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Low-dose valgancyclovir as cytomegalovirus reactivation prophylaxis in allogeneic hematopoietic stem cell transplantation

Basse dosi di valgancyclovir nella profilassi della riattivazione da Citomegalovirus dopo trapianto allogenico di cellule staminali emopoietiche

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INTRODUCTION

Opportunistic viral infections are important complications in hematopoietic stem cell and solid organ transplant recipients, in subjects receiving chemotherapy for hematological malignancies as well as those undergoing long-lasting immunosuppressive therapy, such as immunomodulatory agents and monoclonal antibodies (MAb), for hematological and non hematological diseases [1-7].

Despite advances in antiviral therapy, viral infections still cause high morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). The most relevant viruses in the allogeneic HSCT setting include the herpes virus family, particularly cytomegalovirus (CMV), Epstein-Barr virus and human herpesvirus 6, as well as human adenoviruses and the polyoma virus BK (BKV) [8,9]. CMV is the most common viral infection after allogeneic HSCT due to its high prevalence in the normal population [10].

The high morbidity and mortality of CMV infections among allogeneic HSCT recipients are related not only to their direct effects such as hepatitis, gastrointestinal disease, pneumonia and retinitis, but also to their indirect effects, such as higher susceptibility to opportunistic infections, graft rejection, myelosuppression and nephrotoxicity.

Pre-transplant CMV donor/recipient (D/R) serostatus is the most important independent determinant of CMV reactivation after allogeneic HSCT [11]. Several studies have shown that CMV-seropositive patients (R) receiving both CMV negative and positive donor (D) graft (D-/R+ or D+/R+) are at high risk for CMV reactivation and have a substantial higher susceptibility to delayed CMV-specific immune reconstitution, repeated CMV reactivations, late CMV recurrence, and development of CMV disease [11, 12].

Other risk factors for CMV infection after allogeneic HSCT include T-cell depletion, reduced intensity conditioning (RIC) regimens, acute and chronic graft-versus-host disease (GVHD) as well as the use of matched unrelated, mismatched related and cord blood donors [13-15]. Without and with prophylaxis, the incidence of CMV reactivation after allogeneic HSCT has been reported in approximately 80% and 40% of CMV-seropositive patients, respectively [16, 17]. Since gancyclovir (GCV) introduction and

its rapid administration at the earliest signs of infections based on DNA polymerase chain reaction (PCR) and/or pp65 antigenemia detection assays, the incidence of CMV disease has been successfully reduced [18, 19]. Oral valganciclovir (VGCV), a prodrug of intravenous GCV, has been reported as useful alternative for the management of CMV preemptive therapy in solid organ transplant and HCST recipients [20].

Limited data are available on the efficacy and safety of oral VGCV in the prevention of CMV reactivation after allogeneic HSCT. We prospectively evaluated safety and efficacy of low-dose oral VGCV in the prevention of CMV reactivation in 32 consecutive patients who underwent allogeneic HSCT.

SUBJECTS AND METHODS

Patients

Thirty-two consecutive patients (15 females, 17 males) treated with VGCV as CMV prophylaxis entered in the study. Informed consent was obtained from all patients in accordance with institutional guidelines and the study design was made in accordance with the Helsinki II Declaration [21].

The age at HSCT recipients ranged between 18 and 59 years (mean±SD, 40±12 years) and their post-HSCT follow-up lasted from 3 to 56 months (mean±SD, 30±12 months). Primary diseases were acute myeloid leukemia (AML; n=19), acute lymphoblastic leukemia (ALL; n=4), non Hodgkin's lymphoma (NHL; n=3), multiple myeloma (MM; n=3) and myelodysplastic syndrome (MDS; n=3). Seventeen of twenty-three (74%) acute leukemia (AL) patients were transplanted in first complete remission (1th CR; AML, *n*=15; Philadelphia positive ALL, n=2). The remaining AL patients (AML, n=4; ALL, n=2) were transplanted in 2nd CR. Five patients (16%) suffered from AML secondary to long-lasting MDS (n=3) or Hodgkin disease (n=1) and breast cancer (n=1). At the time of allogeneic HSCT, one LNH and all MM patients had advanced disease after multiple previous treatments including autograft [22, 23]. Stem cell source was bone marrow (BM) (n=3) or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (PBSC) (n=29), which were infused without manipulation. Patients' characteristics are detailed in Table 1.

Table 1 - Patients and pretrasplant characteristics.

Characteristics	*N*
Median age	40±12 range 18-59
Female/Male	15/17
Disease	
AML	19
ALL	4
NHL	3
MM	3
MDS	3
Disease phase	
1st CR	17
2nd CR	4
Previous MDS or neoplasia	5
Advanced disease	6
Conditioning regimen	
Myeloablative	
- BUCY	15
RIC	17
- THIO-CY	2
- THIO- FLU-MEL	3
- BU-FLU	10
- TLI	2
GVHD prophylaxis	
without ATG	21
with ATG	11
Donor	
Sibling	30
Unrelated	2
Graft source	
PBSC	29
BM	3
CMV serostatus	
R-/D-	2
R+/D-	3
R+/D+	27

Abbreviations. AML = acute myeloid leukemia; ALL = acute lymphoblastic leukemia; NHL = non Hodgkin's lymphoma; MM = multiple myeloma; MDS = myelodysplastic syndrome; BUCY = busulphan + cyclophosphamide; RIC = reduced intensity conditioning; TLI = total lymphoid irradiation; BU-FLU = busuphan + fludarabine; THIO-CY = tiothepa + cyclophophamide; THIO-FLU-MEL = tiothepa + fludarabine + melphalan; GVHD = graft-versus-host disease; ATG = anti-thymocyte globulin; PBSC = peripheral blood stem cells; BM = bone marrow; CMV = cytomegalovirus; R = recipient; D = donor.

Conditioning regimen

Fifteen (46%) HSCT recipients (11 AML and 4 ALL) received the same myeloablative conditioning regimen BU-CY2 including intravenous (i.v.) busulphan (BU, Busilvex: total dose 12.8 mg/kg in 4 days) with cyclophosphamide (CY: total dose 120 mg/kg in 2 days) (Table 1). The remaining 17 patients (54%) received reduced intensity conditioning (RIC) regimens:

a) i.v. BU (cumulative dose 12.8 mg/kg in 4 days) plus Fludarabine (FLU: cumulative dose 120 mg/m^2 in 4 days) (BU-FLU) in 10 patients (8 AML and 2 MDS);
b) thiotepa (THIO: 15 mg/kg from day -6 to day -5) plus CY (50 mg/kg; total dose 100 mg/kg on days -3 and -2) (THIO-CY) in 2 patients (1 MDS, 1 NHL);
c) THIO (5 mg/kg on day -6) plus FLU (120 mg/m^2 from day -6 to day -2) and melphalan (MEL: 80 mg/m^2 on day -2 from day -6 to day -2) (THIO-FLU-MEL) in 3 MM patients;
d) total lymphoid irradiation (TLI: cumulative dose 8 Gy from day -11 through -1) with antithymocyte globulin (ATG: 1.5 mg/kg/day from day -11 through -7) (TLI-ATG) in 2 NHL patients [24-26].

GVHD and CMV prophylaxis

Acute GVHD prophylaxis was performed with cyclosporin A (CsA; (1 mg/kg intravenously from day -1 to +21, then 8 mg/kg orally for at least 6 months) and short-course methotrexate (MTX; 10 mg/kg in four doses on days+1, +3 +6 and +11) in AL, MDS and MM transplanted patients. In NHL, GVHD prophylaxis consisted of CsA and mycophenolate mofetil (MMF; 15 mg/kg twice a day from day +1 to +28) instead of MTX. Rabbit anti-ATG (Thymoglobuline, Genzyme, Boston, MA, USA) was administered as a part of the conditioning regimen at 10 mg/kg in three doses (days -3, -2 and -1) in 11 HSCT recipients (6 AL receiving BUCY2 conditioning, 2 hypoplastic MDS and 1 unrelated HSCT receiving BU-FLU, except 2 NHL receiving TLI-ATG as described above).

CMV serostatus of the HSCT recipients and donors was assessed prior to conditioning. CMV immunoglobulin G (IgG) was checked by enzyme linked fluorescent assay. CMV and other herpes virus prophylaxis consisted of acyclovir (ACV) at 5 mg/kg every 8 hours a day in all HSCT recipients (except for unrelated HSCT receiving foscarnet at 30 mg/kg twice a day), associated with i.v. immunoglobulin (IVIG) at 0.4 g/kg weekly, from day -7 to hematopoietic engraftment [25]. After engraftment, all the patients received oral VGCV at a dose of 450 mg daily for at least 6 months as CMV prophylaxis.

CMV monitoring was based on a real-time TaqMan CMV-DNA polymerase chain reaction (PCR) assay twice weekly for the first 100 days, then twice a month until month 6, and finally monthly until the end of the first year or longer if any complication occurred.

CMV surveillance was stopped in patients not receiving any immunosuppressive therapy for chronic GVHD.

CMV reactivation or infection was defined in case of CMV-DNA PCR positivity >1000 copies/ml. Prophylaxis was defined as a course of anti-CMV agents at the time of or soon after transplantation, and preemptive therapy as administration of anti-CMV agent upon detection of CMV-DNA PCR positivity. CMV disease was defined as end-organ disease such as pneumonia, gastrointestinal (gut) disease, hepatitis, etc., with a documented CMV etiology. Late-onset of CMV infection or disease was defined as occurring after 100 days from HSCT.

Statistical analysis

All the data for the analysis, expressed in the tables as mean ± SD, were collected from a computerized data base and chart review. Data of HSCT receiving VGCV prophylaxis (VGCV cohort) were retrospectively compared with a matched gender, disease phase, CMV serostatus cohort of 32 HSCT recipients treated with ACV and IVIG prophylaxis (ACV- IVIG cohort).

Variables were compared using a non-parametric paired Student's *t*-test for the inter-group differences. A P value of ≤ .05 was considered to be statistically significant.

RESULTS

Patients' characteristics

A retrospective observational study evaluating CMV reactivation rates was performed in 32 consecutive patients, with a median age of 40 years, which underwent mostly (94%) allogeneic matched related HSCT after both myeloablative (47%) or RIC regimens (53%) (Table 1). Fifty percent of HSCT recipients had high risk disease, defined as those with advanced age, relapsed/refractory disease, previous MDS and neoplasia (Table 1).

Graft source was mainly mobilized PBSC (90%) and all patients achieved hematological full engraftment in a mean time of 21 days (range 17-35 days). The median follow-up post-HSCT was 30±12 months (Table 1). GVHD prophylaxis included standard dose CsA and short-course MTX in 70% of cases, while the remaining (31%) received CsA, MTX plus ATG as a part of their conditioning regimens.

The pretransplantation CMV serostatus of the donor and/or recipient remains an important risk factor for post-HSCT outcome despite the use of antiviral prophylaxis and preemptive therapy [11, 12]. CMV serostatus of patients and donors was determined as part of the standard diagnostic routine prior to conditioning. CMV serologic status of donors (D) and recipients (R) was D+/R+ in 27 (84%) patients, D-/R+ in 3 (10%) and D-/R- in 2 (6%). Based upon CMV serostatus, 94% of HSCT recipients were classified as high risk (D-/R+ or D+/R+) for CMV reactivation and disease and only 6% of cases as low risk (D-/R-); none of the patients was in the intermediate risk group (D+/R-) (Table 1).

Incidence of CMV infection, CMV disease and GVHD

Starting from time of engraftment, low dose oral VGCV at a dose of 450 mg daily was given prophylactically for at least 6 months. CMV DNA PCR was performed in high risk seropositive recipients. CMV reactivations was categorized on the basis of the peak viral load exceeding 1000 copies/ml in two consecutive plasma samples, which was also the threshold for starting preemptive therapy.

CMV-DNA PCR enabled the identification of 4 early and 2 late CMV reactivations, which occurred only in high risk seropositive HSCT recipients after a median of 59±16 days post-transplant; in all these patients preemptive therapy with oral VGCV at a dose of 900 mg twice daily for at least 21 days determined prompt clearance viremia in a median time of 12 days (range 7-25 days), except for one case developing late fatal gut CMV disease resistant to GCV, foscarnet and cidofovir.

HSCT receiving VGCV (VGCV cohort) was retrospectively compared with a matched gender, disease phase, graft source, CMV serostatus cohort of 32 HSCT recipients treated after hematopoietic engraftment with oral ACV and high dose IVIG (15 mg/kg daily and 0.4 gr/kg wekly for at least 6 months, respectively) (ACV-IVIG cohort). In this cohort, twelve patients experienced CMV-DNA PCR positivity (37% *vs* 18% in ACV and VGCV cohort, respectively; p≤.05) after a mean of 53±13 days post-transplant. Preemptive therapy with GCV or foscarnet suppressed viremia in all reactivated cases except 6 developing CMV disease (18%: 5 gut, 1 pneumonitis); among them 1 died of pneumonitis (data not shown).

In addition to pretrasplant CMV serostatus, RIC transplants, ATG administration, unrelated donor and GVHD have been reported as predictors of CMV reactivation. CMV reactivation was observed in 2 myeloablative (13%) and 4 RIC (23%) transplants, five of them using PB-SC as stem cell source (Table 2). Only 3 of 11 HSCT recipients receiving ATG, as part of conditioning regimen, experienced CMV reactivation (27%).

According to the Glucksberg scoring system [27, 28], grade I-II and III-IV acute GVHD occurred in 13 (41%) and 2 (6%) of the patients, respectively. Based on clinical severity and target organ involvement, limited (n=6, 18%) and extensive (n=3, 10%) chronic GVHD was documented in 9 (28%) patients (Table 2). Grade I

Table 2 - Follow up of HSCT recipients prophylaxed with valgancyclovir.

Follow-up and outcome	*N*
CMV reactivation	6
CMV disease	1
aGVHD	
Grade I-II	13
Grade III-IV	2
cGVHD	
Limited	6
Extensive	3
Other infections	
Bacterial pneumonia	3
Fungal pneumonia	2
Cystitis	3
Outcome	
OS	21
Relapse-related mortality	6
TRM	3
Hematological toxicity	7

Abbreviations. CMV = cytomegalovirus; aGVHD = acute graft-versus-host disease; cGVHD = chronic graft versus-host disease; OS = overall survival; TRM = transplant-related mortality

acute and grade II-IV GVHD were treated by low- (1 mg/kg for 15 days) or high-dose methylprednisolone (2-5 mg/kg for 10 days), respectively, followed by slow-dose tapering as tolerated [29, 30].
Chronic GVHD was treated with prednisolone at doses of 1-2 mg/kg plus CsA at doses ranging from 1 to 8 mg/kg per day combined in 2 cases with MMF (15 mg/kg twice a day). All patients who experienced CMV reactivation in this VGCV cohort showed grade I-II acute (n=4) or limited chronic (n=2) GVHD (Table 2). At the time of CMV reactivation, 4 patients had been affected by grade II-IV acute GVHD and 2 had been affected by an extensive chronic GVHD.

Toxicity and outcome
None of the patients required discontinuation of the oral VGCV secondary to specific gastrointestinal intolerance.
Hematologic toxicity, such as mild anemia, neutropenia and thrombocytopenia was documented in seven cases (22%), but did not required drug discontinuation. The rate of non CMV-related infections was 25% and was similar in both groups with and without CMV reactivation (Table 2).
At the end of the follow-up, 18 of 32 (56%) patients were alive with a median follow up of 31 months (range 2-56). Relapsed-related mortality was 20%, transplant-related mortality (TRM) was 9% and did not differ between group with and without CMV reactivation.

DISCUSSION

CMV is a common viral infection associated with significant morbidity and mortality in hematologic malignancies and non receiving therapy leading to prolonged lymphopenia or T-cell dysfunction. CMV reactivation has been reported in about 10% of lymphoid and leukemia patients with the higher rates among those with lymphoid leukemia.
Despite the development of effective antiviral therapies and the advancement in antigen and DNA PCR monitoring for early diagnosis, CMV infection continues to be a major cause of morbidity and mortality especially in HSCT recipients [1, 8-10, 16-19, 31].
Among HSCT recipients, CMV may induce multiorgan disease with pneumonia still remaining the highest cause of mortality: 30-50% of pneumonia-related deaths in contrast to 85% in pre-CMV prophylaxis and preemptive era with GCV and foscarnet [1, 25, 19]. Pretransplant CMV seropositivity in HSCT recipients is associated with the highest risk of CMV reactivation; CMV reactivation has been reported in 70–80% and 30% of seropositive patients in the pre- and post-CMV prophylaxis and preemptive era, respectively, with approximately one-third of these patients developing symptomatic CMV disease [11, 12, 32].
Many studies using prophylaxis and preemptive therapy to prevent CMV disease have been performed in HSCT recipients. Historically, ACV has been reported to be effective as prophylaxis against CMV viremia and to decrease CMV-related mortality rate, without significantly affecting CMV disease incidence. Currently, intravenous GCV is still considered the drug of choice for preemptive CMV reactivation or CMV disease treatment.
Although foscarnet has shown efficacy similar to GCV and less hematologic toxicity as preemptive therapy, it is generally used as second-line therapy for CMV reactivation or disease [9, 19, 25]. However, both antiviral agents are given intravenously and often require hospitalization for specific complications related to their administration, further increasing the high health-care costs of HSCT patients.
VGCV, an oral prodrug of ganciclovir, with excellent bioavailability, at standard daily dose of 900 mg has been documented highly effective for prophylaxis of CMV reactivation in patients receiving alemtuzumab [33-37].
Recently, we have documented that standard dose of oral VGCV is also effective as CMV prophylaxis for patients suffering from aplastic anemia or single-lineage bone marrow failure disorders treated with an alemtuzumab-based immunosuppressive therapy [38-40].
Standard dose of VGCV has shown a similar efficacy to intravenous GCV for pre-emptive CMV treatment in solid organ and HSCT transplant recipients, including those with gut GVHD and using an unrelated graft source. More recently, VGCV at standard dose has been also proved to be effective for CMV prophylaxis in the setting of HLA-matched and cord blood HSCT.
However, VGCV at standard dose increases the risk of myelosuppression in solid organ and HSCT recipients [34-37].
Pharmacokinetic studies have shown that daily low dose of 450 mg VGCV provide plasma con-

centrations equivalent to oral 3 gr GCV [41, 42]. In addition, it has been reported similar efficacy of low and standard dose of VGCV in preemptive CMV treatment after solid organ transplantation [43]. Based on these evidences, we retrospectively assessed the efficacy of low dose oral VGCV as CMV reactivation prophylaxis in 32 consecutive HSCT recipients, fifty and ninety-four percent of them at high risk for CMV reactivation due to their high risk disease phase and their pretransplant CMV seropositivity, respectively.

In this cohort of high risk HSCT recipients, low-dose VGCV, given after hematopoietic engraftment, was effective to prevent CMV reactivation. Indeed, asymptomatic early and late CMV-DNA PCR reactivation occurred only in 17% of high risk seropositive HSCT recipients, in contrast to 37% and 18% of early and late CMV reactivation observed in matched cohort of HSCT recipients treated prophylactically with ACV-IVIG.

In addition, preemptive therapy with high dose oral VGCV (1800 mg daily) treated with low dose VGCV for CMV prophylaxis resulted in a rapid viremia clearance, except one developing recurrent CMV reactivation leading to a late fatal gut CMV. However, similar numbers of CMV related-death was observed in both group of patients.

In addition to pretransplant CMV seropositivity, the main risk factors for CMV reactivation and disease in HSCT recipients are ATG administration and GVHD [24, 29, 30]. In HSCT patients treated with low dose VGCV as CMV prophylaxis, less than 30% of HSCT patients receiving ATG-based conditioning regimens or developing acute and chronic GVHD experienced CMV reactivation.

A major concern for the use of prophylactic VGCV is the enhanced risk of myelosuppression and of serious bacterial and fungal infections over that seen with ACV prophylaxis alone [19, 25, 44]. In our study, few patients showed mild hematological toxicity related to VGCV treatment, but none of them required drug discontinuation.

The rate of proven [44-48] non CMV-related infections was 26% and was similar in both group with and without CMV reactivation. No other significant specific VGCV-related toxicity was encountered.

To our knowledge, no other studies have been reported on the use of low-dose VGCV as CMV reactivation prophylaxis in HSCT recipients. Despite various limitations, including a small number of patients, retrospective analysis and the lack of a control arm, this study provides evidence that low dose oral VGCV is safe and effective as CMV-prophylaxis in HSCT recipients. These results require further validation in prospective larger studies.

Keywords: allogeneic stem cell transplant, CMV reactivation, CMV prophylaxis.

Conflict of interest disclosure
The authors declare that the article has not been sponsored, that no financial support has been given and finally that there is no conflict of interest.

SUMMARY

The efficacy and safety of low dose oral valgancyclovir (VGCV) as cytomegalovirus (CMV) reactivation prophylaxis was retrospectively evaluated in 32 consecutive patients which underwent allogeneic HLA-matched related and unrelated hematopoietic stem cell transplantation (HSCT).

Thirty HSCT recipients showed pretransplant CMV seropositivity. Fifteen received a myeloablative conditioning regimen, while seventeen patients received a reduced-intensity conditioning regimen. Twenty-one patients received graft-versus-host disease (GVHD) prophylaxis with cyclosporin A (CsA) and methotrexate (MTX), and the others CsA with MTX and anti-thymocyte globulin. CMV infection was monitored weekly using polymerase chain reaction (PCR). VGCV was administered orally at a dose of 450 mg daily for six months. Six patients developed a positive CMV-PCR on average 56 days after HSCT successfully treated with VGCV at 1800 mg/day, except one who developed fatal gastrointestinal CMV disease. At the time of CMV reactivation, four patients had been affected by grade II-IV acute GVHD and two by an extensive chronic GVHD. No significant specific VGCV-related toxicity was encountered. Seven patients presented hematological toxicity which did not require drug discontinuation. Our data suggest that low dose VGCV is safe and effective as CMV reactivation prophylaxis in allogeneic HSCT recipients. These results require further validation in prospective randomized studies.

RIASSUNTO

L'efficacia e la sicurezza del vanganciclovir (VGCV) nella profilassi della riattivazione del citomegalovirus (CMV) è stata valutata in 32 pazienti dopo trapianto allogenico di cellule staminali emopoietiche (TCSE) da donatore consanguineo (n=30) e non consanguineo (n=2). I soggetti trapiantati nel 94% dei casi presentavano sieropositività per il CMV. Quindici pazienti hanno ricevuto condizionamento mieloablativo e 17 non mieloablativo. Ventuno pazienti hanno ricevuto profilassi per la graft-versus-host disease (GVHD) con ciclosporina-A (CsA) e metotrexate (MTX), undici con CsA+MTX e globulina anti-timocitaria. La riattivazione del CMV è stata monitorata settimanalmente mediante "polymerase chain reaction" (PCR). Il VGCV è stato somministrato oralmente alla dose di 450 mg/die per sei mesi. Sei pazienti hanno presentato CMV-PCR positività trattata con successo con VGCV alla dose di 1800 mg/die. Un paziente ha sviluppato una tardiva malattia da CMV.

Al tempo della riattivazione del CMV, 4 pazienti erano affetti da GVHD acuta di grado II-IV e 2 da una forma estensiva di GVHD cronica. Sette pazienti hanno presentato tossicità ematologica non richiedente la sospensione del farmaco. Questi dati suggeriscono che il VGCV a basse dosi è efficace nella prevenzione della riattivazione del CMV in pazienti sottoposti a TCSE. Tali risultati richiedono ulteriori conferme in studi prospettici randomizzati.

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