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Infectious Disease

Cytomegalovirus Infection Incidence and Risk Factors Across Diverse Hematopoietic Cell Transplantation Platforms Using a Standardized Monitoring and Treatment Approach: A Comprehensive Evaluation from a Single Institution

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A B S T R A C T

Human cytomegalovirus (CMV) infection and disease remains a significant cause of morbidity and mortality for hematopoietic cell transplantation (HCT) recipients. Disruption of or weak reconstitution of virus-specific cellular immune function, such as with certain HCT approaches, poses significant risk for CMV-related complications. The incidence of and risk factors for CMV infection and the nature of CMV disease were evaluated retrospectively among 356 consecutive HCT recipients transplanted at the National Institutes of Health using all graft sources, including bone marrow, peripheral blood stem cell (PBSC), and umbilical cord blood (UCB), and a range of in vivo and ex vivo approaches for graft-versus-host disease (GVHD) prophylaxis. The cumulative incidence of CMV infection was higher for CMV-seropositive recipients at 33%, regardless of donor CMV serostatus. Patients transplanted with CMV-seropositive donors had a significantly shorter duration of antiviral therapy. Among graft sources UCB was associated with the highest cumulative incidence of CMV infection at 65% and significantly longer treatment duration at a median of 36 days, whereas PBSC HCT was associated with the lowest incidence at 26% and the shortest CMV treatment duration at a median of 21 days. There were significant differences in the cumulative incidence of CMV infection by T cell manipulation strategy when systemic steroids were included as a risk-modifying event. Over one-third of CMV infections occurred in the setting of systemic steroid administration. CMV disease occurred in 5% of HCT recipients, with 70% of cases in the setting of treatment for GVHD. Although factors related to serostatus, graft source, and GVHD prophylaxis were associated with varied CMV infection incidence, unplanned post-HCT corticosteroid therapy contributed greatly to the incidence of both CMV infection and disease across HCT approaches, highlighting this post-HCT intervention as a key time to potentially tailor the approach to monitoring, preemptive therapy, and even prophylaxis.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative therapy for a variety of benign and malignant hematologic and immunologic conditions. Despite diagnostic and therapeutic advances in the management of post-HCT opportunistic infections, human cytomegalovirus (CMV) remains associated with significant

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morbidity, organ toxicity, and transplant-related mortality [1–3]. Uncontrolled CMV infection can lead to pneumonitis or enterocolitis and, more rarely, hepatitis, retinitis, and encephalitis. CMV infection increases the risk of secondary bacterial and fungal infections [4,5], whereas antiviral therapies incur morbidity from myelotoxicity (ganciclovir) or nephrotoxicity (foscarnet). In addition, CMV infection increases the risks of graft failure and graft-versus-host disease (GVHD), whereas treating CMV infection in the presence of poor graft function or in conjunction with immunosuppressive agents (especially steroids) for GVHD increases the risk from infections [4,6,7]. Finally, the treatment of CMV-associated complications post-HCT is associated with longer hospital length of stay, and CMV therapy carries a substantial financial burden [8,9].

Although letermovir is now approved for CMV prophylaxis in all CMV-seropositive recipients in the first 3 months post-HCT, the benefit is seen mainly in recipients at high risk for CMV disease. Administering letermovir to all CMV-seropositive recipients is expensive and increases pill burden and the chance of drug interactions. Therefore, determining which HCT recipients are most appropriate for letermovir is an important question [10]. Furthermore, identifying recipients who need monitoring for CMV infection beyond day +100 post-HCT and require extended prophylaxis requires a better understanding of pre- and post-HCT factors that affect the risk from CMV.

Post-HCT CMV infection risk increases at times when cellular immunity is hindered or compromised, such as with GVHD or post-HCT relapse and their treatments, or in the setting of graft failure or poor immune reconstitution [11]. This risk period varies with the HCT regimen and with the pace of immune reconstitution and post-HCT events such as GVHD and its treatment. Thus, although grafts depleted of donor T cells may carry an increased risk of viral infection, they incur less risk of GVHD and treatment with systemic steroids, which particularly impair immune reconstitution.

At the National Institutes of Health (NIH) the HCT platforms used within the Clinical Center vary widely, including umbilical cord blood (UCB) HCT, ex vivo T cell depletion (TCD), in vivo TCD with proximal serotherapy, calcineurin inhibitor (CNI) or mammalian target of rapamycin inhibitor (mTORi)-based pharmacologic GVHD prophylaxis, and in vivo immunomodulation with high-dose post-transplantation cyclophosphamide (PTCy). Despite this diversity, all NIH Clinical Center HCT recipients are uniformly monitored for CMV in the blood using quantitative PCR (qPCR) performed in a central clinical laboratory, and patients with CMV infection or disease are treated under NIH-wide HCT guidelines. This unique combination of diverse HCT approaches within a single center that applies a uniform approach to CMV monitoring and treatment offers an opportunity to comprehensively evaluate the cumulative incidence (CInc) of and risk factors for post-HCT CMV infection and disease across different HCT regimens and across all graft sources. Additionally, this framework allowed the evaluation of the impact of systemic steroids after HCT on the CInc of CMV infection, accounting for differences in the incidence of this risk-modifying event within individual HCT platforms.

METHODS

Clinical Record Review

After Institutional Review Board approval we retrospectively evaluated the incidence and risk factors for CMV infection among patients undergoing allogeneic HCT at the NIH between August 10, 2011 and February 11, 2017. All patients transplanted at the NIH were enrolled on prospective clinical trials, and enrollment lists from all HCT trials actively accruing during the study interval were used to generate a list of all HCT recipients. The Biomedical Translational Research Information System and the Clinical Research Information System were queried for HCT and CMV data. Of 390 consecutive HCT recipients transplanted during the study period, recipients were included if the following criteria were met: first allogeneic HCT, clinical follow-up of survivors through 1 year post-HCT, and at least 64% of weekly CMV qPCRs, equating to at least 9 of 14 weekly specimens for survivors through day +100 or fewer specimens but meeting at least the 64% threshold for those with death before day +100. Recipients on single-patient HCT protocols, an HCT protocol that included prophylactic post-HCT virus-specific cytotoxic T cell therapy, and those who died before receipt of donor cells on day 0 were excluded.

All HCT recipients at the NIH remained under direct local NIH care for at least 100 days post-HCT. The approaches to monitoring, preemptive therapy, and treatment of CMV infection and disease are uniform across HCT protocols, as formalized by NIH Bone Marrow Transplant Consortium Infectious Disease guidelines. All recipients were monitored at least weekly with blood CMV qPCR through day +100 post-HCT, with twice weekly monitoring for UCB HCT recipients, ex vivo TCD HCT, and haploidentical (haplo) recipients with hemoglobinopathy/thalassemia. Standard blood components for HCT recipients were leukoreduced to reduce the risk of CMV transmission, but CMV-seronegative recipients with CMV-seronegative HCT donors were not exclusively given blood components from CMV-seronegative donors.

Recipients were considered to be at risk for CMV infection if either donor or recipient were CMV-seropositive. For recipients of dual HLA- haplo $CD34^+$-selected peripheral blood stem cell (PBSC) and UCB (haplo-UCB) grafts, recipients were considered at risk for CMV infection only if the recipient was CMV-seropositive. UCB HCTs were classified to include all HCTs using UCB as a graft source and included haplo-UCB because the engrafting component of the haplo-UCB almost always comes from the UCB component.

T cell manipulation strategies to prevent GVHD were grouped into 4 strategies: PTCy, proximal serotherapy (alemtuzumab starting day −12 or more proximal to day 0 or antithymocyte globulin days −5 to −2), ex vivo TCD, and CNI/mTORi-based. HCTs using both PTCy and proximal serotherapy were designated as serotherapy transplants, because serotherapy so proximal to the graft infusion was considered to have the dominant effect on graft immunomodulation. Within the serotherapy group the approach to HCT conditioning was distinct for recipients with hemoglobinopathy and thalassemia (minimal-intensity conditioning with serotherapy and radiation) as compared with recipients with primary immunodeficiency disease (PID) or malignancy who received a mostly myeloablative chemotherapy–based conditioning. Thus, the serotherapy group was further subdivided by recipient diagnosis (hemoglobinopathy/thalassemia versus other) for some exploratory analyses.

Definitions of Endpoints

CMV infection (synonymous with CMV reactivation) was defined as any of the following: 2 qPCR values between 3.08 and 4.11 $\log_{10}$ IU/mL separated by 1 week, 1 qPCR value > 4.11 $\log_{10}$ IU/mL, or sufficient clinical suspicion for CMV disease to prompt therapy, regardless of CMV qPCR level. Before October 10, 2014 CMV qPCR was reported as $\log_{10}$ copies/mL (minimum level of detection < 2.40 $\log_{10}$ copies/mL, equivalent to <250 copies/mL); as of October 10, 2014 CMV qPCR was reported as $\log_{10}$ IU/mL (same minimum level of detection, reported as <3.08 $\log_{10}$ IU/mL). For results before this change values were converted to $\log_{10}$ IU/mL by adding 1.1 to the $\log_{10}$ copies/mL value.

The CMV qPCR is a laboratory developed assay designed and validated by the Molecular Lab of the NIH Department of Laboratory Medicine. Recurrent infection was defined according to a published definition as a subsequent episode of CMV infection meeting the above criteria, after prior post-HCT CMV infection followed by at least a 4-week period of no virus detection on weekly monitoring [6]. Recipients were designated as having CMV disease if the treating HCT team at the time documented such a diagnosis in the chart or based on autopsy findings. CMV infection data were captured through day +100 post-HCT, and disease data were captured through 1 year post-HCT, because it was presumed that the occurrence of CMV disease in the first year post-HCT would be known to the HCT team, even if diagnosed and treated at another hospital. Treatment of CMV was defined as the administration of ganciclovir, valganciclovir, foscarnet, or cidofovir for CMV infection or disease.

Competing risks (CRs) for CMV infection included death from any cause and the risk-modifying events of graft failure, malignancy relapse requiring systemic therapy, second HCT, donor lymphocyte infusion, and systemic steroid use. Systemic steroid use was chosen as a CR given that it is an objective event that would be expected to modulate CMV infection risk and was an event that captured all patients with GVHD requiring first-line therapy, as well as the use of steroids for other post-HCT complications. Graft failure was defined as the absence of hematopoietic recovery post-HCT or, in the setting of autologous recovery or secondary graft failure, myeloid chimerism < 5% donor cells. Malignancy relapse was defined as post-HCT recurrence or progression of a hematologic malignancy requiring systemic therapy. Systemic steroid use was defined as the post-HCT administration of ≥.5 mg/kg/day of prednisone or prednisone equivalent, excluding hydrocortisone, based on patient weight on starting steroid therapy. Data were locked for analysis on March 1, 2018.

Statistical Analyses

Descriptive statistics were used for HCT characteristics and the timing and duration of CMV therapy. Comparisons of HCT characteristics across subgroups were performed using the chi-squared test for categorical variables and the Kruskal-Wallis test for continuous variables.

One hundred–day post-HCT CMV-free survival was defined as survival of recipients who did not have CMV infection or death occur within 100 days post-HCT, with CMV-infection-free survivors censored at 100 days post-HCT. CMV infection-free, steroid-free, graft failure–free survival was defined as survival of recipients who did not have CMV infection, graft failure, or systemic steroid use within 100 days post-HCT, with event-free survivors censored at 100 days post-HCT. The corresponding survival curves were constructed using the Kaplan-Meier method and compared using the log-rank *P* value.

CInc curves of CMV infection were constructed using the method of Fine and Gray and compared using K-sample tests [12,13]. Death, malignancy relapse requiring systemic therapy, graft failure, second HCT, and donor lymphocyte infusion were considered CRs in every analysis. A second set of CInc curves were constructed including systemic steroid use as an additional CR to account for the risk modification of increased systemic immunosuppression, most often in the setting of acute GVHD.

Survival curves were generated using GraphPad Prism, version 7.01 (GraphPad Software, La Jolla, CA; www.graphpad.com). CInc curves were generated using R program, version 3.3.3 (R Core Development Team, Vienna, Austria).

Multiple logistic regression analyses to evaluate risk factors for CMV infection were performed. Univariate associations between a set of parameters and CMV infection were initially determined before performing multiple logistic regression analyses. After the initial univariate screening analyses, multiple logistic regression analyses were used to determine the association between HCT characteristics and CMV infection. These analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

For all the above non-CInc analyses, results were considered statistically significant if 2-tailed $P < .05$. For the CInc analyses, because of the exploratory nature of some of the analyses and the varying degrees of independence of the tests performed, no formal adjustment for multiple tests was performed and *P* values are presented without any correction. However, in view of the number of tests performed, to provide proper interpretation *P* values are interpreted such that $P < .01$ would be considered statistically significant but *P* between .01 and .05 would be considered to be associated with a trend toward statistical significance.

RESULTS

Recipient, Donor, and HCT Characteristics

Of 390 HCT recipients transplanted during the study period, 356 (91%) met inclusion criteria. Reasons for exclusion included <64% of CMV qPCRs despite survival through 100-days post-HCT (n = 14), second HCT (n = 8), survivor with less than 1 year follow-up (n = 8), death before day 0 (n = 2), single-patient protocol (n = 1), and receipt of prophylactic virus-specific cytotoxic T cells (n = 1). Of the 356, 263 (74%) were at risk for post-HCT CMV infection, with no significant difference among T cell manipulation strategies.

The recipient, donor, and HCT characteristics for the 356 HCTs are shown in Table 1, grouped by T cell manipulation strategy. There were significant differences among groups with respect to these characteristics, as expected given that different T cell manipulation strategies at the NIH were used in the setting of clinical trials, which were largely disease- and HCT approach-specific.

Survival and Freedom from CMV Infection

Survival point estimates are shown in Table 2. There was no difference in 1-year overall survival by donor/recipient (D/R)

Table 1
Recipient, Donor, and Transplant Characteristics

	Patients (n = 356)	CNI/mTORi- based (n = 145)	Serotherapy (n = 82)	PTCy-based (n = 58)	TCD (n = 46)	UCB (n = 25)	*P*
Male	215 (60)	82 (56)	61 (74)	28 (49)	29 (63)	15 (60)	.02
Age at HCT, yr, median (range)	31 (3-71)	38.5 (8-71)	28 (4-68)	22 (6-66)	30 (6-70)	20 (3-52)	<.0001
Diagnosis							<.0001
Malignancy	178 (50)	112 (77)	9 (11)	10 (17)	44 (96)	2 (8)	
Primary immunodeficiency	97 (27)	20 (14)	29 (35)	48 (83)	0	1 (4)	
Hemoglobinopathy/thalassemia	44 (12)	0	44 (54)	0	0	0	
Aplastic anemia	37 (10)	13 (9)	0	0	2 (4)	22 (88)	
Intensity of conditioning							<.0001
NMA/RIC	196 (55)	92 (63)	53 (65)	15 (26)	12 (26)	24 (96)	
Myeloablative	160 (45)	53 (37)	29 (35)	43 (74)	34 (74)	1 (4)	
Graft source							<.001
PBSC	264 (74)	118 (81)	80 (98)	20 (35)	46 (100)	0	
Bone marrow	67 (19)	27 (19)	2 (2)	38 (65)	0	0	
UCB or $CD34^+$ selected haplo+UCB	25 (7)	0	0	0	0	25 (100)	
Donor type							<.0001
HLA-matched related	183 (51)	107 (74)	33 (40)	6 (10)	38 (83)	0	
HLA-matched unrelated	84 (24)	33 (23)	32 (39)	12 (21)	7 (15)	0	
HLA-haplo	56 (16)	0	15 (18)	40 (69)	0	0	
UCB or $CD34^+$ selected haplo+UCB	25 (7)	0	0	0	0	25 (100)	
HLA-mismatched unrelated	8 (2)	5 (3)	2 (2)	0	1 (2)	0	
Donor age, yr, median (range)*	32 (4-71)	38 (5-71)	30 (4-67)	34 (5-65)	30 (12-69)	n/a	.02
Female –> male HCT	89 (25)	32 (22)	29 (35)	8 (14)	11 (24)	9 (36)	.03
At risk for post-HCT CMV infection †	263 (74)	118 (81)	55 (67)	43 (74)	30 (65)	17 (68)	NS
D/R CMV serostatus‡							<.001
+/+	161 (45)	78 (54)	33 (40)	27 (47)	22 (48)	0	
–/–	88 (24)	27 (18)	26 (32)	12 (21)	15 (33)	8 (32)	
–/+	67 (19)	23 (16)	14 (17)	7 (12)	6 (13)	17 (68)	
+/–	31 (9)	17 (12)	8 (10)	5 (9)	1 (2)	0	
T cell dose, $\times 10^7$/kg, median (IQR)§	10.5 (2.0-22.7)	12.1 (3.9-19.3)	31.5 (21.9-38.8)	6.17 (3.8-20)	.09 (.06-.13)	.74 (.5-1.1)	<.0001

Values are n (%) unless otherwise defined. NMA indicates nonmyeloablative; RIC, reduced-intensity conditioning; IQR, interquartile range.

* Excluding UCB and haplo-UCB because engrafting cells are UCB.

† Recipients were considered at risk for CMV infection if either donor or recipient were CMV-seropositive, including the haplo donor of haplo-UCB HCTs.

‡ D/R unknown for 10 recipients (3%); for HCT using haplo+UCB donors, UCB was presumed CMV-seronegative and haplo donor CMV serostatus was used to determine at-risk designations for recipients, because it was presumed that a CMV-seropositive $CD34^+$ selected haplo product could transmit CMV to the recipient. However, haplo donor serostatus was not used to designate donor CMV serostatus for use as a variable for CMV infection risk analyses, because the T cell immunity was provided by the UCB donor.

§ T cell dose unknown in 18 recipients (5%).

Table 2
Kaplan-Meier Survival Curve Point Estimates

	1 Year Overall Survival	*P*	Day +100 CMV-Free Survival	*P*	Day +100 CMV-Free, Steroid-Free, GF-Free Survival	*P*
D/R serostatus		NS		<.0001		<.0001
−/−	86% (77-92%)		93% (85-97%)		51% (40-61%)	
−/+	76% (64-85%)		39% (28-51%)		27% (17-38%)	
+/−	84% (65-93%)		84% (66-93%)		55% (36-70%)	
+/+	78% (71-84%)		41% (33-49%)		25% (19-32%)	
Graft source		NS		.01		.08
Bone marrow	83% (72-90%)		55% (42-66%)		35% (24-46%)	
PBSCs	79% (74-84%)		62% (56-67%)		37% (31-43%)	
UCB	84% (63-94%)		40% (21-58%)		20% (7-37%)	
T-cell manipulation strategy		NS		.08		NS
PTCy-based	84% (72-91%)		57% (43-69%)		39% (27-52%)	
Serotherapy	91% (83-96%)		67% (56-76%)		41% (31-52%)	
TCD	87% (73-94%)		60% (44-73%)		29% (17-42%)	
CNI/mTORi-based	78% (70-84%)		58% (44-65%)		35% (28-43%)	

95% confidence interval is in parantheses.
GF indicates graft failure.

CMV serostatus, graft source, or T cell manipulation strategy. Among graft sources UCB was associated with lower CMV infection–free survival as compared with bone marrow and PBSC grafts ($P = .01$) (Figure 1). There were no differences in CMV infection–free, steroid-free, graft failure–free survival estimates by graft source or T cell manipulation strategy.

Impact of CMV Serostatus and Graft Dose on CMV Infection

The 100-day CInc of CMV infection differed by donor and recipient CMV serostatus ($P < .001$), with CMV infection CInc the highest for CMV-seropositive recipients, regardless of donor serostatus (Table 3). When evaluating the impact of T cell graft dose, not accounting for the effect of the various in vivo T cell manipulation strategies, there was no difference in the 100-day CInc of CMV infection among at-risk recipients (n = 263) who received 1 to 9.9×10^8 CD3 cells/kg compared with those who received 1, 2, or >2 log fewer T cells/kg, although the 100-day CInc of CMV infection trended upward with each grouped decrease in T cell graft dose (Table 3).

Impact of Systemic Steroids and HCT Approach on CMV Infection

The CInc of CMV infection, with and without steroids as a CR, are shown in Table 3. For the at-risk cohort the estimated 100-day CInc of CMV infection was 47% (95% confidence interval [CI], 40% to 52%) without steroids as a CR and 30% (95% CI, 24% to 35%) with steroids as a CR, demonstrating that many CMV infections occurred after the initiation of systemic steroid therapy. Of the 124 recipients with CMV infection in the first 100 days post-HCT, 45 recipients (36%) developed CMV infection after initiation of steroids. UCB grafts were associated with the highest CInc of CMV infection among graft sources (Figure 2). By T cell manipulation strategy there were significant differences between groups with regard to the estimated

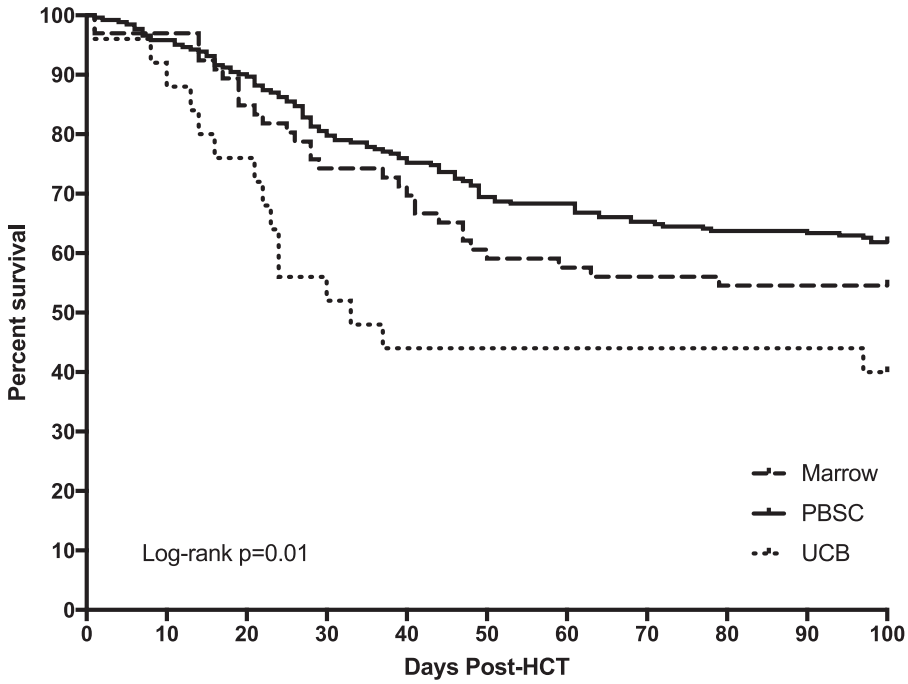

Figure 1. Kaplan-Meier curve of CMV infection-free survival through day +100 post-HCT by graft source.

Table 3
CInc of CMV Infection and CR Events

	100-DAY CInc of CMV Infection, Including Steroids as CR	*P*	100-Day CInc of CMV Infection, Excluding Steroids as CR	*P*
Entire at-risk cohort	30% (24-35%)		47% (40-52%)	
D/R serostatus		<.0001		<.0001
−/−	2% (0-7%)		4% (0-9%)	
−/+	33% (22-44%)		52% (40-63%)	
+/−	9% (2-21%)		12% (4-25%)	
+/+	33% (26-40%)		53% (45-60%)	
Graft source		.001		.0001
Bone marrow	33% (20-46%)		54% (39-67%)	
PBSCs	26% (20-33%)		43% (36-49%)	
UCB	65% (34-84%)		82% (51-95%)	
T-cell manipulation strategy		.007		NS
PTCy-based	26% (14-40%)		49% (32-63%)	
Serotherapy	36% (24-49%)		44% (30-56%)	
TCD	47% (28-64%)		60% (40-75%)	
CNI/mTORi-based	19% (13-27%)		40% (31-49%)	
Graft T cell dose		NS		NS
$<1 \times 10^6$ CD3/kg	41% (23-57%)		56% (37-72%)	
1-9.9 $\times 10^6$ CD3/kg	33% (11-58%)		53% (25-75%)	
1-9.9 $\times 10^7$ CD3/kg	33% (23-43%)		52% (40-62%)	
1-9.9 $\times 10^8$ CD3/kg	24% (17-31%)		40% (32-49%)	

95% confidence interval is in parantheses.

100-day CInc of CMV infection but only when including steroids as a CR, highlighting the significant impact of steroids in changing the CMV risk associated with specific HCT approaches (Figure 2).

Figure 3 shows the CInc estimates of CMV infection and CRs, including steroids, by T cell manipulation strategy to demonstrate the overall rates of experiencing CMV infection or a CR event across HCT approaches. Because PTCy-based and serotherapy-based approaches were associated with some of the more moderate rates of both CMV infection and CRs but the HCT approaches varied by HCT indication/disease, these subgroups are also shown in Figure 3. The CInc of CMV infection was further explored comparing HCT using PTCy without serotherapy for recipients with PID (PTCy alone, n = 34 at risk), HCT using serotherapy with or without PTCy for recipients with hemoglobinopathy/thalassemia (serotherapy-based, n = 34 at risk), and HCT using serotherapy with or without PTCy for PID or malignancy (n = 21 at risk). Including steroids as a CR, there were no significant difference in 100-day CInc of CMV infection, estimated at 29% (95% CI, 15% to 45%) for recipients of serotherapy-based HCT for hemoglobinopathy/thalassemia, 29% (95% CI, 15% to 45%) for recipients of PTCy alone HCT for PID, and 48% (95% CI, 25% to 67%) for recipients of serotherapy with or without PTCy for PID or malignancy. Among pharmacologic, non−PTCy-based and non−serotherapy-based approaches to GVHD prophylaxis, the CInc of CMV infection between CNI-based (n = 57) versus CNI+mTORi-based (n = 61) approaches were compared among at-risk recipients. The estimated 100-day CInc of CMV infection was significantly higher in the CNI-based group as compared with the CNI+mTORi-based group, both with (32% versus 8%, $P = .001$) and without (60% versus 21%, $P < .001$) steroids as a CR. The incidence of CMV infection and CRs for CNI-based approaches and CNI+mTORi-based approaches are also shown in Figure 3.

Risk Factors for CMV Infection and Impact of HLA Mismatch

Based on univariate analyses of factors predicting CMV infection, recipient CMV serostatus, D/R CMV serostatus, T cell dose of the graft as a continuous variable, graft source, and transplant indication (recipient disease) were included in the multiple logistic regression model, whereas conditioning intensity, T cell manipulation strategy, and recipient age were parameters not carried forward to the multiple logistic regression model. Across all approaches this resulted in a classification rule that ultimately included recipient CMV serostatus alone to successfully predict 94.1% of recipients who developed CMV infection by day +100. However, this rule only predicted 52.1% of recipients who did not develop CMV infection by day +100.

Given that HLA mismatching has been associated with increased risk of CMV infection and that a recipient may often have multiple haplo donor options of different CMV serostatuses [14,15], we specifically evaluated the CInc of CMV infection among PTCy-treated HCT recipients, because recipients of haplo grafts (excluding haplo-UCB) all received PTCy. Among PTCy-treated recipients an identical multiple logistic regression analysis was run. The only parameters carried forward to the multiple logistic regression model were recipient CMV serostatus and D/R CMV serostatus. The model reduced down to just using D/R CMV serostatus to result in classification rule where, in contrast to the findings across all approaches, both donor and recipient CMV serostatus were necessary to include to optimally predict 85.7% of recipients who developed CMV infection by day +100 and 68.3% of those who did not develop CMV infection by day +100. For exploratory purposes given the multiple logistic regression model findings, we evaluated the impact of D/R CMV serostatus on the CInc of CMV infection among at-risk recipients of haplo grafts. Including steroids as a CR, CMV infection only occurred among CMV-seropositive recipients with CMV-seropositive donors, with a 100-day CInc of CMV infection of 45%, compared with 0% in both D/R +/− and −/+ groups ($P = .03$). We also evaluated the impact of HLA match on the CInc of CMV infection for PTCy-treated recipients, comparing at-risk recipients of HLA-matched related or unrelated grafts (n = 13) with at-risk recipients of haplo grafts (n = 46). There was no difference in the CInc of CMV infection or the CInc of CRs, with and without steroids as a CR, for haplo graft recipients as compared with matched graft recipients, with 100-day CInc of CMV infection of 38% versus 47%, respectively, without steroids as a CR and 15% versus 30%, respectively, with steroids as a CR.

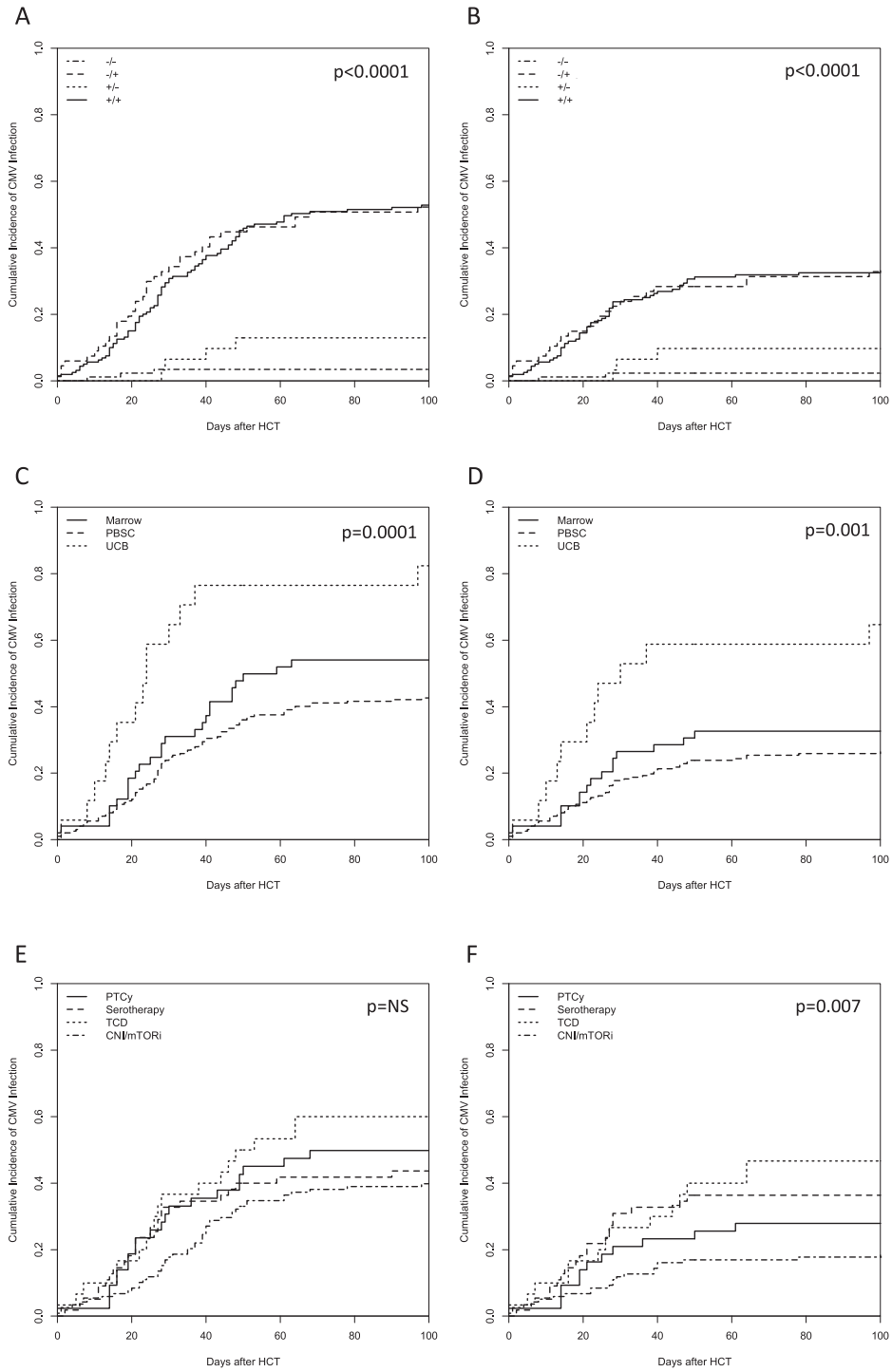

Figure 2. CInc of CMV infection, not including (A, C, and E) and including (B, D, and F) systemic steroids as a CR. (A and B) One hundred–day CInc of CMV infection by D/R serostatus. (C and D): One hundred–day CInc of CMV infection by graft source. (E and F) One hundred–day CInc of CMV infection by T cell manipulation strategy.

Timing and Duration of CMV Infection

By T cell manipulation strategy there was no difference among groups in the timing of CMV infection post-HCT or the duration of therapy for infection, where the median day post-HCT of CMV infection ranged from day +21 for PTCy, day +21 for serotherapy, day +23 for UCB, day +25 for TCD, and day +35 for CNI/mTORi-based approaches. Treatment duration was longer for recipients of UCB grafts at a median of 36 days, as compared with 27 days for bone marrow and 21 days for PBSCs (P =.03). Treatment duration was longer for recipients of grafts from CMV-seronegative donors with a median duration of 30 treatment days, as compared with recipients of grafts from CMV-seropositive donors with a median duration of 21 treatment days (P = .02). When non-UCB CMV-seronegative grafts were considered as a separate group and compared with UCB grafts and CMV-seropositive grafts, the treatment duration was different between groups with median of 21 days with CMV-seropositive grafts; 29 days for non-UCB, CMV-

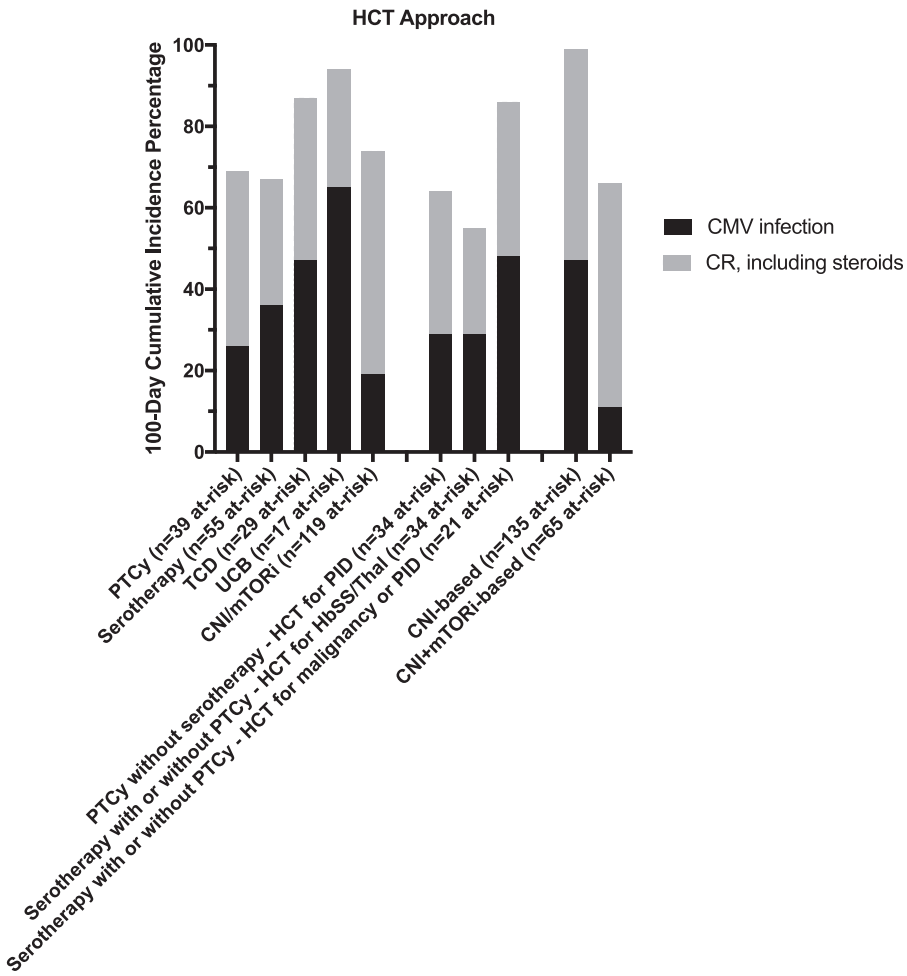

Figure 3. One hundred–day CInc of CMV infection and CRs, including steroids, by HCT approach, shown as a percentage where the remainder to reach 100% are event-free through day +100. Subgroups of the serotherapy-based and PTCy-based approaches, by recipient diagnosis, and subgroups of CNI-based and CNI+mTORi-based pharmacologic approaches to GVHD prophylaxis are also shown.

seronegative grafts; and 36 days for UCB grafts (P = .04). The timing of CMV infection requiring treatment post-HCT did not differ by donor serostatus or whether the seronegative donor was UCB.

At-risk recipients of HCT for hemoglobinopathy/thalassemia received CMV treatment significantly less frequently as compared with recipients of HCT for malignancy, PIDs, or aplastic anemia (chi-square P = .0005). Among recipients of serotherapy-based HCT, those transplanted for a diagnosis of hemoglobinopathy/thalassemia had a shorter treatment duration (median, 16 days) as compared with those transplanted for malignancy or PID (median, 31 days; P = .008).

Antiviral resistance mutation testing was performed in 9 HCT recipients. In 4 recipients with CMV infection, no resistance mutations were found. In 5 recipients, all with CMV disease, resistance mutations were found (Table 4).

CMV Disease

In the year post-HCT 19 recipients (5%) had 21 CMV disease events (colon, 7; lungs, 7; upper gastrointestinal tract, 5; retinitis, 1; central nervous system, 1) at a median of 88 days post-HCT, although 10 recipients were diagnosed beyond day +100 (Table 4). Two cases were resistant to ganciclovir, foscarnet, and cidofovir; 2 cases were resistant to ganciclovir and foscarnet; and 1 case was resistant to foscarnet. Three cases of CMV disease developed without prior CR, whereas 12 cases were in the setting of GVHD, 3 in the setting of relapse and GVHD, 2 in the setting of relapsed hematologic malignancy requiring systemic therapy, and 1 in the setting of second HCT for graft failure. One case was a D/R −/− recipient who had received granulocytes post-HCT, whereas 2 were D/R −/+ and 2 D/R +/−. All but 2 cases occurred in recipients of PBSC grafts. One patient, after developing resistance to antiviral therapy, received multiple infusions of CMV-specific cytotoxic T cells without response.

DISCUSSION

In the modern era of improved methods to monitor, treat, and even prevent CMV infection after HCT, understanding the circumstances in which HCT recipients are or become at risk of CMV infection post-HCT remains an important focus [16]. Our results are consistent with those from a Center for International Blood and Marrow Transplant Research study demonstrating that recipient CMV serostatus is the main determinant of CMV infection incidence post-HCT across HCT approaches. Our data also indicate that among PTCy-treated recipients, donor CMV serostatus also may inform CMV infection risk [17]. The mechanism behind donor CMV seropositivity contributing to the prediction of CMV infection risk among PTCy-treated recipients is unclear but might relate to differences in

Table 4
Recipient, Transplant, and Clinical Characteristics of Cases of CMV Disease

Days Post - HCT of Diagnosis	Graft	HCT Indication (Disease)	HCT Approach	Organ	D/R CMV Serostatus	Setting of CMV Disease	CMV Infection in First 100 Days	Day of Post-HCT Death	Antiviral Resistance	Other Infectious Issues	Cause of Death
+34	PBSC	Malignancy	CNI/mTORi	Colon	+/+	Relapse	Day +31	+38		None	Relapse
+84	PBSC	Malignancy	CNI/mTORi	Stomach	+/+	GVHD, relapse	Day +61	+258		Giardia	Relapse
+29	BM	Malignancy	CNI/mTORi	Lung	+/−	No events	Day +29	Alive		None	N/A
+212	PBSC	Malignancy	CNI/mTORi	Colon	+/+	GVHD	Day +49	+372	Ganciclovir (F412L, V812L in UL54 gene), foscarnet (V812L), cidofovir (F412L, V812L)	*Paecilomyces* sinusitis, VRE bacteremia, *C. difficile* colitis, BK-associated cystitis	GVHD with infectious complications
+254	PBSC	Malignancy	TCD	Retina	+/+	After second HCT for graft failure		+897		HHV6 reactivation, EBV elevation in blood, *Klebsiella* and *E. coli* UTIs, *S. pneumoniae* pneumonia	CNS relapse after second HCT
+237	PBSC	Malignancy	CNI/mTORi	Lung	+/−	Relapse, GVHD	Day +42	+255	Foscarnet (S585A in UL54 gene)	*Aspergillus* pneumonia	Secondary malignancy
+88	PBSC	PID	Serotherapy	Lung	−/−	GVHD	Day +8	+93	Ganciclovir and foscarnet (A809V in UL54 gene)	*Pyrenochaeta* liver abscess, *P. romeroi* pneumonia, *Klebsiella* pneumonia and bacteremia	GVHD with infectious complications
#1: +22	PBSC	Malignancy	CNI/mTORi	#1: Stomach	+/+	#1: CMV just before GVHD	Day +6	+710		#1: *H. pylori* gastritis, BK viremia, *E. faecalis* bacteremia, *S. epidermidis* bacteremia	Relapse
#2: +85				#2: Colon		#2: GVHD				#2: Cholecystitis	
+30	PBSC	Malignancy	CNI/mTORi	Esophagus	+/+	Relapse - second HCT, then relapse again	Day +30	+261		HHV6 reactivation	Relapse
+42	PBSC	PID	PTCy	CNS	+/+	GVHD	Day +43	+83		HHV6 reactivation, EBV elevation in blood, BK-associated cystitis, *Cryptosporidium*, *S. aureus* bacteremia, *Pseudomonas* bacteremia, disseminated *Basidiomycetes* infection	GVHD with infectious complications
+237	UCB	Aplastic Anemia	UCB	Lung	−/+	GVHD	Day +22	+416	Ganciclovir (T503I, A809V in UL54 gene), foscarnet (A809V), cidofovir (T503I); CMV-CTLs	HHV6 reactivation, RSV LRTI, *Cryptococcal* sinusitis, MAC pneumonia, *Aspergillus* pneumonia	CMV pneumonitis
+15	PBSC	Malignancy	Serotherapy	Colon	+/+	GVHD	Day +21	+83		HSV viremia, EBV elevation in blood	Relapse
+269	PBSC	Malignancy	CNI/mTORi	Lung	+/+	GVHD	Day +51	Alive		Adenoviremia	N/A
+108	PBSC	PID	Serotherapy	Colon	+/+	GVHD	Day +91	+277		Adenoviremia, EBV elevation in blood, BK-associated cystitis, *Legionella* peritonitis, *Candida glabrata* fungemia, VRE bacteremia, *C. difficile* colitis	GVHD with infectious complications
+346	PBSC	Malignancy	TCD	Colon	+/+	Relapse - second HCT, then GVHD		+595		Disseminated *Fusarium*, *S. mitis* bacteremia, *Klebsiella* pneumoniae bacteremia	Relapse with infectious complications
+145	PBSC	Malignancy	CNI/mTORi	Colon	+/+	GVHD		Alive		BK-associated cystitis	
+340	PBSC	Malignancy	Serotherapy	Lung	−/+	GVHD	Day +12	+398	Ganciclovir (C603W in UL97 gene), foscarnet (V715M in UL54 gene)	HHV6 reactivation, BK-associated cystitis	GVHD, uncontrolled CMV, TMA
#1: +48	PBSC	PID	PTCy	#1: Stomach	+/+	#1 and 2: GVHD	Day +49	+414		BK-associated cystitis, primary EBV infection	GVHD with uncontrolled CMV
#2: +385				#2: Lungs							
+25	PBSC	Hemoglobinopathy	Serotherapy	Colon	+/+	No events	Day +19	Alive		None	N/A

BM, indicates bone marrow; CMV-CTLs, CMV-specific cytotoxic T lymphocytes; VRE, vancomycin-resistant enterococcus; BK, BK virus; HHV6, human herpesvirus-6; EBV, Epstein-Barr virus; UTI, urinary tract infection; RSV, respiratory syncytial virus; LRTI, lower respiratory tract infection; MAC, *M avium complex*; HSV, herpes simplex virus; TMA, thrombotic microangiopathy.

immunomodulation of donor-derived antiviral immunity by PTCy that depend on donor/graft serostatus. Furthermore, our finding that UCB was associated with high rates of CMV infection and prolonged treatment duration are consistent with a prior study [18]. Unique to our study is how post-HCT events, particularly steroid use, modify the known pre-HCT predictive factors. Furthermore, given that CMV diagnosis and treatment as well as other supportive care elements were standardized across patients, this study eliminates confounding influences present in the Center for International Blood and Marrow Transplant Research registry study. Although the level of detail available to investigate CMV infection and risk modifiers exceeds a registry-based approach, our study is limited by the fact that the variables evaluated across the HCT approaches investigated herein such as disease type, graft source, and T cell manipulation strategy were determined in aggregate by protocol design and not as independent variables.

The degree of HLA matching and donor relatedness are known determinants of CMV infection among at-risk recipients [14], although it is difficult to separate the GVHD prophylaxis approach and the risk for developing GVHD determined by HLA match from differences in post-HCT CMV-specific immunity related directly to the degree of HLA matching. Among recipients of haplo grafts, others have shown that in vivo TCD with antithymocyte globulin carries higher rates of CMV infection and viral infection–related mortality when compared with PTCy-based approaches to haplo HCT [15]. In an exploratory analysis we found no difference in CMV infection incidence between PTCy-treated recipients by degree of HLA match, even when accounting for post-HCT steroid use as a CR. These findings likely are related at least in part to overall low incidences of severe acute and chronic GVHD and thus low immunosuppressive burdens in patients treated with PTCy. This is further supported by our overall data illustrating the impact of post-HCT interventions, such as steroid therapy for GVHD, on CMV infection incidence, regardless of other specifics of the approach [19,20].

In comparing CNI-based GVHD prophylaxis with CNI +mTORi-based approaches, the latter was associated with less CMV infection. There are data to suggest that mTORi may protect against CMV infection [21], although 2 randomized trials of CNI/methotrexate versus CNI/mTORi reported no differences in CMV infection incidence between treatment arms, with similar rates of GVHD between the arms [22,23]. However, the relationship between GVHD onset/treatment and CMV infection was not examined for differences between arms in either trial [22,23]. Because mTORi have been shown to not directly inhibit CMV replication [24], any protective effect of mTORi, if real, would seem to be indirect and related to reconstitution of CMV-specific cellular immunity. Additionally, recipients transplanted for hemoglobinopathy/thalassemia received alemtuzumab during conditioning and post-HCT mTORi without CNI, with a relatively low incidence of CMV infection despite the proximal serotherapy they received. Our finding of lower CMV incidence in recipients of mTORi is interesting in that it suggests mTORi may indirectly mitigate CMV infection risk, perhaps related to the early mixed chimerism often observed in these recipients of mTORi-based GVHD prophylaxis [25–27].

Our data support that unplanned, post-HCT events, particularly steroid use, contribute greatly to the incidence of CMV infection. Analyses with and without steroids as a CR quantify the added risk posed by this very common post-HCT intervention. Of the recipients in this study who had CMV disease, post-HCT risk-modifying events were a co-factor in most cases. As reported by others [28] and also illustrated here, CMV disease resulting in death was often in the seen with multiple concurrent infectious complications arising in the setting of steroid-refractory GVHD.

These data illustrate the utility in tailoring the monitoring and preemptive treatment, strategies in a dynamic manner, based on the nature and severity of post-HCT risk-modifying events and on an individual level. Our analysis from the pre-letermovir era should serve as an historical comparison as new prophylactic approaches are incorporated into practice. Durable restoration of CMV-specific cellular immune function should mark the point post-HCT when risk for clinically significant CMV infection and disease ends for a given individual. However, post-HCT factors may weaken or incapacitate CMV-specific cellular immunity and make patients again at risk for CMV infection or disease. Although assays to quantify CMV-specific cytotoxic T cells are now clinically available, what represents a protective, functional level of cellular immunity is still largely investigational, and the clinical application of these tests post-HCT remains very much uncharted [29,30]. Further work may provide insight into whether these assays are more informative when applied to recipients recovering from a risk-modifying event, such as tapering off unplanned immunosuppression or recovering from chemotherapy plus donor lymphocyte infusion for relapse, to provide additional insight at that time into CMV-specific immunity and guide CMV monitoring approach moving forward. Letermovir is now approved for CMV prophylaxis in CMV-seropositive recipients through day +100 [10]; however, the recipients most vulnerable to significant CMV infection and disease may be best identified not just by their serostatus pre-HCT or the HCT platform used, but by post-HCT events that impair immune reconstitution, even after day +100.

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