because correlating the amounts of mtDNA deletions, depletion, and point mutations with clinical signs and symptoms would require invasive biopsies of all affected tissues (e.g. peripheral nerves, muscle, brain, and gastrointestinal tract) in patients who are already medically compromised. Furthermore, because there is regional variability of the pathology in affected tissues, sampling a severely or mildly affected area might show greater or lesser pathology than is present overall in the affected organ or peripheral nerves.

We have been able to demonstrate that the clinical manifestations of MNGIE improved significantly over time, even in patients with very severe disease manifestations. None of the patients surviving more than 1 year showed progression of MNGIE-related manifestations while objective improvements of clinical manifestations were observed only after 2 years. Organ recovery may continue over several more years, but the extent of restoration of organ function remains uncertain. Recovery of the gastrointestinal tract seems to be slow and at the time of analysis, incomplete. Weight gain occurred only slowly, even in patients who resumed oral feeding and were able to ingest high caloric nutrition. Complementary parenteral nutrition, e.g. overnight feeding, can support nutritional intake and benefits of prolonged use should be carefully weighed against risks such as infection or catheter-related thrombosis. While leukoencephalopathy is typically asymptomatic, neurological problems in MNGIE are primarily due to peripheral neuropathy. Poor recovery of the peripheral neuropathy is probably due to irreversible chronic axonal damage. Nevertheless, because the neuropathy is primarily demyelinating, partial improvements were evident in our patients. Due to the frailty of the patients, transplant- or MNGIE-related complications remained persistent threats that can trigger further declines in performance score and organ function.

Mortality in this series of 24 patients was substantial. Both transplant-related causes as well as pre-existing MNGIE-related comorbidities contributed to this unfavourable overall outcome. This suggests that risks and benefits have to be carefully balanced. Of note, no patient above the age of 30 and no patients with a less than 10/10 HLA-antigen matched donor survived. The presence of liver disease and a history of gastrointestinal pseudo-obstruction can be used as markers for disease stage in a similar manner as conventional co-morbidity risk scores (Sorror *et al.*, 2007).

Patients with MNGIE tolerate drugs differently in part due to their poor physical condition, but also due to the relative reduction of mitochondria. This issue was appreciated at an early stage by some groups and resulted in a consensus statement for future transplants performed in patients with MNGIE (Halter *et al.*, 2011). The small number of patients precludes a definitive statement about the value of these recommendations.

Reduction of transplant-related toxicity is important as well as a reduction of the high rate of primary graft failure observed. Toxicity can be reduced by limiting the number of pre-transplant risk factors, risk of rejection can be lowered by careful selection of HLA-matched donors, an adequate number of cells in the graft (Halter *et al.*, 2011) and strict monitoring of chimerism to detect graft-failure early and guide therapeutic approaches to prevent graft loss. Of note, the five patients who underwent unrelated cord blood transplantation died of transplant-related causes. None of them had an HLA-identical transplant, suggesting that other than well-matched unrelated cord blood cannot yet be considered routinely for HSCT in MNGIE as it is also true for non-malignant haematological disorders (Gluckman *et al.*, 2007; Bizzetto *et al.*, 2011; Boelens *et al.*, 2013). To date, it remains a challenge to determine the optimal time point for HSCT. A failed early transplantation could deprive patients of years of good quality of life; HSCT attempted too late may result in an even higher mortality risk and leave the patient with irreversible neurological and gastrointestinal deficits. Despite these complicating factors, some guidance for future transplantation in MNGIE can be derived from the experience gained to date. First, when detectable, TP level did not correlate with clinical outcome, i.e. partial restoration was adequate. Second, because the majority of post-transplant complications have been related to pre-transplant risk factors, immunosuppressive treatment and gastrointestinal problems due to MNGIE, our data suggest that HSCT should be considered, preferably in younger patients before severe gastrointestinal dysmotility and liver disease develop if an ideal donor can be identified. Similarly, HSCT should not be recommended for patients with advanced disease and lacking a donor with at least a 10/10 HLA antigen match.

In conclusion, allogeneic HSCT can alter the natural course of MNGIE, an otherwise debilitating and inevitably fatal disease. In view of the substantial transplant-related mortality, HSCT should currently be considered for carefully selected patients before severe organ damage has occurred. It will be critical to optimize this treatment for MNGIE in the future until other therapeutic approaches for enzyme replacement become established and more widely

Table 3 Complications and outcome

Patient	aGvHD grading	cGvHD grading	Gastrointestinal function	Peripheral neuropathy	Last follow-up (days after HSCT) survival status	Cause of death
1	II	0	Improved	Improved. Pre-transplant difficulties in walking and climbing stairs, both normalized at last follow-up.	2535 +, alive	n.a.
2	0	0	Worsening of GI dysmotility	No change	86	After graft failure progessive MNGIE-related complications with septicaemia
3	0	0	Improved. Less diarrhea and pseudo-obstruction; stopped TPN	Dysaesthesias improved; tendon reflexes returned	2450 +, alive	n.a.
4	0	0	Worsened	Worsened	281	After graft failure progressive MNGIE-related complications with liver and pancreatic failure
5	0[a] IV[b]	0[a] -[b]	n.d.	n.d.	331[c] 40	Transplant-related toxicity: alveolar haemorrhage, infection
6	0	0	Improved. Less abdominal pain but only able to have minimal oral intake. TPN ongoing	Improved Crawls, but unable to walk	1196	Central line infection (need for ongoing parenteral nutrition due to MNGIE-related gastrointestinal disorder)
7	0[a] I[b]	0[a] Moderate[b]	Improved	Improved	473[c] 1285 +, alive	n.a.
8	0[a] 0[b]	0[a] -[b]	n.d.	n.d.	247[c] 56	Transplant-related toxicity: multiorgan failure, infection
9	II	Mild	Improved, intermittent self-limiting gastroparesis	Improved	1598 +, alive	n.a.
10	I	0	n.a.	n.d.	84	MNGIE-related intestinal perforation with infection
11	0	0	Improved. Able to tolerate low residue diet. Abdominal pain still present. TPN ongoing	Improved. Pretransplant: bed-bound, walks now unaided around the room and mobilizes with a gutter frame. Regained movement in wrists, thumbs and ankles, increased power in elbows and knees, increase in sensory level	947 +, alive	n.a.
12	0	0	Improved. Normalization of GI symptoms	Improved Muscle strength improved	815 +, alive	n.a.
13	I	-	Worsened. Gastroparesis with need for gastrostomy, spontaneous small bowel perforation with need for surgery	n.d.	150	MNGIE-related intestinal perforation with postsurgical infectious complications
14	IV	-	n.a.	n.d.	77	Transplant-related aGvHD
15	0	-	n.a.	n.d.	18	Transplant-related veno-occlusive disease of the liver with multiorgan failure (post-mortem diagnosis of liver cirrhosis)
16	III-IV	Severe	Vomiting/diarrhea, abdominal cramps/pain started to improve 6 months after HSCT. Resumed partial oral nutrition, but remained TPN dependent	n.d.	453	Septic shock after intestinal perforation. Transplant-related GvHD.
17	0	Mild	Abdominal discomfort markedly improved, still recurrent episodes of pseudo-obstruction Oral nutrition significantly improved but still on TPN at last follow up	n.d.	246	MNGIE-related intestinal pseudo-obstruction, septic shock

(continued)

Table 3 Continued

Patient	aGvHD grading	cGvHD grading	Gastrointestinal function	Peripheral neuropathy	Last follow-up (days after HSCT) survival status	Cause of death
18	0	0	Daily abdominal pain with gastroparesis, need for TPN and evacuation by gastrostoma	No change	540 +, alive	n.a.
19	2	Severe	Worsened, repeated bacterial and viral infections	Initially improved strength, regained ability to walk short distances, but due to infections and GvHD, lost ability to walk	606 +, alive	n.a.
20	III-IV	Severe	n.d.	n.d.	166	Transplant-related GvHD, infection
21	IV	-	n.d.	n.d.	170	Transplant-related GvHD, infection, multiorgan failure
22	0	0	n.d.	n.d.	21	Transplant-related toxicity: idiopathic pneumonia syndrome
23	II	-	n.d.	n.d.	105	Transplant-related infection (reactivation of tuberculosis)
24	II	0	n.d.	n.d.	283 +, alive	n.a.

aGvHD = acute graft-versus-host disease; cGvHD = chronic graft-versus-host disease (NIH consensus criteria); n.d. = not determined; n.a. = not available.
[a]After first transplant.
[b]After second transplant.
[c]Censored at the time of second HSCT.

available (Torres-Torronteras *et al.*, 2011; Bax *et al.*, 2013).

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Supplementary material

Supplementary material is available at *Brain* online.

References

Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant 2009; 15: 1628–33.

Bakker JA, Schlesser P, Smeets HJ, Francois B, Bierau J. Biochemical abnormalities in a patient with thymidine phosphorylase deficiency with fatal outcome. J Inherit Metab Dis 2010; 33 (Suppl3): S139–43.

Bax BE, Bain MD, Scarpelli M, Filosto M, Tonin P, Moran N. Clinical and biochemical improvements in a patient with MNGIE following enzyme replacement. Neurology 2013; 81: 1269–71.

Bizzetto R, Bonfim C, Rocha V, Socié G, Locatelli F, Chan K, et al. Eurocord and SAA-WP from EBMT. Outcomes after related and unrelated umbilical cord blood transplantation for hereditary bone marrow failure syndromes other than Fanconi anemia. Haematologica 2011; 96: 134–41.

Boelens JJ, Aldenhoven M, Purtill D, Ruggeri A, Defor T, Wynn R, et al. Eurocord; Inborn Errors Working Party of European Blood and Marrow Transplant group; Duke University Blood and Marrow Transplantation Program; Centre for International Blood and Marrow Research. Outcomes of transplantation using various hematopoietic cell sources in children with Hurler syndrome after myeloablative conditioning. Blood 2013; 121: 3981–7.

Filosto M, Scarpelli M, Tonin P, Lucchini G, Pavan F, Santus F, et al. Course and management of allogeneic stem cell transplantation in patients with mitochondrial neurogastrointestinal encephalomyopathy. J Neurol 2012; 259: 2699–706.

Finkenstedt A, Schranz M, Bösch S, Karall D, Bürgi SS, Ensinger C, et al. MNGIE Syndrome: Liver Cirrhosis Should Be Ruled Out Prior to Bone Marrow Transplantation. JIMD Rep 2013; 10: 41–4.

Garone C, Tadesse S, Hirano M. Clinical and genetic spectrum of mitochondrial neurogastrointestinal encephalomyopathy. Brain 2011; 134: 3326–32.

Halter J, Schüpbach WM, Casali C, Elhasid R, Fay K, Hammans S, et al. Allogeneic hematopoietic SCT as treatment option for patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): a consensus conference proposal for a standardized approach. Bone Marrow Transplant 2011; 46: 330–7.

Gluckman E, Rocha V, Ionescu I, Bierings M, Harris RE, Wagner J, et al. Eurocord-Netcord and EBMT. Results of unrelated cord blood

transplant in fanconi anemia patients: risk factor analysis for engraftment and survival. Biol Blood Marrow Transplant 2007; 13: 1073–82.
Hirano M, Silvestri G, Blake DM, Lombes A, Minetti C, Bonilla E, et al. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. Neurology 1994; 44: 721–7.
Hirano M, Martí R, Casali C, Tadesse S, Uldrick T, Fine B, et al. Allogeneic stem cell transplantation corrects biochemical derangements in MNGIE. Neurology 2006; 67: 1458–60.
la Marca G, Malvagia S, Casetta B, Pasquini E, Pela I, Hirano M, et al. Pre- and post-dialysis quantitative dosage of thymidine in urine and plasma of a MNGIE patient by using HPLC-ESI-MS/MS. J Mass Spectrom 2006; 41: 586–92.
Lara MC, Weiss B, Illa I, Massuet L, Andreu AL, Valentino ML, et al. Infusion of platelets transiently reduces nucleoside overload in MNGIE. Neurology 2006; 67: 1461–3.
Nishigaki Y, Martí R, Copeland WC, Hirano M. Site-specific somatic mitochondrial DNA point mutations in patients with thymidine phosphorylase deficiency. J Clin Invest 2003; 111: 1913–21.
Nishigaki Y, Marti R, Hirano M. ND5 is a hot-spot for multiple atypical mitochondrial DNA deletions in mitochondrial neurogastrointestinal encephalomyopathy. Hum Mol Genet 2004; 13: 91–101.
Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. Science 1999; 283: 689–92.
Papadimitriou A, Comi GP, Hadjigeorgiou GM, Bordoni A, Sciacco M, Napoli L, et al. Partial depletion and multiple deletions of muscle mtDNA in familial MNGIE syndrome. Neurology 1998; 51: 1086–92.
Poulton J, Hirano M, Spinazzola A, Arenas Hernandez M, Jardel C, Lombès A, et al. Collated mutations in mitochondrial DNA (mtDNA) depletion syndrome (excluding the mitochondrial gamma polymerase, POLG1). Biochim Biophys Acta 2009; 1792: 1109–12.
Schüpbach WM, Vadday KM, Schaller A, Brekenfeld C, Kappeler L, Benoist JF, et al. Mitochondrial neurogastrointestinal encephalomyopathy in three siblings: clinical, genetic and neuroradiological features. J Neurol 2007; 254: 146–53.
Sicurelli F, Carluccio MA, Toraldo F, Tozzi M, Bucalossi A, Lenoci M, et al. Clinical and biochemical improvement following HSCT in a patient with MNGIE: 1-year follow-up. J Neurol 2012; 259: 1985–7.
Sorror ML, Giralt S, Sandmaier BM, De Lima M, Shahjahan M, Maloney DG, et al. Hematopoietic cell transplantation specific comorbidity index as an outcome predictor for patients with acute myeloid leukemia in first remission: combined FHCRC and MDACC experiences. Blood 2007; 110: 4606–13.
Spinazzola A, Marti R, Nishino I, Andreu AL, Naini A, Tadesse S, et al. Altered thymidine metabolism due to defects of thymidine phosphorylase. J Biol Chem 2002; 277: 4128–33.
Thomas née Williams SA, Segal MB. Identification of a saturable uptake system for deoxyribonucleosides at the blood-brain and blood-cerebrospinal fluid barriers. Brain Res 1996; 741: 230–9
Torres-Torronteras J, Gómez A, Eixarch H, Palenzuela L, Pizzorno G, Hirano M, et al. Hematopoietic gene therapy restores thymidine phosphorylase activity in a cell culture and a murine model of MNGIE. Gene Ther 2011; 18: 795–806.
Valentino ML, Martí R, Tadesse S, López LC, Manes JL, Lyzak J, et al. Thymidine and deoxyuridine accumulate in tissues of patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). FEBS Lett 2007; 581: 3410–4.
Yavuz H, Ozel A, Christensen M, Christensen E, Schwartz M, Elmaci M, et al. Treatment of mitochondrial neurogastrointestinal encephalomyopathy with dialysis. Arch Neurol 2007; 64: 435–8.

Appendix 1

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