DRUG ADVANCES

blood advances

The use of defibrotide in blood and marrow transplantation

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Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a potentially life-threatening complication of conditioning during hematopoietic stem cell transplantation (HSCT) or chemotherapy without HSCT, with a historically reported mean incidence of 13.7% post-HSCT. Typical symptoms of VOD/SOS may include hyperbilirubinemia, painful hepatomegaly, weight gain, and ascites. Defibrotide, a polydisperse mixture of predominantly single-stranded polydeoxyribonucleotides, is currently the only therapy approved to treat hepatic VOD/SOS with pulmonary/renal dysfunction (ie, multiorgan dysfunction/multiorgan failure [MOD/MOF]) following HSCT in the United States and to treat severe hepatic VOD/SOS post-HSCT in the European Union. In preclinical and human studies, defibrotide has demonstrated profibrinolytic, antithrombotic, anti-inflammatory, and angio-protective actions, thus promoting an anticoagulant phenotype of the endothelium that protects and stabilizes the function of endothelial cells. In a phase 3, historically controlled, multicenter trial in adults and children with VOD/SOS and MOD/MOF (defibrotide: n = 102; controls treated before defibrotide availability: n = 32), defibrotide resulted in significantly greater day +100 survival following HSCT (38.2%) vs controls (25.0%; propensity analysis-estimated between-group difference: 23%; $P = .0109$). The most common adverse events (AEs) were hypotension and diarrhea; rates of common hemorrhagic AEs were similar in the defibrotide and historical control group (64% and 75%, respectively). In a phase 3 prophylaxis trial, defibrotide was found to lower incidence of VOD/SOS in children (not an approved indication) and reduce the incidence of graft-versus-host disease. This review describes the development and clinical applications of defibrotide, focusing on its on-label use in patients with VOD/SOS and MOD/MOF after HSCT.

The development of defibrotide

Origins and drug profile

Defibrotide was introduced as part of research programs begun in the 1950s for developing compounds derived from mammalian organs, which could be used for the treatment of coagulation and thrombotic disorders, and had a lower risk for hemorrhage than other contemporary anticoagulant therapies.[1] Defibrotide was first identified in 1968 as a phosphorous-containing fraction derived from bovine lung, which was called "fraction P" and later found to be a fragment of DNA.[2,3] Renamed defibrotide, this substance was determined to be a polydisperse mixture of predominantly single-stranded polydeoxyribonucleotide sodium salts[4,5] and is currently derived via controlled depolymerization of porcine intestinal mucosal DNA.[6-9]

Compared with other oligonucleotides, defibrotide has a particularly complex mechanism of action.[8] Oligonucleotides have been observed to mimic features of heparin and interact with heparin-binding proteins, including basic fibroblast growth factor (bFGF), a key angio-protective protein that promotes microvessel

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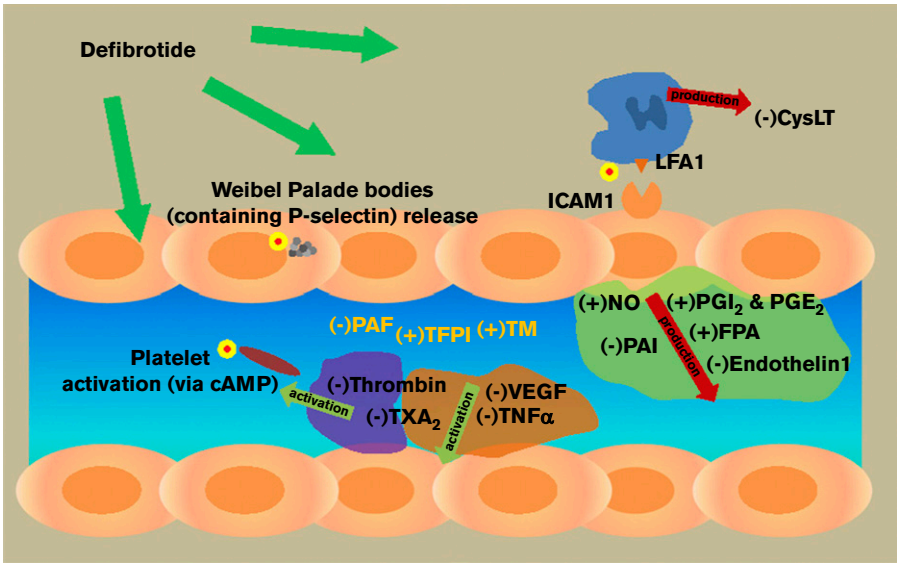

Inflammation and vascular permeability • ↓CysLT release • ↓NFκB activation (bone marrow) • ↓Platelet activation	Cytokine production • ↑PGE_2 and PGI_2 • ↓IL-6, VEGF, TXA_2, LTB4, TNF_α
Leukocyte adhesion molecules • ↓P-selectin expression • Interferes with ICAM1/LFA1 system	HLA molecules • ↓MHCI molecule expression • ↓MHCII expression
Thrombosis • ↑tPA, TFPI, TM • ↓PAI-1, PAF, thrombin	Vascular tone • Antagonizes endothelin 1 • ↑NO and NOS

Figure 1. Proposed pharmacologic actions of defibrotide. Factors involved in coagulation are depicted in yellow, factors released from endothelial cells are in the green area, factors involved in endothelial cell activation are in the orange area, and factors involved in platelet activation are in the purple area. Yellow circle with red center, inhibition, increase (+) or decrease (–); green arrow, activation; red arrow, production. cAMP, cyclic adenosine monophosphate; CysLT, cysteinyl leukotriene; FPA, fibrinogen peptide A; ICAM, intracellular adhesion molecule; IL-6, interleukin 6; LFA1, leukocyte function-associated antigen 1; LTB4, antileukotriene B4; MHC, major histocompatibility complex; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; NOS, nitric oxide synthase; PAI-1, plasminogen activator inhibitor-1; PAF, platelet-activating factor; PGE_2, prostaglandin E2; PGI_2, prostaglandin I2; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; TNF_α, tumor necrosis factor-α; TXA_2, thromboxane A2; VEGF, vascular endothelial growth factor. Reprinted from Pescador et al[2] with permission.

formation.[8,10] Addition of bFGF to endothelial cells (ECs) also induces vascular endothelial growth factor in the same model system, which in turn further promotes angiogenesis and endothelial stabilization.[8] Defibrotide has been shown in vitro to bind to and protect bFGF and to promote EC mitogenesis.[8,10]

Through these and other diverse mechanisms of action, defibrotide has, overall, demonstrated endothelial-protective properties, with profibrinolytic, antithrombotic, anti-ischemic, anti-inflammatory, and antiadhesive activities, but no significant systemic anticoagulant effects.[2,8,11-13] Defibrotide appears to exert several anti-inflammatory and antioxidant effects through interaction with the EC membrane, as shown in an endothelial cell line of hepatic origin.[14]

Key pharmacologic actions and characteristics

Defibrotide was initially considered primarily an antithrombotic and profibrinolytic agent.[3,15] Fibrinolysis was among the first pharmacologic actions of defibrotide observed in in vitro, animal, and clinical studies,[2,3,15-18] whereas aptamers from defibrotide have been identified and shown to inhibit thrombin in vitro.[19] Mechanisms theorized to be involved in defibrotide's profibrinolytic and antithrombotic effects, many of which are thought to contribute to the treatment of veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS), are shown in Figure 1.

Defibrotide has been further shown to reduce platelet adhesion and aggregate formation in humans[20]; inhibit platelet activation, possibly through inhibition of cathepsin-G in vitro[21]; and normalize excessive platelet activity in rabbits.[22] However, defibrotide has demonstrated no significant systemic anticoagulant effects in pharmacologic studies.[4,11,15,23]

Findings of in vitro and animal studies suggest that defibrotide may protect EC from toxic, inflammatory, and reperfusion damage; reduce activation of EC; and modulate their function.[4,18,24-28] In combination, these effects appear to promote an anticoagulant phenotype specific to the endothelium.[4,14] In individual studies, defibrotide was shown in vitro to protect EC, but not tumor cells, from tumor necrosis factor[29]; preserve and increase activity of anti-inflammatory and vasodilatory factors such as nitric oxide in in vitro and animal studies[2,25,30]; modulate endothelial vasodilatory function while inhibiting response to contractile factors in vitro[31]; and inhibit leukocyte–EC interaction, in part by attenuating P-selectin expression, thus reducing leukocyte rolling and adherence to the microvasculature, in animal and in vitro studies.[26,27,30]

Defibrotide also was shown in vitro to protect EC against chemotherapy-induced apoptosis without compromising the antitumor effects of cytotoxic therapy[28] and prevent activation of macro- and microvascular endothelia associated with hematopoietic stem cell transplantation (HSCT).[12] A recent study using human hepatic sinusoidal EC found that defibrotide stimulated angiogenesis dose-dependently and reduced calcineurin inhibitor-induced apoptosis.[32] Other studies in vitro and in perfused rabbit hearts suggested defibrotide may prevent intracellular calcium overloading in cardiac tissue, which further suggested cytoprotective and anti-ischemic effects.[33,34]

Therapeutic investigations and potential use

Based on its pharmacologic actions, the therapeutic potential of defibrotide has been explored in various circulatory disorders. In an early clinical study in 10 patients with thrombophlebitis, defibrotide 200 mg administered IV 3 times daily for 15 days significantly improved leg blood pressure measures and profibrinolytic activity, with rapid disappearance of symptoms.[35] A later trial randomized 288 patients (159 completed the study) with chronic venous insufficiency resulting from deep vein thrombosis (DVT) and/or reflux (chronic deep vein insufficiency) to oral defibrotide 800 mg/d or matching placebo for 1 year.[36] Defibrotide significantly reduced mean ankle circumference ($P < .01$), mean scores for pain ($P = .05$), and the number of thrombotic events such as DVT (2 events with defibrotide vs 10 with placebo; $P = .04$). In multiple, randomized, controlled DVT prophylaxis trials in postsurgery patients, defibrotide also demonstrated significant preventive efficacy vs placebo,[37,38] and similar prophylactic effect vs heparin.[39-41] Moreover, in a large open-label, multicenter study in patients following diverse surgeries (N = 4810), those given defibrotide (n = 2810) had approximately one-half the incidence of postsurgical DVT (1.17%) and pulmonary embolism (0.53%) vs 2000 patients given heparin (2.35%, $P = .002$; 1.15%, $P = .025$, respectively).[42] Postsurgical recovery was normal in both groups.

In small clinical studies in patients with peripheral arterial disease, IV or oral defibrotide treatment improved rheological and hemodynamic parameters of blood flow[43,44] and increased absolute walking distance.[45,46] A large randomized, placebo-controlled study in 310 patients with intermittent claudication showed that defibrotide 800 mg/d (n = 104) or 1200 mg/d (n = 105) significantly improved absolute walking distance at both doses vs placebo (n = 101) during a 1-year follow-up ($P < .01$).[47]

Defibrotide also has been investigated in animal and human studies for a wide variety of other conditions, although these data are generally preliminary. Such conditions include acute myocardial infarction[48,49] and coronary reocclusion after thrombolysis in acute myocardial infarction,[50] ischemia and ischemia-reperfusion injury,[6,51-53] ischemic liver damage,[54] diabetic microangiopathy[55] and diabetic retinopathy,[56] Raynaud phenomenon,[57] frostbite,[58] multiple myeloma,[59,60] and malaria.[61]

Defibrotide has been most extensively investigated for the treatment and prevention of VOD, also known as SOS, in patients undergoing HSCT[2,62,63] (see "Clinical use of defibrotide in bone marrow transplantation").

Pharmacokinetics/pharmacodynamics

In animal and phase 1 studies, defibrotide has demonstrated a half-life of ~10 to 13 minutes and bioavailability of approximately 58%, with excretion predominantly in urine.[64,65] Oral, intramuscular, and IV formulations of defibrotide have been developed, with similar half-lives. Parenteral administration is used for VOD/SOS and may have other applications in relatively short-term regimens, whereas oral administration may be preferred for longer term treatments to increase patient convenience and acceptability[11]; oral administration has not been studied in VOD/SOS. A pharmacokinetic study in 20 healthy male subjects of defibrotide 25 mg/kg IV daily, administered in 4 doses of 6.25 mg/kg (the currently approved dose),[7] reported mean (standard deviation) maximum observed plasma concentrations of 20.59 (4.11) ng/mL, total area under the plasma concentration-time curve of 42.32 (6.95) ng/h per milliliter, half-life of 0.47 (0.10) hours, and total plasma clearance of 9.629 (1.175) L/h.[66] Given this favorable profile, preparations for subcutaneous delivery are being clinically investigated.

Key development milestones and approvals granted

The following timeline describes major milestones in the development of defibrotide for commercial use.

- 1976: Activation of fibrinolytic process by "fraction P" described[67]
- 1983-1986: Defibrotide was first produced from bovine lung, with development of oral and injectable formulas; marketing authorization granted in Italy for prophylaxis of DVT and treatment of thrombophlebitis (later broadened to include "vascular disease associated with the risk of thrombosis")[9,68]
- 1995: The first reported use of defibrotide (IV administration) for treatment of VOD/SOS[62]
- 2003-2007: Defibrotide (now derived from porcine intestinal mucosa pursuant to a request by the Italian Medicines Agency to eliminate use of bovine tissues because of concerns regarding bovine spongiform encephalopathy) received an orphan designation for the prevention and treatment of VOD/SOS in patients undergoing HSCT in the United States (2003 and 2007)[69] and European Union (2004)[9]
- 2009: The phase 3 historically controlled study for treatment of hepatic VOD/SOS with MOD/MOF[70] was completed
- 2011: Initial applications were submitted to the US Food and Drug Administration and the Committee for Medicinal Products for Human Use of the European Medicines Agency for defibrotide treatment of hepatic VOD/SOS with MOD/MOF.[9,71]
- 2013: The Committee for Medicinal Products for Human Use recommended marketing authorization for defibrotide[9] for the treatment of severe hepatic VOD/SOS post-HSCT in adults and in children aged >1 month[72]; the European Commission also granted orphan drug designation to defibrotide for the prevention of graft-versus-host disease (GVHD)[73]
- 2015: Defibrotide was granted marketing authorization in Israel[71]
- 2016-2017: Defibrotide was approved in the United States for the treatment of adult and pediatric patients with hepatic VOD/SOS with renal or pulmonary dysfunction following HSCT[7]; it also received approvals in South Korea and Canada[74]

Clinical use of defibrotide in bone marrow transplantation

Disease profile of VOD/SOS

Hepatic VOD/SOS is a potentially life-threatening complication of conditioning regimens for HSCT. VOD/SOS also may be caused in

Table 1. Diagnostic criteria for VOD/SOS in adults and children

Criteria set	Age groups	Time of signs/symptoms onset*	Signs/symptoms
Baltimore (adopted for EBMT adult criteria as "classical")[80,83]	Adult and pediatric	≤21 d post-HSCT	Bilirubin ≥2 mg/dL plus 2 or more of hepatomegaly, ascites, and weight gain >5%
Modified Seattle[81]	Adult and pediatric	≤20 d post-HSCT	Two or more of bilirubin >2 mg/dL, hepatomegaly or right upper quadrant pain, and weight gain >2% (weight gain threshold of >5% sometimes used)
Late onset (defined for adults only)[83]	Adult	>21 d post-HSCT	"Classical" VOD/SOS (Baltimore criteria) **OR** histologically proven VOD/SOS **OR** 2 or more of bilirubin ≥2 mg/dL, hepatomegaly, ascites, and weight gain >5% **AND** hemodynamic and/or ultrasound evidence of VOD/SOS
Pediatric[82]	Pediatric	No time frame post-HSCT	Either Baltimore ("classical") or Seattle criteria with the following variations/exceptions: Increased bilirubin from patient's baseline for 3 consecutive days or bilirubin ≥2 mg/dL within 72 h may replace bilirubin levels specified for adults (see previous). Weight gain on 3 consecutive days replaces the 2% weight gain required in the Seattle criteria; weight gain >5% above baseline (Baltimore criteria) is maintained as a criterion for children Addition of transfusion-refractory thrombocytopenia as 1 of the possible signs of VOD/SOS (≥2 still required for diagnosis)

*Where specific time frames are given, onset of signs/symptoms must occur within the period indicated to meet the diagnostic criteria.

non-HSCT settings by toxic injury to the hepatic vasculature caused by primary chemotherapy, radiation, and other sources of toxicity, including treatment with granulocyte colony-stimulating factor or calcineurin inhibitors and influx of cytokines and lipopolysaccharides.[75,76] VOD/SOS with MOD/MOF is associated with a mortality rate typically >80%.[77] Common signs and symptoms of VOD/SOS may include painful hepatomegaly, ascites and/or weight gain, and hyperbilirubinemia,[76,78] not attributed to other common post-HSCT complications (eg, sepsis, cholestatic liver disease, acute GVHD).[76,79]

For more than 20 years, VOD/SOS has been diagnosed clinically based on Baltimore and modified Seattle criteria (Table 1).[80-83] (Although histologic examination can establish the diagnosis, it is associated with bleeding risk.[82]) The European Society for Blood and Marrow Transplantation (EBMT) recently provided important updates to these diagnostic criteria (Table 1)[82,83] to help physicians identify less common disease presentations specific to adults and children (eg, absence of hyperbilirubinemia in some children with VOD/SOS).[84,85]

The overall incidence of VOD/SOS in patients undergoing HSCT was estimated to be 13.7% (range, 0%-62%) in a pooled analysis of 135 studies.[77] In a single-institution study, the reported incidence was 11.5% (Baltimore criteria) and 13.8% (modified Seattle criteria)[75]; MOD/MOF occurred in ~36% of patients with VOD/SOS diagnosed by Baltimore criteria and 22% by modified Seattle criteria.[75] Risk factors for post-HSCT VOD/SOS may be patient related (eg, age, history of liver diseases, active hepatitis, prior treatment, primary disease) or transplant related (eg, type of transplant, conditioning regimen intensity, GVHD prophylaxis).[86] Specific chemotherapies observed to increase risk of VOD/SOS include inotuzumab ozogamicin[87] and gemtuzumab ozogamicin in patients with and without HSCT.[88,89] Reduced intensity conditioning may decrease the risk of VOD/SOS post-HSCT, although VOD/SOS has still been reported in ~2% to 9% of allogeneic-transplant patients who received reduced intensity conditioning.[75,90]

The pathophysiology of VOD/SOS begins with toxic injury to sinusoidal cells of the endothelium and hepatocytes of zone 3 of the liver acinus (Figure 2); this may trigger a complex pathophysiologic cascade.[76,78,79,91] The damage to EC impairs their regulation of thrombo-fibrinolytic balance, results in reduced nitric oxide production and increased levels of matrix metalloproteinase,[92] and may be associated with multiple prothrombotic biomarkers, although these may vary by source of toxicity.[12,83,92-95] These actions are accompanied by inflammatory cytokine release from the injured tissue and structural deterioration of the sinusoidal endothelium.[75,76] The degradation of the vascular structure (ballooning of the sinusoidal cells) with increased metalloproteinase activity in the extracellular space opens gaps in the endothelial lining that permit the passage of cellular and extracellular debris, as well as platelets, and their deposition and aggregation in the space of Disse.[63,79,96,97] Activated stellate cells may be prominent in zone 3 in patients with VOD/SOS and may have a substantial role in development of sinusoidal fibrosis.[96,98,99] These factors are associated with a progressive detachment of the endothelial lining and external occlusion of the sinusoidal lumen.[75,79,96] In addition, sinusoidal vessels may become compressed, with thickening of the subintimal zone and narrowing of the lumen, platelet activation, and fibrin-related aggregates, resulting in further reduced sinusoidal flow and the potential for complete sinusoidal occlusion.[76,79,96] These hemodynamic events combine to cause progressive postsinusoidal portal hypertension, with possible hepatorenal syndrome, characterized by sodium and fluid retention with edema and development of azotemia, and potential evolution to MOD/MOF, which may in turn result in death.[76,79,81]

General management of VOD/SOS

Before the introduction of defibrotide, treatment of VOD/SOS had traditionally been supportive, primarily aimed at maintaining intravascular volume and organ perfusion.[63,100] Although such measures may suffice for mild cases, moderate to severe VOD/SOS, which can also progress from mild VOD/SOS rapidly and unpredictably, generally requires much more active treatment.[83,101] However, clinicians

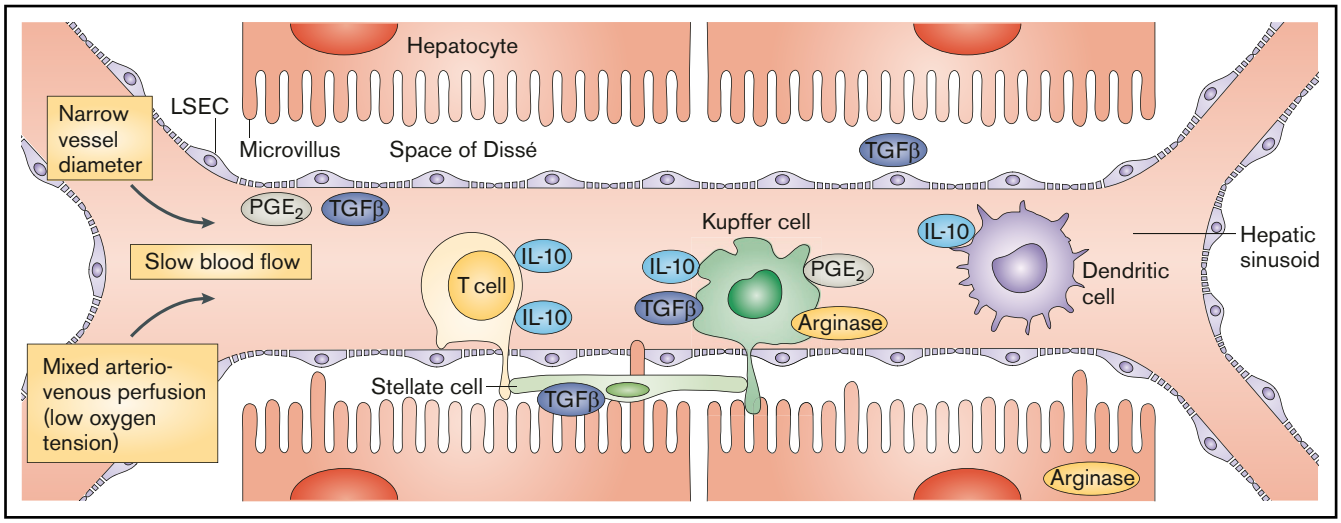

Figure 2. Anatomy of the hepatic sinusoid. The hepatic sinusoids are lined by a population of microvascular LSECs that separate hepatocytes and stellate cells from leukocytes circulating through the liver in the blood. Fenestrations in the LSEC lining allow the passive exchange of molecules between the space of Dissé and the blood, as well as direct contact of lymphocyte filopodia with hepatocyte microvilli. The liver interstitium is highly enriched in cells of the innate immune system (such as antigen-presenting dendritic, Kupffer, natural killer, and NKT cells [not shown]) and in T cells, which participate in adaptive immune responses. Mediators produced by both parenchymal and nonparenchymal cells, including IL-10, TGFβ, arginase, and PGE_2, regulate immune function within the liver. LSEC, liver sinusoidal endothelial cells; TGFβ, transforming growth factor-β. Reprinted from Thomson et al[91] with permission.

traditionally had little guidance for judging VOD/SOS severity and prognosis.[83] Consequently, severity was primarily assessed based on the presence of MOD/MOF and/or retrospectively on outcomes.[77,81,83,100] The EBMT has proposed new criteria for grading VOD/SOS severity in adults (Table 2)[82,83,101,102] and children (Table 3)[82] based on clinical and pathologic factors.[82,83]

Aside from defibrotide, the most widely used and investigated medical therapies for VOD/SOS include methylprednisolone and tissue plasminogen activator (tPA). Limited methylprednisolone data include a retrospective study of 9 pediatric patients (4 also treated with defibrotide), 8 of which had MOD; 7 survived.[84] The British Committee for Standards in Hematology/British Society for Blood and Marrow Transplantation (BCSH/BSBMT) guideline for VOD/SOS management recommends caution when using methylprednisolone for VOD/SOS because of the risk of infections.[100] Use of tPA with or without heparin has been assessed in a number of small studies.[100] One of the larger studies of tPA (n = 42) retrospectively found 25% day +100 survival but a 24% rate of severe hemorrhage.[103] Fibrinolytic treatments are generally not recommended by BCSH/BSBMT because of the associated risk of hemorrhage.[100]

Therapeutic agents investigated for prevention of VOD/SOS in HSCT patients include heparin, low-molecular weight heparin, prostaglandin E1, and ursodeoxycholic acid.[63,100,104] However, evidence supporting these prophylactic approaches is also limited, and heparin is associated with increased risk of serious bleeding.[100,104] Treatment with ursodeoxycholic acid has been suggested as prophylaxis for VOD/SOS[63,100,105,106]; however, clinical results have been mixed. A systematic review showed a benefit, with a relative risk of VOD/SOS of 0.34 (95% confidence interval [CI], 0.17-0.66) to 0.36 (95% CI, 0.15-0.90) in adults compared with no treatment,[105] whereas other studies did not, including a prospective, controlled study in 242 HSCT patients that found no difference in incidence of VOD/SOS.[106]

Development of defibrotide for treatment of VOD/SOS with MOD/MOF

Based on defibrotide's identified profibrinolytic, antithrombotic, and anti-inflammatory actions,[11] it was first considered for use in VOD/SOS in the mid-1990s as the result of a search for promising therapies that was part a research project designed to identify novel agents targeting endothelial injury.[107] The first use of defibrotide for VOD/SOS treatment was reported in 1995.[62] The subsequent clinical development program (Table 4)[70,102,107-112] demonstrated a consistently favorable risk/benefit profile, and defibrotide became the first—and currently only—drug approved for the treatment of any form hepatic VOD/SOS.

Clinical study data: phase 1/2 trials using CR and day +100 survival as primary outcomes

The first published evidence of defibrotide's efficacy and safety was reported for 19 patients who had developed VOD/SOS with MOD/MOF following HSCT and were given defibrotide as part of a compassionate use program (CUP) in the United States (Table 4).[107] Defibrotide dose escalated from 5 to 60 mg/kg per day for ≥14 days resulted in complete response (CR; bilirubin <2 mg/dL with improvement in other symptoms and signs) in 8 (42%) patients and day +100 survival in 6 of the responders; no severe hemorrhages were reported. A CUP follow-up in 88 defibrotide recipients reported a 36% CR rate and a 35% day +100 survival rate, with no serious toxicities reported (Table 4).[85]

A phase 2 multicenter, dose-finding trial of 149 adult and pediatric patients with VOD/SOS and MOD/MOF following HSCT were

Table 2. Proposed EBMT scale for grading VOD/SOS severity in adults

Clinical measure: highest grade with ≥2 symptoms	Mild	Moderate	Severe	Very severe (all patients with MOD/MOF)
Time since first VOD/SOS symptoms*	>7 d	5–7 d	≤4 d	Any time
Bilirubin				
mg/dL	≥2 and <3	≥3 and <5	≥5 and <8	≥8
μmol/L	≥34 and <51	≥51 and <85	≥85 and <136	≥136
Kinetics	—	—	Doubling within 48 h	—
Transaminases	≤2× normal	>2 and ≤5× normal	>5 and ≤8× normal	>8× normal
Weight above baseline	>5%	≥5% and <10%	≥5% and >10%	≥10%
Renal function	<1.2× baseline at transplant	≥1.2 and <1.5× baseline at transplant	≥1.5 and <2× baseline at transplant	≥2× baseline at transplant or other signs of MOD/MOF
Risk factor adjustment†		Mild + ≥2 risk factors	Moderate + ≥2 risk factors	
Treatment options to consider[101]	Maintain fluid and sodium balance Avoid hepatotoxic/nephrotoxic drugs Careful use of diuretics Symptomatic treatment: analgesia, oxygen, thoracentesis, paracentesis (remove <1 L/d ascites to avoid reduced renal flow) Progression of symptoms justifies pharmacologic VOD/SOS therapy	Mild treatments plus: If symptoms/signs persist or progress after 2 d, start pharmacologic VOD/SOS therapy If hemodynamic data are available, start pharmacologic VOD/SOS therapy for patients with hepatic venous gradient pressure ≥10 mmHg	Moderate treatments plus: Start pharmacologic VOD/SOS therapy	Severe treatments plus: Hemodialysis/hemofiltration if required

*Time from the date when the first signs/symptoms of VOD/SOS began to appear (retrospectively determined) and the date when the symptoms fulfilled VOD/SOS diagnostic criteria.
†In the presence of ≥2 risk factors (see Table 1), severity is considered 1 grade higher.
Adapted from Richardson et al[102] with permission.

Table 3. EBMT scale for grading VOD/SOS severity in pediatric patients

Clinical measure	Mild	Moderate	Severe	Very severe (all patients with MOD/MOF)
CTCAE	1	2	3	4
Liver function tests (ALT, AST, GLDH)*	≤2× normal	>2 and ≤ 5× normal	>5	>5
Persistent RT*	<3 d	3-7 d	≥7 d	≥7 d
Bilirubin, mg/dL*†	<2	<2	≥2	≥2
Bilirubin, μmol/L	<34	<34	≥34	≥34
Ascites*	Minimal	Moderate	Necessity for paracentesis (external drainage)	
Bilirubin kinetics				Doubling within 48 h
Coagulation	Normal	Normal	Impaired coagulation	Impaired coagulation with need for replacement of coagulation factors
Renal function GFR, mL/min	89-60	59-30	29-15	<15 (renal failure)
Pulmonary function (oxygen requirement)	Absent or <2 L/min	>2 L/min	Need for ventilator support (including CPAP)	
CNS	Normal	Normal	Normal	New onset cognitive impairment

Patients who fulfill criteria in different categories must be classified in the most severe category; the kinetics of the evolution of cumulative symptoms within 48 h predicts severe disease.
Adapted from Corbacioglu et al.
RT, refractory thrombocytopenia.
*Presence of ≥2 of these criteria qualifies for an upgrade to very severe VOD/SOS.
†Preexistent hyperbilirubinemia resulting from primary disease was excluded.

randomized to receive defibrotide 25 (n = 75) or 40 mg/kg per day (n = 74) in 4 divided doses every 6 hours for ≥14 days (Table 4).[108] The day +100 survival rate was numerically, but not significantly, higher in the 25-mg/kg per day group (49%) vs the 40-mg/kg per day group (39%), which was consistent with CR rate results (46% and 42%, respectively; the primary study end point). Previous exposure to gemtuzumab ozogamicin, which is associated with increased risk for VOD/SOS,[88] did not significantly affect rates of survival or CR. Rates of treatment-related AE were also similar in the 25- and 40-mg/kg per day dose groups (7% vs 10%, respectively). All-grade bleeding events were nonsignificantly higher in the 40- vs 25-mg/kg per day group (57% vs 46%, respectively). Based on the similar efficacy between the doses, and slight reduction in AE with the 25-mg/kg per day dose, this dose was chosen for phase 3 evaluation.

In a large, international CUP (1998-2009), defibrotide dosing was originally titrated from 10 to 60 mg/kg per day; during the final 5 years, it was fixed at 25 mg/kg per day (Table 4).[108,113] Among 710 patients who received ≥1 dose of defibrotide and had outcomes data in the final sample, including 429 (60.4%) with either severe VOD/SOS (Bearman criteria[114]) or with MOD/MOF, Kaplan-Meier estimated survival at day +100 was 54% across all doses and 58% in the 272 patients treated at the approved 25-mg/kg per day dose (70% in pediatric patients and 46% in adults). AE occurred in 53% of patients, most commonly MOD/MOF, progression of hepatic VOD/SOS, sepsis, and GVHD, consistent with AE expected for this population.[113]

Phase 3 and expanded-access trials

The phase 3 trial of defibrotide for treatment of VOD/SOS with advanced MOD/MOF enrolled 102 adults and children at 35 centers in the United States, Canada, and Israel (Table 4).[70] The investigators rejected a randomized design based on ethical concerns regarding the high mortality risk of VOD/SOS with MOD/MOF coupled with prior, substantial research evidence of improved survival with defibrotide. Therefore, a historical control group was used for this study, which was selected by an independent medical review committee (MRC) whose members were blinded to outcomes. Eligibility for both groups required unequivocal diagnosis of VOD/SOS, based on the Baltimore criteria (Table 1) or biopsy, and MOD/MOF, determined via specific clinical indications of renal and/or pulmonary dysfunction. For selection of historical controls, the MRC reviewed the charts of 123 patients with possible hepatic VOD/SOS with MOD/MOF screened from 6867 medical records of patients treated at study sites before defibrotide availability. The MRC eliminated all patients thought to have possible differential diagnoses or protocol-defined exclusions, and selected 32 historical controls with unequivocal VOD/SOS and MOD/MOF. The primary study outcome was rate of survival at day +100 using a propensity-adjusted analysis. Defibrotide was administered at 25 mg/kg per day in 4 divided doses for >21 days or until resolution of VOD/SOS or hospital discharge.

Demographic and baseline clinical characteristics were similar between the treatment and historical control groups. Day +100 survival was 38.2% in defibrotide-treated patients vs 25.0% in historical controls (Figure 3). The propensity-adjusted analysis showed an estimated between-group difference of 23% (95.1% CI, 5.2-40.8; $P = .0109$). Based on this result, the number needed to treat (1/absolute risk reduction) to prevent 1 death was 5. CR, defined as total bilirubin <2 mg/dL and resolution of MOD/MOF, occurred in 25.5% of defibrotide-treated patients vs 12.5% of historical controls (propensity-adjusted difference of 19% [95% CI, 3.5-34.6; $P = .0160$]); the number needed to treat to achieve 1 CR was 6. The most common AE were hypotension and diarrhea. Incidence of common hemorrhagic AE and fatal AE were similar in the defibrotide-treated and historical control groups (64% and 75% and 64% and 69%, respectively).[70]

Similar results were shown by a retrospective analysis of observational data for 8341 patients with hepatic VOD/SOS and MOD/MOF by the Center for International Blood and Marrow Transplant Research.[115] In this study, survival at day +100 (primary outcome) was 39% (95% CI, 24.8-54.3) in the defibrotide group (n = 41)

Table 4. Key clinical studies of defibrotide for treatment or prophylaxis of VOD/SOS

Phase	Condition	Design	Key end points	Other results	Conclusions
Phase 1 (N = 19)[107]	VOD/SOS with MOD/MOF post-HSCT	CUP DF 5-60 mg/kg/d (dose escalation until response/toxicity)	CR: 42%; minimal toxicity at doses tested	Day +100 survival: 32%	CR rate, day +100 survival, and absence of significant DF-associated toxicity was encouraging and warranted further evaluation
Phase 2 (N = 149)[108]	VOD/SOS with MOD/MOF post-HSCT	Randomized, dose-finding, ≥14 d Arm A, DF 25 mg/kg/dl Arm B, DF 40 mg/kg/d	Day +100 CR: arm A, 49%; arm B, 43%	Day +100 survival: arm A, 44%; arm B, 39% Overall TRAE incidence: 8% (greater at 40 vs 25 mg/kg/d)	DF appears effective in treating VOD/SOS with MOD/MOF post-HSCT with low rates of TRAE 25 mg/kg/d selected as phase 3 dose
Phase 3 historically controlled (N = 134); DF, 102; controls, 32[70]	VOD/SOS with advanced MOD/MOF post-HSCT	Nonrandomized comparison of DF 6.25 mg/kg IV every 6 h (25 mg/kg/d) for ≥21 d vs controls	Day +100 CR: DF, 24%; controls, 9% (P = .0131)	Day +100 survival: DF, 38%; controls, 25% (P = .0341, propensity adjusted) Hemorrhagic AE: DF, 65%; controls, 69%	DF improves survival and CR by day +100 in patients with VOD/SOS and MOD/MOF post-HSCT; DF is generally well tolerated
Phase 3 T-IND (N = 867 interim); post-HSCT, 766 (10 not treated); post-CT, 101[110,111]	VOD/SOS with and without MOD/MOF	Open-label T-IND DF 6.25 mg/kg IV every 6 h (25 mg/kg per day) for ≥21 d	Day +100 survival in post-HSCT patients: 51.2%	Day +100 survival in post-CT patients: 68.3%	Survival rate with DF is approximately 50% in diverse post-HSCT population
Phase 3 pediatric prophylaxis (N = 356); DF, 180; controls, 176[112]	Pediatric patients (aged <18 y) receiving HSCT	Open-label, randomized, controlled trial of DF 6.25 mg/kg IV every 6 h (25 mg/kg/d) for prophylaxis of VOD/SOS	VOD/SOS incidence: DF, 12% controls, 20% (−7.7% risk difference)	Incidence of AEs, serious AEs, and AEs leading to discontinuation were similar between groups	DF may be effective for preventing VOD/SOS

CT, chemotherapy; CUP, compassionate use program; DF, defibrotide.
Adapted from Richardson et al[102] with permission.

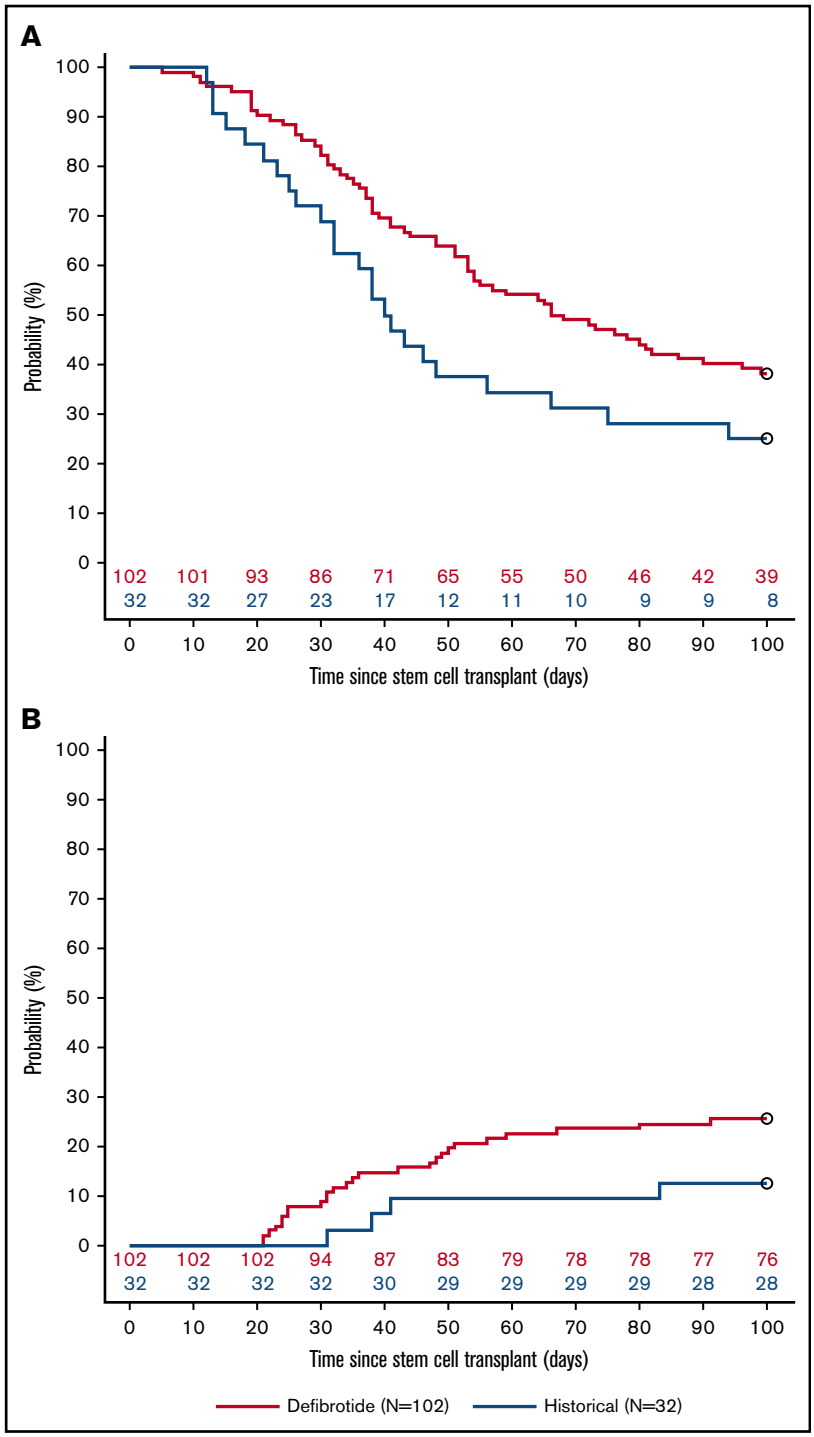

Figure 3. Primary end points of phase 3 study in defibrotide-treated patients vs historical controls receiving supportive treatment. (A) Kaplan-Meier estimates of overall survival distribution and (B) complete response (defined as total bilirubin <2 mg/dL and resolution of MOD/MOF) at day +100 following HSCT. Reprinted from Richardson et al[70] with permission.

and 30.9% (95% CI, 19.5-43.6) in patients who did not receive defibrotide (n = 55; corresponding absolute difference: 8.1%; 95% CI, −11.2 to 27.4). Compared with controls, baseline characteristics of the defibrotide-treated patients included younger age, higher Karnofsky scores, and lower prevalence of a significant fungal infection before HSCT, which may be associated with improved survival.

In addition, an open-label, single-arm, protocol-directed, expanded-access treatment (T-IND) protocol was conducted in the United States in patients with VOD/SOS with and without MOD/MOF, and post-HSCT or post-chemotherapy without HSCT, representing a broader patient population than that for which defibrotide is indicated (Table 4).[110] An interim analysis (n = 642; 573 post-HSCT and 69 post-chemotherapy without HSCT who received ≥1 dose of defibrotide) found that the day +100 survival after HSCT was 50.3% overall. Post hoc interim analyses in HSCT patients also found that earlier initiation of defibrotide treatment after VOD/SOS diagnosis correlated with significantly higher day +100 survival

rates for each cutoff before or after days 1, 2, 3, 4, and 7 ($P \leq .002$, Fisher's exact test), which was confirmed by a trend test ($P < .001$, Cochran-Armitage trend test). No specific day for defibrotide initiation marked a cutoff for improved outcomes, and a consistent pattern of greater benefit with earlier treatment was evident. Of all 573 post-HSCT patients in the interim dataset, 69.6% had ≥1 AE and 21.6% had ≥1 AE that was possibly treatment related. A more recent post hoc subgroup analysis of the final dataset for postchemotherapy patients (ie, without HSCT) treated by day 30 after start of chemotherapy (n = 82), found a day +70 survival rate of 74.1%; 65.9% had ≥1 AE, and 26.8% had an AE that was possibly treatment related.[116]

Phase 3 prevention trials

A phase 3 VOD/SOS prevention trial of defibrotide was conducted in 356 pediatric patients (aged <18 years) undergoing HSCT (not an approved indication), who were considered at high risk for VOD/SOS (Table 4).[112] This open-label, controlled study randomized 180 patients to defibrotide 25 mg/kg per day in 4 divided doses of 6.25 mg every 6 hours starting on the same day as the pretransplant conditioning regimen and continuing for 30 days post-HSCT or ≥14 days if hospital discharge occurred in <30 days; the 176 patients randomized to the control arm (no preventive treatment) would receive defibrotide if they developed VOD/SOS. Incidence of VOD/SOS at 30 days post-HSCT was 12% in the defibrotide group and 20% among controls (risk difference: −7.7%; 95% CI, −15.3 to −0.1; z test for competing risk analysis: $P = .0488$; log-rank test: $P = .0507$). Rates of AE overall, serious AE, and AE leading to discontinuation were similar between the defibrotide-treated and control groups (87.0% vs 88.2%, 61.0% vs 58.5%, and 9.6% vs 11.9%, respectively). Per the study protocol, the small groups of patients in either arm developing VOD/SOS received treatment with defibrotide. VOD/SOS-associated mortality was numerically lower in the defibrotide arm compared with the control arm. This difference did not reach significance (4 [2%] of 180 patients in the defibrotide group and 10 [6%] of 176 controls; $P = .10$).[112]

Based on these data, the BCSH/BSBMT recommended defibrotide for prophylaxis of VOD/SOS in pediatric patients undergoing HSCT with multiple specified risk factors,[100] although this is not an approved indication. An ongoing phase 3, randomized, adaptive multicenter study is evaluating the efficacy, safety, and tolerability of defibrotide prophylaxis for VOD/SOS vs best supportive care in high- or very high-risk adult and pediatric patients undergoing HSCT (Harmony; NCT02851407).[109]

Interestingly, the pediatric prevention trial found that the incidence and severity of GVHD, analyzed as an exploratory end point, was lower at days +30 and +100 in defibrotide-treated patients vs controls, significantly among patients who received allogeneic HSCT ($P = .0046$ for incidence, $P = .0034$ for severity at day +100).[108] Similar GVHD findings have been reported in adults,[117] and a phase 3 trial of defibrotide for prevention of GVHD is being initiated (NCT03339297),[118] with a mechanistic link provided by clinical observations and the potential role of heparanase expression in this setting.[59,119] In addition, a 2014 expert consensus statement recommended the investigation of defibrotide for its potential to improve outcomes of transplant-associated thrombotic microangiopathy.[120] A retrospective, multicenter survey of pediatric patients (n = 22) reported by EBMT working parties and compared with adult patients (n = 17) from a single center, also found that defibrotide treatment resulted in resolution of transplant-associated thrombotic microangiopathy (≥ 2 schistocytes/high-power microscopic field, serum lactate dehydrogenase increase, thrombocytopenia, anemia with negative direct Coombs test, decreased haptoglobin, and no coagulopathy) following HSCT in 77% of treated patients (17/22 pediatric; 13/17 adult).[121]

Clinical notes on defibrotide in patients undergoing HSCT

Close monitoring of patients following HSCT for signs and symptoms of VOD/SOS is essential to its effective management, which may depend on prompt treatment.[101,111] Signs and symptoms of potential VOD/SOS include rapid weight gain, edema, and ascites; hepatomegaly, which may be painful; hyperbilirubinemia; and other indicators, including thrombocytopenia, pleural effusion, pulmonary infiltrates, hypoxia, renal insufficiency or failure, confusion, and encephalopathy.[101]

Following diagnosis of VOD/SOS (Table 1), gauging its potential severity is essential for initiation of appropriate treatment, preferably as soon as possible. The new EBMT criteria for gauging VOD/SOS severity in adults (Table 2)[83] and pediatric patients (Table 3)[82] provide useful guidelines. We recommend immediate treatment with defibrotide for patients with severe VOD/SOS (ie, with MOD/MOF or severe grade VOD/SOS per EBMT guidelines) or moderate VOD/SOS if signs/symptoms persist or progress after 2 days of observation, despite adequate fluid/sodium balance and without hepatotoxic or nephrotoxic drugs.[101] In patients with mild signs/symptoms, or moderate, apparently nonprogressive symptoms and with <2 risk factors, vigilance is critical because of the unpredictability of VOD/SOS.[82,83,101]

VOD/SOS outcomes may be improved with earlier vs later defibrotide treatment, although if treatment must be delayed, defibrotide initiation at any time point might be expected to be beneficial.[111] However, increased dosing above the recommended defibrotide 25 mg/kg per day does not appear to improve efficacy.[108,122]

Conclusions

Defibrotide has been extensively studied since its discovery ~50 years ago as a first-in-class oligonucleotide. Although all aspects of its precise mechanism of action remain to be completely elucidated, defibrotide is understood to have profibrinolytic, anti-thrombotic, anti-inflammatory, and angio-protective actions that include protection of EC from damage and promotion of their function. The therapeutic value of these actions has been demonstrated in experimental and clinical studies for a wide range of conditions. Defibrotide is currently the only approved treatment of post-HSCT patients with VOD/SOS and MOD/MOF in the United States and severe VOD/SOS in the European Union. Defibrotide is being evaluated in additional disease states, including prevention of VOD/SOS and GVHD.

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