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Human herpesvirus type 6 reactivation after haematopoietic stem cell transplantation

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ABSTRACT

Human herpesvirus type 6 (HHV6) is known to reactivate after hematopoetic stem cell transplantation (HSCT) and has been suggested to be associated with increased mortality and severe clinical manifestations, including graft versus host disease (GvHD). The exact etiological role of HHV6 reactivation in increased morbidity and mortality after HSCT remains unclear. This review will focus on the current available evidence of HHV6 reactivation after HSCT and its immuno-modulatory capacities, with particular emphasis on the severe complication GvHD. At present, no effective specific antiviral treatment for HHV6 reactivation has been identified. The currently available antiviral agents are outlined, as well as possible future strategies for the treatment of HHV6 reactivation. Non-toxic, specific treatment or prevention of HHV6 reactivation might improve the safety and efficacy of the HSCT procedure.

1. Introduction

Human herpesvirus type 6 (HHV6) is a member of the beta herpesvirus subfamily (genus *Roseolovirus*) and two distinct variants have been described: HHV6 type A and B (75–95% nucleotide sequence identity). HHV6 infection is recognized as the cause of a febrile disease and exanthem subitum in early childhood. Over 90% of the population is infected within the first 18 months of life.[1,2] After primary infection, HHV6 persists in the host and is detectable in multiple tissues, similar to other herpesviruses (e.g. Cytomegalovirus (CMV)).[3] HHV6 infection rarely causes severe disease in healthy children, but viral reactivation in immuno-compromised patients is associated with severe clinical manifestations and increased mortality.[4]

Allogenic haematopoietic stem cell transplantation (HSCT) is used as treatment for an expanding range of disorders (malignancies, haematological and immunological diseases and inborn errors of metabolism). Bone marrow (BM) stem cells or peripheral blood stem cells (PBSC) are the most frequently used cell source for HSCT, although the use of cord blood (CB) stem cells as donor source is emerging over the last decade. Prior to HSCT, patients receive a myeloablative (MA) regimen or non-myeloablative (NMA) conditioning regimen. The selection for MA or NMA conditioning regimen depends on patient characteristics such as age and comorbidities. The MA conditioning regimen, which includes rigid immuno suppressive/ablative agents, virtually eliminates all pre-existing immunity and results in more severe immuno-toxicity compared with NMA schedules. Following HSCT, patients are treated with immunosuppressive therapy to prevent rejection of the graft and acute graft versus host disease (GvHD). Due to the pre-transplant conditioning treatment as well as immunosuppressive therapy after HSCT, stem cell recipients are severly immunosuppressed, resulting in an increased susceptibility for frequent opportunistic infections. Herpesvirus reactivations, especially CMV, herpes simplex virus (HSV) and varicella zoster virus (VZV) are well known post-transplant complications. Moreover, these viral reactivations have been described to be associated with acute GvHD, allograft rejections and increased non-relapse mortality.[5–7]

Abbreviations: HHV6, human herpesvirus type 6; GvHD, graft versus host disease; CMV, cytomegalovirus; BM, bonemarrow; PBSC, peripheral blood stem cells; CB, cord blood; MA, myeloablative; NMA, non-myeloablative; HSV, herpes simplex virus; VZV, varicella zoster virus; EBV, Epstein Barr virus; TBI, total body irradiation; CNS, central nervous system.

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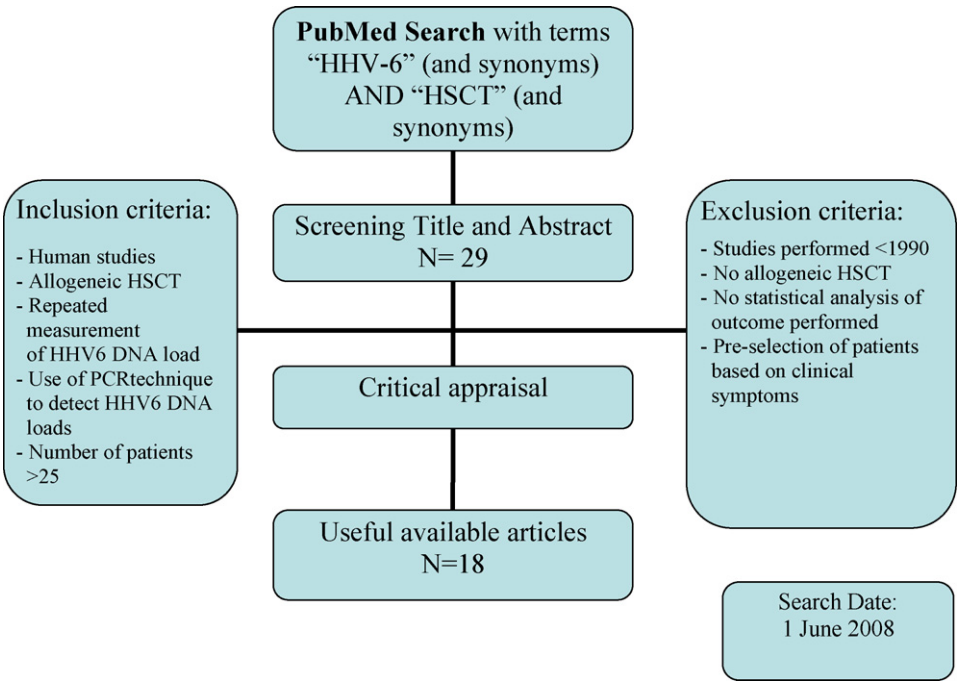

Fig. 1. Flowchart of selection procedure.

The role of HHV6 reactivation after HSCT, however, has scarcely been studied. This review addresses the current knowledge of the role of HHV6 reactivation after HSCT and the consequences for clinical outcome.

1.1. Literature search

A comprehensive literature search was carried out in PubMed library for articles comprising information on HHV6 reactivation in patients after HSCT published between 1990 to June 2008. To identify other possible eligible papers, reference lists from identified publications were screened and peer-reviewed articles published in English were retrieved. After a systematic search (Fig. 1), 29 informative publications were obtained and after critical appraisal, 18 peer-reviewed reports were selected (Table 1).

1.2. Incidence of HHV6 reactivation and clinical manifestations after HSCT

Reactivation of latent herpesviruses, like CMV, EBV, HSV and VZV in the immunocompromised host is associated with increased morbidity and mortality after HSCT. All herpes viral reactivations are known to cause severe disease contributing to this increased mortality after HSCT.[5,8] Implementation of regular monitoring of viral DNA loads and the introduction of prophylactic (for HSV and VZV) or pre-emptive (for EBV and CMV) therapies, have resulted in a significant reduction of clinical complications.[8]

Since over 90% of healthy children contracts HHV6 during the first 18 months of life,[2,9] virtually all HSCT patients over 18 months should be considered as HHV6 infected preceding HSCT treatment. Literature on HHV6 reactivation after HSCT and clinical outcome however is relatively scarce. In Table 1 the selected literature on the association between HHV6 reactivation and clinical outcomes is summarized. All these studies applied HHV6 DNA viral load monitoring after HSCT by PCR technique. Despite the high sensitivity to detect HHV6 reactivation by PCR monitoring, one needs to take into account that the HHV6 viral load assays lack international standardization and that different sample types (e.g. whole blood samples, peripheral blood lymphocytes or plasma) are used by the various laboratories. Therefore, comparison of absolute viral load levels between laboratories is not possible. We focused on comparing clinical associations with HHV6 reactivation data and longitudinal changes in HHV6 viral load of patients after HSCT.

In 12 of 18 studies, HHV6 reactivation within the first month after HSCT was reported. A few additional studies reported later HHV6 reactivations, but this might be due to the less frequent monitoring strategy applied (Table 1). In the prospective study of Zerr et al., HHV6 reactivation was observed in 47% of 110 adult patients and was significantly associated with severe GvHD (grades 3–4) and all cause mortality,[4] confirmed by others.[10] A more recent retrospective study among adult HSCT patients from our center confirmed these associations (Fig. 2A and B).[11] In contrast, Hentrich et al reported a similar association of HHV6 reactivation with severe GvHD, but not with mortality.[12] In other studies, high HHV6 reactivation rates (48–72%) were observed, but without any association with increased GvHD and non-relapse mortality.[13–15] This might be due to the fact that a majority of these patients received NMA conditioning (59%, 55% and 41%, respectively) and this might have lowered the risk of HHV6 reactivation. This is illustrated by our recent study in adult HSCT patients where MA conditioning appeared to be the only significant risk factor for the development of HHV6 DNA positive PCR observations following HSCT in a multivariate Cox proportional hazard model (HR 4.8; 95% CI 1.6–14.8, $p = 0.006$).[11] Zerr et al. did not analyze the association of MA versus NMA conditioning regimen with HHV6 reactivation after HSCT.[4] Cord-blood derived stem cells, mismatched graft (gender disparity, HLA-mismatch) and total body irradation (TBI)-based conditioning regimen are also suggested to be risk factors for HHV6 reactivation.[4] In addition, donor versus recipient mismatch as well as serotherapy (e.g. ATG, Campath) are well known risk factors for viral reactivations after HSCT.[8] Although Chan et al. did not find any significant risk factors for HHV6 reactivation, a systematic statistical analysis lacked in this study.[16] The different conclusions of the various studies may be explained by the differences in conditioning reg-

Table 1
Overview of HHV6 DNA monitoring studies after HSCT

Ref.	Type	Patients group (median age, range, in yrs)	Samples/ Procedure	Incidence of HHV6reactivation	Median time HHV6reactivation after HSCT (wks)	Risk factors for HHV6 reactivation	Significant association with clinical manifestations
11	R	49 allogeneic HSCT patients (40;r 18-66)	plasma; w q rt PCR	14/25 (56%) in MA patients	3 (r 2-5)	MA conditioning	acute GvHD, NRM, overall mortality
29	P	72 allogeneic HSCT patients (28;r 8-58)	PBMC; w q rt PCR	35/72 (49%)	3 (r 1-12)	n.p.	early skin rash, CMV reactivation
14	P	46 allogeneic HSCT patients (47; r 20-63)	plasma; w q rt PCR	22/46 (47.8%)	3 (r 2-5)	CB graft, HLA mismatched donor, tacrolimus prophylaxis, low HHV6 IgG titer	CMV reactivation
4	P	110 allogeneic HSCT recipients; (42; r 15-67)	plasma; w q rt PCR	52/110 (47%)	3 (r 2.7-12.9)	sex-mismatched graft, haematologic malignancy >1 remission, younger age	delayed platelet and monocyte engraftment, overall mortality, grade >II aGvHD, CNS dysfunction
12	P	228 allogeneic HSCT patients (40; r 14-63)	PBL; w q rt PCR	69/ 228 patients (42.1%)	4 (r 1-20)	unrelated donor graft	GvHD, EBV reactivation
15	P	50 allogeneic BM HSCT patients (41; r 12-59)	plasma; w q rt PCR	24/50 (48%)	3 (r 0-7)	HLA mismatched donor, steroids-use	delayed platelet engraftment
13	R	82 allogeneic HSCT patients (39; r 17-64)	plasma; w q rt PCR	HHV6A:24/82(29%) HHV6B:35/82 (43%)	HHV6A: 5 (r 1-33) HHV6B: 6 (r 1-30)	HHV6B: early acute GvHD, bonemarrow derived graft	HHV6B: delayed platelet engraftment
20	P	38 allogeneic HSCT recipients (33; r 6-54)	PBL, qPCR		3 (0-24)	Bonemarrow derived graft	Delayed platelet engraftment #
17	P	74 allogeneic HSCT patients (38; r 4-55)	PBMC; every other week q PCR	58/74 (78%)	-	unrelated donor graft, HLA mismatched family donor, IvIg prophylaxis	delayed platelet engraftment
18	P	60 allogeneic HSCT patients (8; r 2-20)	whole blood; serology + PCR	27/49 (55%)	3 (r 0-24)	conditioning regimen with TBI	delayed platelet and erythrocyte engraftment
22	P	41 allogeneic HSCT recipients (31; r 16-49)	PBL; w PCR	19/41 (46%)	n.p.	n.p.	Vascular endothelial injury
10	P	57 HSCT recipients (21;r 2-53); 36 allogeneic, 24 autologous	W PCR; PBL	36/57 (63%)	5 (r 2-10)	n.s.	Acute GvHD
33	P	57 HSCT recipients; 34 allogeneic, 23 autologous (	PBL; PCR	26/34 (76%) allogeneic recipients	n.p.	n.p.	Acute GvHD
21	P	58 HSCT patients (8; r 0-18)	plasma; w q rt PCR	67%	2 (r 0-17)	n.s.	acute and chronic GvHD, NRM and overall mortality
19	P	92 HSCT patients; 28 allogeneic, 64 autologous transplants (45 ; r 3-65)	PBMC/plasma; w PCR	39/92 patients, 42%	2.4 (r 1-17)	bone marrow stem cell graft	fever, anemia, delayed neutrophil and platelet engraftment
26	P	27 allogeneic HSCT patients (7; r 1-17)	PBMC/plasma w s.q. PCR	16/27 (59%)	4 (r 2-6)	HHV6 reactivation in PBMCs: cord blood stem cell graft	n.s.
16	R	61 HSCT recipients; 50 allogeneic, 11 autologous; (33; r 3-50)	PBL; w PCR	17/61 (28%)	3 (r 1-12)	n.s.	n.s.
23	P	26 HSCT patients; 15 allogeneic and 11 autologous (40; r 22-60)	PMBCs; w isolation, PCR, serology	12/26, 46%	7 (r 1-28)	cytomegalovirus infection, sinusitis	n.s.

P: prospective study-design, R: retrospective study-design, BM: bonemarrow, CB: cord blood, HHV6: human herpesvirus type 6, HSCT: haematopoietic stem cell transplantation, NRM: non-relapse mortality, MA: myeloablative conditioning regimen, n.p.: not performed; n.s.: no significant association found, PBL: peripheral blood lymphocytes, PBMC: peripheral blood mononunclear cells, q rt PCR: quantitative realtime PCR assay, w: weekly.
[a] Associations only for HHV6 reactivation within the first month after HSCT.

imens and underlying diseases. Autologous transplanted patients, included in several studies, without severe post-HSCT immunosuppressive treatment regimens and associated risk factors for viral reactivations may have had large impact on overall associations. So far, all studies reported are relatively small and risk factors have not been accorded for in all studies.

Delayed platelet engraftment associated with HHV6 reactivation have been described in several studies,[4,13,15,17–20] but this was not confirmed by others.[21–23] This is possibly caused by the low number of patients and variation in the conditioning regimens. In vitro data showed that HHV6 can infect hematological progenitor cells and reduce colony formation, which may explain the delayed engraftment after HSCT.[24,25]

For studies concerning children after HSCT, 3 studies have been published.[18,21,26] Now, significant associations of HHV6 reactivation with severe GvHD, delayed platelet engraftment, poor survival and non-relapse mortality (NRM) were found, but probably due to the low number of patients, the influence of HHV6 reactivation might even have been underestimated.

HHV6 type B is the viral type described in the majority of HHV6 reactivations of HSCT patients.[4,27] Only 2 studies reported HHV6 type A DNA viral loads after HSCT.[4,13] This might be due to geographical differences among populations or later acquisition of the more uncommon HHV6 type A variant.[28]

With respect to disease, HHV6 encephalitis has been described in detail over 40 HSCT recipients[13,14,29,30] and central nervous system (CNS) dysfunction in patients with high HHV6 loads was reported in 2 studies.[4,31] The relatively low incidence of HHV6 encephalitis and the small sizes of HHV6 studies as well as the lack of systematic screening of CNS dysfunction limit the possibilities for interpretation of the consequences of HHV6 reactivation for CNS dysfunction.[32] More invasive diagnostics (e.g. viral load monitoring in liquor or tissues and histopathological examinations) might detect HHV6 and possible associated disease more frequently, as described for other viruses as well.[3]

1.3. HHV6 reactivation and the immune system after HSCT

Although associations of HHV6 reactivation with clinical symptoms have been identified, the causative role of HHV6 is not completely clear. The association of HHV6 reactivation with acute GvHD has been described by several authors.[4,11,12,21] Appleton et al. demonstrated that HHV6 reactivation preceded the development of acute GvHD.[33] Tissue damage, attributable to previous therapy, underling disease and conditioning regimens, are assumed to act as triggers for the development of acute GvHD.[34,35] HHV6 reactivation may enhance tissue damage by inflammatory responses due to the lytic infection and lympho-proliferation. In this way, HHV6 reacti-

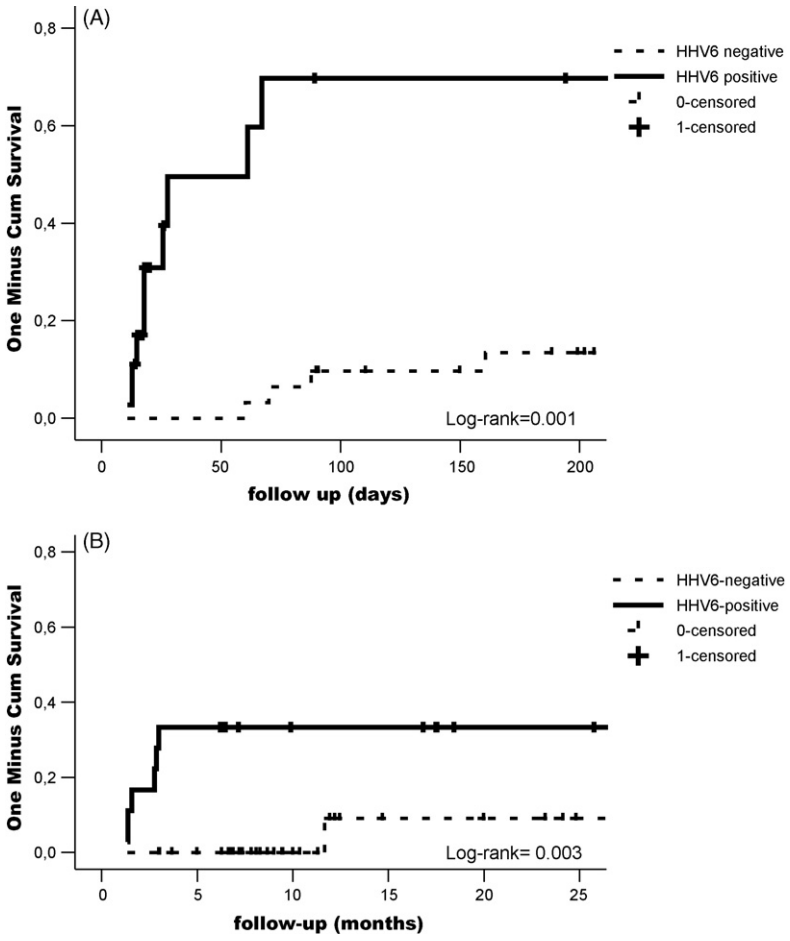

Fig. 2. (A) Acute GvHD after HSCT and (B) non-relapse mortality after HSCT.

vation may directly or indirectly play a role in the development of acute GvHD.

HHV6 is also considered to be immunomodulatory and immunosuppressive by itself. The modulated immune reconstitution may facilitate complications, such as superinfections, other viral reactivations and GvHD. HHV6 can interfere with the immune system through a variety of mechanisms.[36] CD4+T-cells and monocytes are the primary targets for HHV6 replication.[37] During this lytic phase HHV6 can induce alterations in and influence the associated immune response.[38,39] Furthermore, different immunomodulatory effects seem to be mediated by the engagement of the primary HHV6 receptor, CD46 (a member of the regulator of complement activation protein family).[40] This ubiquitous CD46 receptor, expressed on all nucleated human cells, prevents spontaneous activation of complement on autologous cells. HHV6 infection, however, dramatically downregulates CD 46 expression and T-cell activity and modulates cytokine and chemokine expression and excretion (e.g. IL-10) to create a favorable environment for viral survival and latency state throughout life.[41] The early exposure of the reconstituting immune system to large amounts of HHV6 antigen after HSCT and the immuno-modulatory effects of HHV6 at this time might dramatically influence immune reconstitution. In the early period after HSCT, when HHV6 reactivation occurs, the majority of reacting T-cells will be peripheral proliferating T-cells. This may not only lead to HHV6-specific immune responses, but, due to the (pro)inflammatory environment, also to direct or indirect proliferation and activation of alloreactive T-cell clones, as was also suggested for immune responses against CMV.[42] Studying the role of HHV6 specific immune responses after HSCT might provide more information regarding the association with GvHD and NRM after HSCT. Future studies should reveal this hypothesis.

1.4. Prevention and therapy of HHV6 reactivation

Antiviral treatment of HHV6 has not been extensively studied. In line with associations observed between CMV-reactivation and GVHD, pre-emptive or prophylactic therapy might improve clinical outcome in patients with HHV6 reactivation. In the absence of antiviral therapy with specific activity against HHV6,[43] treatment of HHV6 disease after HSCT currently relies on relatively broad-spectrum anti-herpetic drugs, like (val) ganciclovir and foscarnet. Evidence for clinical efficacy of these drugs has not been proven yet and can only be derived from case reports of patients with highly variable backgrounds (Table 2). In most reports, some antiviral activity is reported for ganciclovir although in certain cases of fulminant HHV6 infection showed no response.[44–48] Use of ganciclovir is complicated by severe bone marrow suppression which is an unwanted side-effect early after HSCT.[49] As in almost all patients suffering from HHV6 reactivation, prophylactic acyclovir was prescribed to prevent VZV/HSV reactivation, acyclovir appears to be ineffective against HHV6. This was also confirmed in vitro.[50] Foscarnet on the other hand appears to be active against HHV6 both in vitro and in vivo,[51,52] but can cause severe nephrotoxicity when administered at therapeutic levels.[53] Cidofovir showed excellent anti HHV6 activity in vitro,[54] and seems to be the most effective

Table 2
Clinical used antiviral therapy against HHV6

Antiviral drug	Type of antiviral agent	In vitro evidence	EC 50 (μM)[a]	In vivo data (case reports)
Ganciclovir[44–48]	Acyclic nucleoside analog	Good activity	69	Effective in majority of patients
Acyclovir[50]	Acyclic nucleoside analog	Poor effectivity	185	Not effective
Foscarnet[51–53]	Pyrophosphate analog	Excellent effectivity	25	Effective
Cidofovir[54,55]	Acyclic nucleoside phosphonate analog	Excellent effectivity	9.8	Secondline treatment, due to nephrotoxicity
Maribavir[57]	Nucleoside analog	Ineffective	>100	N/A
Cyclopropavir[58,59]	Methylenecyclopropane analog	Good activity	7.8	Preclinical phase

[a] EC50: 50% antiviral effective concentration. All EC50 concentrations are determined in MOLT-3 T-cell lines, infected with laboratory strain HHV6-B, variant Z-29.[61] Only cyclopropavir was tested in HHV-6 Z-29 infected cord blood lymphocytes.[58]

compound available for the treatment of HHV6 reactivation. However, the clinical use of cidofovir for HSCT patients is restricted due to the risk of developing nephrotoxicity.[55] Combination therapies of foscarnet and cidofovir or ganciclovir have also been described and resulted in successful treatment of HHV6 encephalitis, except for remaining short term memory dysfunction.[56] A more recently developed anti-herpetic drug, maribavir, developed for the treatment of cytomegalovirus, is not active against HHV-6 in vitro,[57] but cyclopropavir, another recently developed antiviral drug demonstrated to be active against HHV-6 in vitro. Cyclopropavir is still in the preclinical stage.[58]

For the present, all available evidence regarding antiviral therapy is based on therapies started at the onset of HHV6 disease. For instance, Ogata et al. prospectively studied pre-emptive ganciclovir therapy in 6 of 29 HSCT patients. These 6 patients developed high HHV6 DNA loads and 4 patients were pre-emptive treated with ganciclovir therapy. Two of these patients developed encephalitis, together with a further increase of the plasma HHV6 DNA load. Due to the dynamic kinetics of HHV6 plasma load and -encephalopathy, the pre-emptive therapy might have been started too late and prophylactic therapy might have been of benefit to prevent HHV6 disease.[59] However, prophylactic therapy against HHV6 reactivation after HSCT has not been studied yet. Clinical trials with antiviral therapeutics should be undertaken to identify effective antiviral therapy for HHV6 reactivation and can be used to further elucidate the etiological role of HHV6 in clinical manifestations. Elucidating the pathogenesis of HHV6 reactivation might also give additional clues for preventive or pre-emptive treatment of HHV6 reactivation. As previously described, prevention of HHV6 reactivation might also prevent the development of GvHD and improve outcome after HSCT.

In addition to anti-herpetic drugs, specific cellular therapies may be of benefit. By infusing matched HHV6 specific T-cells to patients after HSCT, HHV6 reactivation could be prevented or treated. These cellular therapies with ex vivo proliferated HHV6 specific cytotoxic lymphocytes might be part of prophylactic or pre-emptive therapy against HHV6 reactivation as well, as also used for other herpesvirus reactivation treatments.[60]

1.5. Conclusion/future directions

HHV6 reactivation is common in immunosuppressed patients after HSCT, mainly in the myelo-ablative setting, and seems to be associated with severe clinical complications (e.g. GvHD and CNS dysfunction) and mortality. Due to the heterogeneous patient populations, variations in HHV6 monitoring strategies and the use of different sample types (e.g. whole blood samples, PBMC or plasma), in addition to lack of standardization of HHV6 DNA load calculation, it is difficult to compare available data. Further studies are needed to elucidate the causal relationship of HHV6 and outcome after HSCT and to identify the effect of HHV6 reactivation on immune reconstitution and/or GvHD after HSCT. A future HHV6-specific non-toxic therapy might provide opportunities to treat HHV6 disease in the critical period early after HSCT.

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