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Review

Defibrotide, a Polydisperse Mixture of Single-stranded Phosphodiester Oligonucleotides with Lifesaving Activity in Severe Hepatic Veno-occlusive Disease: Clinical Outcomes and Potential Mechanisms of Action

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ABSTRACT

Veno-occlusive disease of the liver (VOD) remains a troubling and potentially fatal complication of high-dose chemotherapy and hematopoietic stem cell transplantation conditioning regimens. No effective therapy has been available for these patients to date, and the best supportive care measures remain woefully inadequate. Defibrotide (DF) (Gentium, S.p.A., Como, Italy), a polydisperse mixture of all the single-stranded phosphodiester oligodeoxyribonucleotides that can be obtained from the controlled depolymerization of porcine intestinal mucosal genomic DNA, seems to offer a safe and effective treatment for some patients suffering from severe VOD, a condition for which no accepted standard therapy currently exists. Early clinical studies evaluating the efficacy of DF for the treatment of severe VOD in patients undergoing hematopoietic stem cell transplantation have been very encouraging. Approximately 45% of the patients treated in multiple initial phase II clinical trials achieved a complete response at day +100, demonstrating normalization of serum bilirubin and resolution of the clinical syndrome. However, although multi-institutional, these represented single arm studies. A large, FDA-approved, pivotal, prospective, multi-institutional, global phase III trial of DF vs. historical controls (best available therapy) commenced in the first quarter of 2006 and should provide further validation of DF's efficacy. The drug seems to have few significant side effects, and almost all test subjects who have received this treatment have tolerated it well. Although the mechanism of action remains unclear, the drug exerts minimal systemic anticoagulant effects yet appears to induce numerous antithrombotic and profibrinolytic effects both *in vitro* and *in vivo*. It may function as an adenosine receptor agonist and causes increased concentrations of endogenous prostaglandins, which modulate thrombomodulin, platelets, and fibrinolysis. It also appears to block lipopolysaccharide (LPS)-induced tissue factor (TF) expression. However, despite the fact the DF is composed of oligonucleotides, its mechanism of action, which at the present time is unclear, is not related to Watson-Crick base pair-dependent downregulation of gene expression but is rather likely a result of its polyanionic nature.

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INTRODUCTION

HEPATIC VENO-OCCLUSIVE DISEASE (VOD) represents a clinicopathologic entity initially described in the beginning of the last century (Wilmot and Robertson, 1920). Early cases involved toxic ingestion of certain bush teas and foods rich in pyrrolizidine alkaloids (DeLeve et al., 2002). With the introduction of modern chemotherapy, immunosuppressive drugs, and drug combinations, case reports of VOD increased markedly (Katzka et al., 1986). Today, VOD remains a serious and often life-threatening regimen-related toxicity of hematopoietic stem cell transplantation (SCT). The incidence reported in the transplant literature, in the range of 10%–60%, appears highly variable, possibly because of the clinical nature of the diagnosis (Richardson and Guinan, 2001; Wadleigh et al., 2003).

Numerous chemotherapeutic drugs, biologic agents, and immunosuppressive medications may cause VOD at conventional doses. These include gemtuzumab ozogamicin (Mylotarg, Wyeth Pharmaceuticals, Madison, N.J.), actinomycin D, dacarbazine, cytosine arabinoside, mithramycin, 6-thioguanine, and certain chemotherapy combinations (Boula et al., 2005; DeLeve et al., 2002).

High-dose cytoreductive conditioning regimens used during SCT and bone marrow transplant (BMT) protocols, often in combination with total body irradiation (TBI), remain the most common cause of VOD (DeLeve et al., 2002; Bearman, 1995). High doses of cyclophosphamide or busulphan appear to pose the greatest risk for development of VOD.

CLINICAL FEATURES OF VOD

Patients undergoing either allogeneic or autologous hematopoietic SCT remain at risk for developing hepatic VOD. Patients typically experience painful hepatomegaly, jaundice, and fluid retention, often with rapid and substantial weight gain (McDonald et al., 1984).

Onset generally begins within the first 30 days after SCT, although delayed development may occur up to 100 days after therapy (Lee et al., 1999). Severity ranges from mild reversible disease to a severe life-threatening syndrome (McDonald et al., 1993). Extreme renal sodium avidity may develop early in severe cases of VOD. These patients frequently develop acute oliguric renal failure, that may progress to the hepatorenal syndrome, leading to multisystem organ failure and death (McDonald et al., 1993).

A number of clinical characteristics appear to increase an individual's risk of developing hepatic VOD. These include older age, poor performance status, female sex, advanced disease, prior radiotherapy, and pretreatment elevations in aspartate aminotransferase (AST) level and HLA disparity between allograft donor and recipient (Carreras et al., 1998; McDonald et al., 1993; Bearman, 1995). Treatment with norethisterone, a medication frequently used to suppress menses in women undergoing SCT (where risk of significant uterine bleeding secondary to chemotherapy-induced thrombocytopenia exists), also appears to dramatically increase the risk for developing hepatic VOD in the posttransplant setting.

DIAGNOSIS OF VOD

The diagnosis of VOD requires the ability to differentiate this clinical entity from others that share similar features. Examples include acute graft-versus-host disease (GVHD), congestive heart failure, infectious and noninfectious hepatitis, hepatic venous thrombosis, intraabdominal sepsis, and several other clinical conditions that may arise in the post-SCT period (Ho et al., 2004; Baglin, 1994; Rollins, 1986).

Laboratory test abnormalities, when present, are generally nonspecific. Increased serum levels of bilirubin and alanine aminotransferase (ALT) occur frequently (Baglin, 1994). The rise in serum plasminogen activator inhibitor-1 (PAI-1) levels parallels the initial rise in serum bilirubin. PAI-1 levels frequently increase more than 10-fold compared with pretreatment levels (M. Pihusch et al., 2005). Researchers evaluated the utility of PAI-1 levels for confirmation of the diagnosis of VOD. Using a cutoff level of 200 ng/mL yielded a sensitivity of PAI-1 of 85.7% for diagnosis of VOD, with a specificity of 100% (Salat et al., 1997). Although it remains unclear if PAI-1 plays a role in the pathogenesis of VOD, recently published experimental evidence using a novel murine model of VOD provides evidence that it might do so (Smith et al., 2006). Additionally, though PAI-1 levels predictably rise as part of the clinical spectrum of VOD, the clinical diagnosis of VOD does not require determination of PAI-1 levels.

Modern radiologic imaging techniques have contributed to our understanding of the complex process of VOD but are generally not a vital part of the diagnostic evaluation. Liver ultrasound or CT scan imaging typically reveals a congested liver, ascites, and enlarged portal veins and collateral vessels. Portal vein Doppler ultrasound assessment may reveal decreased portal flow, but again, these findings are nonspecific and certainly not diagnostic of VOD (Pegram and Kennedy, 2001).

Controversy exists as to the utility of liver biopsy with or without determination of the wedged hepatic venous pressure gradients (WHVPG). Some authorities argue that transvenous liver biopsy with hepatic venous pressure determinations remains the gold standard for diagnosis of VOD (Richardson and Guinan, 2001). One investigative group reported that finding a WHVPG of

>10 mm Hg correlated with a 91% specificity, 86% positive predictive value, and 52% sensitivity for the diagnosis of VOD (Shulman et al., 1995). Although liver biopsy with histologic evaluation may substantially contribute to the diagnostic workup, VOD generally is diagnosed clinically (Rollins, 1986). In fact, liver biopsy may pose an unacceptably high risk of serious bleeding for patients who frequently experience significant thrombocytopenia in the posttransplant setting. Transvenous biopsy approaches are favored over percutaneous techniques, as the latter may pose an unacceptably high bleeding risk (Carreras et al., 1993). Liver biopsy may ultimately become necessary for those patients whose hepatic dysfunction cannot be clinically distinguished from other causes (Bearman, 1995). Similarly, although several authorities have advocated the use of transvenous intrahepatic portosystemic shunt (TIPS) placement for treatment of some patients with severe VOD, this also may create substantial bleeding complications. The procedure remains controversial (Senzolo et al., 2005).

At least two clinical criteria for the diagnosis of VOD have been published. These are the modified Seattle criteria and the Baltimore criteria (McDonald et al., 1993; Jones et al., 1987).

Modified Seattle criteria

Presence before day 20 after SCT of at least two of the following:

Bilirubin ≥2 mg/dL (~34 μmol/L)
Hepatomegaly, right upper quadrant pain
Ascites ± unexplained weight gain of > 2% from baseline

Baltimore criteria

Presence of hyperbilirubinemia ≥ 2 mg/dL before day 21 after SCT and at least two of the following:

Hepatomegaly (usually painful)
Ascites
Weight gain > 5% from baseline

Clinicians generally rely on the presence of the triad of sudden unexplained weight gain with ascites and painful hepatomegaly to make the diagnosis of VOD.

PATHOGENESIS OF VOD

Histologic evaluation of liver biopsy and autopsy specimens taken from patients with VOD has substantially contributed to our understanding of the subsequent structural changes and ensuing liver injury. Typically, a two-stage pattern occurs, with early sinusoidal injury and endothelial disruption occurring first, followed by hepatic stellate cell proliferation and resultant deposition of varying amounts of collagen and other connective tissues. In severe VOD, the collagen deposition within the hepatic sinusoids and venules may be severe. VOD characteristically involves injury to the area surrounding the hepatic central vein, leading to damage involving hepatic sinusoidal cells and especially hepatocytes located in zone 3 of the liver acinus (Richardson and Guinan, 2001). Disruption of hepatic sinusoidal endothelial cells with subsequent sinusoidal luminal obstruction appears so central to the pathogenesis of VOD that one leading group of researchers in the area proposed an alternative name for the condition, sinusoidal obstruction syndrome (DeLeve et al., 2002).

The first identifiable changes occur 6–8 days after the toxic liver insult (DeLeve et al., 2002). These early changes include swelling and widening of the sinusoidal endothelium and its associated subendothelial matrix zone, located between the basement membrane and the adventitia of the central and sublobular veins (Shulman et al., 1980, 1987; DeLeve et al., 2002). Disruption of endothelial integrity allows accumulation of cellular debris and red blood cells within the subendothelial matrix. Cytokines, including transforming growth factor-β1 (TGF-β1) released from activated platelets, appear to induce sinusoidal endothelial PAI-1 and tissue factor (TF) expression and may contribute to the development of a hemostatic imbalance within the liver (V. Pihusch et al., 2005). Hepatic sinusoids become dilated and engorged, and the space of Disse and sinusoidal lumens fill with red cells and necrotic cellular debris, with resultant sinusoidal obstruction. Ischemia ensues, causing damage to hepatocyte cords, with frank necrosis of perivenular hepatocytes (Shulman et al., 1980, 1987; Wantanabe et al., 1996).

Free-flowing groups of hepatocytes may become clogged in damaged central veins or embolize into portal veins. Additionally, deposition of fibrinogen and factor VIII/von Willebrand factor, demonstrated by immunostaining, occurs along the widened subendothelial spaces of venules and likely physically obstructs the small drainage channels that normally flow between the hepatic sinusoids and venules. In fact, electron microscopy analysis has shown closed sinusoidal endothelial cell fenestrae, with increased extracellular material, including collagen, obstructing sinusoidal pores (Vonnahme, 1993).

Later-stage histologic changes in the liver typically arise within 2 weeks after the onset of the clinical diagnosis of VOD (DeLeve et al., 2002). Increased numbers of hepatic stellate cells occupy the lining of the sinusoids and are likely responsible for the resultant exaggerated connective tissue deposition and fibrosis. In severe cases, the degree of fibrosis mirrors that seen in certain forms of cirrhosis.

PROGNOSIS OF VOD

Disease prognosis generally depends on the degree of liver injury and the severity of the patient's hepatic dysfunction. Patients with mild disease who do not manifest evidence of hepatic dysfunction will almost always improve and can expect to experience complete resolution of the signs and symptoms of VOD.

Moderate VOD includes evidence of liver dysfunction. Patients usually require treatment with diuretics for fluid retention and often need analgesic medications for significant right upper quadrant pain (Richardson and Guinan, 2001).

Severe VOD represents a serious and life-threatening event. These patients display the most brisk rise in serum bilirubin, the fastest weight gain, and the highest rates of ascites and suffer the most significant hepatic dysfunction. Supportive care measures generally fail to provide benefit to these patients. Their clinical course often worsens quickly, and many patients display intense renal sodium avidity, with worsening edema, ascites, and pulmonary infiltrates. Oliguric renal failure may rapidly ensue, and as many as half of the patients develop the hepatorenal syndrome. Curiously, these patients rarely die secondary to liver failure but generally succumb to severe renal and cardiac failure, and multiorgan failure eventually ensues. The day +100 post-SCT mortality for these patients approaches 100% (McDonald et al., 1984, 1993; Bearman et al., 1993).

Bearman's group performed regression analysis on data taken from a large number of patients enrolled in the Seattle Transplant Registry to determine clinically useful parameters for the risk of developing severe VOD (McDonald et al., 1993). They found that both the rate and degree of rise in bilirubin and weight gain help identify those patients at greatest risk for developing severe VOD. Additionally, the development of ascites remains ominous, present in nearly 50% of patients who will eventually progress to severe VOD (Table 1).

VOD of the liver remains a serious and often life-threatening regimen-related toxicity associated with commonly employed SCT conditioning regimens. To date, treatment has generally been supportive in nature, and clinical outcomes often are disappointing. Significant experimental therapeutics research in this area continues, with the agent Defibrotide (DF) (Gentium, S.p.A., Como, Italy) soon to enter phase III clinical trials based on significant success in treating patients with the most severe VOD.

DEFIBROTIDE IN THE TREATMENT OF VOD

DF belongs to the nucleic acid class of pharmacologic agents (Falanga et al., 2003). It consists of a highly complex polydisperse mixture of single-stranded phosphodiester oligodeoxyribonucleotides derived from the controlled depolymerization of porcine intestinal mucosal DNA. The average length of DF is approximately 50 mer (range 9–80 mer), and the average molecular mass is approximately 16.5 kDa (Palmer and Goa, 1993). It is not possible, by any known physicochemical technique (e.g., capillary gel electrophoresis), to separate the individual molecules that comprise DF. Whereas the majority of the individual oligomers that comprise DF are single stranded, approximately 10% are double stranded (e.g., hairpins, lariats, concatemers). Commercial DF is contaminated with only trace amounts of protein, but very small amounts of heparin can be found, which are insufficient to produce anticoagulant activity. Due most likely to complex patterns of hydrogen bonding, DF will undergo hysteresis, which can be observed clearly in its temperature-dependent circular dichroism spectrum. Like all DNA, DF is stable at ambient temperature and neutral pH and is highly sensitive to nuclease digestion. It has long been known that 3′,5′-exonuclease activity in plasma is the most important mechanism of digestion of phosphodiester oligonucleotides and that clearance rates from the plasma for such molecules are on the order of minutes. In fact, it was this observation that was the *fons et origo* of the dogma that phosphodiester oligonucleotides could not be used as drugs and that led to the development of phosphorothioates. However, for the VOD indication, this dogma appears inaccurate, given the clinical data regarding this agent.

Table 1. Clinical Features of VOD According to Severity of Disease

	Mild	*Moderate*	*Severe*
Weighted gain, % increase	7.0	10.1	15.5
Maximum total bilirubin (after day 20)	4.7	7.9	26.2
% patient with edema	23	70	85
% patient with ascites	5	16	48
Day 100 mortality (all cause), %	3	20	98

McDonald et al., 1993; Wadleigh et al., 2003.

DF exerts minimal systemic anticoagulant effects yet appears to induce numerous antithrombotic and profibrinolytic effects both *in vitro* and *in vivo*. It functions as an adenosine receptor agonist and causes increased concentrations of endogenous prostaglandins, which modulate thrombomodulin, platelets, and fibrinolysis (Pegram and Kennedy, 2001).

Experiments using human microvascular endothelial cell (HMEC-1) and macrovascular endothelial cell (HUVEC) lines reveal that DF blocks lipopolysaccharide (LPS)-induced TF expression, an important observation given that LPS is possibly implicated in the onset of VOD (Falanga et al., 2003). Additionally, TF represents the most important activator of the coagulation cascade. Reduction in TF expression may help reduce microvascular fibrin deposition, a known feature of clinical VOD, which probably contributes to the eventual development of organ dysfunction.

The control of fibrinolysis occurs via a normal homeostatic balance between its activation, mediated by tissue plasminogen activator (tPA), and its inhibition, controlled by PAI-1. LPS stimulation of endothelial cells causes a moderate increase in tPA and a large increase in PAI-1. The net result is a shift in this balance and an overall reduction in fibrinolysis. DF enhances LPS-induced increases in tPA and, more importantly, blocks the LPS-induced PAI-1 increase. The net effect is that DF inhibits endothelial cell-mediated PAI-1 release, resulting in increased fibrinolytic activity (Falanga et al., 2003). These effects appear consistent with *in vivo* observations of increased fibrinolytic activity and decreased PAI-1 levels in human subjects treated with DF (Violi et al., 1992).

Observations that DF possessed a powerful ability to modulate thrombotic and fibrinolytic pathways without exerting meaningful systemic anticoagulant effects led to the hypothesis that DF might hold promise for the treatment of VOD and other thrombotic vascular disorders.

CLINICAL EXPERIENCE WITH DF IN THE TREATMENT OF VOD

The early clinical experience using DF to treat severe VOD is limited to small case series of patients who received the drug on a compassionate-use basis. These trials are summarized in Table 2.

Richardson et al. (1998) retrospectively analyzed data from 19 patients treated with DF from March 1995 through August 1997. Patients were eligible for consideration for treatment if they were undergoing SCT and their referring physicians had made a clinical diagnosis of VOD based on the Baltimore criteria. Patients who did not meet clinical criteria but had a liver biopsy confirming the diagnosis were also eligible. Additionally, patients were required to have a predicted risk of $\geq$40% for severe VOD based on the Bearman model (1995). All patients had to have failed prior treatment with tPA and heparin (increasing bilirubin or the presence of multiple organ failure [MOF]) or have been deemed inappropriate for such treatment based on risk of excessive bleeding.

Patients received DF via i.v. infusion in normal saline in four divided doses, each over 2 hours. Initial daily doses started at 10 mg/kg and incrementally increased by 10 mg/kg every 24–48 hours to a maximum potential daily dose of 60 mg/kg. The planned treatment course was a minimum of 14 days.

Patients received daily history and physical examinations, and underwent detailed laboratory evaluation, including serial evaluation of their coagulation profile through measurements of prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen measurements. Each patient also had abdominal ultrasound scans performed.

Response to treatment was defined as evidence of improvement in VOD-related symptoms, with a concomitant or subsequent decrease in serum bilirubin to $<$2 mg/dL. Toxicities were graded according to the National Cancer Institute (NCI) common toxicity criteria.

Forty-two percent of the patients (8 of 19) achieved complete responses (CR). Of the 8 responders, 6 had complete resolution of evidence of end-organ dysfunction, in addition to achieving a decrease in bilirubin to $<$2 mg/dL. All 11 patients who failed to meet the definition of response died of progressive VOD and MOF. Three of the 8 responders died of other causes between 2 and 130 days after discontinuation of DF, and 5 patients remain alive and well at the time of publication.

DF was generally well tolerated. Documented grade 3–4 adverse events included 7 cases of sepsis, 5 cases of pulmonary edema, 2 cases of cytomegalovirus (CMV) infection, 2 cases of hypotension, and 2 cases of respiratory failure. Mild documented toxicities (grade 1 or 2)

TABLE 2. CLINICAL TRIALS CONDUCTED ON DEFIBROTIDE IN SEVERE VOD

Author	*Patients (*n*)*	*CR rate (%)*	*Day + 100 survival*
Richardson et al., 1998	19	8/19 (42)	30%
Chopra et al., 2000	28	10/28 (36)	36%
Richardson et al., 2002	88	32/88 (36)	35%

during the DF therapy included nausea, transient mild systolic hypotension, fever, abdominal cramping, and vasomotor symptoms. A single case of alveolar hemorrhage occurred in a patient with documented CMV pneumonitis. This patient also experienced a bleeding rectal ulcer. No significant hemorrhage occurred in any other patients. The etiologies of the documented grade 3–4 toxicities were difficult to characterize based on the complex medical status of the study patients. In 8 patients in whom DF was temporarily withheld per treatment guidelines and subsequently restarted at equivalent or decreased dose, a causative temporal relationship to any grade 3–4 toxicity could not be shown. Indeed, many of these severe adverse events appear commonly in patients undergoing SCT, and were not directly attributable to DF administration.

Chopra et al. (2000) reported on 40 patients meeting established criteria for VOD who received treatment with DF in 19 European centers as part of the European multicenter compassionate-use program; 28 of these 40 patients met risk criteria predicting progression of VOD and fatality or had evidence of MOF and were labeled as poor risk.

Patients were eligible for treatment if they carried a clinical diagnosis based either on the Baltimore or Seattle criteria. Those patients diagnosed within 16 days of receiving a transplant were evaluated according to their predicted risk of developing severe VOD based on the Bearman model. Patients who developed clinical features of VOD longer than 16 days after the transplant were deemed eligible for treatment if it was thought that VOD constituted their major clinical problem.

Patients received DF administered by i.v. infusion mixed with normal saline. The dosage consisted of either 200–3600 mg in four divided doses daily or was calculated on a per kilogram basis from 10 to 40 mg/kg. Dose adjustments were provided to patients based on rate and degree of response. The dose was reduced or the medication was discontinued altogether if significant potentially attributable toxicity occurred. CR was defined as bilirubin falling to <34.2 μmol/L and complete resolution of all other end-organ dysfunction. Definition of partial response (PR) required a fall in bilirubin but persistence or occurrence of other end-organ toxicity.

Of the 40 patients who met the diagnostic criteria for VOD, 22 achieved a CR. Of the 22 CR, 17 were alive at day $+100$ post-transplant. The remaining 5 patients who achieved a CR died either from progression of their malignancy or from pulmonary viral infection. Among the poor-risk group, 10 of 28 (36%) patients demonstrated CR and were alive at day $+100$ posttransplant. Overall, 17 of 40 patients (42.5%) demonstrated a complete response to DF and survived beyond 100 days after the transplant.

The authors report no significant toxicity directly attributable to DF. The drug was administered safely, and in general, no significant side effects occurred. DF was discontinued in 1 patient who experienced rectal bleeding, thrombocytopenia, and hemolysis, but this was not clearly attributed to DF. The study authors (Chopra et al., 2000) conclude that their data suggest that the use of DF in patients with severe VOD causes significant response rates, with no toxicity, and are consistent with the data presented initially by Richardson et al. (1998). They further suggest a possible role for DF in the prophylactic setting.

Following the initial successes of these early case series, eight transplantation programs in the United States formed a working group that enrolled 69 further patients for prospective study from August 1997 through May 2001 (Richardson et al., 2002). These data represent the largest experience to date of DF administration to patients with severe hepatic VOD and MOF following SCT.

All centers followed the same inclusion criteria based on the initial Richardson study. Patients were eligible if they carried a clinical diagnosis of VOD based on Baltimore criteria or met at least two criteria and had a diagnostic liver biopsy. Those patients eligible to be evaluated according to the Bearman model were required to have a predicted risk of severe VOD of at least 30%. Patients ineligible for evaluation by Bearman criteria (e.g., onset VOD $>$ 16 days posttransplant) were deemed eligible if VOD was considered their major clinical problem and organ failure was present in at least one other organ system.

Patients received DF administered i.v. in either normal saline or 5% dextrose in water. Treatment was typically divided into four doses, each infused over 2 hours at an initial starting dose of 10 mg/kg. The drug was mixed to a maximum concentration of 4 mg/mL, and dosages were incrementally increased by 10 mg/kg every 2–4 days to a maximum potential total daily dose of 60 mg/kg. The planned treatment course was 14 days. Toxicities were graded according to the NCI's common toxicity criteria. CR was defined as evidence of improvement in VOD-related symptoms and concurrent MOF and a decrease in bilirubin to <2 mg/dL (34.2 μM). Patients who failed to achieve CR were defined as having no response (NR).

Patients ranged in age from 8 months to 62 years, with a median age of 35 years. There were 47 males and 41 females. Sixty patients underwent allogeneic SCT, and 28 received autografts. Seventy-five percent of patients received cytoxan-based conditioning regimens, and most had hematologic malignancies.

CR occurred in 32 (36%) of 88 patients. Thirty-one of 32 patients achieving CR survived to day $+100$ post-transplant, and of those, 18 patients (60%) were alive as of October 2001. No mortality from VOD occurred beyond day $+134$. The most common cause of death was late relapse. The median duration of DF treatment was 15 days, and the duration ranged from 1 to 139 days. Most

of the responses occurred at doses of between 20 and 40 mg/kg/day.

No major toxicities attributable to DF were recorded. Patients who did experience serious grade 3–4 toxicity were believed to be manifesting complications commonly observed in critically ill patients in the posttransplantation setting. Their treating physicians did not attribute these problems (such as sepsis, acute renal failure, pulmonary edema) to their DF treatment. In patients who had DF held and subsequently restarted per treatment guidelines, no causal temporal relationship of any grade 3 or 4 toxicity to DF was demonstrated. Mild to moderate toxicities (grades 1–2) noted included nausea, transient mild systolic hypotension, fever, abdominal cramping, and vasomotor symptoms, such as hot flashes. These are accepted as the recognized side effects of DF treatment. Most notably, no life-threatening hemorrhage attributable to DF occurred in this high-risk population. The finding that DF posed essentially no risk for severe adverse events is consistent with previous treatment experience in patients with severe thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) (Bonomini et al., 1985). Phase III placebo-controlled data on aged individuals with peripheral vascular disease also clearly demonstrated the well-established safety profile of DF (Violi et al., 2000).

ADDITIONAL POTENTIAL CLINICAL APPLICATIONS FOR DF

Although DF has generated the most interest and excitement for its potential use in the treatment or prevention of posttransplant VOD, this unique compound may have other important applications. One group of researchers from Italy and The Netherlands investigated the possible role of DF as an adjunct to standard cytokine stimulation protocols for mobilization and collection of peripheral blood progenitor cells (PBPCs).

Researchers from the "Cristina Gandini" Bone Marrow Transplantation Unit (Milan, Italy) investigated the use of granulocyte colony-stimulating factor (G-CSF), with and without DF, stimulation for collection of PBPCs in mice. After demonstrating that a 5-day treatment course of DF alone resulted in no effect on white blood cell (WBC) counts or on other primitive early white cell forms, the team compared mice injected with recombinant human G-CSF (rHuG-CSF) (5 μg/mouse/day) alone with those injected with both rHuG-CSF (5 μg/mouse/day) and DF (15 mg/mouse/day). Compared with rHuG-CSF treatment alone, the addition of DF caused a 2-fold increase in the long-term culture initiating cell (LTC-IC) frequency and a 4-fold increase in the absolute numbers of committed colony-forming cells (CFC) (CarloStella et al., 2002). DF synergized with rHuG-CSF to significantly increase the mobilization of a broad spectrum of PBPCs, including primitive and committed progenitor cells. In addition, transplantation of progenitor cells collected after DF/rHuG-CSF mobilization resulted in bone marrow rescue and reconstitution of hematopoiesis in 71% of recipient mice compared with 43% of mice transplanted with progenitor cells collected after rHuG-CSF stimulation alone (Violi et al., 2000).

The possible mechanisms for these impressive results remain unclear. DF treatment alone does not trigger cytokine production or release, nor does it induce a proliferative effect in blood progenitor cells. Instead, the authors (Violi et al., 2000) offer an alternative explanation. Earlier reports detail DF's ability to alter the expression and functional activity of endothelial cell adhesion molecules and to exert an antifibrin effect by enhancing fibrinolysis (Scalia et al., 1998; Pellegatta et al., 1996). It is possible that DF enhances stem cell mobilization by modulating the expression pattern of adhesion receptors involved in stem cell trafficking. Further studies will be required to further elucidate the cellular and molecular mechanisms that underlie these important findings.

The initial successes of the DF progenitor cell mobilization studies performed in mice led the same research group to evaluate the capacity of DF to enhance cytokine-induced hematopoietic mobilization in nonhuman primates (Carlo-Stella et al., 2004). Rhesus monkeys received stem cell mobilization with rHuG-CSF (100 μg/kg/day s.c. for 5 days), followed by remobilization with DF (15 mg/kg/hour continuous infusion [CI] for 5 days) and rHuG-CSF (100 μg/kg/day s.c. for 5 days) following a washout period of 4–6 weeks. Hematopoietic mobilization was evaluated by complete blood count (CBC), frequency and absolute numbers of CFCs, high proliferative potential CFCs (HPP-CFCs), and LTC-ICs. Treatment with rHuG-CSF both alone and in combination with DF markedly increased the efficacy of progenitor cell mobilization as measured by multiple outcomes. The addition of DF to rHuG-CSF increased circulating CFCs, HPP-CFCs, and LTC-ICs by 1.4-fold, 6-fold, and 5-fold, respectively. Similar benefits were observed when DF given over 2 days was added to the same 5-day regimen of rHuG-CSF. In fact, 2 days of treatment with DF was as effective as 5 days. The benefits of adding DF to rHuG-CSF included both higher values of blood progenitors and improvements in mobilization kinetics (earlier progenitor cell release). Although these results are intriguing, however, we still do not fully understand the mechanism by which DF enhances rHuG-CSF induced progenitor cell mobilization.

CONCLUSIONS

VOD of the liver remains a troubling and potentially fatal complication of high-dose chemotherapy and hemato-

poietic SCT conditioning regimens. No effective therapy has been available for these patients to date, and our best supportive care remains woefully inadequate. DF, a polydisperse mixture of phosphodiester oligonucleotides, seems to offer a safe and effective treatment for some patients suffering from severe VOD, a condition for which no accepted standard therapy currently exists. Early clinical studies evaluating the efficacy of DF for the treatment of severe VOD in patients undergoing hematopoietic SCT have been very encouraging. Approximately 45% of the patients treated in multiple initial phase II clinical trails achieved a CR at day +100, demonstrating normalization of serum bilirubin and resolution of the clinical syndrome. Although these initial studies appear encouraging, they represent single arm studies, where patient selection may unduly influence the results. Larger studies will be required to further investigate the true efficacy of DF.

A large, FDA-approved, pivotal, prospective phase III trial of DF vs. historical controls (best available therapy) commenced in the first quarter of 2006 and should provide further data on this potentially useful medication. The drug seems to have no significant side effects, and almost all test subjects who have received this treatment tolerated it well. Although the mechanism of action of DF remains unclear, the drug exerts minimal systemic anticoagulant effects yet appears to induce numerous antithrombotic and profibrinolytic effects both *in vitro* and *in vivo*. Regardless of mechanism, however, it is difficult to believe that the activity of DF has anything to do with Watson-Crick base pair hybridization, RNase H activity, or selective gene silencing. Its clinical activity is probably more related to its polyanionic character than to any other property it possesses. This does not mean that all polyanions will behave in a similar manner, but at this time, the molecular determinants of DF that engender its remarkable clinical activity are not known but are a fascinating area of continuing study.

FINANCIAL DISCLOSURE

C.A. Stein is a member of the Scientific Advisory Board of Gentium, S.p.A.

REFERENCES

BAGLIN, T.P. (1994). Veno-occlusive disease of the liver complicating bone marrow transplantation. Bone Marrow Transplant. **13,** 1–4.

BEARMAN, S.I., ANDERSON, G.L., MORI, M., HINDS, M.S., SHULMAN, H.M., and McDONALD, G.B. (1993). Veno-occlusive disease of the liver: Development of a model predicting fatal outcome after marrow transplantation. J. Clin. Oncol. **11,** 1729–1736.

BEARMAN, S.I. (1995). The syndrome of hepatic veno-occlusive disease after marrow transplantation. Blood **85,** 3005–3020.

BONOMINI, V., FRASCA, G.M., RAIMONDI, C., D'ARCANGELO, G.L., and VANGELISTA, A. (1985). Effect of a new antithrombotic agent (Defibrotide) in acute renal failure due to thrombotic microaniopathy. Nephron **40,** 195–200.

BOULA, A.M., MANTADAKIS, E., XILOURI, I.M., CHRISTOFORIDOU, A.V., FOUDOULAKIS, A.M., and SAMONIS, G. (2005). Veno-oclusive disease of the liver associated with chronic myelomonocytic leukemia treated with vincristine and standard doses of cytarabine. Am. J. Hematol. **79,** 216–219.

CARLO-STELLA, C., DI NICOLA, M., LONGONI, P., MILANI, R., MILANESI, M., GUIDETTI, A., HAANSTRA, K., JONKER, M., CLERIS, L., MAGNI, M., FORMELLI, F., and GIANNI, A.M. (2004). Mobilization of primitive and committed hematopoietic progenitors in nonhuman primates treated with defibrotide and recombinant human granulocyte colony-stimulating factor. Exp. Hematol. **32,** 68–75.

CARLO-STELLA, C., DI NICOLA, M., MAGNI, M., LONGONI, P., MILANESI, M., STUCCHI, C., CLERIS, L., FORMELLI, F., and GIANNI, M.A. (2002). Defibrotide in combination with granulocyte colony-stimulating factor significantly enhances the mobilization of primitive and committed peripheral blood progenitor cells in mice. Cancer Res. **62,** 6152–6157.

CARRERAS, E., BERTZ, H., ARCESE, W., VERNANT, J.P., TOMAS, J.F., HAGGLUND, H., BANDINI, G., ESPEROU, H., RUSSELL, J., DE LA RUBIA, J., DI GIROLAMO, G., DEMUYNCK, H., HARTMANN, O., CLAUSEN, J., RUUTU, T., LEBLOND, V., IRIONDO, A., BOSI, A., BENBASSAT, I., KOZA, V., GRATWOHL, A., and APPERLEY, J.F. (1998). Incidence and outcome of hepatic veno-occlusive disease after blood or marrow transplantation: A prospective cohort study of the European Group for Blood and Marrow Transplantation. European Group for Blood and Marrow Transplantation Chronic Leukemia Working Party. Blood **92,** 3599–3604.

CARRERAS, E., GRANENA, A., NAVASA, M., BRUGUERA, M., MARCO, V., SIERRA, J., TASSIES, M.D., GARCIA-PAGAN, J.C., MARTI, J.M., and BOSCH, J. (1993). Transjugular liver biopsy in BMT. Bone Marrow Transplant. **11,** 21–26.

CHOPRA, R., EATON, J.D., and GRASSI, A. (2000). Defibrotide for the treatment of hepatic veno-occlusive disease: Results of the European compassionate-use study. Br J Hematol **111,** 1122–1129.

DELEVE, L.D., SHULMAN, H.M., and McDONALD, G.B. (2002). Toxic injury to hepatic sinusoids: sinusoidal obstruction syndrome (veno-occlusive disease). Semin. Liver Dis. **22,** 27–40.

FALANGA, A., VIGNOLI, A., MARCHETTI, M., and BARBUI, T. (2003). Defibrotide reduces activity and increases fibrinolytic properties of endothelial cells. Leukemia **17,** 1636–1642.

HO, V., MOMTAZ, P., DIDAS, C., WADLEIGH, M., and RICHARDSON, P. (2004). Posttransplant hepatic veno-oc-

clusive disease: Pathogenesis, diagnosis and treatment. Rev. Clin. Exp. Hematol. **8,** E3.

JONES, R.J., LEE, K.S., BESCHORNER, W.E., VOGEL, V.G., GROCHOW, L.B., BRAINE, H.G., VOGELSANG, G.B., SENSENBRENNER, L.L., SANTOS, G.W., and SARAL, R. (1987). Veno-occlusive disease of the liver following bone marrow transplantation. Transplantation **44,** 778–783.

KATZKA, D.A., SAUL, S.H., JORKASKY, D., SIGAL, H., REYNOLDS, J.C., and SOLOWAY, R.D. (1986). Azathioprine and hepatic venocclusive disease in renal transplant patients. Gastroenterology **90,** 446–454.

LEE, J.L., GOOLEY, T., BENSINGER, W., SCHIFFMAN, K., and McDONALD, G.B. (1999). Veno-occlusive disease of the liver after busulfan, melphalan, and thiotepa conditioning therapy: Incidence, risk factors, and outcome. Biol. Blood Marrow Transplant. **5,** 306–315.

McDONALD, G.B., HINDS, M.S., FISHER, L.D., SCHOCH, H.G., WOLFORD, J.L., BENAJI, M., HARDIN, B.J., SHULMAN, H.M., and CLIFT, R.A. (1993). Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: A cohort study of 355 patients. Ann. Intern. Med. **118,** 255–267.

McDONALD, G.B., SHARMA, P., MATTHWES, D.E., SHULMAN, H.M., and THOMAS, E.D. (1984). Venocclusive disease of the liver after bone marrow transplantation: Diagnosis, incidence, and predisposing factors. Hepatology **4,** 116–122.

PALMER, K.J., and GOA, K. (1993). Defibrotide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in vascular disorders. Drugs **45,** 259–294.

PEGRAM, A., and KENNEDY, L. (2001). Prevention and treatment of veno-occlusive disease. Ann. Pharmacother. **35,** 935–942.

PELLEGATTA, F., LU, Y., RADAELLI, A., ZOCCHI, M.R., FERRERO, E., CHIERRCHIA, S., GAJA, G., and FERRERO, M.E. (1996). Drug-induced *in vitro* inhibition of neutrophil-endothelial cell adhesion. Br. J. Pharmacol. **118,** 471–476.

PIHUSCH, M., WEGNER, H., GOEHRING, P., SALAT, C., PIHUSCH, V., HILLER, E., ANDREESEN, R., KOLB, H.J., HOLLER, E., and PIHUSCH, R. (2005). Diagnosis of hepatic veno-occlusive disease by plasminogen activator inhibitor-1 plasma antigen levels: A prospective analysis in 350 allogeneic hematopoietic stem cell recipients. Transplantation **80,** 1376–1382.

PIHUSCH, V., PIHUSCH, M., PENOVICI, M., KOLB, H.J., HILLER, E., and PIHUSCH, R. (2005). Transforming growth factor beta-1 released from platelets contributes to hypercoagulability in veno-occlusive disease following hematopoietic stem cell transplantation. Thromb. Res. **116,** 233–240.

RICHARDSON, P., ELIAS, A., KRISHNAN, A., WHEELER, C., NATH, R., HOPPENSTEADT, D., KINCHLA, N.M., NEUBERG, D., WALLER, E.K., ANTIN, J.H., SOIFFER, R., VREDENBURGH, J., LILL, M., WOOLFREY, A.E., BEARMAN, S.I., IACOBELLI, M., FAREED, J., and GUINAN, E.C. (1998). Treatment of severe veno-occlusive disease with defibrotide: Compassionate use results in response without significant toxicity in a high-risk population. Blood **92,** 737–744.

RICHARDSON, P., and GUINAN, E. (2001). Hepatic veno-occlusive disease following hematopoietic stem cell transplantation. Acta Haematol. **106,** 57–68.

RICHARDSON, P., MURAKAMI, C., JIN, Z., WARREN, D., MOMTAZ, P., HEPPENSTEADT, D., ELIAS, A.D., ANTIN, J.H., SOIFFER, R., SPITZER, T., AVIGAN, D., BEARMAN, S.I., MARTIN, P.L., KUTZBERG, J., VREDENBURGH, J., CHEN, A.R., ARAI, S., VOGELSANG, G., McDONALD, G.B., and GUINAN, E.C. (2002). Multi-institutional use of defibrotide in 88 patients after stem cell transplant with severe veno-occlusive disease and multi-system organ failure: Response without significant toxicity in a high-risk population and factors predictive of outcome. Blood **100,** 4337–4343.

ROLLINS, B.J. (1986). Hepatic veno-occlusive disease. Am. J. Med. **81,** 297–306.

SALAT, C., HOLLER, E., KOLB, H.J., REINHARDT, B., PIHUSCH, R., WILMANNS, W., and HILLER, E. (1997). Plasminogen activator inhibitor-1 confirms the diagnosis of hepatic veno-occlusive disease in patients with hyperbilirubinemia after bone marrow transplantation. Blood **89,** 2184–2188.

SCALIA, R., KOCHILAS, L., CAMPBELL, B., and LEFER, A.M. (1998). Effects of defibrotide on leukocyte-endothelial cell interaction in the rat mesenteric vascular bed: Role of P-selectin. Methods Find. Exp. Clin. Pharmacol. **18,** 669–676.

SENZOLO, M., CHOLONGITAS, E., PATCH, D., and BURROUGHS, A.K. (2005). TIPS for veno-occlusive disease: Is the contraindication real? Hepatology **42,** 240–241.

SHULMAN, H.M., GOOLEY, T., DUDLEY, M.D., KOFLER, T., FELDMAN, R., DWYER, D., and McDONALD, G.B. (1995). Utility of transvenous liver biopsies and wedged hepatic venous pressure measurements in sixty marrow transplant recipients. Transplantation **59,** 1015–1022.

SHULMAN, H.M., GOWN, A.M., and MUGENT, D.J. (1987). Hepatic veno-occlusive disease after bone marrow transplantation. Immunohistochemical identification of the material within occluded central venules. Am. J. Pathol. **127,** 549–558.

SHULMAN, H.M., McDONALD, G.B., MATTHEWS, D., DONEY, K.C., KOPECKY, K.J., GAUVREAU, J.M., and THOMAS, E.D. (1980). An analysis of hepatic venocclusive disease and centrilobular hepatic degeneration following bone marrow transplantation. Gastroenterology **79,** 1178–1191.

SMITH, L.H., DIXON, J.D., STRINGHAM, J.R., EREN, M., ELOKDAH, H., CRANDALL, D.L., WASHINGTON, K., and VAUGHAN, D.E. (2006). Pivotal role of PAI-1 in a murine model of hepatic vein thrombosis. Blood **107,** 132–134.

VIOLI, F., FERRO, D., ALIOLA, M., QUINTARELLI, C., BASILI, S., and BALSANO, F. (1992). Effect of oral defibrotide on tissue-plasminogen activator and tissue-plasminogen activator inhibitor balance. Eur. J. Clin. Pharmacol. **42,** 379–383.

VIOLI, F., MARUBINI, E., COCCHERI, S., and NENCI, G. (2000). Improvement in walking distance by defibrotide in

patients with intermittent claudication—Results of a randomized, placebo-controlled study (the DICLIS study). Defibrotide Intermittent Claudication Italian Study. Thromb. Haemost. **83,** 672–677.

VONNAHME, F.-J. (1993). [The Human Liver. A Scanning Electron Microscopic Atlas, 1st ed.] Freiburg, Germany: Karger.

WADLEIGH, M., HO, V., MOMTAZ, P., and RICHARDSON, P. (2003). Hepatic veno-occlusive disease: Pathogenesis, diagnosis and treatment. Curr. Opin. Hematol. **10,** 451–462.

WANTANABE, K., IWAKI, H., SATOH, M., IKEDA, T., ICHIMIYA, S., SUZUKI, N., KUDOH, T., ODA, T., MATSUURA, A., MORI, M., and KIKUCHI, K. (1996). Veno-occlusive disease of the liver following bone marrow transplantation: A clinical-pathological study of autopsy cases. Artif. Organs. **20,** 1145–1150.

WILMOT, F.C., and ROBERTSON, G.W. (1920). Senecio disease, or cirrhosis of the liver due to senecio poisoning. Lancet **2,** 848–849.

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