Prior gemtuzumab ozogamicin exposure significantly increases the risk of veno-occlusive disease in patients who undergo myeloablative allogeneic stem cell transplantation

Martha Wadleigh, Paul G. Richardson, David Zahrrieg, Stephanie J. Lee, Corey Cutler, Vincent Ho, Edwin P. Alyea, Joseph H. Antin, Richard M. Stone, Robert J. Soiffer, and Daniel J. DeAngelo

Gemtuzumab ozogamicin (GO), a monoclonal antibody used in the treatment of acute myelogenous leukemia (AML), has been linked to the development of veno-occlusive disease (VOD). We conducted a retrospective study of 62 patients with previously treated AML/MDS (myelodysplastic syndrome) who underwent allogeneic stem cell (SC) transplantation at our institution from December 2000 to October 2002 to determine whether GO exposure prior to allogeneic SC transplantation increases the risk of developing VOD.

Fourteen patients received GO prior to SC transplantation. Of 62 patients, 13 (21%) developed VOD; 9 (64%) of 14 with prior GO exposure developed VOD compared with 4 (8%) of 48 without prior GO exposure (P < .0001). Logistic regression controlling for sex, disease status, donor type, and graft-versus-host disease prophylaxis identified prior treatment with GO as a significant risk factor for VOD (odds ratio [OR], 21.6; 95% confidence interval [CI], 4.2-112.2). Nine of 10 patients who underwent SC transplantation 3.5 months or less following GO developed VOD compared with none of 4 patients who underwent SC transplantation more than 3.5 months from GO administration. Three of 14 patients who received GO prior to SC transplantation died of VOD. We conclude that patients undergoing SC transplantation within a short interval from GO administration are at increased risk of developing VOD.

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Veno-occlusive disease. VOD was diagnosed using the standard Baltimore clinical criteria of hyperbilirubinemia (bilirubin, > 34.2 µM [> 2 mg/dL]),
accompanied by 2 or more of the following: painful hepatomegaly, fluid retention or ascites, or sudden weight gain (> 5% of baseline weight) in the absence of another explanation. In 10 of 13 patients the diagnosis was supported by pathologic evidence of VOD on liver biopsy or autopsy. Of the remaining 3 patients, all met clinical criteria for VOD, and 1 had evidence of reversal of portal flow on Doppler ultrasound, with no clinical evidence of another causative condition in such patients of graft-versus-host disease (GVHD). Severe VOD was defined prospectively by a risk of 30% or more if the patient was addressed by the Bearman model⁶ or the presence of multigene failure (MOF), which was defined as organ dysfunction in one other system in addition to the liver with renal dysfunction (doubling of baseline creatinine level and/or dialysis dependence), or both; oxygen requirement (oxygen saturation < 90%, ventilatory support, or both); or encephalopathy.⁶

**Time from GO to SC transplantation.** Time from GO to SC transplantation was defined as the number of months between the last dose of GO given and day 0 of SC transplantation. If the exact date of GO administration could not be determined, then the middle of the month it was administered was estimated as the date of GO administration.

**Outcome of VOD**

Resolution of VOD was defined as bilirubin level less than 34.2 μM (< 2 mg/dL), and resolution of VOD-associated symptoms and signs, with discontinuation of analgesics and diuretics, by day +100 following SC transplantation. Time of onset of VOD was defined as the time when a patient met clinical criteria for VOD. Survival at day +100 from SC transplantation as well as death as a result of VOD was also documented.

**Statistical methods**

The primary end point was the incidence of VOD. The outcome for the patients with prior GO exposure was compared with that for patients without prior GO exposure, first in univariate analyses and then in multivariable logistic regression analyses. Odds ratios (ORs), 95% confidence intervals, and P values were estimated. The Wald test was used to evaluate significance of the explanatory variables as predictors of developing VOD. Factors included in the regression analyses were sex, disease status, donor type, and GVHD prophylaxis. We explored all the logistic regression models that included prior GO exposure, 1 of the 4 factors, and the 2-way interaction between that factor and prior GO exposure. Our goal was to ascertain whether there were factors for which the magnitude of the effect of prior GO exposure differs according to the level of the factor.

Associations between categorical variables were assessed by a Fisher exact test. The Wilcoxon rank-sum test was used for testing differences between continuous variables. The methods of Kaplan and Meier were used to estimate survival curves, and significance of difference between curves was tested using a log-rank test. Two-sided P value less than .05 was considered to be statistically significant. No adjustment was made for performing multiple tests.

**Results**

**Patient characteristics**

Between December 2000 and October 2002, 62 patients with previously treated AML or AML arising from MDS underwent allogeneic SC transplantation at the Dana-Farber Cancer Institute/Brigham and Women’s Hospital. Clinical characteristics of the patients are shown in Table 1. There were 31 women and 31 men, with a median age of 43 years (range, 18-57 years). At the time of SC transplantation, 20 patients were in their first complete response (CR), whereas 42 had more advanced disease (second CR, primary refractory, or active, relapsed AML). Thirty-eight patients had human leukocyte antigen (HLA)–matched sibling donors; 24 had alternative donors (17 matched unrelated, and 7 mismatched). GVHD prophylaxis included tacrolimus plus depletion of CD8+ T cells in 16 patients, whereas the remaining 46 patients received tacrolimus in conjunction with methotrexate or sirolimus or both. All but 3 patients were conditioned with cyclophosphamide (Cy) and total body irradiation (TBI). The others received busulphan and cyclophosphamide (Bu/Cy). There was no statistically significant difference in baseline characteristics between the group of patients who received GO and those who had not. Fourteen patients received GO prior to SC transplantation (7 for de novo disease and 7 for relapsed disease). Eight patients received GO as a single dose of either 6 mg/m² (n = 6) or 9 mg/m² (n = 2) on day 4 in combination with daunomycin and cytarabine. The remaining 6 patients received GO as a single agent for relapsed disease at the Food and Drug Administration (FDA)–approved dose and schedule of 9 mg/m² days 1 and 15 (n = 4), or day 1 only (n = 2). Of the 14 GO-treated patients, data on infusional hepatotoxicity was available on 12. None of the 14 patients had grade 2 or greater bilirubin abnormalities, and 4 patients had grade 2 or greater AST and/or alanine aminotransferase (ALT) abnormalities. None of the patients who had received GO had AML arising from MDS. A slightly higher proportion of patients in the GO group had unrelated or mismatched donors compared with those patients in the non-GO group (57% versus 33%, P = .12). The median interval between GO and SC transplantation for the 14 patients was 3.2 months (range, 0.7-17.6 months). Baseline liver function tests were similar in the 2 groups prior to transplantation.

**Incidence of VOD**

Thirteen (21%) of 62 patients were diagnosed with VOD. In 10 of 13 patients the diagnosis was supported by pathologic evidence of VOD on liver biopsy or at autopsy. Of the remaining 3 patients, all met clinical criteria for VOD, and 1 had evidence of reversal of portal flow on Doppler ultrasound, with no clinical evidence of
Table 2. Univariate analysis of risk factors for VOD

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>VOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes, n</td>
</tr>
<tr>
<td>GO exposure</td>
<td>9</td>
</tr>
<tr>
<td>relapsed/active disease</td>
<td>8</td>
</tr>
<tr>
<td>unrelated or mismatched donor</td>
<td>8</td>
</tr>
<tr>
<td>non-T-cell depleted</td>
<td>5</td>
</tr>
<tr>
<td>female</td>
<td>6</td>
</tr>
</tbody>
</table>

NS indicates not significant.
*P < .05 are deemed statistically significant. P value determined by Fisher exact test.

After controlling for all 4 factors of sex, relapsed/active disease, mismatched/unrelated donor, and CD8 T-cell depletion, the only factor associated with VOD development was GO, with an odds ratio of 21.6 (95% confidence interval [CI], 4.2-112.2). This finding is slightly higher than what was observed in the univariate analysis. None of the other 4 factors were statistically significant in the presence of each other.

Clinical characteristics and outcome of VOD

The clinical presentation of VOD was similar whether or not a patient had prior exposure to GO (Table 3), except that the median peak bilirubin level for the GO group was lower than those who had not received GO (196.65 μM [range, 22.23-501.03 μM] [11.5 mg/dL; range, 1.3-29.3 mg/dL] vs 576.27 μM [range, 220.59-1185.03 μM] [33.7 mg/dL; range, 12.9-69.3 mg/dL]), but this did not achieve significance (*P = .10). Similarly, there was a trend toward a shorter time of onset of VOD in the 9 patients who received GO compared with the 4 who did not (median, day +13 [range, day 7-21] versus day +22 [range, day 10-27]; *P = .06). However, median day of onset of VOD for all 13 patients was day +15 after SC transplantation (range, day 7-27).

We attempted to determine which factors would be predictive of the development of VOD in GO-treated patients. Neither dose of GO nor the presence of grade 2 or greater AST or ALT abnormality was predictive of VOD. Timing of SC transplantation in relation to GO exposure appeared to be an important factor for those who developed VOD. For the 9 patients who had received GO and developed VOD, the median time between GO administration and SC transplantation was 2.3 months (range, 0.7-3.5 months) versus 4.1 months (range, 3.1-17.6 months) for the 5 patients who did not develop VOD (*P = .007, by Wilcoxon rank-sum test). Nine of 10 patients who underwent SC transplantation 3.5 months or less from GO development died of VOD, whereas none of the 4 patients who underwent transplantation 3.5 months or less from GO development died of VOD, whereas none of the 4 patients who underwent transplantation more than 3.5 months from GO administration developed VOD.

Table 3. Clinical characteristics and outcomes of patients with VOD

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y/sex</th>
<th>Status at conditioning</th>
<th>GO exposure</th>
<th>Clinical features</th>
<th>Biopsy proven</th>
<th>Delbrotide therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51/F</td>
<td>Active relapse</td>
<td>Yes (relapse)</td>
<td>Elevated bilirubin; RUQ pain; weight gain</td>
<td>No</td>
<td>No</td>
<td>Died of leukemia</td>
</tr>
<tr>
<td>2</td>
<td>45/M</td>
<td>1st CR</td>
<td>No</td>
<td>Elevated bilirubin; RUQ pain; weight gain; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died of VOD/MOF</td>
</tr>
<tr>
<td>3</td>
<td>20/M</td>
<td>Active relapse</td>
<td>Yes (induction)</td>
<td>Elevated bilirubin; RUQ pain; weight gain; reversal of portal flow on US; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Alive; recovered</td>
</tr>
<tr>
<td>4</td>
<td>45/M</td>
<td>1st CR</td>
<td>Yes (induction)</td>
<td>Elevated bilirubin; weight gain; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Alive; recovered</td>
</tr>
<tr>
<td>5</td>
<td>19/F</td>
<td>Active relapse</td>
<td>Yes (relapse)</td>
<td>Elevated bilirubin; hepatomegaly; RUQ pain; weight gain</td>
<td>No</td>
<td>No</td>
<td>Died of leukemia</td>
</tr>
<tr>
<td>6</td>
<td>45/F</td>
<td>2nd CR</td>
<td>Yes (relapse)</td>
<td>Elevated bilirubin; RUQ pain; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died of VOD/MOF</td>
</tr>
<tr>
<td>7</td>
<td>48/M</td>
<td>Primary refractory</td>
<td>No</td>
<td>Elevated bilirubin; hepatomegaly; RUQ pain; weight change; reversal of portal flow on US; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died of VOD/MOF</td>
</tr>
<tr>
<td>8</td>
<td>47/F</td>
<td>1st CR</td>
<td>Yes (induction)</td>
<td>Elevated bilirubin; hepatomegaly; RUQ pain; weight change; reversal of portal flow on US; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died of VOD/MOF</td>
</tr>
<tr>
<td>9</td>
<td>43/M</td>
<td>Primary refractory</td>
<td>Yes (relapse)</td>
<td>Elevated bilirubin; RUQ pain; weight change; reversal of portal flow on US; MOF</td>
<td>No</td>
<td>Yes</td>
<td>Died of VOD/MOF</td>
</tr>
<tr>
<td>10</td>
<td>42/F</td>
<td>2nd CR</td>
<td>Yes (relapse)</td>
<td>Elevated bilirubin; hepatomegaly; RUQ pain; weight gain; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died of GVHD</td>
</tr>
<tr>
<td>11</td>
<td>24/F</td>
<td>Primary refractory</td>
<td>No</td>
<td>Elevated bilirubin; RUQ pain; Weight gain</td>
<td>No</td>
<td>No</td>
<td>Died of diffuse alveolar hemorrhage</td>
</tr>
<tr>
<td>12</td>
<td>43/M</td>
<td>1st CR</td>
<td>Yes (induction)</td>
<td>Hepatomegaly; ascites; RUQ pain; weight change; reversal of flow on US; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died of VOD/MOF</td>
</tr>
<tr>
<td>13</td>
<td>47/M</td>
<td>1st CR</td>
<td>No</td>
<td>Elevated bilirubin; hepatomegaly; RUQ pain; reversal of flow on US; MOF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died of VOD/MOF</td>
</tr>
</tbody>
</table>

Elevated bilirubin indicates > 2 mg/dL of baseline; RUQ, right upper quadrant; weight gain, > 5% of baseline; MOF, multiorgan failure; and US, ultrasound/Doppler.
Survival at 100 days after SC transplantation for the entire cohort was 73%. Day +100 survival was lower for those patients who had received GO prior to SC transplantation compared with those who had not (46% versus 81%, \( P = .003 \)). Six (46%) of 13 patients diagnosed with VOD died as a consequence of VOD. VOD-related mortality for patients with a prior exposure to GO was 21% (3 of 14).

Ten (7 with and 3 without prior GO treatment) of 13 patients with severe VOD and MOF were treated with delibrotide (DF) as part of a large randomized, multicenter prospective phase 2 trial to determine an optimal dose of DF for patients with severe VOD and MOF. Three (42%) of 7 patients with prior GO exposure achieved complete response (defined as bilirubin level < 34.2 \( \mu \)M [< 2 mg/dL] and resolution of VOD-related symptoms, signs, and MOF) with DF therapy, compared with none of those without prior GO exposure, and all of these patients had received GO as part of induction chemotherapy.

Discussion

Our results indicate that patients exposed to GO prior to SC transplantation are at increased risk of developing VOD. After logistic regression, controlling for multiple variables such as sex, disease status, unrelated or mismatched donor, and GVHD prophylaxis, the relationship between prior GO exposure and VOD remained statistically significant.

High rates of VOD (47%) have been documented in patients who received GO for relapse after SC transplantation. However, for patients receiving GO prior to SC transplantation, Stevens et al.\(^{17}\) reported a VOD rate of only 17%, but in that series 16 of 46 patients had received autologous SC transplantation, which is associated with a lower incidence of VOD.\(^{8,10}\) All of the patients in our cohort underwent allogeneic SC transplantation, and nearly all were conditioned with Cy/TBI.

The higher rate of GO-associated VOD seen in our study cannot be attributed to patients with more advanced AML. Five patients in their first CR developed VOD during SC transplantation. Three of these patients received GO as part of induction chemotherapy for de novo disease. Seven patients received GO during their induction chemotherapy. After controlling for active or relapsed disease, the adjusted odds of developing VOD with a prior exposure to GO was still extremely high with an OR of 21. More patients in the GO group had matched unrelated or mismatched donors, but on univariate analysis, as well as in logistic regression, this was not a significant predictor of VOD in our cohort.

VOD is a clinical syndrome characterized by hyperbilirubinemia, jaundice, painful hepatomegaly, and/or fluid retention with ascites, encephalopathy, and MOF, as later events are typically seen in severe disease.\(^{11}\) In VOD, the exact pathogenesis is incompletely understood, but injury to the sinusoidal endothelial cells (SECs) and hepatocytes appear to be key initial events with subsequent damage to the central veins in zone 3 of the liver acinus characteristic of the syndrome.\(^{12,13}\) Fibrinogen, factor VIII, and fibrin are deposited within venular walls and sinusoids; subendothelial edema occurs; and stellate cells proliferate, triggering intense fibrosis.\(^{14}\) All of this leads to sinusoidal obstruction, hepatocellular necrosis, centrlobular congestion, and venular occlusion, which ultimately results in portal hypertension and the clinical syndrome of VOD.\(^{14}\) Animal studies in a rat model of VOD have demonstrated that the earliest changes occur in the sinusoids of the liver.\(^{15}\)

In this rat model, the first event appears to be rounding up of the SECs, which allows for red blood cells in the space of Disse to flow under the SEC and dissect them from the parenchymal cells.\(^{15}\) These SEC cells then embolize and obstruct sinusoidal blood flow.\(^{15}\) Moreover, this effect is abrogated by intraportal administration of glutathione.\(^{15,16}\) and it is noteworthy that glutathione depletion in the SECs by conditioning regimens or other agents may also be involved in the pathogenesis of human VOD.\(^{12}\) In GO-related VOD, sinusoidal obstruction and fibrosis are especially marked, and thus the term sinusoidal obstructive syndrome (or SOS) has been proposed for this variant, but the mechanism of GO’s toxicity on the liver is yet to be elucidated.\(^{3,17}\)

Calicheamicin is a cytotoxic enediyene antibiotic. Once internalized in cells it binds to DNA in the minor groove, causing DNA double-strand breaks and cell death.\(^{18}\) In preclinical studies, free calicheamicin caused liver toxicity in animals, and GO has been shown to be distributed within the liver parenchyma.\(^{19}\) However, a direct toxic effect on hepatocytes and sinusoidal endothelium by calicheamicin is unlikely, as the concentration of unconjugated calicheamicin derivatives in the final drug product is less than 20 \( \mu \)g/dose, which is below the level of toxicity observed in preclinical studies.\(^{3,20}\) Another possible explanation for GO-related hepatotoxicity is receptor-mediated targeting of CD33\(^+\) cells in the liver. Studies have shown that CD33 is indeed expressed in the liver on hematopoietic progenitors during fetal development,\(^{21}\) but other studies have not found it to be present in adult hepatocytes or in normal SECs.\(^{22}\) Kupffer cells and stellate cells may also be targets of GO, especially the former, and future studies will be important in defining the respective roles of these components and their contribution to hepatic injury.\(^{21,22}\) Finally, the role of GO on the depletion of glutathione in SECs is unknown, but there is evidence that increased glutathione results in less DNA damage inflicted by calicheamicin, suggesting a possible link.\(^{23}\)

The risk of VOD associated with GO has significant implications for treatment-related decisions. Our data suggest that careful consideration should be given prior to administration of GO for de novo AML, in patients likely to proceed to SC transplantation in first remission. Moreover, those patients with a shorter interval between GO administration and SC transplantation had a higher likelihood of developing VOD. Thus, the use of GO for relapsed disease just prior to SC transplantation as a temporizing measure may result in increased risk of hepatic injury. Strategies should be developed to minimize the incidence of VOD in these patients. These strategies may include less intensive ablative regimens and prophylaxis against VOD with agents such as n-acetylcysteine with the goal of replenishing hepatocyte glutathione,\(^{24}\) ursodiol,\(^{25}\) or DF.\(^{26,28}\)

DF is of particular interest in this context.\(^{29}\) DF is an adenosine receptor agonist which has anti thrombotic, anti-ischemic, and thrombolytic properties, with selective activity for small vessels demonstrated in a lipopolysaccharide (LPS)–mediated microvascular injury model and no significant systemic anticoagulant effects.\(^{30,32}\) Clinical studies have shown significant DF activity in patients with severe VOD and MOF after SC transplantation.\(^{26,28}\) In the largest reported multicenter study to date, DF treatment achieved a CR rate of 36% with a 35% day +100 survival in 88 patients with severe VOD and MOF (versus < 10% expected) without significant toxicity.\(^{30}\) In our study, 3 patients who had a prior GO exposure and developed severe VOD and MOF achieved CR with protocol-directed DF therapy. In contrast, the 3 patients who developed severe VOD and MOF without prior exposure to GO did not respond to treatment with DF. This raises the question as to whether there is a difference between GO-mediated VOD
versus non-GO-mediated VOD, perhaps through a predominance of sinusoidal injury and SOS in GO-exposed patients, in which DF’s activity at the SEC level may potentially be greatest.\textsuperscript{34} DF could thus have a role in the treatment of GO-associated VOD as therapy for the established clinical syndrome, or as prophylaxis, as has been tried in Europe with reported success (R. Chopra, personal communication, September 25, 2002).

In conclusion, our data suggest that prior exposure to GO significantly increases the risk of VOD in subsequent SC transplantation. VOD was pathologically documented by liver biopsy in most of the cases in this study. We found that if patients underwent SC transplantation 3.5 months or less from GO exposure, they were more likely to develop VOD. Furthermore, VOD resulted in death in 3 of 9 patients with prior GO exposure. Therefore, we advise caution in using GO in patients with AML immediately prior to SC transplantation. Given the impressive antileukemic activity of GO, further study is warranted to elucidate the mechanism of hepatic injury by GO, as well as to determine effective strategies to minimize the development of this potentially fatal complication in this high-risk group of patients.

References