High Rate of Cytomegalovirus Drug Resistance Among Patients Receiving Preemptive Antiviral Treatment After Haploidentical Stem Cell Transplantation

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We examined the rate, clinical impact, and risk factors of cytomegalovirus (CMV) drug resistance in 561 patients who underwent 616 hematopoietic stem cell transplantations (HSCTs) over 5 years. Drug resistance was exclusively identified in haploidentical (haplo)-HSCT recipients receiving preemptive therapy, among whom the rate was 14.5%. Resistance appeared after prolonged treatment (median, 70 days), was associated with higher preceding viral load (P < .001), and was the strongest predictor for disease by multivariate analysis. The high rate of drug resistance as interlinked with severe disease in haplo-HSCT recipients suggests the potential advantage of prophylactic over preemptive treatment in high-risk patients and highlights the need for better-tolerable anti-CMV drugs.

Keywords. cytomegalovirus; antiviral drug resistance; hematopoietic stem cell transplantation.

Despite diagnostic and therapeutic advances, cytomegalovirus (CMV) infection has remained a significant complication after hematopoietic stem cell transplantation (HSCT) [1, 2]. In recent years, the natural history of CMV infection in the transplantation setting has been drastically changed by the routine implementation of preventive antiviral therapy, involving either universal prophylaxis or preemptive treatment [1, 2]. The latter approach, potentially limiting treatment-related toxicity, is widely used in the majority of HSCT centers [1–3].

The widespread use of preemptive therapy has reduced the occurrence of early CMV disease, but the development of late disease and drug resistance is increasingly recognized [1, 2, 4]. Ganciclovir resistance results mainly from mutations in the CMV UL97 kinase and, less frequently, from mutations in the viral DNA polymerase (UL54 gene) [4]. Foscarnet and cidofovir resistance mutations are found in the UL54 gene [4]. UL54 mutations have been associated with higher-level and multidrug resistance [5].

Thus far, reports of drug resistance following HSCT have remained anecdotal, and the current general notion is that drug resistance is rare in this setting [4, 6–9]. Yet, in recent years, the scope of HSCT has expanded to include more patients receiving transplants from mismatched, haploidentical, and unrelated donors—conditions associated with protracted immune suppression and high infection rates [10]. These trends, along with advances in effective T-cell depletion and reduced-intensity conditioning, have led to improved survival among high-risk patients requiring prolonged antiviral treatment.

The changing circumstances, along with the limited existing data in HSCT recipients, have prompted us to examine the rate and risk factors of antiviral drug resistance in HSCT recipients receiving preemptive antiviral treatment, with particular attention to the growing population of high-risk patients. We have further evaluated the clinical impact of resistance in this setting and its relation to CMV disease.

PATIENTS AND METHODS

Study Design and Patient Population
This 5-year prospective study included all patients receiving HSCT at Hadassah University Hospital from April 2003 through March 2008, a period after the introduction of CMV load surveillance. The cohort included 561 patients receiving 616 HSCTs (538 were receiving their first, 73 were receiving their second, and 5 were receiving their third HSCT), including 102 HSCTs from haploidentical (haplo) donors.

The conditioning protocol for haplo-HSCT included fludarabine, thiopeta, anti-thymocytic globulin, and total body irradiation. Standard prophylactic treatments for Pneumocystis (carinii) jirovecii and Toxoplasma infection were used, along
with acyclovir (500 mg/m² 3 times daily, from day 9 before to day 100 or longer after transplantation) prophylaxis against herpesvirus and varicella-zoster virus. A matched donor was defined by matching of HLA-A, -B, -C, and -DRB1. A mismatched donor was defined as an individual with allele mismatches or antigenic mismatch on either HLA loci. Haplo-HSCT was defined as use of a transplant from a related donor with at least 2 HLA antigen disparities at loci A, B, or DRB1 in the donor-recipient pair.

The demographic, clinical, and laboratory data were prospectively collected by our data management team, verified by review of medical files, and analyzed retrospectively. The study was approved by the institutional review board, and was performed according to the Declaration of Helsinki, good clinical practice guidelines, and the human-experimentation guidelines of the Israeli Ministry of Health. Studied variables included donor and recipient age, underlying disease, donor source and degree of donor-recipient matching, HSCT conditioning regimen, CMV donor and recipient serostatus, engraftment, acute and chronic graft-versus-host disease (GVHD), treatment parameters, CMV infection and disease, CMV drug resistance, coinfections, clinical course, outcome, and mortality.

CMV Surveillance and Preemptive Therapy
Patients were monitored weekly for the presence of CMV infection by testing for viral load in peripheral blood until day 100, after which surveillance was continued weekly or biweekly, depending on the medical condition. Preemptive treatment with ganciclovir (5 mg/kg twice daily) was given for at least 2 weeks or until no viral DNA was detected to patients with a viral load of ≥1000 DNA copies/mL or patients with >0.5 log viral load increase between 2 consecutive samples. An induction dose was followed by maintenance dose (5 mg/kg daily) for at least 2 weeks. Exceptions to the uniform criteria included patients receiving anti-T-cell therapies, for whom preemptive treatment was initiated at any viral load, and autologous HSCT recipients, for whom preemptive treatment was not routinely used (unless rapidly increasing viral load was observed). Ganciclovir was substituted with foscarnet in patients with neutropenia.

CMV Infection and Disease
CMV load was determined as described elsewhere [11]. CMV infection was defined by the detection of viral DNAemia (≥50 copies/mL). The presence of CMV disease was determined on the basis of standardized diagnostic criteria [12].

Assessment of Drug Resistance
Patients with persistent or recurrent DNAemia (≥1000 copies/mL), increasing viral load, or CMV disease after >2 weeks of antiviral treatment (“clinically suspected resistance”) underwent genotypic analysis of drug-resistance mutations, performed as described elsewhere [11].

Statistical Analysis
Statistical analysis was performed using SPSS, version 18.0. Variables potentially associated with the development of CMV infection, drug resistance, and disease were first analyzed using univariate analyses, where the association between 2 categorical variables was tested using the χ² test or the Fisher exact test. To compare quantitative (continuous) variables between 2 independent groups, the t test was used, whereas comparison between ≥3 groups was performed using analysis of variance. Risk factors identified by univariate analyses were included in a multivariate logistic regression model and the Cox regression model. The identification of an intersection point with high specificity and sensitivity to differentiate patients with infection and patients without infection on the basis of viral load was performed using receiver operating characteristic analysis. All tests applied were 2 tailed, and a P value of ≤ .05 was considered statistically significant.

RESULTS

CMV Infection Rate
The overall infection rate after HSCT was 54.1% (333/616). Infection rates were significantly higher in allogeneic HSCT recipients than in autologous HSCT recipients (271/410 vs 62/206) and did not differ between the subcategories of allogeneic HSCT (Supplementary Figure 1).

Rate and Distribution of Drug Resistance
Clinically suspected resistance was observed in 20 patients (3.2%). Of those, drug-resistance mutations (hereafter, “drug resistance”) were identified in 10 (50%). Overall, drug resistance was detected in 1.6% of the cohort and in 3.0% of patients with CMV infection. Importantly, clinically suspected resistance and drug-resistance mutations were exclusively identified in haplo-HSCT recipients, with the latter occurring in 9.8% of the total patient population and 14.5% of the 69 patients with CMV infection.

Variables Associated With CMV Infection in Haplo-HSCT Recipients
In multivariate analysis, high-dose steroid treatment (adjusted odds ratio [OR], 6.21; 95% confidence interval [CI], 2.00–19.26; P = .002) and recipient seropositivity (adjusted OR, 4.86; 95% CI, 1.02–19.63; P = .027) were associated with infection. In stepwise Cox regression, only acute GVHD was associated with infection (hazard ratio, 1.67; 95% CI, 1.02–2.73; P = .043).

Variables Associated With Drug Resistance Among Haplo-HSCT Recipients
A higher median peak viral load in the period before the emergence of resistance was associated with the development of resistance (P < .001). Advanced donor age closely approached statistical significance (P = .058). While 26/102 haplo-HSCT
patients had received a previous transplant, these patients were not at greater risk for developing resistance (1/26 vs 9/76). Additional details are available in Supplementary Table 1.

**Characteristics of Haplo-HSCT Recipients With Drug Resistance**

Nine patients had resistance mutations in the UL97 gene (Table 1). One patient who received prolonged foscarnet treatment (patient 3) had a multidrug-resistance mutation in the UL54 gene [11]. Resistance mutations appeared after a median cumulative treatment duration of 70 days (range, 39–330 days).

Six patients developed CMV disease. Nine died within a median duration of approximately 1 month (range, 3–203 days) from the detection of resistance.

**Rate and Distribution of CMV Disease Among HSCT Recipients**

CMV disease was diagnosed in 9 patients (1.5% of the total). Importantly, 8 received haplo-HSCT. Thus, CMV disease developed in 11.6% of haplo-HSCT recipients with infection (8/69), compared with a very low rate of disease among all other HSCT recipients with infection (1/264; \( P < .0001 \); Supplementary Figure 1).

**Characteristics of Haplo-HSCT Recipients With CMV Disease**

Disease developed at a median time of 128 days after HSCT (range, 59–379 days), with most cases (6/8 [75.0%]) occurring at late time (>100 days; Table 1). Disease developed in all cases during antiviral treatment (median cumulative duration, 94 days; range, 43–334 days). Manifestations were mostly severe and included pneumonia (4 cases), encephalitis (1), disseminated disease (1), colitis (1), and retinitis (1), and the associated mortality was 87.5%.

**Variables Associated With the Development of CMV Disease in Haplo-HSCT Recipients**

Advanced donor age, higher peak viral load, and the presence of drug resistance were associated with CMV disease by univariate analysis (Table 2). On multivariate analysis, drug resistance was the only independent predictor for disease (adjusted OR, 171.8; 95% CI, 3.25–9080; \( P = .011 \)).

**DISCUSSION**

We have prospectively determined the rate and distribution of CMV drug resistance and disease, as well as their associated risk factors, in a large and unselected cohort of HSCT recipients receiving preemptive antiviral therapy.

Drug resistance was detected in 1.6% of the total cohort of patients undergoing 616 HSCTs over a 5-year-period and in 3.0% of those who developed CMV infection and received preemptive treatment. This overall low rate is in accordance with the 0%–4% incidence range reported in previous, smaller studies [4, 6–9]. Notably, we have exclusively identified the
emergence of drug resistance in the subset of HSCT recipients undergoing haplo-HSCT. Haplo-HSCT has been increasingly performed in recent years, representing an alternative treatment option for high-risk patients lacking an HLA-identical donor [10]. Because considerable experience in this field has been gained at our center, with >100 haplo-HSCTs performed over the study period, an important finding of our study was the high rate of resistance (14.5%) among haplo-HSCT recipients treated for CMV infection. Of note, the increased rate of drug resistance was observed despite a similar rate of CMV infection, compared with the rate in patients undergoing other allogeneic HSCTs. Moreover, the major predictors for CMV infection in haplo-HSCT recipients, including recipient CMV seropositivity, acute GVHD, and high-dose steroid treatment, were not unique to this patient subset [2, 13]. Thus, susceptibility of haplo-HSCT recipients to CMV infection per se could not account for the high incidence of drug resistance. Rather, the delayed immune reconstitution, combined with impaired cross-talk between the disparate donor T cells and recipient antigen-presenting cells (characteristic of haplo-HSCT), could allow for continued virus replication, facilitating the emergence of resistant strains under drug pressure. Indeed, we have identified a significant association between drug resistance and higher preceding viral load, reflecting uncontrolled viral

Table 2. Univariate Analysis of Variables Associated With Development of Cytomegalovirus (CMV) Disease in Haploidentical Hematopoietic Stem Cell Transplant (HSCT) Recipients

<table>
<thead>
<tr>
<th>Variable</th>
<th>CMV Disease (n = 8)</th>
<th>No CMV Disease (n = 61)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age, y</td>
<td>19.19 (6.6–57.5)</td>
<td>17.66 (0.2–64.0)</td>
<td>.822</td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
<td></td>
<td>.382</td>
</tr>
<tr>
<td>Congenital immunodeficiency</td>
<td>1 (12.5)</td>
<td>3 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Severe aplastic anemia</td>
<td>0 (0.0)</td>
<td>2 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Other congenital disease</td>
<td>1 (12.5)</td>
<td>2 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td>6 (75.0)</td>
<td>48 (78.7)</td>
<td></td>
</tr>
<tr>
<td>Solid tumor</td>
<td>0 (0.0)</td>
<td>6 (9.8)</td>
<td></td>
</tr>
<tr>
<td>HSCT conditioning protocol</td>
<td></td>
<td></td>
<td>.596</td>
</tr>
<tr>
<td>Myeloablative</td>
<td>7 (87.5)</td>
<td>55 (90.2)</td>
<td></td>
</tr>
<tr>
<td>Nonmyeloablative</td>
<td>1 (12.5)</td>
<td>4 (6.6)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0 (0.0)</td>
<td>2 (3.3)</td>
<td></td>
</tr>
<tr>
<td>CMV serostatus</td>
<td></td>
<td></td>
<td>.613</td>
</tr>
<tr>
<td>R−/D+</td>
<td>1/6 (16.7)</td>
<td>4/58 (6.9)</td>
<td></td>
</tr>
<tr>
<td>R−/D−</td>
<td>0/6 (0.0)</td>
<td>0/58 (0.0)</td>
<td></td>
</tr>
<tr>
<td>R+/D+</td>
<td>5/6 (83.3)</td>
<td>50/58 (86.2)</td>
<td></td>
</tr>
<tr>
<td>R+/D−</td>
<td>0/6 (0.0)</td>
<td>4/58 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Engraftment</td>
<td>8 (100.0)</td>
<td>51 (83.6)</td>
<td>.592</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>4 (50.0)</td>
<td>34 (55.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>1 (12.5)</td>
<td>16 (26.2)</td>
<td>.669</td>
</tr>
<tr>
<td>High-dose steroid treatmentb</td>
<td>4 (50.0)</td>
<td>32 (52.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Coinfection</td>
<td>8 (100.0)</td>
<td>58 (95.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Bacterial</td>
<td>7 (87.5)</td>
<td>55 (90.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Viral</td>
<td>3 (37.5)</td>
<td>22 (36.1)</td>
<td>1.00</td>
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<tr>
<td>Fungal</td>
<td>7 (87.5)</td>
<td>35 (57.4)</td>
<td>.136</td>
</tr>
<tr>
<td>CMV DNAemia before HSCT</td>
<td>1 (12.5)</td>
<td>6 (10.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Donor age, y</td>
<td>46.5 (39–59)</td>
<td>37 (14–66)</td>
<td>.009</td>
</tr>
<tr>
<td>Peak viral load, copies/mL</td>
<td>309 725 (3100–11 636 250)</td>
<td>2795 (30–1 800 000)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Antiviral drug resistancec</td>
<td>6 (75.0)</td>
<td>4 (6.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Time to viral load of at least 1000 copies/mL, d</td>
<td>29 (2–76)</td>
<td>39 (0–135)</td>
<td>.113</td>
</tr>
</tbody>
</table>

Data are no. (%) of HSCT recipients, proportion (%) of HSCT recipients, or median value (range).

Abbreviations: D, donor; GVHD, graft-versus-host disease; R, recipient; −, negative; +, positive.

* Values ≤.05 were considered statistically significant.

b ≥2 mg prednisone/kg per day.

c Statistically significant in multivariate analysis.

d Counted from the first HSCT.
replication during treatment. Interestingly, the second variable that was closely associated with resistance was advanced donor age, known by itself to be related to delayed immunologic recovery [14].

In accordance with the temporal pattern of resistance in solid-organ transplant recipients [2, 4], drug resistance in haplo-HSCT recipients appeared after prolonged antiviral treatment (median duration, 70 days). Yet, in contrast to the minimal-to-moderate clinical significance of drug resistance in most affected solid-organ transplant recipients [4, 15], the clinical impact of resistance in haplo-HSCT recipients was profound, and the outcome was grave (Table 1). These findings highlight the disparate clinical consequences of resistance in different settings.

Overall, CMV disease occurred in 1.5% of our cohort, reflecting the general efficacy of preemptive treatment. Importantly, however, haplo-HSCT recipients remained highly vulnerable to the development of disease, which still occurred in 11.6% of patients with CMV infection, despite preemptive treatment. This finding was in line with the high rate of resistance in these patients. In fact, drug resistance was the best predictor for CMV disease in haplo-HSCT patients on multivariate analysis.

To our knowledge, this is the largest cohort of HSCT recipients studied for the development of CMV drug resistance. The large cohort size and the unselected patient population allowed us to gain an unbiased insight into the occurrence and distribution of resistance and disease during preemptive therapy. Yet, our findings should be interpreted with caution because of the single-center nature and heterogeneity of our cohort, some exceptions in the use of preemptive therapy, and the small sample size of patients with resistance and disease.

In summary, despite the overall low rate of drug resistance and disease among HSCT recipients receiving preemptive therapy, our findings reveal a high rate of drug resistance as interlinked with severe CMV disease in haplo-HSCT recipients. These remaining complications argue for the potential advantage of prophylactic rather than preemptive treatment in high-risk HSCT recipients and underscore the need for better-tolerable anti-CMV drugs with different mechanisms of action.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**References**