Neutropenia in Allogeneic Marrow Transplant Recipients Receiving Ganciclovir for Prevention of Cytomegalovirus Disease: Risk Factors and Outcome

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To determine risk factors, frequency, time patterns, and outcome of ganciclovir-related neutropenia in allogeneic marrow transplant recipients, 278 consecutive patients receiving ganciclovir from engraftment until day 100 were studied. In this cohort, 159 patients (57%) had absolute neutrophil counts (ANC) less than 1,500/μL, 112 (41%) had an ANC less than 1,000/μL, 87 (31%) less than 750/μL, and 56 (21%) less than 500/μL for at least 2 consecutive days. Statistically significant risk factors for neutropenia in a Cox model were low marrow cellularity between day 21 and 28 (relative risk [RR] 2.4, $P = .0002$), hyperbilirubinemia ≥6 mg/dL during the first 20 days (RR 2.5, $P = .0001$), and elevation of serum creatinine ≥2 mg/dL after day 21 after transplant (RR 2.1, $P = .001$). Restriction to factors present at engraftment resulted in a similar model with low marrow cellularity, hyperbilirubinemia ≥6 mg/dL, and elevated serum creatinine as significant risk factors. Patients with no risk factor had an incidence of neutropenia of 21%, an incidence of 31% for one risk factor, and of 57% for two or more risk factors (RR 3.8, $P = .001$). Neutropenia was a negative predictor of overall (RR 2.0, $P = .0001$) and event-free survival (RR 2.1, $P < .0001$), and a predictor of relapse (RR 1.7, $P = .03$) and nonrelapse mortality (RR 2.1, $P = .003$). Thus, early liver dysfunction, elevated serum creatinine, and low marrow cellularity are risk factors for ganciclovir-related neutropenia. Neutropenia in ganciclovir recipients after marrow transplantation is an independent risk factor for mortality.

GANCICLOVIR IS HIGHLY effective in preventing cytomegalovirus (CMV) disease in allogeneic marrow transplant recipients.[1-4] This has led to widely accepted prophylactic or preemptive use of ganciclovir in CMV-seropositive patients, either at time of engraftment or at first evidence of CMV-infection.[5,6] The most common adverse effect of ganciclovir is neutropenia. In marrow transplant recipients the frequency of neutropenia, defined as absolute neutrophil count (ANC) less than 1,000/μL, ranges from 41% to 58% in ganciclovir-treated patients. This is approximately twice the rate of neutropenia in untreated patients.[3,4] Neutropenia with an ANC of less than 750/μL occurs in 30% of ganciclovir recipients and has been associated with increased rates of bacterial sepsis and invasive fungal infections in marrow transplant recipients.[3,6]

The frequency, severity, and duration of ganciclovir-related neutropenia varies widely between HIV-infected patients, solid organ, and marrow transplant recipients and is further influenced by duration of therapy. Patients with advanced HIV infection have a high frequency of severe neutropenia (defined as an ANC < 500/μL) with an incidence of 34% in ganciclovir-treated patients, compared to 14% in foscarnet-treated patients.[7] In solid organ transplant patients its frequency is considerably lower with 10%, defined by a neutrophil count of less than 1000/μL.[8-10] Although the mechanism of neutropenia in ganciclovir recipients is well described as a dose-dependent inhibition of DNA-polymerase in hematopoietic progenitor cells,[11] the differences in the incidence of neutropenia between different populations have not yet been studied and explained.

Identifying the risk factors for neutropenia in ganciclovir recipients could be useful in lowering the incidence of neutropenia, and in designing alternative strategies for high-risk patients.

The purpose of this study is to determine risk factors, frequency, and time patterns of neutropenia in marrow transplant recipients receiving ganciclovir. In addition, the relation of neutropenia to the long-term outcome was studied.

PATIENTS AND METHODS

Patients. All consecutive CMV-seropositive patients of all ages undergoing allogeneic marrow transplantation between December 1990 and March 1994 who received ganciclovir for prevention of CMV disease from engraftment (ANC ≥ 750/μL for 2 consecutive days) until day 100 were included in this analysis. At engraftment, hematopoietic growth factors were discontinued in patients who received them during the pre-engraftment period. Patients were either treated in two randomized studies[3,6] or outside randomized studies. All clinical and laboratory data were extracted from the computerized clinical database and from the research charts. Time periods for analysis of laboratory values were divided into two time periods, day 0 after transplant until day 20 as the period before engraftment, and day 21 until day 100 after transplant as the period after engraftment. This was done to differentiate between factors influencing neutropenia present before ganciclovir therapy and during ganciclovir therapy. Conditioning for marrow transplantation,[12-14] prophylaxis,[15,16] and treatment[15] of graft-versus-host disease (GVHD) was done according to the current protocols. Additional supportive care included fluconazole, 400mg/d for prevention of fungal infection from the start of conditioning until day 75 after transplant (introduced in June 1992, in 121 of 278 patients), acyclovir 250 mg/m^2 intravenously every 12 hours in patients seropositive for herpes-simplex-virus from day 7 until start of ganciclovir therapy, and trimethoprim-sulfamethoxazole (one double-strength tablet twice daily for 2 consecutive days per week) after engraftment for the prophylaxis of *Pneumocystis carinii* pneumonia.

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Underlying disease was classified as advanced for all patients not in remission, patients with acute nonlymphocytic leukemia in second or later remission, and patients with acute lymphocytic leukemia in third remission or later. Patients with chronic myelogeneous leukemia were classified as having advanced disease when in blast crisis at time of transplant. All other patients were classified as not having advanced disease. Assessment and staging of GVHD was made according to the established categories for acute and chronic GVHD.[17,18] Growth factor use after engraftment was analyzed descriptively, because no controlled or standardized intervention protocol existed and therapy was initiated at the discretion of the treating physician. Growth factors used before engraftment were not analyzed. This study was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center.

Marrow examination. Marrow exams were routinely performed between day 21 and 28 after transplant. Marrow cellularity was determined and adjusted for age categories from biopsy specimens obtained from the posterior iliac crest.[19] To examine a correlation between marrow cellularity and neutropenia, data were classified semiquantitatively into three groups of equal size. Patients in the lowest third had age-adjusted marrow cellularity below 35%, patients in the medium third were between 35% and 50%, and patients in the upper third had more than 50% cellularity.

Neutropenia. ANCs were calculated from daily blood counts, taken as the sum from absolute polymorphonuclear cells and bandforms per microliter. Frequency, time of onset, and duration of neutropenia were analyzed at different levels of neutropenia. These levels were defined as ANC of 1,500/μL, 1,000/μL, 750/μL, 500/μL, and 200/μL. Data of patients were categorized by the lowest level of neutropenia. To be categorized to one of these levels, a patient had to have a neutrophil count below this level for at least 2 consecutive days. The end of a period of neutropenia was defined by return to the next higher level for 2 or more days. Complete resolution of neutropenia was defined as return of the ANC to more than 1,500/μL for at least 2 consecutive days.

For all analyses of risk factors and for the influence of neutropenia on outcome, an ANC level of 750/μL was chosen. This was the level where drug was to be discontinued by standard-of-care protocol. Irreversible graft failure was defined as ANC less than 500/μL without recovery of neutrophils.

Ganciclovir treatment and dose reduction. Ganciclovir was started in all patients at engraftment with a dosage of 5 mg/kg intravenously, every 12 hours for 5 days and then continued at 5 mg/kg once daily, 6 days per week. Dose reductions according to creatinine clearance were made on the basis of calculated creatinine clearance according to adjusted ideal body weight,[20] with no dose adjustment above 50 mL/min. Ganciclovir was stopped when ANCs fell to 750/μL for 2 consecutive days and was reinstituted when neutrophils increased again above 750/μL.

CMV infection and disease. CMV disease was defined as detection of CMV by biopsies from visceral sites, by culture or histology,[1] or if CMV was detected by culture or direct fluorescent antibody stain in bronchoalveolar lavage (BAL) in the presence of new or changing pulmonary infiltrates.[1,3] CMV infection was defined by positive CMV DNA-polymerase chain reaction (PCR) and/or positive antigenemia and/or positive surveillance culture from blood, urine, or throat swabs.[6] Subclinical infection was defined as CMV infection without signs or symptoms of CMV disease. In a subset of patients, the CMV antigenemia assay and PCR was performed weekly.[6] These patients were additionally analyzed for the influence of CMV infection on neutropenia.[21]

Bacterial and fungal infections. Bacterial bloodstream infections[22] and invasive fungal infections (either from bloodstream, from otherwise sterile sites and Aspergillus sp. isolated from BAL, or sinus biopsies with radiological signs of disease)[23] occurring between day 30 and 150 were included in the analysis of neutropenia-associated infections.

Statistical analysis. For determination of risk factors of neutropenia, a Cox proportional hazards model was formulated with neutropenia as the outcome.[24] Variables were first analyzed for their association with the hazard of neutropenia in a univariate model and later in a multivariate model. The multivariate model was formulated by stepwise introduction of variables found significant ($P < .05$) in univariate analysis. Variables were introduced in a stepwise fashion on the basis of likelihood ratios; entry and removal levels of significance were .05 and .1, respectively. For multivariate analysis, a significance level of less than .05 was considered significant.

A second model was constructed to study the influence of neutropenia at less than 750/μL as a time-dependent covariate on overall and event-free survival, relapse, and nonrelapse mortality. Event-free survival was defined as time to either death, relapse, or second transplant, whichever occurred first. Time to relapse was calculated as time to first relapse. Patients dying from any cause after relapse were assigned to have died because of relapse. Relapse rate and nonrelapse mortality were calculated as the simple rates of relapse or nonrelapse mortality.[25] Other factors included in this model were age at transplant, underlying disease (as strata, because the proportional hazards assumption was not valid), advanced versus nonadvanced disease and acute and chronic GVHD (defined as acute GVHD grades 0-2 *v* grades 3-4 and clinical extensive de novo or progressive chronic GVHD *v* otherwise time-dependent covariates). No adjustments were made for multiple comparisons, all *P* values are two-sided. All correlation coefficients were calculated as Spearman's rank coefficients. All statistical analyses were performed using SPSS 6.1.1 (SPSS Inc, Chicago, IL).[26]

RESULTS

Two hundred seventy eight consecutive patients were analyzed; patient characteristics are shown in Table 1. Follow-up data including intercurrent infections, late complications, and cause of death were available for all patients with a median follow-up of 1,083 days (dead n = 142, alive n = 136; surviving 226 to 1,697 days) through July 31, 1995. Prophylaxis with ganciclovir was initiated between days 10 and 44, median day 23. The median day of evaluation for marrow cellularity was day 23 after transplant.

Frequency, time pattern, and degree of neutropenia. Neutropenia of less than 1,500/μL was found in 159 patients, 112 patients developed neutropenia of less than 1,000/μL, 87 of less than 750/μL, 56 of less than 500/μL, and 19 patients had ANC less than 200/μL (Table 2). Nineteen patients (7%) had irreversible secondary graft failure. Seventeen of these died during neutropenia and 2 received a second transplant and were alive at last contact date. Duration of neutropenic episodes and rates of progression and recovery are shown in Table 2.

Risk factors for the development of neutropenia. In univariate analysis, patients with advanced disease, with low marrow cellularity between day 21 and 28, and with late platelet engraftment (later than median time of platelet engraftment) had a higher risk of developing neutropenia. Furthermore, patients with GVHD grade 3 or 4, patients with a peak bilirubin of $\geq$6 mg/dL either during the first 20 days or after day 20 after transplantation, and patients with elevated creatinine during the first 20 days or after day 20 were at

Table 1. Patients Characteristics

	N	%
Total no.	278	100
Age (median, range)	37 (1-66)	N/A
Gender		
Female	130	47
Male	148	53
Diagnosis (advanced disease/relapse)		
Acute leukemias	88 (52)	32 (12)
CML	107 (13)	38 (14)
Myelodysplasia	37 —	13
Non-Hodgkin's-lymphoma/Hodgkin's disease/multiple myeloma	35 (30)	13 (5)
Nonmalignant diseases	11 —	4
Conditioning regimen		
Cyclophosphamide + TBI	148	53
Cyclophosphamide + busulfan + TBI	31	11
Other with TBI	17	6
Busulfan + cyclophosphamide	69	25
Other without TBI	13	5
Donor type		
Matched related	147	53
Mismatched related	41	15
Unrelated	90	32
GVHD prophylaxis		
Cyclosporine	14	5
Cyclosporine + steroids	28	10
Cyclosporine + MTX	183	66
Cyclosporine + steroids + MTX	10	4
Other	43	15
Acute GVHD		
Grade 0-1	70	26
Grade 2-4	208	75
Grade 3-4	102	36

Abbreviation: TBI, total body irradiation.

Table 3. Risk Factors for Neutropenia in Multivariate Analysis

	RR	95% CI	*P* Value
Factors significant in the final model			
Marrow cellularity day 21			
0-35%	2.44	1.53-3.89	.0002
>35%*	1†		
Max. bilirubin before day 20			
<6 mg/dL*	1†		
≥6 mg/dL	2.49	1.59-3.91	.0001
Max. serum-creatinine after day 20			
<2 mg/dL*	1†		
≥2 mg/dL	2.12	1.43-3.37	.0013
Factors not significant‡			
Disease status			
Not advanced	1†		
Advanced	1.31	0.83-2.09	.24
GVHD prophylaxis			
Steroids			
No	1†		
Yes	.81	0.62-1.06	.14
MTX			
No	1†		
Yes	.62	0.37-1.04	.08
Time of platelet engraftment			
<Median	1†		
>Median	1.13	0.89-1.45	.31
Max. serum-creatinine < day 20			
<1.2 mg/dL*	1†		
>1.2 mg/dL*	1.33	0.76-2.21	.33
Max. serum-bilirubin > day 20			
<2 mg/dL	1†		
≥6 mg/dL	1.19	0.58-2.44	.63
Acute GVHD			
Grade 0-2	1†		
Grade 3-4	1.41	0.89-2.25	.14

* Categories pooled in multivariate analysis.

† Reference category.

‡ Values if factor added to final model.

higher risk of developing neutropenia. GVHD prophylaxis was associated with a higher rate of neutropenia in patients who were not treated with steroids and in patients who were not treated with methotrexate (MTX). Age, gender, donor match, underlying disease, use of total body irradiation, transplanted cell dose, and time of neutrophil engraftment were not significant in univariate analysis and therefore not incorporated in the multivariate analysis.

After stepwise introduction into the multivariate model, only low marrow cellularity between day 21 and 28, serum creatinine ≥ 2.0 mg/dL after day 20, and hyperbilirubinemia ≥ 6 mg/dL during the first 20 days after transplant were significant risk factors for the development of neutropenia in the Cox model (Table 3).

To focus on risk factors at the time when ganciclovir was initiated, the model was further restricted to factors present in the first 20 days after transplant. In addition to low marrow cellularity and hyperbilirubinemia, early creatinine ≥ 1.2

Table 2. Neutropenia: Progression, Duration, and Recovery

Degree of Neutropenia (ANC/μL)	Patients Reaching Level (n = 278)	Median Day of Onset (range)	Median Duration (range) in Days	Died Without Recovery	Progress Down to Next Level	Recovered
<1,500	159 (57%)	64 (18-100)	13 (2-67)	1	112 (70%)	44
<1,000	112 (40%)	66 (18-100)	9 (2-53)	0	87 (78%)	25
<750*	87 (31%)	69 (25-102)	6 (2-40)	5	56 (64%)	26
<500	56 (21%)	72 (31-105)	3 (2-38)	7	19 (34%)	30
<200	19 (7%)	74 (35-96)	7 (2-20)	6	—	13

* Ganciclovir was discontinued when the ANC was less than 750/μL for 2 consecutive days.

mg/dL was a significant risk factor (relative risk [RR] 1.67, 95% CI 1.03 to 2.69, $P = .0365$). Incidence of subsequent neutropenia (<750 ANC/μL) in this model was 21% for patients with no risk factor (n = 54), 31% for patients with one risk factor (n = 117), and 57% for patients with two or more risk factors (n = 71). Patients with two or more risk factors had a relative risk of neutropenia of 3.8 (95% CI 1.94 to 7.47, $P = .0001$), compared with patients without any risk factor in this model.

Neutropenia and outcome. At the end of follow-up, the median survival for the cohort was 976 days and the median event-free survival was 413 days. Of 73 patients with a relapse after transplant, 55 (75%) died. Nonrelapse related deaths were found in 87 patients.

In multivariate analysis, neutropenia (ANC < 750/μL) was an independent negative risk factor for overall survival (RR 2.03, 95% CI 1.41 to 2.93, $P = .0001$) and for event-free survival (RR 2.09, 95% CI 1.47 to 2.97, $P < .0001$), and a risk factor for nonrelapse mortality (RR 2.05, 95% CI 1.28 to 3.28, $P = .0028$). Neutropenia was also associated with a higher risk of relapse (RR 1.71, 95% CI 1.05 to 2.78, $P = .029$). For overall survival and event-free survival as outcome, age at transplant, advanced disease and acute GVHD (≥ grade 3) or clinical extensive chronic GVHD were additional significant risk factors. For relapse, GVHD and age were not statistically significant risk factors.

Underlying disease had a strong influence on the frequency of different outcomes. In patients with acute leukemias, 28 of 39 patients (72%) died from relapse, whereas in patients with CML, transplant-related mortality (24 of 35 deaths) was the most important cause of death (69%). When these groups were analyzed separately, two different patterns emerged. In patients with acute leukemia, neutropenia was independently associated with relapse (RR 3.08, 95% CI 1.48 to 6.41, $P = .003$) and nonrelapse mortality (RR 2.45, 95% CI 0.89 to 6.77, $P = .08$). Because relapse was diagnosed early in many patients with acute leukemias (median, 91 days after transplant), neutropenia in these patients could have been influenced by residual disease. In contrast, neutropenia in patients with CML was only predictive for nonrelapse mortality, whereas there was no significant influence on relapse.

CMV infection and neutropenia. Subclinical reactivation of CMV was frequent in the 91 monitored patients. Positive antigenemia was found in 35 patients (38.5%) and a positive DNA-PCR of peripheral blood leukocytes was found in 52 patients (57.1%). Only 31 patients (34.1%) remained negative for both markers. For patients with CMV reactivation (a positive result in any one of the two tests), rates and degrees of neutropenia were not statistically significant or different from patients negative for both tests. Furthermore, in a univariate model, CMV-antigenemia and/or positive PCR were not significant risk factors for neutropenia (RR 1.10, 95% CI 0.76 to 1.61, $P = .62$ for any marker positive *v* none).

The overall incidence of CMV disease was low in this cohort with 6 patients developing CMV disease between engraftment and day 100 after transplant (2.2%, 5 CMV pneumonia, 1 CMV gastrointestinal disease). Of the 6 patients with CMV-disease, 4 did not develop any neutropenia.

Hematopoietic growth factor use and neutrophil count. Growth factors were used in 33 patients after engraftment, granulocyte-macrophage colony-stimulating factor (GM-CSF) in 9 patients, granulocyte colony-stimulating factor (G-CSF) in 23 patients, and both in 1 patient. Growth factors were started at a median of 71 days after transplant (range, 20 to 91) for a median of 12 days (range, 3 to 112). Only 24 patients were treated for neutropenia less than 1,500/μL, 2 were started at an ANC of 1,500/μL, 4 at 1,000/μL, 7 at 750/μL, 10 at 500/μL, and 2 at 200/μL. The overall recovery rate of neutropenia below an ANC of 750/μL and median duration of neutropenia on this level did not appear to be different between patients who did and did not receive growth factors (68% recovery rate and median duration of 8 days in patients receiving *v* 79% and 6 days in patients not receiving growth factors).

Neutropenia and bacterial and fungal infections. Bacterial bloodstream and invasive fungal infections were frequent in this cohort (Table 4). A clear trend was observed between the degree of neutropenia and the rate of bacterial infections (Table 4, $P < .0001$). In contrast, an increased incidence of fungal infections was present only in patients with an ANC of less than 200/μL, whereas there was no significant trend over the different degrees of neutropenia. The rates of both bacterial and fungal infections in patients treated with hematopoietic growth factors were similar to the overall rates on the different levels of neutropenia (Table 4).

DISCUSSION

In this study, we identified three factors associated with a high frequency of neutropenia in marrow transplant recipients receiving ganciclovir prophylaxis, ie, hyperbilirubinemia in the first 20 days after transplant, low marrow cellularity early after engraftment, and elevated serum-creatinine. Additionally, it was shown that neutropenia is an independent risk factor for overall poor survival, for relapse and nonrelapse mortality, and that it increases the risk for fungal and bacterial infections.

Hyperbilirubinemia has not previously been described as a risk for ganciclovir-associated neutropenia in this setting. Although a correlation between maximum levels after and before day 20 existed (r = .5, $P < .0001$), hyperbilirubinemia in the later transplant period was less significant in influencing neutropenia. Hyperbilirubinemia in the first 20 days after transplant most commonly reflects the development of veno-occlusive disease (VOD).[27] VOD results from toxic effects of the conditioning regimen on liver tissue and presumably, other organs. Additionally, VOD has recently been shown to influence renal function significantly, predominantly with tubular changes without concomitant changes in serum creatinine levels.[28] As tubular secretion is a significant factor in ganciclovir-clearance,[29] and ganciclovir doses are adjusted on calculated creatinine clearance only, one hypothesis for the link between hyperbilirubinemia and neutropenia would be an alteration of ganciclovir pharmacokinetics by VOD-associated tubular dysfunction, leading to higher drug levels

Table 4. Degree of Neutropenia and Infections Between Day 30 and 150

Neutropenia	Bacteremias	Rate*	Rate in Patients Treated With G-CSF or GM-CSF*	Invasive Fungal Infections	Rate*	Rate in Patients Treated With G-CSF or GM-CSF*
None (n = 119)	19	1.48	—	17	1.33	—
<1,500 μL (n = 47)	11	2.03	—	5	0.92	—
<1,000/μL (n = 25)	4	1.30	—	2	0.65	—
<750/μL (n = 31)	11	3.28	2.77 (n = 6)	4	1.19	—
<500/μL (n = 37)	14	3.36	4.69 (n = 12)	6	1.44	2.00 (n = 12)
<200/μL (n = 19)	11	5.98	6.8 (n = 6)	7	3.81	3.44 (n = 6)

* Number of episodes per 1,000 patient days.

than anticipated. Because of apparent differences in the ganciclovir pharmacokinetics between different patient settings[30] and a lack of sufficient pharmacokinetic data of ganciclovir in marrow transplant recipients,[31] pharmacokinetic studies are needed to examine this hypothesis.

Low marrow cellularity at day 21 is most likely a marker for poor engraftment and was in this analysis more highly correlated to neutropenia than other markers of engraftment such as time of neutrophil or platelet engraftment. Patients with poor engraftment might have a lower threshold for drug toxicity.

The influence of elevated serum creatinine on the hazard of neutropenia raises concern about adequate dose adjustments. Although ganciclovir dosing was adjusted to calculated creatinine clearance on a daily basis, patients may already have been exposed to higher drug levels by the time of adjustment. A correlation between maximum levels of serum creatinine and serum bilirubin existed ($r = .42$, $P < .001$), linking renal failure to impaired hepatic function. Therefore, patients with elevated serum creatinine might well have had impaired tubular secretion before development of overt renal impairment.

To determine whether patients can be identified as at risk for development of neutropenia before initiation of ganciclovir treatment, the model was further restricted to factors only present at or before engraftment. Whereas early hyperbilirubinemia and low marrow cellularity remained significant with virtually unchanged risks, elevated serum creatinine in the early transplant period became additionally significant. Rates of subsequent neutropenia of less than 750/μL increased from 21% without any, to 57% for two or more risk factors. Thus these patients may represent a subgroup that might benefit from alternative treatment strategies.

Neutropenia was found to be an independent risk factor for poor long-term outcome. This was shown as a negative effect on overall survival and nonrelapse mortality. In addition, in patients with acute leukemias, neutropenia was statistically associated with relapse. As relapse occurred early (median 91 days after transplant) in this group, it seems unlikely that neutropenia in these patients was due to ganciclovir at all. Instead, in patients with acute leukemias and a high risk of relapsing disease, neutropenia might be an early sign of marrow suppression by expanding residual disease.

Surprisingly, neither CMV disease nor subclinical CMV infection diagnosed by positive antigenemia or positive CMV DNA-PCR were associated with a higher rate of neutropenia and neither marker was a risk factor for neutropenia. Marrow suppression and especially neutropenia can be associated with CMV-infection[21]; however, the proportion of CMV infections causing neutropenia might have been too low to be identified as an independent factor in this study.

In conclusion, this study has identified three risk factors for ganciclovir-related neutropenia in allogeneic marrow transplant recipients, ie, low marrow cellularity, early hyperbilirubinemia, and elevated serum creatinine. Importantly, neutropenia during the first 100 days after transplant is an independent predictor for overall survival and nonrelapse mortality. In patients with acute leukemia, neutropenia may also be a sign of early relapse. These results have important implications. First, patients who have two or more risk factors at engraftment may be good candidates for alternative antiviral strategies such as alternative dose regimens of ganciclovir, antivirals without marrow and renal toxicity, or concomitant use of growth factors. An evaluation of these strategies is warranted. The renal toxicity associated with the use of foscarnet and cidofovir may limit its use in these patients too. Second, pharmacokinetic studies are required to determine whether patients with VOD and/or subclinical tubular damage have an altered ganciclovir clearance that leads to toxic drug levels. Therapeutic drug monitoring and targeted ganciclovir dosing in high-risk patients may be required and might significantly reduce the incidence of neutropenia after marrow transplantation. Although empiric low-dose maintenance regimens of ganciclovir (eg, 5 mg/kg 3 times per week) seem to be associated with less marrow suppression and may be effective in patients without acute GVHD or in non—T cell–depleted transplant recipients, these regimens have led to a high incidence of CMV disease in patients after an unrelated or T-cell–depleted transplant.[32-34] Thus, drug monitoring may be required in these patients for optimal antiviral activity and minimal toxicity. Finally, because an ANC of less than 1,000/μL is predictive for both progression of neutropenia and mortality, ganciclovir should be discontinued when the ANC reaches 1,000/μL rather than at an ANC of 750/μL.

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REFERENCES

1. Goodrich JM, Mori M, Gleaves CA, Du Mond C, Cays M, Ebeling DF, Buhles WC, DeArmond B, Meyers JD: Early treatment

with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. N Engl J Med 325:1601, 1991

2. Schmidt GM, Horak DA, Niland JC, Duncan SR, Forman SJ, Zaia JA: A randomized, controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants; The City of Hope-Stanford-Syntex CMV Study Group. N Engl J Med 324:1005, 1991

3. Goodrich JM, Bowden RA, Fisher L, Kelley C, Schoch G, Meyers JD: Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. Ann Intern Med 118:173, 1993

4. Winston DJ, Ho WG, Bartoni K, Du Mond C, Ebeling DF, Buhles WC, Champlin RE: Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients. Results of a placebo-controlled, double-blind trial. Ann Intern Med 118:179, 1993

5. Einsele H, Ehninger G, Hebart H, Wittkowski KM, Schuler U, Jahn G, Mackes P, Herter M, Klingebiel T, Löffler J, Wagner S, Müller CA: Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood 86:2815, 1995

6. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA: Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation—A randomized double-blind study. Blood 88:4063, 1996

7. Anonymous: Morbidity and toxic effects associated with ganciclovir or foscarnet therapy in a randomized cytomegalovirus retinitis trial. Studies of ocular complications of AIDS Research Group, in collaboration with the AIDS Clinical Trials Group. Arch Intern Med 155:65, 1995

8. Merigan TC, Renlund DG, Keay S, Bristow MR, Starnes V, O'Connell JB, Resta S, Dunn D, Gamberg P, Ratkovec RM, Richenbacher WE, Millar RC, DuMond C, De Amond B, Sullivan V, Cheney T, Buhles W, Stinson EB: A controlled trial of ganciclovir to prevent cytomegalovirus disease after heart transplantation. N Engl J Med 326:1182, 1992

9. Winston DJ, Wirin D, Shaked A, Busuttil RW: Randomised comparison of ganciclovir and high-dose acyclovir for long-term cytomegalovirus prophylaxis in liver-transplant recipients. Lancet 346:69, 1995

10. Duncan SR, Grgurich WF, Iacono AT, Burckart GJ, Yousem SA, Paradis IL, Williams PA, Johnson BA, Griffith BP: A comparison of ganciclovir and acyclovir to prevent cytomegalovirus after lung transplantation. Am J Respir Crit Care Med 150:146, 1994

11. Sommadossi J-P, Carlisle R: Toxicity of 3′-azido-3′-deoxythymidine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine for normalhuman hematopoietic progenitor cells in vitro. Antimicrob Agent Chemother 31:452, 1987

12. Buckner CD, Clift RA, Appelbaum FR, Storb R, Petersen FB, Sanders JE, Thomas ED, Hansen JA: Effects of treatment regimens in patients allografted for acute and chronic myelogenous leukemia. Bone Marrow Transplant 7:6, 1992 (suppl 2)

13. Clift RA, Buckner CD, Thomas ED, Bensinger WI, Bowden R, Bryant E, Deeg HJ, Doney KC, Fisher LD, Hansen JA, Martin P, McDonald, Sanders JE, Schoch G, Singer J, Storb R, Sullivan KM, Witherspoon RP, Appelbaum FR: Marrow transplantation for chronic myeloid leukemia: A randomized study comapring cyclophosphamide and total body irradiation with busulfan and cyclophosphamide. Blood 84:2036, 1994

14. Lynch MH, Peterson FB, Appelbaum FR, Bensinger WI, Clift RA, Storb R, Sanders JE, Hansen JA, Buckner CD: Phase II study of busulfan, cyclophosphamide and fractionated total body irradiation as a preparatory regimen for allogeneic bone marrow transplantation in patients with advanced myeloid malignancies. Bone Marrow Transplant 15:59, 1995

15. Deeg HJ, Lin D, Leisenring W, Boeckh M, Anasetti C, Appelbaum FR, Chancey TR, Doney K, Flowers M, Martin P, Nash R, Schoch G, Sullivan KM, Witherspoon RP, Storb R: Cyclosporine or cyclosporine plus methylprednisolone for prophylaxis of graft-versus-host disease—A prospective, randomized trial. Blood 89:3880, 1997

16. Storb R, Leisenring W, Deeg HJ, Anasetti C, Appelbaum F, Bensinger W, Buckner CD, Clift RA, Doney K, Hansen J, Martin P, Sanders J, Stewart P, Sullivan K, Thomas ED, Witherspoon R: Long-term follow-up of a randomized trial of graft-versus-host disease prevention by methotrexate/cyclosporine versus methotrexate alone in patients given marrow grafts for severe aplastic anemia. Blood 83:2749, 1994 (letter)

17. Przepiorka D, Weisdorf D, Martin P, Klingemann H-G, Beatty P, Hows J, Thomas ED: Consensus conference on acute GVHD grading. Bone Marrow Transplant 15:825, 1995

18. Atkinson K, Horowitz MM, Gale RP, Lee MB, Rimm AA, Bortin MM: Consensus among bone marrow transplanters for diagnosis, grading and treatment of chronic graft-versus-host disease. Committee of the International Bone Marrow Transplant Registry. Bone Marrow Transplant 4:247, 1989

19. Hartsock RJ, Smith EB, Petty CS: Normal variations with aging of the amount of hematopoietic tissue in bone marrow from the anterior iliac crest. Am J Clin Pathol 43:326, 1965

20. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. Nephron 16:31, 1976

21. Torok-Storb B, Fries B, Myerson D: Cytomegalovirus mediated cytopenias, in Guignon M (ed): The Negative Regulation of Hematopoiesis, vol 229. John Libbey Eurotext Ltd, 1993, p 383

22. Sayer HG, Longton G, Bowden R, Pepe M, Storb R: Increased risk of infection in marrow transplant patients receiving methylprednisolone for graft-versus-host disease prevention. Blood 84:1328, 1994

23. Bowden RA, Cays M, Gooley T, Mamelok R, van Burik J-A: Phase I study of amphotericin B colloidal dispersion for the treatment of invasive fungal infections after marrow transplant. J Infect Dis 173:1208, 1996

24. Kalbfleisch JD, Prentice RL: The statistical analysis of failure time data. New York, NY, Wiley, 1980

25. Pepe MS, Longton GM, Pettinger M, Mori M, Fisher LD, Storb R: Summarizing data on survival, relapse and chronic graft-versus-host disease after bone marrow transplantation: Motivation for and description of new methods. Br J Haematol 83:602, 1993

26. SPSS I: SPSS 6.1.1. Chicago, SPSS Inc, 1995

27. McDonald GB, Hinds MS, Fisher LD, Schoch HG, Wolford JL, Banaji M, Hardin BJ, Shulman HM, Clift RA: Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: A cohort study of 355 Patients. Ann Intern Med 118:255, 1993

28. Fink JC, Cooper MA, Burkhart KM, McDonald GB, Zager RA: Marked enzymuria after bone marrow transplantation: A correlate of veno-occlusive disease induced ''hepatorenal syndrome''. J Am Soc Nephrol 6:1655, 1995

29. Fletcher C, Sawchuck R, Chinnock B, de Miranda P, Balfour HH: Human pharmakokinetics of the antiviral drug DHPG. Clin Pharmacol Ther 40:281, 1986

30. Yuen GJ, Drusano GL, Fletcher C, Capparelli E, Connor JD, Lalezari JP, Drew L, Follansbee S, Busch D, Jacobson M, Spector SA, Squires K, Buhles W: Population Differences in Ganciclovir Clearance as Determined by Nonlinear Mixed Effects Modelling. Antimicrob Agents Chemother 39:2350, 1995

31. Shepp DH, Dandliker PS, de Miranda P, Burnette TC, Cederberg DM, Kirk LE, Meyers JD: Activity of 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine in the treatment of cytomegalovirus pneumonia. Ann Intern Med 103:368, 1985

32. Atkinson K, Downs K, Golenia M, Biggs J, Marshall G, Dodds A, Concannon A: Prophylactic use of ganciclovir in allogeneic bone marrow transplantation: Absence of clinical cytomegalovirus infection. Br J Haematol 79:57, 1991

33. Przepiorka D, Ippoliti C, Panini A, Goodrich J, Giralt S, van Besien K, Mehra R, Deisseroth AB, Andersson B, Luna M, Tarrand JJ, Champlin RE: Ganciclovir three times per week is not adequate to prevent cytomegalovirus reactivation after T cell-depleted marrow transplant. Bone Marrow Transplant 13:461, 1994

34. Atkinson K, Arthur C, Bradstock K, Dale B, Downs K, Gibson J, Golenia M, Ho J, Joshua D, Juttner C, Shaw P, Sheridan W, Szer J, Vowels M and the Australasian Bone Marrow Transplant Study Group: Prophylactic ganciclovir is more effective in HLA-identical family member marrow transplant recipients than in more heavily immune-suppressed HLA-identical unrelated donor marrow transplant recipients. Australasian Bone Marrow Transplant Study Group. Bone Marrow Transplant 16:401, 1995