

# Safety and efficacy of defibrotide for the treatment of severe hepatic veno-occlusive disease

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**Abstract:** Hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome, is a potentially life-threatening complication of chemotherapeutic conditioning used in preparation for hematopoietic stem-cell transplantation (SCT). VOD may occur in up to 62% of patients undergoing SCT, with onset generally within the first month after SCT. In severe cases, 100-day mortality is in excess of 80%. Current management consists of best supportive care, with no agents to date approved for treatment in the USA or the EU. Defibrotide, a polydisperse oligonucleotide, has been shown in phase II and III trials to improve complete response and survival in patients undergoing SCT with severe VOD. This article reviews our current understanding of VOD, and examines recent clinical findings on defibrotide for the treatment and prophylaxis of VOD.

**Keywords:** defibrotide, sinusoidal obstruction syndrome, veno-occlusive disease

## Introduction

Hepatic veno-occlusive disease (VOD) is a serious condition that usually arises within the first 30 days after hematopoietic stem-cell transplantation (SCT), and is believed to be the result of endothelial cell damage and hepatocellular injury from high-dose conditioning regimens prior to SCT [Bearman, 1995; Kumar *et al.* 2003]. VOD is characterized by rapid weight gain, painful hepatomegaly, hyperbilirubinemia/jaundice, and ascites/fluid retention, without other identifiable causes for liver disease; VOD is fatal in 20–50% of patients [Bearman, 1995; Kumar *et al.* 2003]. Clinical criteria for the diagnosis of VOD have been developed; Seattle criteria stipulate that at least two or more clinical features including jaundice, painful hepatomegaly or ascites, and/or unexplained weight gain must be evident within 30 days of transplantation [Bearman, 1995; McDonald *et al.* 1984]; Baltimore criteria specify an elevated bilirubin level of at least 2.0 mg/dl and two or more of the following characteristics: hepatomegaly, ascites, or at least 5% weight gain by day 21 post-SCT [Bearman, 1995; Jones *et al.* 1987]. Although not included in the formal

diagnostic criteria in the past, it has also been found that rapid and unexpected elevations in the blood levels of specific anti-graft-*versus*-host-disease (GvHD) drugs (such as sirolimus and tacrolimus) in association with a rise in creatinine as well as fluid avidity and weight gain (but prior to liver function abnormalities emerging) can be a valuable hint that VOD and sinusoidal obstruction may be evolving [Cutler *et al.* 2010].

In this article, we review the incidence and pathogenesis of VOD, and evaluate approaches to the treatment of the disease. In particular, we focus on the safety and efficacy of the investigational drug, defibrotide, a polydisperse mixture of porcine-derived single-stranded oligonucleotides [Bianchi *et al.* 1993], and its potential for the treatment and prophylaxis of VOD in patients undergoing SCT.

## Prevalence of veno-occlusive disease

A recent analysis of retrospective data, prospective cohort studies and clinical trials published between 1979 and 2007 detailed the prevalence

*Ther Adv Hematol*

(2012) 3(4) 253–265

DOI: 10.1177/2040620712441943

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**Table 1.** Variables that can influence incidence of veno-occlusive disease.

	Incidence (%)
All patients with SCT*	14
Diagnostic criteria*	
Baltimore	10
Seattle	17
SCT type*	
Autologous	9
Allogeneic	13
Conditioning chemotherapy\$	
Oral BU + CY	42
Intravenous BU + CY	18

\*[Coppell *et al.* 2010].  
\$[Lee *et al.* 2005].  
BU, busulfan; CY, cyclophosphamide; SCT, stem-cell transplantation; VOD, veno-occlusive disease.

and severity of VOD [Coppell *et al.* 2010]. This comprehensive analysis found that VOD was reported in up to 62% (with a mean of 13.7%) of patients undergoing SCT following myeloablative conditioning. Prevalence varied according to the conditioning regimen, the type of transplantation, and the diagnostic criteria used [Coppell *et al.* 2010] (Table 1). Similarly, in an important recent retrospective study of 845 patients who received an allogeneic SCT over the past 24 years, the cumulative incidence of VOD was found to be 13.8% using Seattle criteria and 8.8% using Baltimore criteria [Carreras *et al.* 2011]. The incidence of VOD in children undergoing SCT also varies, ranging from 11% to 20% [Cesaro *et al.* 2005; Lee *et al.* 2010]. VOD ranges from mild, reversible disease to severe illness with multiple organ failure (MOF) and death [Kumar *et al.* 2003]. In the Carreras retrospective study, approximately two-thirds of patients had mild or moderate VOD and one-third had severe VOD according to Seattle criteria; this was closer to 50% when Baltimore criteria were used [Carreras *et al.* 2011]. In the Coppell analysis, up to 77% of all cases of VOD proved severe, with severe disease in turn associated with a mortality rate in excess of 80% [Coppell *et al.* 2010]. For severe VOD, the death rate in a large single-centre study (from Seattle) was reported as 98% [McDonald *et al.* 1993]. Many alternative conditions that adversely affect the liver after transplantation can mimic the signs and symptoms of VOD; diagnosis of VOD, especially in its early stages, is therefore a clinical challenge [Kumar *et al.* 2003]. In addition to the emergence of MOF, the risk for developing

severe VOD can also be estimated using the Bearman prognostic model, which is based on height of bilirubin elevation, percentage weight gain, and time from transplant (up to day +16), although this model has only been validated for certain regimens [including cyclophosphamide (CY) plus total body irradiation (TBI), busulfan (BU) plus CY, and CY, carmustine plus etoposide] [Bearman *et al.* 1993a].

### Pathogenesis of veno-occlusive disease

Sinusoidal endothelial cell and hepatocyte injury, usually within zone 3 of the hepatic acinus, triggered by intensive chemotherapy conditioning are thought to be pivotal in the development of VOD [Coppell *et al.* 2010; Kumar *et al.* 2003]. Endothelial damage and hepatocellular necrosis with subendothelial edema and microthrombosis are seen (reviewed in [Richardson and Guinan, 1999; Carreras, 2000]). Fibrin-related aggregates and cellular debris occlude the small pores that perforate the endothelial lining, causing obstruction of the venous outflow. This results in intrahepatic portal hypertension, which is responsible for the main clinical manifestations of VOD (reviewed in [Carreras, 2000]). As a consequence of the endothelial cell damage, sinusoidal obstruction is often prominent on microscopy, leading to the alternative terminology sinusoidal obstruction syndrome [Kumar *et al.* 2003]. Cytokines, such as interleukin-1, interleukin-2 and tumor necrosis factor  $\alpha$ , may be elevated due to tissue injury caused by both chemotherapy and allogeneic injury, with their procoagulant effects on endothelial cell surfaces further contributing to the pathogenesis of VOD, with endothelial damage leading to further cytokine release and serine protease activation (e.g. tissue factor) [Baars *et al.* 1992; Carreras, 2000; Holler *et al.* 1990; Nawroth *et al.* 1986]. Conditioning-related injury may be potentiated by agents used for GvHD prophylaxis, for example sirolimus (or a combination of sirolimus and tacrolimus), because sirolimus may accelerate the senescence of hepatic endothelial cells after conditioning-related injury [Cutler *et al.* 2010]. Sirolimus may also reduce endothelial growth factor levels, hindering the healing process [Cutler *et al.* 2010]. Elevated levels of several markers of endothelial injury have been described, including a key inhibitor of fibrinolysis, plasminogen activator inhibitor type-1 (PAI-1), and plasma thrombomodulin [Nurnberger *et al.* 1998; Salat *et al.* 1994]. PAI-1 inhibits fibrinolysis, may contribute to microthrombosis and can exacerbate subsequent

organ failure [Reilly *et al.* 1994]; as a result, PAI-1 has become recognized as both a diagnostic and prognostic biomarker for VOD [Lee *et al.* 2002]. PAI-1 production is stimulated by transforming growth factor  $\beta$  (TGF- $\beta$ ); TGF- $\beta$  released from activated platelets further contributes to hemostatic imbalance at the level of the sinusoidal endothelial surface in patients with VOD via an increase in endothelial cell PAI-1 production [Pihusch *et al.* 2005]. TGF- $\beta$  has been implicated in the pathogenesis of fibrosis [Czaja *et al.* 1989], and elevated levels of TGF- $\beta$  strongly correlate with an increased risk of the disease following SCT [Anscher *et al.* 1993]. Also a reduction in hepatic nitric oxide (NO) levels has been shown to contribute to the development of VOD via disruption in sinusoidal integrity and subsequent disturbance of sinusoidal perfusion [DeLeve *et al.* 2003]. Conversely, NO has been found to play a role in reducing hepatocyte damage and increasing hepatic microcirculation in rats, suggesting a potential role for NO in the prevention of ischemia-reperfusion injuries [Kuroki *et al.* 2004].

### Risk factors for veno-occlusive disease

A number of risk factors, both pre and post SCT, can predispose patients to developing VOD. Pretransplant risk factors include patient-related factors, such as older patient age, female gender, pre-existent liver injury (especially cirrhosis) and advanced malignancy (reviewed in [Ho *et al.* 2008]). Allogeneic SCT is associated with a markedly increased risk of development of VOD compared with autologous SCT (Table 1); this could be due to the use of cyclosporin or methotrexate for the prophylaxis of GvHD, or may indicate an allogeneic immunological element in the development of VOD [Carreras, 2000] via cytokine dysfunction and inflammation [Richardson and Guinan, 1999].

As mentioned above, specific transplant conditioning regimens, including CY followed by TBI [McDonald *et al.* 2003] and BU followed by CY [McCune *et al.* 2007], are associated with the development of VOD, although inter-patient variability can confound correlations [McCune *et al.* 2007].

In an *in vitro* study, BU has been shown to deplete hepatic glutathione and, at high concentrations, induce oxidative stress resulting in BU toxicity [DeLeve and Wang, 2000]. Another *in vitro* study revealed that sinusoidal cells are highly sensitive

to CY metabolites; CY metabolites deplete glutathione by more than 95% and profound depletion of glutathione precedes the onset of toxicity [DeLeve, 1996]. Patients undergoing SCT with a short interval from administration of gemtuzumab ozogamicin, a monoclonal antibody against CD33 used in the treatment of acute myeloid leukemia (AML), have a significantly increased risk of VOD development [Wadleigh *et al.* 2003], possibly due to targeting of CD33+ cells in the sinusoids of the liver, activation of stellate cells, damage to sinusoidal endothelial cells, sinusoidal vasoconstriction and ischemic hepatocyte necrosis [McDonald, 2002]. Prior abdominal irradiation has also been found to increase the risk of VOD compared with patients with no prior abdominal irradiation (13% *versus* 5%, respectively;  $p = 0.009$ ) [Carreras *et al.* 1998].

### Approaches to the prevention of veno-occlusive disease

Modification of conditioning and GvHD prophylaxis regimens may reduce the risk of developing VOD. In patients conditioned with BU plus CY, intravenous BU has been found to significantly decrease the incidence of VOD after allogeneic SCT compared with orally administered BU (Table 1) [Lee *et al.* 2005]. Since CY metabolites are implicated as important contributors to endothelial injury in VOD, substitution of high-dose CY in the conditioning regimen may also ameliorate VOD risk. Indeed, a recent retrospective study comparing oral BU plus CY with targeted intravenous BU plus fludarabine (BU/Flu) as conditioning therapy in patients with AML demonstrated that high-dose BU/Flu was associated with less early toxicity, including VOD [Pidala *et al.* 2010]. In addition, caution has been advised against intensive treatment with gemtuzumab ozogamicin, especially within 3 months of SCT, to reduce the risk of VOD [McKoy *et al.* 2007; Ho *et al.* 2004]. Gemtuzumab ozogamicin (Mylotarg, Wyeth Pharmaceuticals, Philadelphia, PA, USA) was withdrawn from the US market in 2010 following reports of increased incidence of VOD in patients with AML in the absence of SCT as well as during the early post-transplantation period in patients who had been previously treated with the agent.

VOD risk may also be reduced by careful consideration of choice of GvHD prophylaxis in patients undergoing allogeneic SCT. For example, use of *ex vivo* T-cell depletion as GvHD

prophylaxis has been associated with a lower risk of VOD [Moscardó *et al.* 2001]. Conversely, if pharmacological GvHD prophylaxis is employed, the combination of tacrolimus, sirolimus, and methotrexate is associated with a higher risk of VOD compared with tacrolimus and methotrexate [Cutler *et al.* 2008]. Further, the use of sirolimus as GvHD prophylaxis after high-dose BU/CY conditioning appears to further escalate the risk of severe VOD [Cutler *et al.* 2008].

#### *Pharmacological prevention*

As VOD is associated with aberrant fibrin deposition leading to sinusoidal and/or central vein occlusion, antithrombotic or fibrolytic agents have been investigated as prophylaxis for VOD. In an animal model, antithrombin III has been shown to exert anti-inflammatory properties [Okada *et al.* 1999] and potentially reverse pro-coagulant stress at the level of the endothelium [Richardson and Guinan, 2001; Richardson *et al.* 2010b]. However, antithrombin III as prophylaxis has not been found to decrease VOD incidence [Haussmann *et al.* 2006]. Separately, a recent study in 374 children undergoing SCT suggests that prostaglandin E1 may have a protective effect against the development of VOD and may therefore be suitable for prophylaxis, although tolerability proved to be an issue [Lee *et al.* 2010]. Similarly, an earlier study found that significant toxicity occurred with prostaglandin E1 prophylaxis in patients undergoing SCT [Bearman *et al.* 1993b], and its ability to prevent severe VOD therefore remains unproven. Heparin prophylaxis, including combination therapy, has shown some potential to reduce the incidence of VOD [Batsis *et al.* 2006; Forrest *et al.* 2003; Lakshminarayanan *et al.* 2010]. Randomized clinical trials are required to clarify the otherwise mixed data observed to date. Ursodiol (ursodeoxycholic acid) as prophylaxis following allogeneic SCT has been associated with a reduced incidence of VOD [Essell *et al.* 1998; Ohashi *et al.* 2000]. However, a multi-center randomized trial from the Nordic Bone Marrow Transplantation Group comparing patients with or without ursodiol treatment prior to and after allogeneic SCT did not show any benefit in terms of VOD, although improved overall survival and reduced hepatic complications were observed in the ursodiol arm [Ruutu *et al.* 2002].

#### **Management of veno-occlusive disease**

Current standard management of VOD focuses on best supportive care, which includes avoidance of hepatorenal toxic agents, management of fluid overload with diuresis, paracentesis for large volume ascites, ventilator support and hemodialysis as clinically indicated [DeLeve *et al.* 2009]. In rare cases, liver transplantation may be considered [DeLeve *et al.* 2009].

There are currently no standard approved pharmacological agents for the treatment of VOD in the USA or the EU. Systemic anticoagulants or thrombolytics such as tissue plasminogen activator (tPA) have been investigated as treatment options for established VOD, but they have been associated with significant bleeding complications, including fatal events, thus limiting their utility and with no survival benefit observed (reviewed in [Ho *et al.* 2008]).

#### *Defibrotide*

Defibrotide is a promising candidate for the treatment of VOD due to its pleiotropic effects at the endothelial surface, and the relatively low risk of significant systemic bleeding associated with its use [Richardson and Guinan, 1999, 2001]; exciting new data on defibrotide are now available in this setting.

*Mechanisms of action.* Defibrotide is a polydisperse mixture of porcine-derived single-stranded oligonucleotides, which was initially identified as an adenosine receptor agonist [Bianchi *et al.* 1993]. However, subsequent preclinical studies have shown defibrotide to have multiple actions in the micro- and macrovasculature, and have confirmed defibrotide as an attractive candidate agent for the treatment of VOD [Echart *et al.* 2009; Falanga *et al.* 2003].

Studies have revealed that defibrotide confers antithrombotic properties by increasing levels of the fibrinolytic protein plasmin, through increase in tPA, which activates plasminogen to plasmin [Falanga *et al.* 2003], by increasing activity of plasmin itself [Echart *et al.* 2009], and by reducing PAI-1 levels [Falanga *et al.* 2003]. In addition, defibrotide reduces the amount of a key endothelial cell surface procoagulant tissue factor (TF), through blocking TF deposition in the extracellular matrix [Palomo *et al.* 2011] and stimulating release of TF pathway inhibitor [Benimetskaya

*et al.* 2008] as well as other vascular mediators, such as NO [Richardson and Guinan, 2001]. Thus, defibrotide may limit the organ dysfunction and thrombotic microangiopathies associated with fibrin deposition [Falanga *et al.* 2003]. In addition, defibrotide has been shown to protect endothelial cells against apoptosis induced by certain cytotoxic drugs [Eissner *et al.* 2002] and to prevent allogeneic responses of CD8 cells against endothelial targets.

Defibrotide has also been shown to confer anti-inflammatory properties. Preclinical studies have found that the proinflammatory p38 mitogen-activated protein kinase and Akt cell signaling pathways are upregulated in endothelial cells that have been incubated in sera from patients after autologous SCT [Palomo *et al.* 2011]. These signaling pathways were inactivated by defibrotide [Palomo *et al.* 2011]. Moreover, the same group observed that elevated levels of intercellular adhesion molecule-1 (ICAM-1), which are associated with blood vessel inflammation [Lawson and Wolf, 2009], were blocked by defibrotide [Palomo *et al.* 2011]. Previous exposure and continuous incubation of cultured endothelial cells with defibrotide prevented the signs of activation and damage induced by sera from patients following autologous SCT, indicating that defibrotide has protective anti-inflammatory and antithrombotic effects in this setting [Palomo *et al.* 2011].

Furthermore, defibrotide may provide a stimulus to the sinusoidal endothelium of a damaged liver and aid in revascularization. *In vitro*, defibrotide was found to promote endothelial cell proliferation and angiogenesis, in addition to stimulating tubular morphogenesis [Benimetskaya *et al.* 2008].

Defibrotide may also have a protective effect against acute GvHD. Elevated expression of the heparanase gene was identified as a risk factor for acute GvHD following SCT [Ostrovsky *et al.* 2010], and defibrotide has been shown to suppress heparanase expression [Mitsiades *et al.* 2009]. Activation of adenosine receptors has been found to inhibit T-cell function with the potential for prevention and treatment of acute GvHD [Lappas *et al.* 2010; Saldanha-Araujo *et al.* 2011].

**Clinical assessment of defibrotide.** Defibrotide does not interfere with chemotherapeutics *in vitro*, and it has been shown to reduce resistance to chemotherapy *in vitro* and in animal models,

although defibrotide has no antitumor properties of its own [Mitsiades *et al.* 2009]. Defibrotide is well tolerated and can be administered intravenously, intramuscularly, or orally (reviewed by [Palmer and Goa, 1993]).

The first study to evaluate defibrotide for the treatment of severe VOD was performed in the USA and analyzed 19 patients with severe VOD after SCT (defined as bilirubin  $>2$  mg/dl, and two or more of the following clinical features: hepatomegaly and right upper quadrant pain, ascites, or  $>5\%$  weight gain above admission weight), with patients treated on a compassionate-use basis [Richardson *et al.* 1998]. Resolution of VOD ( $<2$  mg/dl bilirubin and improvement in other symptoms) was observed in eight patients (42%), six of whom survived for longer than 100 days, and no significant bleeding was seen. Although this was a retrospective study and of limited sample size, the encouraging findings prompted a European multicenter compassionate-use study, which assessed defibrotide in 40 patients with VOD post SCT (according to either the Baltimore or Seattle criteria) [Chopra *et al.* 2000]. This study reported a complete response (CR) in 22 patients (55%) and 17 patients (43%) were still alive after 100 days [Chopra *et al.* 2000], again with no significant toxicity described. However, the data were based on a retrospective review of eligibility and outcome forms at individual treating centers. Thus, these results must be interpreted with some caution.

The encouraging findings of the initial US experience led to the enrollment of an additional 69 patients with severe VOD into a larger multi-institutional study, to provide a total of 88 patients as part of an emergency use dose escalation protocol. The study reported CR in 36% of patients, with a 100-day survival rate of 35% [Richardson *et al.* 2002]. A decrease in PAI-1 levels during defibrotide treatment was associated with better outcome. In line with the VOD risk factors discussed above, patients who had received autologous SCT responded better than those who had received allogeneic SCT, and improved survival was observed in patients who had not previously received BU-based conditioning compared with those who had [Richardson *et al.* 2002]. Since this study, several case series have reported similar CR rates and improved survival with defibrotide in patients with severe VOD [Bairey *et al.* 2002; Palladino *et al.* 2008; Shah *et al.* 2009].

In a recent, rigorously conducted, prospective phase II, multicenter, randomized, dose-finding trial, adult and pediatric patients with severe VOD were randomized to receive one of two doses of defibrotide (25 mg/kg/day or 40 mg/kg/day) [Richardson *et al.* 2010b]. Patients were eligible if they had at least a 30% chance of developing severe VOD by the Bearman prognostic model [Bearman *et al.* 1993a], or had MOF. Overall, 46% of 141 evaluable patients achieved CR, and the 100-day survival rate was 42% in 149 treated patients, with no significant difference in response between the two dose regimens. Defibrotide was generally well tolerated, with treatment-related adverse events reported in only 8% of patients, although a trend toward more toxicity was seen with the 40 mg/kg/day defibrotide dose compared with the 25 mg/kg/day dose, particularly among the pediatric patients. Based on these results, 25 mg/kg/day was the dose selected for further study.

A pivotal phase III, historically-controlled clinical trial of defibrotide in patients with severe VOD has been recently completed [Richardson *et al.* 2009]. The trial comprised 102 patients with VOD (according to the Baltimore criteria) within 21 days of SCT and with MOF (renal and/or lung dysfunction) by day 28 post transplant. Patients received intravenous defibrotide 6.25 mg/kg every 6 h (total 25 mg/kg/day) for at least 21 days. Due to the life-threatening nature of severe VOD and the absence of any other effective treatment as a comparator, the placebo arm of this phase III study was composed of a matched historical control (HC). Medical charts at each center were reviewed by an independent Medical Review Committee (MRC) to provide 32 suitable HC patients. The MRC was blinded to outcome data from both the defibrotide and HC arms. The primary endpoint of this trial was CR (bilirubin <2 mg/dl and resolution of MOF) by 100 days post SCT; the secondary endpoint was 100-day mortality. The 100-day CR rate was significantly higher in the defibrotide arm compared with the HC arm (24% *versus* 9%, respectively; adjusted  $p = 0.015$ ). There was a substantial decrease in the 100-day mortality rate with defibrotide compared with HC (62% *versus* 75%, respectively; adjusted  $p = 0.051$ ). A strong correlation was observed between CR and survival for both patients receiving defibrotide ( $p < 0.0001$ ) and those in the HC group ( $p = 0.0016$ ). Subgroup analysis revealed that in patients receiving defibrotide compared with those in the HC group, autologous transplantation, age 16 years and younger, and dialysis

independence were associated with a significantly higher CR ( $p = 0.005$ , 0.04 and  $p = 0.027$ , respectively). A similar number of patients in both groups experienced hemorrhage: 65% of patients receiving defibrotide and 69% of patients in the HC group, with treatment discontinued in 18% of patients who received defibrotide due to possible drug-related adverse events. Whilst these results are encouraging, it should be noted that this phase III study was not a prospectively randomized study, and matched HCs were used as the comparator. Although using a HC methodology is not ideal, the absence of any other effective treatment as a comparator in patients with severe disease limits the options available for ethically evaluating the efficacy of defibrotide, and so this approach constituted the most reasonable strategy in this setting.

A meta-analysis combining results from the above phase II and III trials has since been conducted [Richardson *et al.* 2010c]. Of the total 133 patients treated with defibrotide 25 mg/kg/day, 29% achieved a CR by day 100 compared with 9% in the HC group ( $p = 0.0021$ ). The mortality rate at day 100 was 60% in the defibrotide cohort *versus* 75% in the HC cohort ( $p = 0.0408$ ). Hemorrhage and GvHD incidence were lower in patients treated with defibrotide compared with those in the HC group (61% *versus* 72%, respectively, for hemorrhage; 6% *versus* 25% for GvHD). The observations of reduced GvHD seen in both treatment and prophylactic studies of defibrotide are intriguing and imply that endothelial injury is also important in the development of acute GvHD, so warranting further evaluation [Cooke *et al.* 2008].

Due to the recognition of VOD with MOF as a life-threatening condition, defibrotide became available in the USA through an investigational new drug (IND) treatment protocol in December 2007. An interim analysis of 104 patients enrolled on this protocol from multiple centers found that 36 patients (35%) achieved a CR at day 100 post transplant, and the 100-day overall survival was 39% [Richardson *et al.* 2010a]. From this protocol, 69 patients met the eligibility criteria of the pivotal phase III trial, and when compared with HC counterparts in the phase III trial, they achieved a significantly higher CR rate ( $p = 0.0007$ ). Although comparison with matched HCs has limitations, as discussed previously, this is a promising finding and may reflect the fact that this population were marginally less sick in

**Table 2.** Summary of clinical response and overall survival with defibrotide for the treatment of severe hepatic veno-occlusive disease.

	Clinical response (%)	100-day mortality (%)
Phase II* (N = 149)	46 <sup>#</sup>	42
Phase III\$ (N = 102)	24	62
Meta-analysis <sup>†</sup> (N = 133)	29	60
IND protocol <sup>§</sup> (N = 104)	35	61

\*[Richardson et al. 2010b].  
\$[Richardson et al. 2009].  
†[Richardson et al. 2010c].  
§[Richardson et al. 2010a].  
<sup>#</sup>N = 141.  
IND, investigational new drug.

terms of degree of MOF, an observation also seen in other studies of defibrotide therapy where earlier intervention has been associated with better outcome [Corbacioglu *et al.* 2004; Richardson *et al.* 2010b]; Table 2 summarizes comparable data from these larger pivotal studies.

In a recent retrospective analysis of 845 consecutive cases of allogeneic SCT performed in a single center during the last 24 years, the mortality rate due to VOD was 36% before 1997 compared with 14% since 1997, the only relevant change in the management of these patients being the introduction of defibrotide [Carreras *et al.* 2011]. Whilst other changes in the management of patients with VOD have also been associated with improved outcomes, and these include the increasing use of reduced-intensity conditioning regimens [Ramasamy *et al.* 2006], use of high-resolution human leukocyte antigen typing [Petersdorf, 2004], improvement of patient support measures, plus the increasing use of peripheral blood stem cells, this observation was considered compelling [Carreras *et al.* 2011]. Switching administration from oral to intravenous BU and using targeted dose-adjusted BU also decreases the incidence of VOD and may improve outcomes for patients with VOD, although the impact of this change on survival has been at best very modest in patients with established disease [Clopés *et al.* 2006; Kashyap *et al.* 2002].

**Defibrotide as prophylaxis.** The promising results observed in the treatment trials described above provided a good rationale for further investigation of defibrotide as prophylaxis against VOD following SCT. Although no prospective randomized studies in adult SCT have been published to date,

there have been a number of prospective historically controlled trials suggesting that defibrotide is safe and effective in the setting of VOD prevention. One study compared 52 patients receiving defibrotide prophylaxis in addition to heparin with a HC group of 52 patients [Chalandon *et al.* 2004]. The investigators reported that no patients receiving defibrotide and heparin prophylaxis developed VOD compared with 19% of patients in the HC group ( $p = 0.001$ ). There was also a trend towards improved 100-day survival with defibrotide and heparin compared with the HC group ( $p = 0.07$ ). A more recent study evaluated 58 consecutive patients receiving protocol-directed defibrotide prophylaxis without concurrent heparin following allogeneic SCT [Dignan *et al.* 2007]. No patients developed VOD (according to the Baltimore criteria) and no patients died within 100 days of SCT, which was significantly better than expected based upon prior incidence rates at that center (namely, the Royal Marsden Hospital, UK).

The role of defibrotide to prevent VOD in children has been recently evaluated in a prospective multicenter phase II/III study [Corbacioglu *et al.* 2010]. This study randomized 360 children (<18 years) undergoing myeloablative SCT (69% allogeneic, 31% autologous) to either receive prophylactic defibrotide from conditioning to 30 days post SCT or no prophylaxis (control group). In the intent-to-treat analysis ( $n = 356$ ) 12% of patients in the defibrotide arm *versus* 20% in the control group developed VOD by day 30 after SCT ( $p = 0.051$ ). In the per protocol analysis ( $n = 360$ ) VOD incidence was 11% in the defibrotide arm *versus* 20% in the control group ( $p = 0.023$ ). The composite score for morbidity and mortality

was significantly lower with defibrotide compared with the control ( $p = 0.034$ ), and renal failure was observed in 1% of patients in the defibrotide arm *versus* 6% in the control group ( $p = 0.017$ ). Excluding patients with autologous SCT, the incidence (45% *versus* 63%;  $p = 0.004$ ) and severity ( $p = 0.003$ ) of acute GvHD by day 100 was significantly reduced in the patients in the defibrotide arm compared with the control arm, suggesting that endothelial injury is also important in the pathogenesis of GvHD. Results from this prospective randomized trial confirm results from previously reported studies showing an advantage of defibrotide prophylaxis in children at a high risk of developing VOD [Cappelli *et al.* 2009; Corbacioglu *et al.* 2006; Qureshi *et al.* 2008; Versluys *et al.* 2004]. Importantly, defibrotide has been demonstrated to have favorable effects on thrombotic microangiopathy and renal failure [Bonomini *et al.* 1984, 1985; Vangelista *et al.* 1986]; preliminary studies in the SCT population have been favorable, with a positive effect on renal outcome also seen in this large pediatric prevention trial [Corbacioglu *et al.* 2010].

### Future directions

Defibrotide is currently available via an ongoing treatment IND study in the USA and elsewhere via named patient use programmes, with regulatory approval for both treatment and prophylaxis of hepatic VOD being actively sought in the EU and the USA. Additional studies in prophylaxis are planned in adult populations and specific high-risk settings.

With improved understanding of the mechanism of action of defibrotide and pathogenesis of VOD, it may become possible to rationally design treatment and preventative strategies for VOD based on patient and transplant-specific profiles. As an example, subgroup analysis of the defibrotide phase III clinical study has already identified patients at increased risk of VOD, and those most likely to respond to defibrotide [Richardson *et al.* 2009], suggesting that a more personalized approach could further improve treatment and prevention outcomes. Though clinical trials with defibrotide have provided the most promising results as effective therapy for VOD to date, 100-day mortality from severe VOD remains unacceptably high at over 50%. Other therapies, such as antithrombin III, novel plasminogen activator inhibitors, N-acetyl cysteine and other novel antithrombotics may

warrant further investigation in combination with defibrotide [Ho *et al.* 2008; Ringden *et al.* 2000]. If these agents complement defibrotide therapy without increasing the risk of bleeding or other toxicity, superior outcomes and survival may yet be realized.

Importantly, recent observations have found the outcome of early intervention with defibrotide to be superior to delayed treatment [Richardson *et al.* 2010a]. Elevations of von Willebrand factor, thrombomodulin, E-selectin, and soluble ICAM-1 before and early after allogeneic transplantation may be useful in predicting VOD in patients receiving sirolimus [Cutler *et al.* 2010] and could lead to pre-emptive trials based on biomarkers, leading to improved treatment strategies to reverse VOD pathophysiology earlier in specific high-risk groups [Platzbecker *et al.* 2009].

### Acknowledgements

The authors acknowledge Kate Silverthorne and Abigail Marmont for their assistance in writing this manuscript, and the administrative assistance of Katie Redman and Michelle Maglio of the Dana-Farber Cancer Institute.

### Funding

Funding for writing and administrative assistance was provided by Gentium S.p.A and the Rick Corman Multiple Myeloma Research Fund, respectively.

### Conflict of interest statement

V.T. Ho, S. Giralt, S. Arai, S. Mineishi, J.H. Antin and N. Stavitzski have no conflicts of interest to declare. P.G. Richardson is on the advisory board for Gentium S.p.A. C. Cutler is consultant for Sigma Tau and has equity in Gentium S.p.A. D. Niederwieser is a consultant for the speakers bureau, Gentium S.p.A. E. Holler has received honoraria and research grants from Gentium S.p.A. E. Carreras and R. Soiffer have received honoraria from Gentium S.p.A.

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