

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

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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 α , 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 β (TGF- β); TGF- β 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- β has been implicated in the pathogenesis of fibrosis [Czaja *et al.* 1989], and elevated levels of TGF- β 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 procoagulant 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 [Hausmann *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 multicenter 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 [Bailey *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 [#]	42
Phase III [§] (N = 102)	24	62
Meta-analysis [‡] (N = 133)	29	60
IND protocol [§] (N = 104)	35	61

*[Richardson *et al.* 2010b].
[§][Richardson *et al.* 2009].
[‡][Richardson *et al.* 2010c].
[§][Richardson *et al.* 2010a].
[#]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 [Clopes *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].

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Conflict of interest statement

V.T. Ho, S. Giralt, S. Arai, S. Mineishi, J.H. Antin and N. Stavitzki 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.

References

- Anscher, M.S., Peters, W.P., Reisenbichler, H., Petros, W.P. and Jirtle, R.L. (1993) Transforming growth factor beta as a predictor of liver and lung fibrosis after autologous bone marrow transplantation for advanced breast cancer. *N Engl J Med* 328: 1592–1598.
- Baars, J.W., de Boer, J.P., Wagstaff, J., Roem, D., Eerenberg-Belmer, A.J., Nauta, J. *et al.* (1992)

- Interleukin-2 induces activation of coagulation and fibrinolysis: resemblance to the changes seen during experimental endotoxaemia. *Br J Haematol* 82: 295–301.
- Bairey, O., Kirgner, I., Yakobi, M., Hamdan, A., Ben-Ari, Z. and Shaklai, M. (2002) Clinical severe hepatic venoocclusive disease during induction treatment of acute monoblastic leukemia managed with defibrotide. *Am J Hematol* 69: 281–284.
- Batsis, I., Yannaki, E., Kaloyannidis, P., Sakellari, I., Smias, C., Georgoulis, I. *et al.* (2006) Veno-occlusive disease prophylaxis with fresh frozen plasma and heparin in bone marrow transplantation. *Thromb Res* 118: 611–618.
- Bearman, S.I. (1995) The syndrome of hepatic veno-occlusive disease after marrow transplantation. *Blood* 85: 3005–3020.
- Bearman, S.I., Anderson, G.L., Mori, M., Hinds, M.S., Shulman, H.M., McDonald, G.B. (1993a) Venoocclusive disease of the liver: development of a model for predicting fatal outcome after marrow transplantation. *J Clin Oncol* 11: 1729–1736.
- Bearman, S.I., Shen, D.D., Hinds, M.S., Hill, H.A. and McDonald, G.B. (1993b) A phase I/II study of prostaglandin E1 for the prevention of hepatic venoocclusive disease after bone marrow transplantation. *Br J Haematol* 84: 724–730.
- Benimetskaya, L., Wu, S., Voskresenskiy, A.M., Echart, C., Zhou, J.F., Shin, J. *et al.* (2008) Angiogenesis alteration by defibrotide: implications for its mechanism of action in severe hepatic veno-occlusive disease. *Blood* 112: 4343–4352.
- Bianchi, G., Barone, D., Lanzarotti, E., Tettamanti, R., Porta, R., Moltrasio, D. *et al.* (1993) Defibrotide, a single-stranded polydeoxyribonucleotide acting as an adenosine receptor agonist. *Eur J Pharmacol* 238: 327–334.
- Bonomini, V., Frasca, G.M., Raimondi, C., Liviano, D.G. and Vangelista, A. (1985) Effect of a new antithrombotic agent (defibrotide) in acute renal failure due to thrombotic microangiopathy. *Nephron* 40: 195–200.
- Bonomini, V., Vangelista, A. and Frasca, G. (1984) A new antithrombotic agent in the treatment of acute renal failure due to hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura. *Nephron* 37: 144.
- Cappelli, B., Chiesa, R., Evangelio, C., Biffi, A., Rocchia, T., Frugnoli, I. *et al.* (2009) Absence of VOD in paediatric thalassaemic HSCT recipients using defibrotide prophylaxis and intravenous Busulfan. *Br J Haematol* 147: 554–560.
- Carreras, E. (2000) Veno-occlusive disease of the liver after hemopoietic cell transplantation. *Eur J Haematol* 64: 281–291.
- Carreras, E., Bertz, H., Arcese, W., Vernant, J.P., Tomas, J.F., Hagglund, H. *et al.* (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., Diaz-Beya, M., Rosinol, L., Martinez, C., Fernandez-Aviles, F. and Rovira, M. (2011) The incidence of veno-occlusive disease following allogeneic hematopoietic cell transplantation has diminished and the outcome improved over the last decade. *Biol Blood Marrow Transplant* 17: 1713–1720.
- Cesaro, S., Pillon, M., Talenti, E., Toffolutti, T., Calore, E., Tridello, G. *et al.* (2005) A prospective survey on incidence, risk factors and therapy of hepatic veno-occlusive disease in children after hematopoietic stem cell transplantation. *Haematologica* 90: 1396–1404.
- Chalandon, Y., Roosnek, E., Mermillod, B., Newton, A., Ozsahin, H., Wacker, P. *et al.* (2004) Prevention of veno-occlusive disease with defibrotide after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 10: 347–354.
- Chopra, R., Eaton, J.D., Grassi, A., Potter, M., Shaw, B., Salat, C. *et al.* (2000) Defibrotide for the treatment of hepatic veno-occlusive disease: results of the European compassionate-use study. *Br J Haematol* 111: 1122–1129.
- Clopes, A., Sureda, A., Sierra, J., Queralto, J.M., Broto, A., Farre, R. *et al.* (2006) Absence of veno-occlusive disease in a cohort of multiple myeloma patients undergoing autologous stem cell transplantation with targeted busulfan dosage. *Eur J Haematol* 77: 1–6.
- Cooke, K.R., Jannin, A. and Ho, V. (2008) The contribution of endothelial activation and injury to end-organ toxicity following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 14: 23–32.
- Coppell, J.A., Richardson, P.G., Soiffer, R., Martin, P.L., Kernan, N.A., Chen, A. *et al.* (2010) Hepatic veno-occlusive disease following stem cell transplantation: incidence, clinical course, and outcome. *Biol Blood Marrow Transplant* 16: 157–168.
- Corbacioglu, S., Cesaro, S., Faraci, M., Valteau-Couanet, D., Gruhn, B., Boelens, J.J. *et al.* (2010) Defibrotide prevents hepatic VOD and reduces significantly VOD-associated complications in children at high risk: final results of a prospective phase II/III multicentre study. *Bone Marrow Transplant* 45 (Suppl. 2): S1.
- Corbacioglu, S., Greil, J., Peters, C., Wulffraat, N., Laws, H.J., Dilloo, D. *et al.* (2004) Defibrotide

- in the treatment of children with veno-occlusive disease (VOD): a retrospective multicentre study demonstrates therapeutic efficacy upon early intervention. *Bone Marrow Transplant* 33: 189–195.
- Corbacioglu, S., Honig, M., Lahr, G., Stohr, S., Berry, G., Friedrich, W. *et al.* (2006) Stem cell transplantation in children with infantile osteopetrosis is associated with a high incidence of VOD, which could be prevented with defibrotide. *Bone Marrow Transplant* 38: 547–553.
- Cutler, C., Kim, H.T., Ayanian, S., Bradwin, G., Revta, C., Aldridge, J. *et al.* (2010) Prediction of veno-occlusive disease using biomarkers of endothelial injury. *Biol Blood Marrow Transplant* 16: 1180–1185.
- Cutler, C., Stevenson, K., Kim, H.T., Richardson, P., Ho, V.T., Linden, E. *et al.* (2008) Sirolimus is associated with veno-occlusive disease of the liver after myeloablative allogeneic stem cell transplantation. *Blood* 112: 4425–4431.
- Czaja, M.J., Weiner, F.R., Flanders, K.C., Giambrone, M.A., Wind, R., Biempica, L. *et al.* (1989) In vitro and in vivo association of transforming growth factor-beta 1 with hepatic fibrosis. *J Cell Biol* 108: 2477–2482.
- DeLeve, L.D. (1996) Cellular target of cyclophosphamide toxicity in the murine liver: role of glutathione and site of metabolic activation. *Hepatology* 24: 830–837.
- DeLeve, L.D., Valla, D.C. and Garcia-Tsao, G. (2009) Vascular disorders of the liver. *Hepatology* 49: 1729–1764.
- DeLeve, L.D. and Wang, X. (2000) Role of oxidative stress and glutathione in busulfan toxicity in cultured murine hepatocytes. *Pharmacology* 60: 143–154.
- DeLeve, L.D., Wang, X., Kanel, G.C., Ito, Y., Bethea, N.W., McCuskey, M.K. *et al.* (2003) Decreased hepatic nitric oxide production contributes to the development of rat sinusoidal obstruction syndrome. *Hepatology* 38: 900–908.
- Dignan, F., Gujral, D., Ethell, M., Evans, S., Treleaven, J., Morgan, G. *et al.* (2007) Prophylactic defibrotide in allogeneic stem cell transplantation: minimal morbidity and zero mortality from veno-occlusive disease. *Bone Marrow Transplant* 40: 79–82.
- Echart, C.L., Graziadio, B., Somaini, S., Ferro, L.I., Richardson, P.G., Fareed, J. *et al.* (2009) The fibrinolytic mechanism of defibrotide: effect of defibrotide on plasmin activity. *Blood Coagul Fibrinolysis* 20: 627–634.
- Eissner, G., Multhoff, G., Gerbitz, A., Kirchner, S., Bauer, S., Haffner, S. *et al.* (2002) Fludarabine induces apoptosis, activation, and allogenicity in human endothelial and epithelial cells: protective effect of defibrotide. *Blood* 100: 334–340.
- Essell, J.H., Schroeder, M.T., Harman, G.S., Halvorson, R., Lew, V., Callander, N. *et al.* (1998) Ursodiol prophylaxis against hepatic complications of allogeneic bone marrow transplantation. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 128: 975–981.
- Falanga, A., Vignoli, A., Marchetti, M. and Barbui, T. (2003) Defibrotide reduces procoagulant activity and increases fibrinolytic properties of endothelial cells. *Leukemia* 17: 1636–1642.
- Forrest, D.L., Thompson, K., Dorcas, V.G., Couban, S.H. and Pierce, R. (2003) Low molecular weight heparin for the prevention of hepatic veno-occlusive disease (VOD) after hematopoietic stem cell transplantation: a prospective phase II study. *Bone Marrow Transplant* 31: 1143–1149.
- Hausmann, U., Fischer, J., Eber, S., Scherer, F., Seger, R., Gungor, T. (2006) Hepatic veno-occlusive disease in pediatric stem cell transplantation: impact of pre-emptive antithrombin III replacement and combined antithrombin III/defibrotide therapy. *Haematologica* 91: 795–800.
- Ho, V., Momtaz, P., Didas, C., Wadleigh, M. and Richardson, P. (2004) Post-transplant hepatic veno-occlusive disease: pathogenesis, diagnosis and treatment. *Rev Clin Exp Hematol* 8: E3.
- Ho, V.T., Revta, C. and Richardson, P.G. (2008) Hepatic veno-occlusive disease after hematopoietic stem cell transplantation: update on defibrotide and other current investigational therapies. *Bone Marrow Transplant* 41: 229–237.
- Holler, E., Kolb, H.J., Moller, A., Kempeni, J., Liesenfeld, S., Pechumer, H. *et al.* (1990) Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. *Blood* 75: 1011–1016.
- Jones, R.J., Lee, K.S., Beschorner, W.E., Vogel, V.G., Grochow, L.B., Braine, H.G. *et al.* (1987) Venooclusive disease of the liver following bone marrow transplantation. *Transplantation* 44: 778–783.
- Kashyap, A., Wingard, J., Cagnoni, P., Roy, J., Tarantolo, S., Hu, W. *et al.* (2002) Intravenous versus oral busulfan as part of a busulfan/cyclophosphamide preparative regimen for allogeneic hematopoietic stem cell transplantation: decreased incidence of hepatic venoocclusive disease (HVOD), HVOD-related mortality, and overall 100-day mortality. *Biol Blood Marrow Transplant* 8: 493–500.
- Kumar, S., DeLeve, L.D., Kamath, P.S. and Tefferi, A. (2003) Hepatic veno-occlusive disease (sinusoidal obstruction syndrome) after hematopoietic stem cell transplantation. *Mayo Clin Proc* 78: 589–598.
- Kuroki, I., Miyazaki, T., Mizukami, I., Matsumoto, N. and Matsumoto, I. (2004) Effect of sodium

- nitroprusside on ischemia-reperfusion injuries of the rat liver. *Hepatology* 51: 1404–1407.
- Lakshminarayanan, S., Sahdev, I., Goyal, M., Vlachos, A., Atlas, M. and Lipton, J.M. (2010) Low incidence of hepatic veno-occlusive disease in pediatric patients undergoing hematopoietic stem cell transplantation attributed to a combination of intravenous heparin, oral glutamine, and ursodiol at a single transplant institution. *Pediatr Transplant* 14: 618–621.
- Lappas, C.M., Liu, P.C., Linden, J., Kang, E.M. and Malech, H.L. (2010) Adenosine A2A receptor activation limits graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *J Leukoc Biol* 87: 345–354.
- Lawson, C. and Wolf, S. (2009) ICAM-1 signaling in endothelial cells. *Pharmacol Rep* 61: 22–32.
- Lee, J.H., Choi, S.J., Lee, J.H., Kim, S.E., Park, C.J., Chi, H.S. *et al.* (2005) Decreased incidence of hepatic veno-occlusive disease and fewer hemostatic derangements associated with intravenous busulfan vs oral busulfan in adults conditioned with busulfan + cyclophosphamide for allogeneic bone marrow transplantation. *Ann Hematol* 84: 321–330.
- Lee, J.H., Lee, K.H., Lee, J.H., Kim, S., Seol, M., Park, C.J. *et al.* (2002) Plasminogen activator inhibitor-1 is an independent diagnostic marker as well as severity predictor of hepatic veno-occlusive disease after allogeneic bone marrow transplantation in adults conditioned with busulphan and cyclophosphamide. *Br J Haematol* 118: 1087–1094.
- Lee, S.H., Yoo, K.H., Sung, K.W., Koo, H.H., Kwon, Y.J., Kwon, M.M. *et al.* (2010) Hepatic veno-occlusive disease in children after hematopoietic stem cell transplantation: incidence, risk factors, and outcome. *Bone Marrow Transplant* 45: 1287–1293.
- McCune, J.S., Batchelder, A., Deeg, H.J., Gooley, T., Cole, S., Phillips, B. *et al.* (2007) Cyclophosphamide following targeted oral busulfan as conditioning for hematopoietic cell transplantation: pharmacokinetics, liver toxicity, and mortality. *Biol Blood Marrow Transplant* 13: 853–862.
- McDonald, G.B. (2002) Management of hepatic sinusoidal obstruction syndrome following treatment with gemtuzumab ozogamicin (Mylotarg). *Clin Lymphoma* 2 (Suppl. 1): S35–S39.
- McDonald, G.B., Hinds, M.S., Fisher, L.D., Schoch, H.G., Wolford, J.L., Banaji, M. *et al.* (1993) Venous-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., Matthews, D.E., Shulman, H.M. and Thomas, E.D. (1984) Venous-occlusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. *Hepatology* 4: 116–122.
- McDonald, G.B., Slattery, J.T., Bouvier, M.E., Ren, S., Batchelder, A.L., Kalhorn, T.F. *et al.* (2003) Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. *Blood* 101: 2043–2048.
- McKoy, J.M., Angelotta, C., Bennett, C.L., Tallman, M.S., Wadleigh, M., Evens, A.M. *et al.* (2007) Gemtuzumab ozogamicin-associated sinusoidal obstructive syndrome (SOS): an overview from the research on adverse drug events and reports (RADAR) project. *Leuk Res* 31: 599–604.
- Mitsiades, C.S., Rouleau, C., Echart, C., Menon, K., Teicher, B., Distaso, M. *et al.* (2009) Preclinical studies in support of defibrotide for the treatment of multiple myeloma and other neoplasias. *Clin Cancer Res* 15: 1210–1221.
- Moscardó, F., Sanz, G.F. de la Rubia, J., Jiménez, C., Saavedra, S., Regadera, A. *et al.* (2001) Marked reduction in the incidence of hepatic veno-occlusive disease after allogeneic hematopoietic stem cell transplantation with CD34(+) positive selection. *Bone Marrow Transplant* 27: 983–988.
- Nawroth, P.P., Handley, D.A., Esmon, C.T. and Stern, D.M. (1986) Interleukin 1 induces endothelial cell procoagulant while suppressing cell-surface anticoagulant activity. *Proc Natl Acad Sci U S A* 83: 3460–3464.
- Nurnberger, W., Michelmann, I., Burdach, S. and Gobel, U. (1998) Endothelial dysfunction after bone marrow transplantation: increase of soluble thrombomodulin and PAI-1 in patients with multiple transplant-related complications. *Ann Hematol* 76: 61–65.
- Ohashi, K., Tanabe, J., Watanabe, R., Tanaka, T., Sakamaki, H., Maruta, A. *et al.* (2000) The Japanese multicenter open randomized trial of ursodeoxycholic acid prophylaxis for hepatic veno-occlusive disease after stem cell transplantation. *Am J Hematol* 64: 32–38.
- Okada, Y., Zuo, X.J., Marchevsky, A.M., Nicolaidou, E., Toyoda, M., Matloff, J.M. *et al.* (1999) Antithrombin III treatment improves parameters of acute inflammation in a highly histoincompatible model of rat lung allograft rejection. *Transplantation* 67: 526–528.
- Ostrovsky, O., Shimoni, A., Rand, A., Vlodaysky, I. and Nagler, A. (2010) Genetic variations in the heparanase gene (HPSE) associate with increased risk of GVHD following allogeneic stem cell transplantation: effect of discrepancy between recipients and donors. *Blood* 115: 2319–2328.

- Palladino, M., Miele, L., Pompili, M., Forgione, A., Vellone, V., Vecchio, F.M. *et al.* (2008) Severe veno-occlusive disease after autologous peripheral blood stem cell transplantation for high-grade non-Hodgkin lymphoma: report of a successfully managed case and a literature review of veno-occlusive disease. *Clin Transplant* 22: 837–841.
- Palmer, K.J. and Goa, K.L. (1993) Defibrotide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in vascular disorders. *Drugs* 45: 259–294.
- Palomo, M., az-Ricart, M., Rovira, M., Escolar, G. and Carreras, E. (2011) Defibrotide prevents the activation of macrovascular and microvascular endothelia caused by soluble factors released to blood by autologous hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 14: 497–506.
- Petersdorf, E.W. (2004) HLA matching in allogeneic stem cell transplantation. *Curr Opin Hematol* 11: 386–391.
- Pidala, J., Kim, J., Anasetti, C., Kharfan-Dabaja, M.A., Nishihori, T., Field, T. *et al.* (2010) Pharmacokinetic targeting of intravenous busulfan reduces conditioning regimen related toxicity following allogeneic hematopoietic cell transplantation for acute myelogenous leukemia. *J Hematol Oncol* 3: 36.
- 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.
- Platzbecker, U., von Bonin, M., Goekkurt, E., Radke, J., Binder, M., Kiani, A. *et al.* (2009) Graft-versus-host disease prophylaxis with everolimus and tacrolimus is associated with a high incidence of sinusoidal obstruction syndrome and microangiopathy: results of the EVTAC trial. *Biol Blood Marrow Transplant* 15: 101–108.
- Qureshi, A., Marshall, L. and Lancaster, D. (2008) Defibrotide in the prevention and treatment of veno-occlusive disease in autologous and allogeneic stem cell transplantation in children. *Pediatr Blood Cancer* 50: 831–832.
- Ramasamy, K., Lim, Z.Y., Pagliuca, A., Grundy, R., Devereux, S., Ho, A.Y. *et al.* (2006) Incidence and management of hepatic venoocclusive disease in 237 patients undergoing reduced-intensity conditioning (RIC) haematopoietic stem cell transplantation (HSCT). *Bone Marrow Transplant* 38: 823–824.
- Reilly, T.M., Mousa, S.A., Seetharam, R. and Racanelli, A.L. (1994) Recombinant plasminogen activator inhibitor type 1: a review of structural, functional, and biological aspects. *Blood Coagul Fibrinolysis* 5: 73–81.
- Richardson, P. and Guinan, E. (1999) The pathology, diagnosis, and treatment of hepatic veno-occlusive disease: current status and novel approaches. *Br J Haematol* 107: 485–493.
- Richardson, P. and Guinan, E. (2001) Hepatic veno-occlusive disease following hematopoietic stem cell transplantation. *Acta Haematol* 106: 57–68.
- Richardson, P., Tomblyn, M., Kernan, N., Brochstein, J.A., Mineishi, S., Termuhlen, A. *et al.* (2009) Defibrotide (DF) in the treatment of severe hepatic veno-occlusive disease (VOD) with multi-organ failure (MOF) following stem cell transplantation (SCT): results of a phase 3 study utilizing a historical control. *Blood* 114: abstract 654.
- Richardson, P.G., Elias, A.D., Krishnan, A., Wheeler, C., Nath, R., Hoppensteadt, D. *et al.* (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.G., Murakami, C., Jin, Z., Warren, D., Momtaz, P., Hoppensteadt, D. *et al.* (2002) Multi-institutional use of defibrotide in 88 patients after stem cell transplantation with severe veno-occlusive disease and multisystem organ failure: response without significant toxicity in a high-risk population and factors predictive of outcome. *Blood* 100: 4337–4343.
- Richardson, P.G., Smith, A., Arai, S., Grupp, S., Kernan, N.A., Adams, R., *et al.* (2010a). Defibrotide (DF) in the treatment of severe hepatic veno-occlusive disease (VOD) with multi-organ failure (MOF): results of a treatment IND expanded access protocol. *Blood* 116: abstract 906.
- Richardson, P.G., Soiffer, R.J., Antin, J.H., Uno, H., Jin, Z., Kurtzberg, J. *et al.* (2010b) Defibrotide for the treatment of severe hepatic veno-occlusive disease and multiorgan failure after stem cell transplantation: a multicenter, randomized, dose-finding trial. *Biol Blood Marrow Transplant* 16: 1005–1017.
- Richardson, P.G., Steinbach, G., Kernan, N., Guinan, E.C., Chen, A.R., Martin, P.L., *et al.* (2010c) Meta-analysis of defibrotide (DF) in the treatment of severe hepatic veno-occlusive disease (VOD) with multi-organ failure (MOF) with comparison to a historical control (HC). *Blood* 116: abstract 3481.
- Ringden, O., Remberger, M., Lehmann, S., Hentschke, P., Mattsson, J., Klaesson, S. *et al.* (2000) N-acetylcysteine for hepatic veno-occlusive disease after allogeneic stem cell transplantation. *Bone Marrow Transplant* 25: 993–996.
- Ruutu, T., Eriksson, B., Remes, K., Juvonen, E., Volin, L., Remberger, M. *et al.* (2002) Ursodeoxycholic acid for the prevention of hepatic

complications in allogeneic stem cell transplantation. *Blood* 100: 1977–1983.

Salat, C., Holler, E., Reinhardt, B., Kolb, H.J., Seeber, B., Ledderose, G. *et al.* (1994) Parameters of the fibrinolytic system in patients undergoing BMT: elevation of PAI-1 in veno-occlusive disease. *Bone Marrow Transplant* 14: 747–750.

Saldanha-Araujo, F., Ferreira, F.I., Palma, P.V., Araujo, A.G., Queiroz, R.H., Covas, D.T. *et al.* (2011) Mesenchymal stromal cells up-regulate CD39 and increase adenosine production to suppress activated T-lymphocytes. *Stem Cell Res* 7: 66–74.


Shah, M.S., Jeevangi, N.K., Joshi, A. and Khattry, N. (2009) Late-onset hepatic veno-occlusive disease post autologous peripheral stem cell transplantation successfully treated with oral defibrotide. *J Cancer Res Ther* 5: 312–314.

Vangelista, A., Frasca, G.M., Raimondi, C., Liviano-D'Arcangelo, G. and Bonomini, V. (1986) Effects of defibrotide in acute renal failure due to thrombotic microangiopathy. *Haemostasis* 16 (Suppl. 1): 51–54.

Versluys, B., Bhattacharaya, R., Steward, C., Cornish, J., Oakhill, A. and Goulden, N. (2004) Prophylaxis with defibrotide prevents veno-occlusive disease in stem cell transplantation after gemtuzumab ozogamicin exposure. *Blood* 103: 1968.

Wadleigh, M., Richardson, P.G., Zahrieh, D., Lee, S.J., Cutler, C., Ho, V. *et al.* (2003) Prior gemtuzumab ozogamicin exposure significantly increases the risk of veno-occlusive disease in patients who undergo myeloablative allogeneic stem cell transplantation. *Blood* 102: 1578–1582.

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