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Delaying haematopoietic stem cell transplantation in children with viral respiratory infections reduces transplant-related mortality

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Summary

Viral respiratory infections (VRIs) contribute to the morbidity and transplant-related mortality (TRM) after allogeneic haematopoietic stem cell transplantation (HSCT) and strategies to prevent and treat VRIs are warranted. We monitored VRIs before and after transplant in children undergoing allogeneic HSCT with nasopharyngeal aspirates (NPA) and assessed the impact on clinical outcome. Between 2007 and 2017, 585 children underwent 620 allogeneic HSCT procedures. Out of 75 patients with a positive NPA screen (12%), transplant was delayed in 25 cases (33%), while 53 children started conditioning with a VRI. Patients undergoing HSCT with a positive NPA screen had a significantly lower overall survival (54% vs. 79%) and increased TRM (26% vs. 7%) compared to patients with a negative NPA. Patients with a positive NPA who delayed transplant and cleared the virus before conditioning had improved overall survival (90%) and lower TRM (5%). Pre-HSCT positive NPA was the only significant risk factor for progression to a lower respiratory tract infection and was a major risk factor for TRM. Transplant delay, whenever feasible, in case of a positive NPA screen for VRIs can positively impact on survival of children undergoing HSCT.

Keywords: haematopoietic stem cell transplantation, viral infections, nasopharyngeal aspirate, transplant-related mortality.

Haematopoietic stem cell transplantation (HSCT) is curative for several haematological (Peters *et al*, 2010) and immunological disorders (Freeman, 2018), as well as an alternative approach to enzyme replacement therapy in some inborn errors of metabolism (de Ru *et al*, 2011). However, infections are a major cause of treatment failure and contribute significantly to transplant-related mortality (TRM) (Atay *et al*, 2018). Viral infections are difficult to control in immunocompromised hosts, such as transplanted patients, due to impaired humoral and cell-mediated immunity post-HSCT because of *in vivo* (serotherapy) (Lamba *et al*, 2005) or *ex vivo* (alpha/beta and B cells depletion) lymphodepletion (Laberko *et al*, 2017) and immunosuppressants used to treat graft-*versus*-host disease (GvHD). Currently, there are no clear recommendations on screening and monitoring for respiratory viruses in children undergoing HSCT and there is very little evidence on efficacy of anti-viral drugs (Hirsch *et al.*, 2013). Despite a lower prevalence compared to herpes family viruses or adenovirus reactivation, respiratory viruses can be responsible for severe lower respiratory tract infections that can negatively affect transplant outcome (Boeckh, 2008; Dokos *et al*, 2013; Chemaly *et al*, 2014). Epidemiological studies have shown an incidence of upper and lower respiratory infections in children undergoing HSCT of around 20–30% (Bredius *et al*, 2004; Verdeguer *et al*, 2011; Srinivasan *et al*, 2011a, 2013). Progression to a lower respiratory tract infection can be influenced by patients' age and lack of immune reconstitution after transplant (El Saleeby *et al*, 2008; Srinivasan *et al*, 2011b; Gooskens *et al*, 2016). Overall, there are few specific recommended anti-viral treatments, and for a large proportion of respiratory viruses there is no evidence on efficacy for prophylaxis or therapy (Hirsch *et al.*, 2013; Dignan *et al*, 2016).

The impact of viral infections on outcome of children undergoing HSCT has not been fully explored, especially for patients that start a conditioning regimen with an active viral

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respiratory infection (VRI), as screening for respiratory pathogens in asymptomatic patients is not routine clinical practice. VRIs can lead to high early TRM within the first three months after transplant (Hutspardol *et al*, 2015). However, although European Conference on Infections in Leukaemia guidelines recommend considering delaying HSCT in the presence of viral infections (Hirsch *et al.*, 2013), there are no data on the efficacy of this approach on final outcome. In this retrospective study we analysed the prevalence of VRIs in children undergoing allogeneic HSCT and the prognostic significance of a positive nasopharyngeal aspirate (NPA) sample collected as part of the screening protocol prior to transplant.

Patients and methods

We retrospectively included all children who underwent allogeneic HSCT in the Bone Marrow Transplantation Unit at Great Ormond Street Hospital for Children in London, between November 2007 and November 2017. All but two patients had an NPA screen for VRIs before starting the conditioning regimen. The decision to delay the transplant in case of a positive NPA was made according to patients' characteristics and the clinical background. Patients with malignant disorders delayed the transplant according to disease status and donor availability. For patients with primary immunodeficiency disorder (PID), transplant delay was considered if residual T-cell immunity was observed and could reasonably allow viral clearance. We collected data on positive NPAs up to 90 days prior to transplant, in order to evaluate viral clearance in patients whose HSCT was delayed due to a positive NPA. We considered patients with active VRI at the time of transplant when they had a positive NPA before the start of the conditioning regimen. New occurrence of VRIs after HSCT (documented through NPA or bronchoalveolar lavage, BAL) was also recorded, and weekly NPA monitoring was routinely adopted during the early phases post-transplant. Moreover, we also retrieved data on symptomatic patients that were re-admitted or followed-up in the outpatient clinic up to 1 year after transplant.

We aimed to investigate the impact of VRIs on clinical outcome. Patients with severe respiratory symptoms or requiring paediatric intensive care unit (PICU) admission for respiratory distress and evidence of viral infection in the NPA or BAL, without any sign of other possible cause were considered as suffering from a viral LRTI. Mortality was considered to be attributable to VRIs when patients died from respiratory failure with positive specimens (NPA or BAL) for viral infections, and with no other ascertainable causes.

Polymerase chain reaction (PCR) for respiratory viruses

Viral DNA and RNA from NPA and BAL samples were analysed via PCR for the following viruses: cytomegalovirus (CMV); adenovirus (ADV); respiratory syncytial virus (RSV) A and B; influenza virus A and B; parainfluenza virus 1–4; human metapneumovirus. Extended viral PCR, adopted from 2013 included rhinoviruses, enterovirus and human coronavirus strains.

HSCT procedures

Human leucocyte antigen (HLA) typing was performed by molecular typing for HLA class I and II loci; mismatch was defined as ≤9 out of 10 HLA identical for bone marrow and peripheral blood stem cell source and ≤5/6 for cord blood. Myeloablative conditioning included busulfan-based conditioning receiving >8 mg/kg cumulative dose and total body irradiation-based regimens receiving ≥8 Gy fractioned dose. The diagnosis of acute GvHD was made clinically, and confirmed pathologically with skin, mucosal or liver biopsy whenever possible. Grading of acute GvHD was performed according to the Seattle criteria (Martino *et al*, 1999). Immune reconstitution was evaluated through absolute count of total lymphocytes and lymphocyte subsets evaluated through flow cytometry ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16/56^+$ cells) at 1, 3, 6 and 12 months after transplant. Significant CMV, Epstein–Barr virus (EBV) and ADV reactivation were considered when peripheral blood viral loads reached the cut-off of 10 000 iu/ml, 40 000 copies/ml and 1000 copies/ml, respectively.

Statistical analysis

Transplant features and demographic characteristics were included in descriptive statistics. Evaluated outcomes included overall survival (OS), TRM at day +100 after HSCT (defined as patients in complete response deceased up to 100 days after the procedure), progression to lower respiratory tract infection (LRTI) (as previously defined) and rate of admission to ICU. Log rank test (Mantel-Cox) was performed to compare Kaplan–Meier survival curves and cumulative incidence of TRM between groups of patients. Univariate analysis for categorical variables using Fisher's exact test investigated risk factors for final outcome. Variables that showed a significant association with outcome in univariate analysis were included in logistic regression model for multivariate analysis. Threshold for significant results for all the analysis was set at $P < 0{\cdot}05$. Data analysis and statistics were performed using Prism GraphPad software, version 6 (GraphPad Software Inc., La Jolla, CA, USA) and Epi Info™ 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) software.

Results

Transplant characteristics

Features of patients and transplant procedures are summarized in Table I. In this 10-year study period, 586 children

Table I. Transplant characteristics (n = 620) and patient demographics (n = 585) of the study cohort (second column); HSCT (n = 53) and patients' characteristics of children (n = 50) transplanted with a positive NPA (third column); HSCT and patients' characteristics of children (n = 22) who delayed the transplant and started conditioning with a negative NPA (fourth column).

	Study cohort (n = 585)	NPA+ transplanted patients (n = 50)	Delayed NPA+ patients (n = 22)	P value
Median age at BMT, years (range)	4·8 (0·04–17·03)	1·6 (0·07–12)	2·4 (0·2–8·8)	<0·0001
Diagnosis				
Malignant disorders	198/585 (34%)	13/50 (26%)	8/22 (36%)	0·4
ALL	93	7	4	
AML	64	3	2	
Others	41	3	2	
Non-malignant disorders	387/585 (66%)	37/50 (74%)	14/22 (64%)	
PIDs	249	31	9	
Metabolic disorders	47	2	4	
Haematological	41	0	0	
Autoimmunity/inflammatory	50	4	1	
Stem cells source				
BM	296/620 (47·7%)	22/53 (42%)	13/22 (59%)	0·5
PB	215/620 (34·7%)	19/53 (36%)	6/22 (27%)	
UCB	107/620 (17·3%)	12/53 (22%)	3/22 (14%)	
BM + PB	2/620 (0·3%)			
HLA matching				
Full match	397/620 (64%)	31/53 (59%)	18/22 (82%)	0·15
Mis-match	223/620 (36%)	22/53 (41%)	4/22 (18%)	
Conditioning				
None	37/620 (6%)	7/53 (13%)	1/22 (4%)	0·1
Reduced intensity	379/620 (61%)	34/53 (64%)	10/22 (46%)	
Myeloablative conditioning	204/620 (33%)	12/53 (23%)	11/22 (50%)	
Serotherapy				
Alemtuzumab	281/620 (46%)	17/53 (32%)	9/22 (41%)	0·8
ATG	102/620 (16%)	14/53 (26%)	3/22 (13%)	
Muromonab-CD3	5/620 (1%)	1/53 (2%)	0	
None	232/620 (37%)	21/53 (39%)	10/22 (46%)	
Number of transplants				
1st	568/620 (91%)	44/53 (83%)	22/22 (100%)	<0·05
2nd	49/620 (8%)	8/53 (15%)		
3rd	3/620 (1%)	1/53 (2%)		

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; BM, bone marrow; BMT, bone marrow transplantation; HLA, human leucocyte antigen; NPA, nasopharyngeal aspirate; PB, peripheral blood; PID, primary immunodeficiency; UCB, unit cord blood.

underwent 621 allogeneic HSCT procedures. One patient was excluded from the analysis, as NPA samples were not collected at any time before and after transplant. Most patients (249/585, 43%) were referred to HSCT for a PID or haematological malignancy (198/585, 34%). The preferred stem cell source was bone marrow (296/620, 47·7%) and most patients were fully HLA-matched with related (187/620, 30%) or unrelated (210/620, 34%) donors. The median age of patients in the cohort was 4·8 years (range 0·04–17·03). A small proportion of patients with severe combined immunodeficiency (SCID; 37/620, 6%) did not receive any conditioning prior to HSCT.

Viral respiratory infections before HSCT

Screening NPA results before starting the conditioning regimen were available for 618 transplants. Two patients were not investigated for VRIs at any time before transplant but were monitored in the early and late post-transplant follow-up. Overall, 75 patients (12·1%) presented with a viral pathogen in the NPA before transplant. Three patients received two HSCT with a positive NPA. Most of the children presented with symptoms of active upper respiratory tract infection (URTI) (47/75, 63%), while 24 (32%) were asymptomatic. Frequencies of different viral infections are reported in Table II. Eight patients presented pre-transplant with co-infection involving two viruses. The decision to delay HSCT was taken in 25 cases. Most of them were found positive for RSV (11/25, 44%), six had Influenza A or B, five had Parainfluenza viruses, two had ADV and, finally, one had Metapneumovirus. However, three patients did not clear the viral infection and, overall, 50 patients underwent 53 HSCT with an active viral URTI. These were rhinovirus (18/53, 34%), parainfluenza (12/53, 23%), RSV (6/53, 11%), ADV

Table II. Viral pathogens detected in nasopharyngeal aspirates of children screened for respiratory infections before starting conditioning (eight patients presented with a viral co-infection) and *de novo* respiratory viral infections after transplant, caused by viruses that were not detected at the time of pre-transplant screening (seven patients presented with a viral co-infection).

Viral pathogen	Pre-HSCT positive NPA $n = 75$ (%)	Post-HSCT positive NPA $n = 86$ (%)
Rhinovirus	22/75 (29)	22/86 (26)
Parainfluenza (PF1, PF2, PF3)	17/75 (23)	23/86 (27)
RSV	17/75 (23)	19/86 (22)
Influenza (A/B)	10/75 (13)	4/86 (5)
ADV	8/75 (11)	16/86 (19)
CMV	4/75 (5)	1/86 (1)
Metapneumovirus	3/75 (4)	3/86 (3)
Coronavirus	2/75 (3)	4/86 (5)
Enterovirus	0	1/86 (1)

ADV, adenovirus; CMV, cytomegalovirus; HSCT, haematopoietic stem cell transplantation; NPA, nasopharyngeal aspirate; RSV, respiratory syncytial virus; PF, parainfluenza; RSV = respiratory syncytial virus.

(4/53, 7%), influenza A/B (3/53, 5%), metapneumovirus (2/53, 4%) and CMV (1/53, 2%). Seven patients presented with a viral co-infection: coronavirus + rhinovirus (2/53, 4%), CMV + rhinovirus (1/53, 2%), parainfluenza + influenza (1/53, 2%), ADV + rhinovirus (1/53, 2%), RSV + CMV (1/53, 2%), RSV + parainfluenza (1/53, 2%).

De novo *viral infections after HSCT*

After transplant, patients were routinely screened through NPA once a week during admission for HSCT and, following discharge, in case of respiratory symptoms suggestive of URTI. Eighty-six (14%) new VRIs were documented within the first year post-HSCT (Table II) and 60/86 (70%) children had respiratory symptoms. Seven patients were found to be positive with two viruses at the same determination (3/7 were Coronavirus co-infections). Viruses were detected from day +1 and up to 11 months after transplant, although 71% (61/86) of infections occurred within the first 50 days after transplant, and the median time to NPA positivity was 25 days. About half (45/86, 52%) of new VRI occurred in the first 30 days after transplant ("early infections").

Lower airways infections and TRM

Of the 161 patients with URTI at or within the first year after HSCT, 32 (20%) were diagnosed with LRTI, which was documented with a positive BAL in 19 cases and with a positive NPA in 13 patients. The most frequent virus responsible for LRTI was Parainfluenza virus (12/32, 37·5%), followed by RSV (8/32, 25%). Three patients experienced a co-infection with two viruses. Of the 32 patients, 21 (66%) had LRTI due to the same pathogen, detected on pre-transplant screening. Admission to the PICU was necessary for 19 patients with respiratory failure. Overall 17/32 (53%) patients diagnosed with LRTI died (Table SI), and in all but two cases, the cause was attributed to VRI (LRTI-associated mortality 47%, 15/32). A significantly higher rate of LRTI was observed in patients with a positive NPA prior to transplant, compared to those that experienced a new URTI after transplant [36% vs. 15%, odds ratio (OR) 3·13, $P < 0·01$]. Moreover, patients with an active pre-HSCT infection required PICU admission more frequently [30% vs. 8%, OR 4·8, 95% confidence interval (CI 95) 1·9–12·9, $P < 0·005$, Fig 1]. Early infections (from day +1 to day +30) and late infections (after day +30) equally contributed to LRTI (6/48 vs. 7/38, $P = 0·55$) in children with negative pre-transplant NPA.

Risk factors for OS, TRM and progression to LRTI

Overall survival (OS) in our cohort was 78% and 100-day TRM was 8·5%. We stratified survival rates according to four different groups: patients undergoing transplant with a positive NPA (pre-NPA+), patients with a newly positive NPA after transplant (post-NPA+), patients with a negative NPA before and after transplant (NPA−) and a group of patients whose transplant was delayed due to positive NPA and cleared the virus before conditioning (delayed-NPA+). We found a statistically higher survival rate in patients that delayed HSCT and started the conditioning regimen after resolving the VRI (OS = 90·5%) compared to patients that

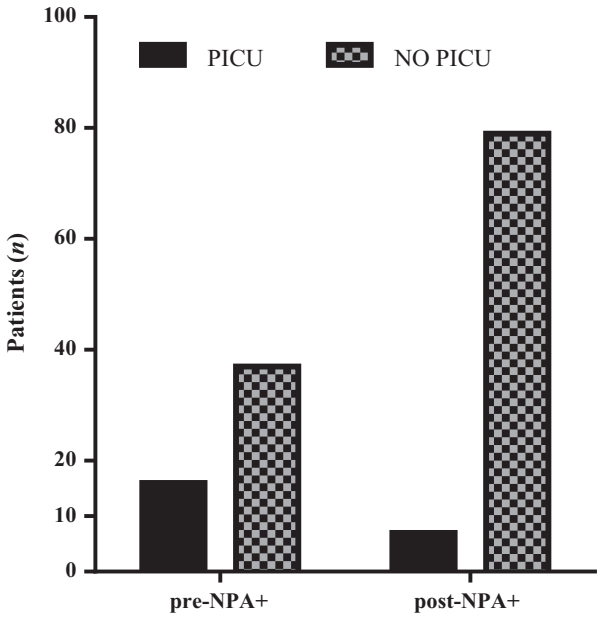

Fig 1. Admission to PICU was more frequent when NPA screening was positive at time of transplant ($P < 0·005$). NPA, nasopharyngeal aspirate; PICU, paediatric intensive care unit.

started conditioning with an active VRI (OS = 53%) [hazard ratio (HR) 4·2, CI 95 1·1–7·7, $P < 0·05$] (Fig 2A). No significant difference in OS was noted between the delayed group and patients diagnosed with a new respiratory infection after transplant or who did not experience any VRI (72·6% and 73·6%, respectively, $P = 0·2$).

We also evaluated the stratification of 100-day TRM in these groups of patients (Fig 2B). Children that presented with a positive NPA screen at the time of transplant had a significantly higher 100-day TRM (26·5%, HR 4·4, CI 95 1·9–12, $P < 0·001$), while no significant difference in early mortality was observed in patients with new VRIs (7%), and no VRIs at the time of transplant due to delay of the procedure (5%) or no occurrence of respiratory infections (7%). Considering patients with a positive NPA pre-HSCT, for 11/14 (79%) deceased patients, the cause of death was attributed to VRI, while two died of fungal pneumonia and one of lung GvHD/transplant-associated microangiopathy. Overall, LRTI-associated mortality was higher in patients with pre-HSCT active URTIs and in children that were NPA positive after transplant (24·6% vs. 8·4%, HR 4·1, CI 95 1·6–12, $P < 0·005$) (Fig 3A). In order to evaluate the impact of different risk factors on patients that died due to transplant complications in the first 100 days after HSCT (100-day TRM) and progression to LRTI we performed a univariate analysis on different variables (Table III). NPA positivity at the time of transplant was a significant risk factor for early mortality related to transplant complications, as well as the use of umbilical cord blood (UCB) as stem cell source and the use of HLA mismatched donors. On multivariate

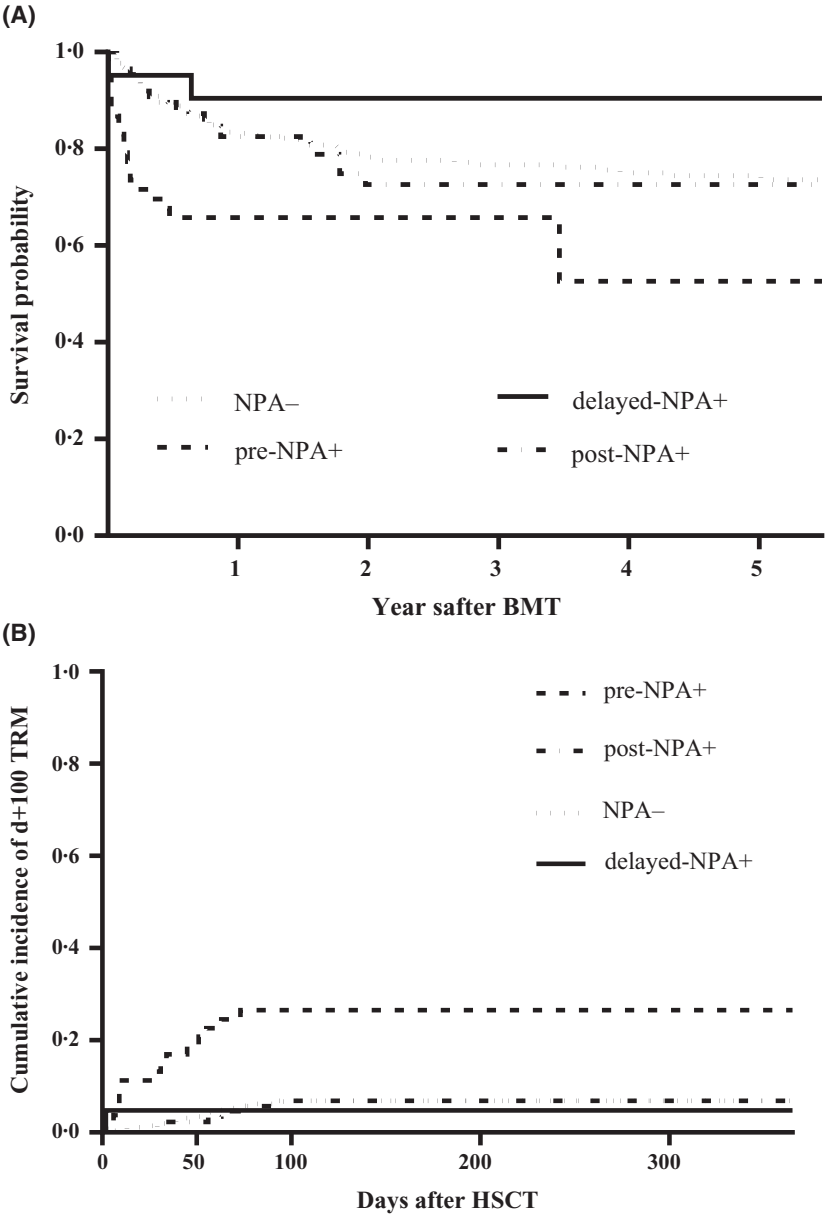

Fig 2. (A) Kaplan–Meier estimator for overall survival (OS) and log rank comparison for patients with pre-HSCT NPA positivity (53%), post-HSCT NPA positivity (72·6%), NPA negativity (73·6%) and patients with delayed HSCT (90·5%) ($P < 0·05$). (B) Cumulative incidence of 100-dayTRM and log rank comparison for patients with pre-HSCT NPA positivity (26·5%), post-HSCT NPA positivity (7%), NPA negativity (7%) and patients with delayed HSCT (5%) ($P < 0·001$). HSCT, haematopoietic stem cell transplantation; NPA, nasopharyngeal aspirate; TRM, transplant-related mortality.

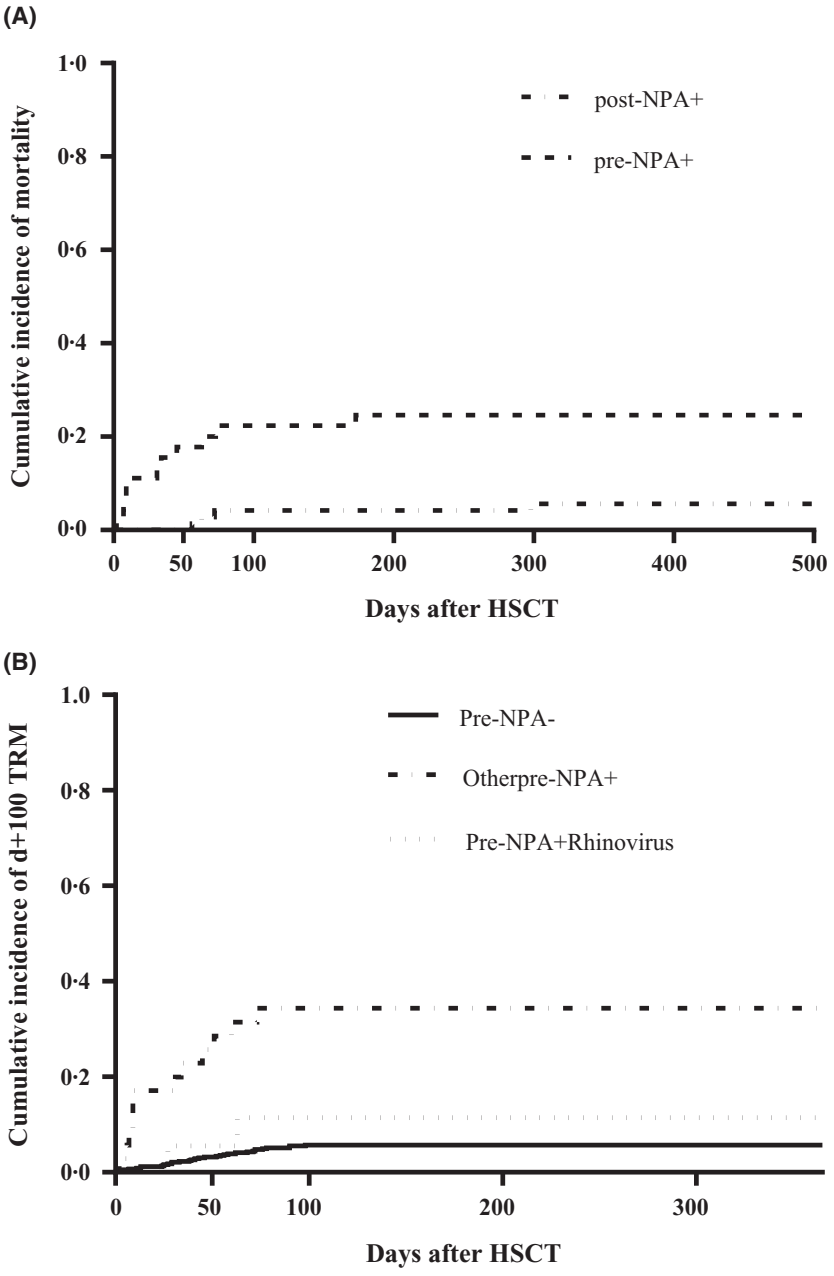

Fig 3. (A) Cumulative incidence of mortality due to lower respiratory tract infection in patients with a positive NPA at the time of transplant was higher than patients who experience a new upper respiratory tract infection (URTI) after bone marrow transplantation ($P < 0{\cdot}005$). (B) Cumulative incidence of 100-day TRM and log rank comparison for patients with pre-HSCT rhinovirus (12%), other pre-HSCT URTI (34%) and no pre-HSCT URTI (6%). HSCT, haematopoietic stem cell transplantation; NPA, nasopharyngeal aspirate; TRM, transplant-related mortality.

analysis, only NPA positivity and the use of HLA mismatched donors were independently associated with early TRM (Table III). We also investigated the impact of rhinovirus infection on outcome (Fig 3B). Early TRM was lower for patients with pre-transplant NPA positive for rhinovirus (12%) as compared with patients with other VRI (34%) starting conditioning, although this did not reach significance ($P = 0{\cdot}07$). However, no differences were noted with patients with pre-HSCT negative NPA (100-day TRM 6%, $P = 0{\cdot}3$). In univariate analysis, the risk of progression to LRTI was significantly higher in children with pre-HSCT positive NPA (Table III).

Discussion

Viral respiratory infections represent a major cause of morbidity and mortality after HSCT, because of the possible evolution to respiratory failure and the lack of effective therapeutic strategies available. Screening for respiratory viruses is not routinely performed in bone marrow transplant (BMT) Units and the presence of an URTI is not universally recognised as a risk factor that merits transplant delay. We extensively analysed the outcome of a sub-group of patients with a positive NPA screen before HSCT over a 10-year period. We found that pre-transplant viral infections had a

Table III. Univariate analysis (Fisher's exact test) of risk factors for 100-days TRM in children receiving HSCT ($n = 620$) and for progression to LRTI in patients with a positive NPA pre-HSCT conditioning ($n = 53$) versus patients with a documented URTI after HSCT ($n = 86$) and multivariate analysis (logistic regression) for 100-days TRM.

	100-day TRM OR (95 CI)	*P* value	LRTI OR (95 CI)	*P* value
Univariate analysis (Fisher's exact test)				
Pre-NPA+ *versus* Post-NPA+	4·3 (1·5–12·5)	<0·001	3·1 (1·3–6)	<0·01
UCB *versus* BM/PBSC	2·3 (1·2–4·2)	<0·05	2·8 (1·1–7)	0·052
aGvHD II-IV *versus* aGvHD 0-I	0·7 (0·4–1·3)	0·3	0·8 (0·3–1·8)	0·7
HLA ≤ 9/10 vs. 10/10	3 (1·5–6)	<0·005	1·3 (0·6–3)	0·5
PID diagnosis *versus* Other	1·2 (0·7–2)	0·7	1·8 (0·8–4)	0·2
Viral reactivation *versus* None viral reactivation	1 (0·6–1·8)	1·0		
$CD3^+$ cells at 1 month $<0{\cdot}2 \times 10^9$/l vs. $>0{\cdot}2 \times 10^9$/l	1·2 (0·6–2·2)	0·6	1·4 (0·6–3)	0·5
2nd/3rd HSCT *versus* 1st HSCT	1·15 (0·43)	0·8		
Logistic regression for 100-days TRM				
pre-NPA+	4·8 (2·4–9·8)	<0·001		
HLA mismatch ≤ 9/10	2·7 (1·4–5)	<0·005		
UCB	1·5 (0·7–2·9)	0·2		

95 CI, 95% confidence interval; aGvHD, acute graft-*versus*-host disease; BM, bone marrow; HLA, human leucocyte antigen; HSCT, haematopoietic stem cell transplantation; LRTI, lower respiratory tract infection; NPA, nasopharyngeal aspirate; OR, odds ratio; PBSC, peripheral blood stem cells; PID, primary immunodeficiency; TRM, transplant-related mortality; UCB, umbilical cord blood.

profound impact on clinical outcomes, leading to lower OS and higher 100-day TRM, compared to patients that experienced a *de novo* viral infection after HSCT. Active viral infection at the time of transplant negatively influenced outcome of these patients who experienced respiratory infection-related mortality in almost 25% of cases.

A significant proportion of children included in our cohort was affected by PID. These patients could be more susceptible to recurrent and difficult to eradicate viral RTI. However, this did not result in an increased prevalence of pre-HSCT VRI as compared to the literature. Indeed, Campbell *et al* (2015) reported a higher prevalence of pre-transplant positivity of respiratory virus screening, especially in children. However, in their study only a minority of patients were <18 years old (52/458, 11%). Of note, in this study, a higher mortality rate in children with pre-BMT viral infections was also reported, compared to adults. In our study, newly occurring VRIs were mainly documented in the early phase after HSCT and prevalence was comparable to the one recently described in children receiving HSCT (16·6%) (Fisher *et al*, 2018). Interestingly, in this study, early infections within the first 60 days after transplant were associated with higher morbidity and mortality, supporting the crucial impact of immune status for the outcome of patients with RVIs.

The main complication related to viral URTI is progression to LRTI. We showed that starting conditioning with an active upper airways respiratory infection can lead more frequently to pulmonary involvement, irrespective of background diagnosis, HLA matching and occurrence of GvHD. Use of UCB was only partially associated with higher rate of LRTI and this could be related to the lower cell dose and delayed lymphocytes engraftment (Nichols *et al*, 2004; Kim *et al*, 2014). Interestingly, a higher rate of intensive care needs for patients with a positive NPA screen was also observed.

Importantly, children who delayed transplant presented similar background features to NPA+ transplanted patients but had a reduced early mortality after transplant compared to those patients that did not delay the transplant despite the active viral infection documented on admission. This difference is unlikely to reflect a selection bias for better risk patients: when we looked at early TRM, progression to LRTI was the main cause of graft failure in the NPA+ group, and three other patients died due to pulmonary complications. Accordingly, risk factors analysis showed that a pre-transplant positive NPA was a major risk factor for early mortality. The more severe clinical course in patients with a respiratory virus documented in the upper airways before transplant could be related to the younger age of this subgroup of patients, given that age <2 years has been associated with dismal outcome of VRI (El Saleeby *et al*, 2008). However, patients who experienced a viral infection after transplant were not significantly older, with a median age <2 years.

European guidelines only provide weak recommendations for postponing conditioning in case of VRI (Hirsch *et al.*, 2013) and transplant delay can be controversial, because

some patients cannot postpone the procedure (e.g. patients affected by aggressive malignant disorders) or do not present immunological competence to clear the virus (e.g. patients with SCID). Nevertheless, in case of influenza or RSV infection, a delay of HSCT can allow pharmacological treatment (i.e. Oseltamivir and Ribavirin, respectively). Unfortunately, for most viruses there is no effective treatment available, and viral clearance is dependent on the efficacy of the host's immune system. Especially for patients with PID, that can more frequently present with chronic/refractory VRIs, balance of risk/benefit ratio is challenging because viral clearance is unlikely in SCID patients but can occur in the presence of a residual T cell immunity. Only one study reported the efficacy of transplant delay in 37 adults and children with RSV URTI before conditioning, showing that survival was significantly improved compared to those who encountered the virus after transplant (Peck *et al*, 2004). Whether to postpone HSCT because of a positive NPA for rhinovirus is more debatable, since LRTI progression is not frequent. However, rhinovirus-associated lower airways infection before transplant can lead to a worsen outcome (Seo *et al*, 2017; Mowrer *et al*, 2018). However, our data, although retrospective and limited to a small cohort of patients, show that pre-HSCT rhinovirus infection is associated with a modestly lower TRM as compared to other viral strains involved in URTI, and is overall comparable to that of patients with a negative NPA screen.

When transplant delay is not feasible, strategies aimed to improve lymphocytes recovery in the first weeks after transplant [e.g. targeted *in vivo* T-cell depletion (Admiraal *et al*, 2017), adoptive immunotherapy (Ciceri *et al*, 2009)] should be considered. Faster immune reconstitution is usually expected when *in vivo* T-cell depletion is omitted or administered early during conditioning (Lindemans *et al*, 2014). GvHD prophylaxis with alemtuzumab results in delayed lymphocytes recovery when compared to anti-thymocyte globulin (Shah *et al*, 2007), and decisions regarding *in vivo* T-cell depletion strategy should balance the risk of GvHD *versus* the burden of active viral infections. *Ex vivo* manipulation of the graft, selectively depleting alloreactive cells and allowing the presence of gamma/delta T cells in the graft, can tackle post-transplant infections (Bertaina *et al*, 2014; Locatelli *et al*, 2017), and turning-off T cell with an inducer of dimerization, have also been developed (Zhou *et al*, 2015). Finally, non-myeloablative and reduced-intensity conditioning regimens could limit organ toxicity and allow transplant also in patients with severe infections (i.e. PID patients), although the risk of engraftment failure with an active viral infection should be contemplated. However, prospective data are necessary to evaluate the impact of *in vivo* and *ex vivo* T-cell depletion on VRIs and no recommendation on the best strategy can be made.

Conclusion

This retrospective study from the largest paediatric HSCT centre in the UK shows that screening for VRIs prior to transplant is of paramount importance, as outcomes are dismal when patients are transplanted with a positive NPA screen. Moreover, patients who delayed HSCT until clearance of VRI had an improved outcome, suggesting that this screening strategy can reduce TRM. Prospective studies are needed to explore novel strategies to treat viral infections in children eligible for HSCT.

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Conflicts of interest

The authors declare no conflict of interest.

Authors contributions

GO and RC designed the study, analysed the data and wrote the manuscript. KR, PA, GL, RE and PV interpreted the results and critically reviewed the manuscript. JSF, JB, OC and AL reviewed the manuscript. All authors reviewed and approved the manuscript.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Features of 15 children who died due to viral LRTI.

References

Admiraal, R., Nierkens, S., de Witte, M.A., Petersen, E.J., Fleurke, G., Verrest, L., Belitser, S.V., Bredius, R.G.M., Raymakers, R.A.P., Knibbe, C.A.J., Minnema, M.C., van Kesteren, C., Kuball, J. & Boelens, J.J. (2017) Association between anti-thymocyte globulin exposure and survival outcomes in adult unrelated haemopoietic cell transplantation: a retrospective, pharmacodynamic cohort analysis. *The Lancet Haematology*, **4**, e183–e191.

Atay, D., Akcay, A., Erbey, F. & Ozturk, G. (2018) The impact of alternative donor types on viral infections in pediatric hematopoietic stem cell transplantation. *Pediatric Transplantation*, **22**, e13109.

Bertaina, A., Merli, P., Rutella, S., Pagliara, D., Bernardo, M.E., Masetti, R., Pende, D., Falco, M., Handgretinger, R., Moretta, F., Lucarelli, B., Brescia, L.P., Li Pira, G., Testi, M., Cancrini, C.,

Kabbara, N., Carsetti, R., Finocchi, A., Moretta, A., Moretta, L. & Locatelli, F. (2014) HLA-haploidentical stem cell transplantation after removal of + T and B cells in children with nonmalignant disorders. *Blood*, **124**, 822–826.

Boeckh, M. (2008) The challenge of respiratory virus infections in hematopoietic cell transplant recipients. *British Journal of Haematology*, **143**, 455–467.

Bredius, R.G.M., Templeton, K.E., Scheltinga, S.A., Claas, E.C.J., Kroes, A.C.M. & Vossen, J.M. (2004) Prospective study of respiratory viral infections in pediatric hemopoietic stem cell transplantation patients. *The Pediatric Infectious Disease Journal*, **23**, 518–522.

Campbell, A.P., Guthrie, K.A., Englund, J.A., Farney, R.M., Minerich, E.L., Kuypers, J., Corey, L. & Boeckh, M. (2015) Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant. *Clinical Infectious Diseases*, **61**, 192–202.

Chemaly, R.F., Shah, D.P. & Boeckh, M.J. (2014) Management of Respiratory viral infections in hematopoietic cell transplant recipients and patients with hematologic malignancies. *Clinical Infectious Diseases*, **59**, S344–S351.

Ciceri, F., Bonini, C., Stanghellini, M.T.L., Bondanza, A., Traversari, C., Salomoni, M., Turchetto, L., Colombi, S., Bernardi, M., Peccatori, J., Pescarollo, A., Servida, P., Magnani, Z., Perna, S.K., Valtolina, V., Crippa, F., Callegaro, L., Spoldi, E., Crocchiolo, R., Fleischhauer, K., Ponzoni, M., Vago, L., Rossini, S., Santoro, A., Todisco, E., Apperley, J., Olavarria, E., Slavin, S., Weissinger, E.M., Ganser, A., Stadler, M., Yannaki, E., Fassas, A., Anagnostopoulos, A., Bregni, M., Stampino, C.G., Bruzzi, P. & Bordignon, C. (2009) Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I–II study. *The Lancet Oncology*, **10**, 489–500.

Dignan, F.L., Clark, A., Aitken, C., Gilleece, M., Jayakar, V., Krishnamurthy, P., Pagliuca, A., Potter, M.N., Shaw, B., Skinner, R., Turner, A., Wynn, R.F., Coyle, P. & on behalf of the Haemato-oncology Task Force of the British Committee for Standards in Haematology & the British Society for Blood and Marrow Transplantation and the UK Clinical Virology Network (2016) BCSH/BSBMT/UK clinical virology network guideline: diagnosis and management of common respiratory viral infections in patients undergoing treatment for haematological malignancies or stem cell transplantation. *British Journal of Haematology*, **173**, 380–393.

Dokos, C., Masjosthusmann, K., Rellensmann, G., Werner, C., Schuler-Lüttmann, S., Müller, K.-M., Schiborr, M., Ehlert, K. & Groll, A.H. (2013) Fatal human metapneumovirus infection following allogeneic hematopoietic stem cell transplantation. *Transplant Infectious Disease*, **15**, E97–E101.

El Saleeby, C.M., Somes, G.W., DeVincenzo, J.P. & Gaur, A.H. (2008) Risk factors for severe respiratory syncytial virus disease in children with cancer: the importance of lymphopenia and young age. *Pediatrics*, **121**, 235–243.

Fisher, B.T., Danziger-Isakov, L., Sweet, L.R., Munoz, F.M., Maron, G., Tuomanen, E., Murray, A., Englund, J.A., Dulek, D., Halasa, N., Green, M., Michaels, M.G., Madan, R.P., Herold, B.C. & Steinbach, W.J. (2018) A multicenter consortium to define the epidemiology and outcomes of inpatient respiratory viral infections in pediatric hematopoietic stem cell transplant recipients. *Journal of the Pediatric Infectious Diseases Society*, **7**, 275–282.

Freeman, A.F. (2018) Hematopoietic stem cell transplantation in primary immunodeficiencies beyond severe combined immunodeficiency. *Journal of the Pediatric Infectious Diseases Society*, **7**, S79–S82.

Gooskens, J., Marijt, W.A.F., van Essen, E.H.R., Rimmelzwaan, G.F. & Kroes, A.C.M. (2016) Host immunity dictates influenza A(H1N1) pdm09 infection outcome in hematology–oncology patients. *Bone Marrow Transplantation*, **51**, 138–141.

Hirsch, H.H., Martino, R., Ward, K.N., Boeckh, M., Einsele, H. & Ljungman, P. (2013) Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. *Clinical Infectious Diseases*, **56**, 258–266.

Hutspardol, S., Essa, M., Richardson, S., Schechter, T., Ali, M., Krueger, J., Fujii, H., Egeler, R.M. & Gassas, A. (2015) Significant transplantation-related mortality from respiratory virus infections within the first one hundred days in children after hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, **21**, 1802–1807.

Kim, Y.-J., Guthrie, K.A., Waghmare, A., Walsh, E.E., Falsey, A.R., Kuypers, J., Cent, A., Englund, J.A. & Boeckh, M. (2014) Respiratory syncytial virus in hematopoietic cell transplant recipients: factors determining progression to lower respiratory tract disease. *The Journal of Infectious Diseases*, **209**, 1195–1204.

Laberko, A., Bogoyavlenskaya, A., Shelikhova, L., Shekhovtsova, Z., Balashov, D., Voronin, K., Kurnikova, E., Boyakova, E., Raykina, E., Brilliantova, V., Pirumova, V., Novichkova, G., Maschan, A. & Maschan, M. (2017) Risk factors for and the clinical impact of cytomegalovirus and epstein-barr virus infections in pediatric recipients of TCR-α/β− and CD19-depleted grafts. *Biology of Blood and Marrow Transplantation*, **23**, 483–490.

Lamba, R., Carrum, G., Myers, G.D., Bollard, C.M., Krance, R.A., Heslop, H.E., Brenner, M.K. & Popat, U. (2005) Cytomegalovirus (CMV) infections and CMV-specific cellular immune reconstitution following reduced intensity conditioning allogeneic stem cell transplantation with Alemtuzumab. *Bone Marrow Transplantation*, **36**, 797–802.

Lindemans, C.A., Chiesa, R., Amrolia, P.J., Rao, K., Nikolajeva, O., de Wildt, A., Gerhardt, C.E., Gilmour, K.C., B Bierings, M., Veys, P. & Boelens, J.J. (2014) Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. *Blood*, **123**, 126–132.

Locatelli, F., Merli, P., Pagliara, D., Li Pira, G., Falco, M., Pende, D., Rondelli, R., Lucarelli, B., Brescia, L.P., Masetti, R., Milano, G.M., Bertaina, V., Algeri, M., Pinto, R.M., Strocchio, L., Meazza, R., Grapulin, L., Handgretinger, R., Moretta, A., Bertaina, A. & Moretta, L. (2017) Outcome of children with acute leukemia given HLA-haploidentical HSCT after αβ T-cell and B-cell depletion. *Blood*, **130**, 677–685.

Martino, R., Romero, P., Subirá, M., Bellido, M., Altés, A., Sureda, A., Brunet, S., Badell, I., Cubells, J. & Sierra, J. (1999) Comparison of the classic Glucksberg criteria and the IBMTR Severity Index for grading acute graft-versus-host disease following HLA-identical sibling stem cell transplantation. *Bone Marrow Transplantation*, **24**, 283–287.

Mowrer, C., Lee, B.R., Goyal, R., Selvarangan, R. & Schuster, J.E. (2018) Outcome of children with rhinovirus detection prior to allogeneic hematopoietic cell transplant. *Pediatric Transplantation*, **22**, e13301.

Nichols, W.G., Guthrie, K.A., Corey, L. & Boeckh, M. (2004) Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. *Clinical Infectious Diseases*, **39**, 1300–1306.

Peck, A.J., Corey, L. & Boeckh, M. (2004) Pretransplantation respiratory syncytial virus infection: impact of a strategy to delay transplantation. *Clinical Infectious Diseases*, **39**, 673–680.

Peters, C., Cornish, J.M., Parikh, S.H. & Kurtzberg, J. (2010) Stem cell source and outcome after hematopoietic stem cell transplantation (HSCT) in children and adolescents with acute leukemia. *Pediatric Clinics of North America*, **57**, 27–46.

de Ru, M.H., Boelens, J.J., Das, A.M., Jones, S.A., van der Lee, J.H., Mahlaoui, N., Mengel, E., Offringa, M., O'Meara, A., Parini, R., Rovelli, A., Sykora, K.-W., Valayannopoulos, V., Vellodi, A., Wynn, R.F. & Wijburg, F.A. (2011) Enzyme replacement therapy and/or hematopoietic stem cell transplantation at diagnosis in patients with mucopolysaccharidosis type I: results of a European consensus procedure. *Orphanet Journal of Rare Diseases*, **6**, 55.

Seo, S., Waghmare, A., Scott, E.M., Xie, H., Kuypers, J.M., Hackman, R.C., Campbell, A.P., Choi, S.-M., Leisenring, W.M., Jerome, K.R., Englund, J.A. & Boeckh, M. (2017) Human rhinovirus detection in the lower respiratory tract of hematopoietic cell transplant recipients:

association with mortality. *Haematologica*, **102**, 1120–1130.

Shah, A.J., Kapoor, N., Crooks, G.M., Weinberg, K.I., Azim, H.A., Killen, R., Kuo, L., Rushing, T., Kohn, D.B. & Parkman, R. (2007) The effects of campath 1H upon graft-versus-host disease, infection, relapse, and immune reconstitution in recipients of pediatric unrelated transplants. *Biology of Blood and Marrow Transplantation*, **13**, 584–593.

Srinivasan, A., Wang, C., Yang, J., Shenep, J.L., Leung, W.H. & Hayden, R.T. (2011a) Symptomatic parainfluenza virus infections in children undergoing hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, **17**, 1520–1527.

Srinivasan, A., Wang, C., Yang, J., Inaba, H., Shenep, J.L., Leung, W.H. & Hayden, R.T. (2011b) parainfluenza virus infections in children with hematologic malignancies. *The Pediatric Infectious Disease Journal*, **30**, 855–859.

Srinivasan, A., Flynn, P., Gu, Z., Hartford, C., Lovins, R., Sunkara, A., Srivastava, D.K., Leung, W. & Hayden, R.T. (2013) Detection of respiratory viruses in asymptomatic children undergoing allogeneic hematopoietic cell transplantation. *Pediatric Blood & Cancer*, **60**, 149–151.

Verdeguer, A., de Heredia, C.D., González, M., Martínez, A.M., Fernández-Navarro, J.M., Pérez-Hurtado, J.M., Badell, I., Gómez, P., González, M.E., Muñoz, A. & Díaz, M.A. (2011) Observational prospective study of viral infections in children undergoing allogeneic hematopoietic cell transplantation: a 3-year GETMON experience. *Bone Marrow Transplantation*, **46**, 119–124.

Zhou, X., Dotti, G., Krance, R.A., Martinez, C.A., Naik, S., Kamble, R.T., Durett, A.G., Dakhova, O., Savoldo, B., Di Stasi, A., Spencer, D.M., Lin, Y.-F., Liu, H., Grilley, B.J., Gee, A.P., Rooney, C.M., Heslop, H.E. & Brenner, M.K. (2015) Inducible caspase-9 suicide gene controls adverse effects from alloreplete T cells after haploidentical stem cell transplantation. *Blood*, **125**, 4103–4113.