CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208114Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA	208114
Submission Date(s):	07/31/2015
Brand Name: **Generic Name:**	DEFITELIO® Defibrotide
Submission Type; Code: **PUDFA Date:**	NME NDA; Priority review 3/31/2016
Sponsor:	Jazz Pharmaceuticals
Formulation; Strength(s):	lyophilized powder, 200 mg/vial, 80 mg/mL solution
Proposed Indication:	For the treatment of patients with hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome, with (b) (4) dysfunction following hematopoietic stem cell transplantation
OND Division:	DHP
OCP Division:	DCP5 DPM

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1 EXECUTIVE SUMMARY

Defibrotide is the sodium salt of a highly complex, polydisperse mixture of predominantly single-stranded polydeoxyribonucleotides. The proposed indication is the treatment of patients with (b) (4) hepatic veno-occlusive disease (VOD), with (b) (4) dysfunction following hematopoietic stem cell transplantation (HSCT). The proposed dosing regimen is 6.25 mg/kg every 6 hours (25 mg/kg/day) given as a 2-hour intravenous (IV) infusion. Patients should be treated for a minimum of 21 days.

To support the proposed dose and indication, the sponsor submitted results from a dose finding Phase 2 study (99-118) and a pivotal Phase 3 study (2005-01) that used a historical control (HC) group for comparison. The study design and main efficacy results of the two trials are outlined in **Table 1** below.

Table 1: Summary of efficacy findings of studies 99-118 and 2005-01

Study	Treatment (number of patients)	Day+100 Survival Rate % (95 %CI)
99-118 Dose finding study	Defibrotide 25 mg/kg/day (n=75)	44.0 (32.8, 55.2)
	Defibrotide 40 mg/kg/day (n=74)	37.8 (26.8, 48.9)
2005-01 Pivotal	Defibrotide 25 mg/kg/day (n=102) Historical Control (n=32)	38.2 (28.8, 48.4) 25.0 (11.5, 43.0)

The Phase 2 data showed that the 25 mg/kg/day dose provided a favorable benefit-risk profile. The Phase 3 trial was thus conducted using defibrotide dose of 25 mg/kg/day and the comparator group was a historical control (HC) receiving best supportive care.

In the pivotal study 2005-01, primary efficacy endpoint of Day+100 survival rate was 38.2% in defibrotide group and 25.0% in the HC group. All grades TEAEs considered to be possibly related to defibrotide including hemorrhage and hypotension did not occur at a higher incidence in the defibrotide group compared to historical controls.

Results from a legacy study IRI-151612 in healthy volunteers using intravenous ^{125}I-Defibrotide suggested that urinary and fecal excretions accounted for 71% and 19% of the administered drug, respectively, with 10 to 14% of the administered drug excreted in the urine as parent drug. In subjects with end stage renal disease (ESRD), urinary excretion of the parent drug decreased to 5%. Overall, the data suggest that defibrotide is mainly eliminated by metabolism followed by renal excretion, and only a small portion of defibrotide is eliminated unchanged by the kidney.

The Phase 2 and the Phase 3 trials enrolled VOD patients with multi-organ dysfunction, including those with renal and hepatic impairments, since organ impairment is the natural history of VOD. Therefore, since the proposed dosing regimen was evaluated in the presence of hepatic and renal impairment, dose-adjustment is not recommended in these populations.

Furthermore, 48.3% of those treated with defibrotide dose of 25 mg/kg/day in both trials were pediatric patients ≤ 16 years (48.6% of them aged 2-11 years), which is supportive of the proposed dose of 25 mg/kg/day for children.

Literature and *in vitro* study data suggest that defibrotide is predominantly degraded by exonucleases in human plasma then by various enzymes involved in DNA degradation.

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Defibrotide is not a substrate, inhibitor, or inducer of any of the known drug metabolizing enzymes or transporters.

1.1 Recommendation

This NDA is acceptable from a clinical pharmacology perspective.

Decision	Acceptable to OCP?			Comment
Overall	Yes ☒	No ☐	NA ☐	
Evidence of Effectiveness†	Yes ☒	No ☐	NA ☐	1 Phase 2 dose finding study and 1 pivotal Phase 3 study
Proposed dose for general population	Yes ☒	No ☐	NA ☐	6.25 mg/kg every 6 hours via a 2-hour iv infusion
Proposed dose selection for others	Yes ☒	No ☐	NA ☐	No dose adjustment is recommended for patients with renal or hepatic impairment or for pediatric patients
Pivotal BE	Yes ☐	No ☐	NA ☒	IV administration
Labeling	Yes ☐	No ☒	NA ☐	Revised labeling is proposed

†Clinical Pharmacology perspective; although dose-response was not apparent.

1.2 Post Marketing Requirements

None.

1.3 Post Marketing Commitment

None.

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DDD – **B Booth**; DD – **A Rahman**

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1.4 Summary of Important Clinical Pharmacology Findings

Defibrotide is a complex, polydisperse mixture of predominantly single-stranded polydeoxyribonucleotides. The mechanism of action (MOA) of defibrotide has not been fully elucidated, especially the actual drug target related to the treatment of hepatic veno-occlusive disease (VOD). Preclinical and clinical data suggest that defibrotide may stabilize hepatic endothelial cells (ECs) and restore the thrombo-fibrinolytic balance.

The proposed dose of 25 mg/kg/day was selected based on the comparison of efficacy (CR and Day+100 survival) and safety of defibrotide 25 mg/kg/day versus 40 mg/kg/day in patients with severe VOD in the Phase 2 dose finding study 99-118. In this study, there was no difference in efficacy as measured by either CR or survival at Day+100. There was a slightly higher incidence (>4%) of treatment-related TEAEs, treatment-related SAEs, fatal TEAEs, hemorrhage and hypotension TEAEs in the 40 mg/kg/day treatment arm. Although these results did support the selection of 25 mg/kg/day over 40 mg/kg/day for the Phase 3 study, it was not clear if a dose lower than 25 mg/kg/day would provide improved benefit-to-risk ratio. To address this concern, the applicant collected and analyzed the efficacy data prospectively at doses ranging from 10 mg/kg/day to 80 mg/kg/day in a compassionate use program. The results suggested that survival at Day+100 increased from 36.5% at 10 mg/kg/day to 44.9% at 25 mg/kg/day, then reached a plateau at higher doses (40 to 80 mg/kg/day), which is consistent with the finding in the Phase 2 dose finding study.

The efficacy and safety of defibrotide at the proposed dose of 25 mg/kg/day were assessed in the open-label pivotal Phase 3 study (Study 2005-01) with a historical control group. In this study, the observed primary efficacy endpoint of survival on Day+100 in the defibrotide group was 38.2% and 25.0% in the historical control group (p = (b) (4). The survival rate on Day+180 in the defibrotide group decreased to 32.4% because of additional 6 deaths while there was no additional death in the historical control group up to Day+180. Hemorrhage and hypotension were considered AE of special interest for defibrotide. There was a higher incidence of fatal hemorrhage in the defibrotide group compared to the historical control (15% vs. 6%). The overall incidence of hypotension was 39% in defibrotide treatment group and 50% in historical control group.

Overall, the proposed dose of defibrotide appears acceptable for the treatment of severe VOD based on the submitted data.

Literature information and sponsor's *in vitro* and *in vivo* study data suggest that defibrotide is predominantly degraded by exonucleases in the human plasma initially, then progressively metabolized to the free 2'-deoxyribose sugar, purine and pyrimidine bases by various enzymes involved in DNA degradation.

In the legacy study (Study IRI-151612), following single intravenous administration of ^{125}I-Defibrotide 400 mg in 3 male healthy volunteers, approximately 71% and 19% of the total administered radioactivity was excreted by the urinary and fecal routes, respectively. The excretion data from other healthy volunteer studies showed that 10-14% of unchanged defibrotide was excreted in the urine.

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The pharmacokinetics (PK) characteristics of defibrotide were evaluated in subjects with ESRD (study VOD-2012-03-PKRen) and subjects with normal renal function following 2-hour infusion of 6.25 mg/kg defibrotide. The ESRD subjects consisted of both dialysis-dependent and dialysis-independent populations. Hemodialysis had no significant effect on the exposure of defibrotide in dialysis-dependent ESRD subjects. Defibrotide AUC and C_{max} increased by about 60% and 35% in dialysis-independent ESRD subjects compared to subjects with normal renal function. In subjects with ESRD urinary excretion of unchanged defibrotide was approximately 5%.

The effect of hepatic impairment on the PK of defibrotide was not investigated in a dedicated study. However, it should be noted that both the Phase 2 and Phase 3 studies included patients with renal and hepatic impairment, since the natural history of VOD includes organ impairment. As such, the target population has renal and hepatic impairments and the proposed dose of 25 mg/kg/day was evaluated in the presence of renal and hepatic impairments. Therefore, no dose adjustments are recommended for patients with hepatic or renal impairment.

Because the Phase 2 and Phase 3 studies included a sufficient number of children , 48.3% of those treated with defibrotide dose of 25 mg/kg/day in both trials were pediatric patients $\leq$ 16 years (48.6% of them aged 2-11 years), the proposed dose of 25 mg/kg/day is also appropriate for children ages 2 to 11 years old.

A population PK (pop PK) model for defibrotide was developed based on pooled PK data from a thorough QT study (Study R09-1425), the renal impairment study and the Phase 2 dose finding study. The PK data were adequately described by a one-compartment model with linear elimination. The total clearance (CL) estimated from the pop PK model was 9.1 L/h in healthy subjects, consistent with the non-compartmental analysis (NCA) estimation. The estimated typical renal clearance CLr was 0.9 L/h, which is approximately 10% of the total CL. Body weight was integrated in the model for allometric scaling of both clearance and volume of distribution. There was insufficient data to assess the effect of demographic factors such as disease status, age, race, and organ impairment on the PK of defibrotide as part of the population PK assessment. As such, there are no labeling statements proposed by the sponsor based on population PK analysis.

Defibrotide does not inhibit or induce any of the known liver metabolism enzymes. It is not a substrate or inhibitor of major drug uptake transporters. Overall, defibrotide is expected to have no drug-drug interaction potential at therapeutic concentrations.

The effects of single therapeutic (6.25 mg/kg) and supra-therapeutic (15 mg/kg) dose of defibrotide on QTc interval were investigated in the thorough QT study R09-1425. The results of this ECG trial showed that defibrotide has no effect on heart rate, AV conduction, or cardiac depolarization.

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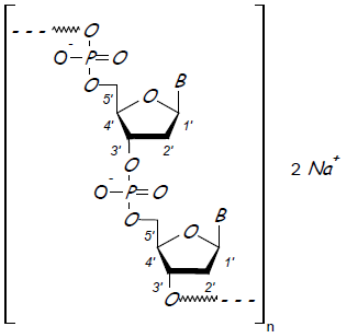
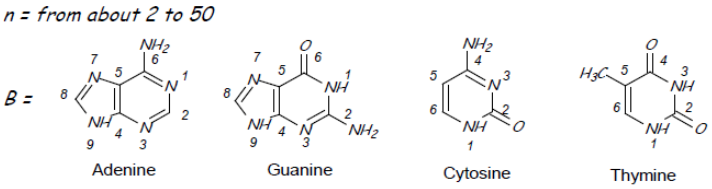
2 QUESTION BASED REVIEW

2.1 General Attributes

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Defibrotide has the following physical and chemical characteristics:





Source: Applicant's Quality Overall Summary (Substance)

Established Name:	Defibrotide
Molecular Weight:	13-20 kDa (b) (4)
Molecular Formula:	NA
Chemical Name:	NA
Description:	(b) (4)
Chirality:	NA
Solubility:	(b) (4)
Log P:	NA
pKa-Values:	(b) (4)
Defibrotide concentrate solution for intravenous infusion contains 80 mg/mL of defibrotide as the sodium salt. One vial contains 200 mg (2.5 mL) of defibrotide.	

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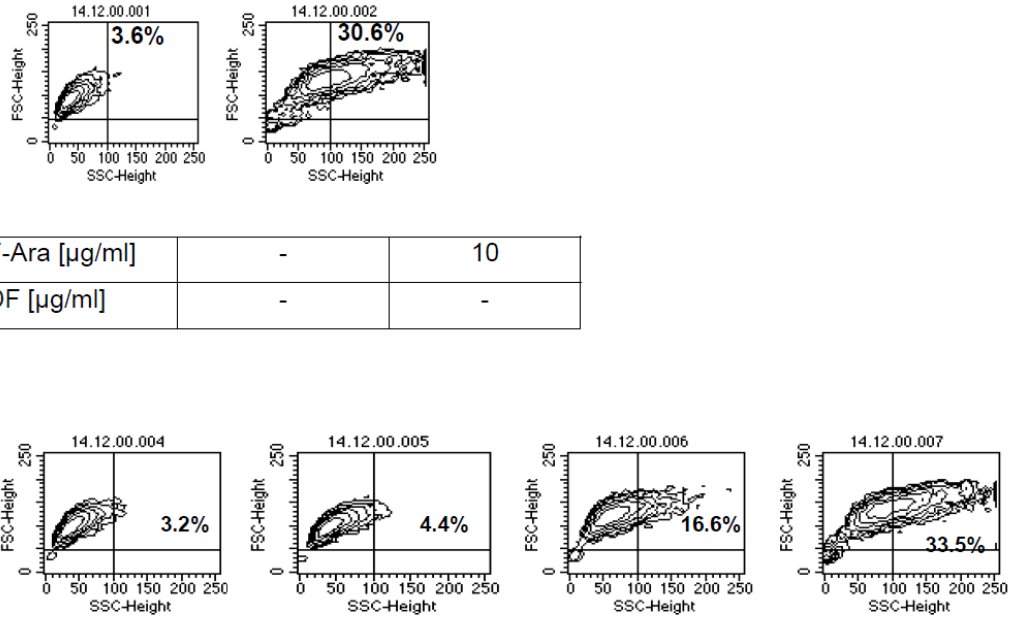
2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?

The mechanism of action (MOA) of defibrotide has not been fully elucidated. The primary cause of VOD is injury to sinusoidal endothelial cells and hepatocytes by high-dose chemotherapeutic agents given during conditioning therapy for HSCT. Characteristics of VOD include the activation, damage, death, detachment of endothelia cells and decreased fibrinolytic activity. As such, the sponsor has conducted several *in vitro* pharmacodynamic studies to address the potential relevant MOA in the treatment of VOD, the key biological activities of defibrotide from these studies are summarized as following:

1) Protection of human hepatic endothelial cells (ECs) from cytotoxic drugs (5-FU and fludarabine) induced apoptosis and cell death, based on results of cell proliferation assay (**Figure 1**), gene expression, and microarray profiling analyses (**Table 2**).

Figure 1: Defibrotide protects human ECs from fludarabin in the flow cytometric proliferation assay



F-Ara [µg/ml]	-	10
DF [µg/ml]	-	-

F-Ara [µg/ml]	10	10	10	10
DF [µg/ml]	100	10	1	0.1

Table 2: Effect of 24-hour defibrotide treatment on suppressing the expression of selected genes induced by fludarabine

Category	Genes
Apoptosis	PDCD6, FAS, APAF-1, MOAP-1, Caspase 3, Caspase 9, BAD
Angiogenesis/Migration	Heparanase, FGFR3, CCL5, MCAM (CD146), PLAU, VEGF, IL8
Inflammation/Innate Immunity	IL-1β, IL-32, IL-11, TRL1, TLR3, ICAM1, MHC Class II

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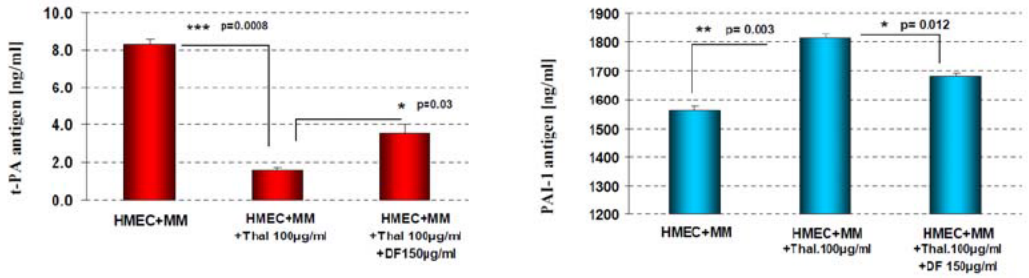
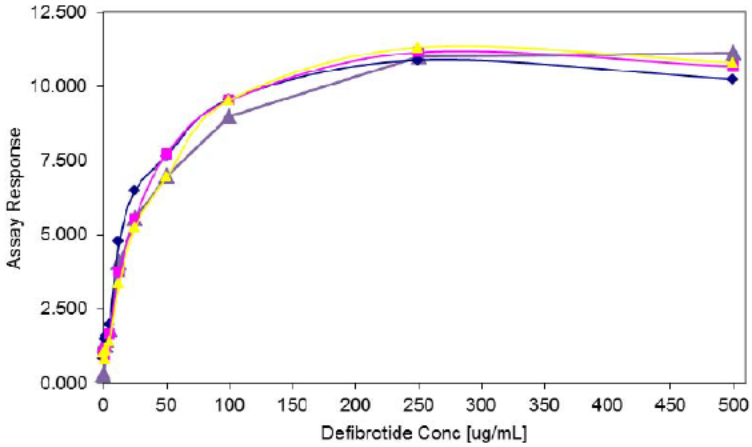
2) Blunting the pro-thrombotic effect of thalidomide in ECs, by increasing the release of t-PA and reducing the level of plasminogen activator inhibitor-1 (PAI-1) (**Figure 2**), and increasing the fibrinolytic activity of ECs.

Figure 2: Defibrotide increased t-PA and reduced PAI-1 in human ECs and MM co-culture system treated with thalidomide



3) Increasing the fibrinolytic and amidolytic activity of plasmin in *in vitro* bioassays (**Figure 3**).

Figure 3: Asymptotic dose-response curve of defibrotide amidolytic activity in the euglobulin assay



In a Phase 2 dose range finding study, treatment with defibrotide also decreased PAI-1 (an inhibitor of t-PA) in plasma of VOD patients (**Figure 4**), but the change was not statistically significant and only observed in those patients who had complete response, and there was no dose-response on PAI-1.

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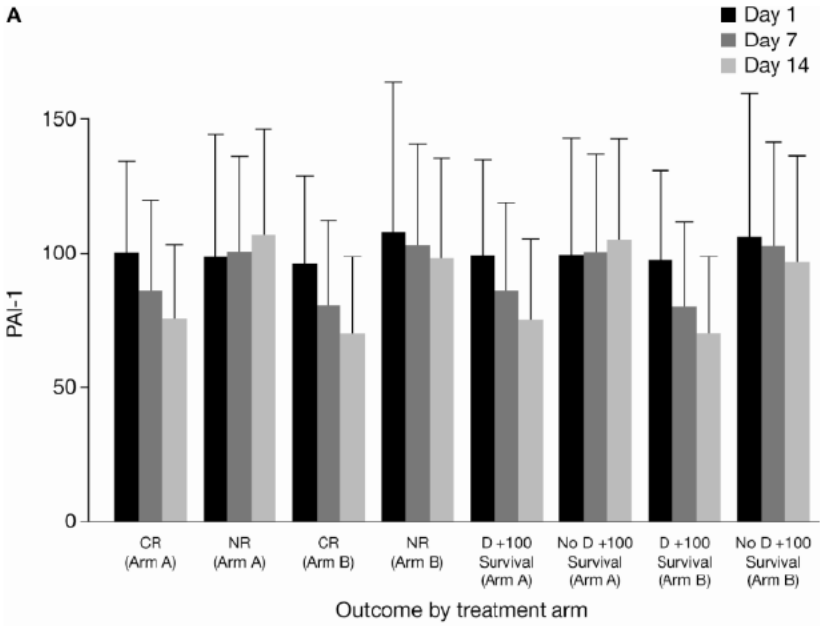
Figure 4: Inhibition of PAI-1 in severe VOD patients who respond to defibrotide treatments in Study 99-118



Together, the sponsor suggests the mechanism of action for defibrotide is the stabilization and protection of ECs, with both direct and EC-mediated restoration of the thrombo-fibrinolytic balance.

The proposed indication for defibrotide is the treatment of patients with hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), with (b) (4) dysfunction following hematopoietic stem cell transplantation (HSCT).

2.1.3 What are the proposed dosage(s) and route(s) of administration?

The recommended dose of defibrotide is 6.25 mg/kg every 6 hours, taken via 2-hour IV infusion, to be administered for a minimum of 21 days.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

Several clinical trials were conducted in severe VOD patients and healthy subjects to support the proposed indication at the proposed dose (**Table 3**).

The Phase 2 study (99-118) was conducted to determine the efficacy, safety and PK of defibrotide in severe VOD patients who were randomized to either the 25 mg/kg/day or the 40 mg/kg/day treatment groups.

Based on the Phase 2 study results, the sponsor conducted an open-label, single-arm, multicenter, Phase 3 trial (2005-01) in 102 VOD patients with multi-organ dysfunction treated with

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defibrotide 25 mg/kg/day. The clinical efficacy and safety of this dosing regimen was then compared with a historical control group of 32 VOD patients. PK Samples were not collected in the Phase 3 trial.

Table 3 Clinical pharmacology and clinical trials conducted to support marketing approval of defibrotide

Study No (N)	Type of Study	Dosing Regimen Evaluated	PK Subset
DFPK 99-118 (149)	Phase 2, dose finding study in patients with severe VOD post HSCT	10 mg/kg/day IV in 4 divided doses on Day 1, then 6.25 and 10 mg/kg every 6 hours via 2-hour IV infusion, for 21 days	13
2005-01 (102) (Pivotal trial)	Phase 3, randomized, single arm study with historical control	6.25 mg/kg every 6 hours via 2-hour IV infusion, for 21 days	no
R09-1425 (52)	Phase 1, double-blind, crossover, through QT study in healthy subjects	6.25 and 15 mg/kg, 2-hour IV infusion, single dose administration	52
DF VOD-2012-03-PKRen (18)	Phase 1, renal impairment study	Part 1 (Dialysis effect): 6.25 mg/kg, 2-hour IV infusion single dose on Day 1 and Day 4 Part 2 (Renal impairment): 6.25 mg/kg, 2-hour IV infusion, 4 doses	18

The effects of therapeutic (6.25 mg/kg) and supra-therapeutic (15 mg/kg) doses of defibrotide on QTc prolongation was assessed in a single-center, double-blind, randomized, placebo- and positive-controlled (moxifloxacin 400 mg) study R09-1425 in healthy volunteers.

To investigate the effects of renal impairment and dialysis on PK of defibrotide, the sponsor conducted a Phase 1, open-label, renal impairment study VOD-2012-03-PKRen with two parts, in non-VOD subjects with severe renal impairment to ESRD and matched healthy subjects.

The sponsor also conducted a population PK analysis using data from the Phase 2 dose finding study and the two clinical pharmacology studies as described in **Table 3** above.

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

Efficacy Endpoint

In the Phase 2 dose finding study (Study 99-118), the primary efficacy endpoint was complete response (CR) of severe VOD, defined as a bilirubin <2 mg/dL after initiation of defibrotide, and, if present at baseline, resolution of multi-organ failure (MOF), as evidenced by renal, pulmonary, and/or CNS dysfunction. This endpoint was chosen as it is a direct measure of the drug's activity and could be objectively assessed based on laboratory and clinical endpoints. Patients should have received a minimum of 3 days of defibrotide to be evaluable for response.

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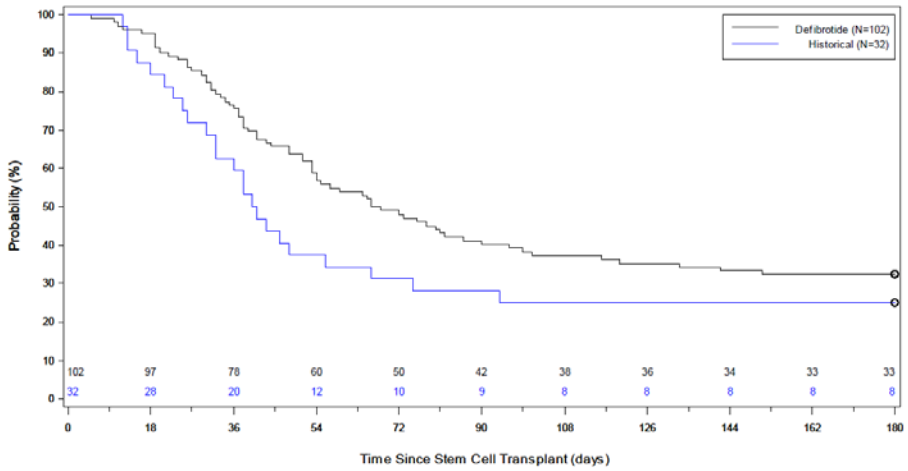
Survival at Day+100 was also assessed as the major secondary endpoint. Results of primary and secondary efficacy endpoints of this study are shown in **Table 4**, which suggest a flat dose-efficacy relationship between the two defibrotide treatment groups.

Table 4 Summary of primary and key efficacy endpoints of defibrotide in clinical trials (sponsor's analysis)

Study	Treatment Group (N)	Complete Response Rate % (N) [95% CI]	Survival Rate at Day+100 % (N) [95% CI]
99-118 **Phase 2**	25 mg/kg/day (75)	46.7 (35) [35.1, 58.6]	44.0 (33) [32.8, 55.2]
	40 mg/kg/day (74)	40.5 (30) [29.3, 52.6]	37.8 (28) [26.8, 48.9]
2005-01 **Pivotal Phase 3**	25 mg/kg/day (102)	25.5 (26) [17.0, 34.0]	38.2 (39) [28.8, 47.7]
	Historical Control (N=32)	12.5 (4) [1.0, 24.0]	25.0 (8) [9.9, 40.1]

In the pivotal Phase 3 study 2005-01, the primary efficacy endpoint was survival at Day+100 post-HSCT, using a historical control group as a comparator. Secondary efficacy endpoints include CR by Day+100 post-HSCT, survival at Day+180 post-HSCT, and overall survival (defined as mortality status at the date of last contact). The results of primary and secondary efficacy endpoints (survival and complete response rate at Day+100) are shown in **Table 4**. The Kaplan-Meier plot by Day+180 post-HSCT for the historical control and defibrotide groups is provided in **Figure 5** below.

Figure 5: Summary of time to death by Day+180 post HSCT (sponsor's analysis)



Defibrotide: Death=69 (67.6%), Censored=33 (32.4%) and Historical Control: Death=24 (75.0%), Censored=8 (25.0%)
Note: Number at risk shown in first line for Defibrotide and in second line for Historical Control.

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Pharmacodynamic Endpoint

In the Phase 2 study 99-118, blood samples were obtained at Baseline, Day 7, Day 14 and at the end of treatment to measure the level of biomarker PAI-1.

Treatment with defibrotide decreased PAI-1 in plasma of VOD patients (**Figure 4**), but the change was not statistically significant and only observed in those patients who had complete response, and there was no dose-response on PAI-1.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

No. The sponsor only collected PK samples from a subgroup in the Phase 2 study, there was no PK collection in the Phase 3 study. Due to the limited PK data available in patient population, exposure-response relationship can't be assessed.

2.2.4 Exposure-Response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy?

Exposure-response analysis was not conducted for defibrotide because insufficient PK data were collected from patients who took part in the Phase 2 and Phase 3 trials. However, the results from the dose finding Phase 2 study 99-118 where CR and Day+100 survival were compared at two defibrotide dose levels (25 mg/kg/day vs. 40 mg/kg/day), no meaningful difference in both CR (46.7% vs. 40.5%, respectively) and Day+100 survival (44.0% vs. 37.8%, respectively) was observed.

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.

Similarly, exposure-response analysis for safety was not conducted because of the data limitation. Dose-safety analysis with pooled data from defibrotide 25 mg/kg/day and 40 mg/kg/day suggested a slightly higher incidence (>4%) of treatment-related TEAEs, treatment-related SAEs, fatal TEAEs, and hemorrhage and hypotension TEAEs reported in the 40 mg/kg/day treatment arm.

2.2.4.3 *Does this drug prolong the QT or QTc interval?* (You must answer this question, unless this is addressed in the question above.)

No. In a thorough QT study, the QTcI mean change from baseline placebo-corrected were -0.1 and 0.3 ms for the therapeutic dose 6.25 mg/kg and supra-therapeutic dose 15 mg/kg groups, respectively. In addition, neither of the 2 defibrotide dose groups demonstrated an upper bound that approached or exceeded 10 ms.

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

Yes, the proposed dose of 25 mg/kg/day in adults and pediatrics is acceptable.

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The proposed dosing regimen is based on the comparison of efficacy (CR and Day+100 survival) and safety of defibrotide 25 mg/kg/day versus 40 mg/kg/day in patients with severe VOD with associated organ dysfunction. In the Phase 2 study, there was no dose-related difference in efficacy and safety (see section 2.2.4.1 and 2.2.4.2). The observation of similar efficacy and safety at both doses suggests a relatively flat exposure-response relationship likely insensitive to intrinsic or extrinsic factors that can modify PK.

Overall, the dose of 25 mg/kg/day selected by the sponsor appears to be consistent with the flat dose-response relationship observed in the Phase 2 study while the safety concerns increased slightly at 40 mg/kg/day.

There is no unresolved dosing or administration issue.

2.2.5 What are the PK characteristics of the drug and its major metabolite?

2.2.5.1 What are the single dose and multiple dose PK parameters?

PK sampling was performed in a subgroup of Phase 2 study 99-118 in VOD patients after single and multiple dosing. There was no PK assessment in the pivotal Phase 3 study 2005-01. **Table 5** below summarizes the PK sampling schemes in the Phase 2 study 99-118.

Table 5 Defibrotide PK sampling schemes in clinical studies

Trial	N_{PK}	Bioanalytical Assay Used	PK Sampling
99-118 (Phase 2 dose finding)	11	UPLC/UV	**Day 1 (run-in):** • 2.5 mg/kg every 6 hours (i.e. 10 mg/kg/day): pre-dose, 30, 60, 90 and 115 minutes after starting of infusion, and 5, 10, 15, 20, 30, 45 and 60 min after end of infusion (EOI) **Day 2:** • Arm A: 6.25 mg/kg every 6 hours (i.e. 25 mg/kg/day): time points same as above • Arm B: 10 mg/kg every 6 hours(i.e. 40 mg/kg/day): time points same as above **Day 7:** • Arm A: 6.25 mg/kg every 6 hours (i.e. 25 mg/kg/day): time points same as above • Arm B: 10 mg/kg every 6 hours (i.e. 40 mg/kg/day): time points same as above
R09-1425 (TQT)	52	UPLC/UV	**Crossover with 3-day washout:** • Treatment A - 6.25 mg/kg single dose IV infusion: pre-dose, 1, 2 hours(immediately prior to EOI) after starting of infusion, and 2.083 (in relation to actual time of EOI), 2.25, 2.75, 3, 3.5, 4, 6, 12, 18 and 23 hours • Treatment B - 15 mg/kg single IV infusion dose: time points same as in treatment A
VOD-2012-03-PKRen (Renal	18	Flurometric	**Dialysis arm (subjects with ESRD on dialysis):** • Day 1 (non-dialysis day) - 6.25 mg/kg single dose IV infusion: pre-dose, 0.25, 0.5, 1, 1.5 and 2 hours (immediately prior to EOI) after

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Trial	N_{PK}	Bioanalytical Assay Used	PK Sampling
impairment)			starting of infusion, and 2.083 (in relation to actual time of EOI), 2.25, 2.5, 2.75, 3, 3.5, 4, 4.5, 5.0, 5.5 and 6 hours • Day 4 (dialysis day) - 6.25 mg/kg single dose IV infusion: time points same as on Day 1 above; hemodialysis started at 1 hour after the starting of infusion and lasts for 4 hours **Main arm:** • Subjects with severe renal impairment to ESRD: 6.25 mg/kg every 6 hours IV infusion for 4 doses: PK collection after the 1st and 4th dose at pre-dose, 0.25, 0.5, 1, 1.5 and 2 hours (immediately prior to EOI) after starting of infusion, and 2.083 (in relation to actual time of EOI), 2.25, 2.5, 2.75, 3, 3.5, 4, 4.5, 5.0, 5.5, 6 hours; in addition, pre-dose PK samples were also collected before the 3rd dose. • Matching healthy subjects: 6.25 mg/kg every 6 hours IV infusion for 4 doses: PK collection time points same as above

In Study 99-118, a group of 16 patients received defibrotide 2.5 mg/kg every 6 hours (10 mg/kg/day) during the PK run-in on Day 1 and PK samples were collected after the first dose on Day 1. Single-dose and multiple-dose PK of defibrotide were then assessed on Day 2 and Day 7 after the first infusion of the day among these 16 patients who were randomized to treatment of 6.25 mg/kg every 6 hours (Arm A) and 10 mg/kg every 6 hours (Arm B). Among these patients, reliable PK parameter estimates were only available from 11 patients. In addition, there were insufficient PK samples from two pediatric patients in Arm B to be included in PK assessment.

Table 6: Summary of single and multiple doses PK parameters of defibrotide (mean, CV%) in Phase 2 study 99-118

Dose	N	C_{max} (µg/mL)	AUC_{0-t} (µg*h/mL)	$T_{1/2}$ (h)	CL (L/h)	V_{ss} (L)
Arm A (25 mg/kg/day)						
Day 1: 2.5 mg/kg	5	46.3 (23%)	60.1 (66%)	1.1* (NA)	3.7* (NA)	5.4* (NA)
Day 2: 6.25 mg/kg	5	45.8 (30%)	87.7 (35%)	1.5 (44%)	3.5 (38%)	7.3 (28%)
Day 7: 6.25 mg/kg	5	48.8 (57%)	99.1 (62%)	1.9 (38%)	3.4 (37%)	8.7 (50%)
Arm B (40 mg/kg/day)						
Day 1: 2.5 mg/kg	5	63.6 (105%)	85.7 (66%)	1.9** (NA)	2.3** (NA)	5.2** (NA)
Day 2: 10 mg/kg	5	53.3 (30%)	110.7 (29%)	1.6 (37%)	5.1 (42%)	9.6 (25%)
Day 7: 10 mg/kg	4	57.6 (29%)	113.7 (30%)	1.1 (25%)	6.1 (34%)	8.3 (35%)

Note: *: N=2; **: N=1

As shown in **Table 6** above, defibrotide C_{max} and AUC on Day 2 and day 7 were similar in both arms, indicating the absence of meaningful drug accumulation following repeated doses. Such a finding is expected because defibrotide has a very short half-life of 1 to 2 hours. The results also

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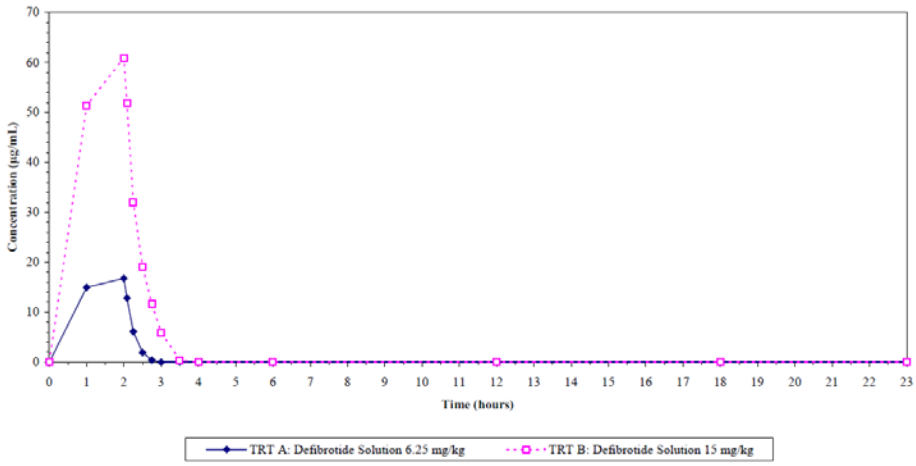
suggested the exposure of defibrotide increased in a less than dose-proportional manner in severe VOD patients within the dose range of 10 mg/kg/day to 40 mg/kg/day. However, this relationship should be interpreted with caution due to the limited number of patients in each dose group, high PK variability and impact of major organ (such as kidney and liver) failure on PK.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

PK of defibrotide in healthy volunteers was characterized in the through QT study R09-1425. The PK sampling scheme in this trial was presented in **Table 5** above.

Typical plasma concentration-time profiles of defibrotide after single 2-hour IV infusion at therapeutic and supra-therapeutic doses in healthy volunteers are shown in **Figure 6**. Plasma concentrations of defibrotide reached maximum at the end of 2-hour infusion and then dropped exponentially. In healthy volunteers, exposures (both C_{max} and AUC) of defibrotide (**Table 7**) appear significantly lower than those in severe VOD patients after a single IV administration of defibrotide at the same dose level (**Table 6**). This is consistent with the observation that total clearance in healthy volunteers ranged from 9.8 to 10.4 L/h, higher than the observed total clearance in severe VOD patients with a range of 3.4 to 6.1 L/h. The half-life of defibrotide in healthy volunteers ranged from 0.45 to 0.71 hours, shorter than the half-life in severe VOD patients with a range of 1.1 to 1.9 hours. The volume of distribution appears similar between healthy volunteers and severe VOD patients since the observed values are largely overlapped.

Figure 6: Mean defibrotide plasma concentrations versus time profile: linear scale (N=52)



Results from healthy volunteers in the TQT study indicated that both C_{max} and $AUC_{0\text{-}t}$ of defibrotide increased more than dose-proportionally between dose of 6.25 mg/kg and 15 mg/kg, while the increase of $AUC_{0\text{-}inf}$ was dose-proportional. This observation in healthy volunteers is different from the less than dose-proportional increase in exposure observed in severe VOD patients.

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Table 7 Summary of single and multiple doses PK parameters of defibrotide (mean, CV%) in study R09-1425

Study	Dose	N	C_{max} (µg/mL)	AUC_{0-t} (µg*h/mL)	AUC_{0-inf} (µg*h/mL)	$T_{1/2}$ (h)	CL (L/h)	Vss (L)
R09-1425	**Treatment A (Therapeutic Dose)**							
	Day 1: 6.25 mg/kg	52	17.3 (22%)	26.9 (32%)	48.1* (13%)	0.71* (49%)	10.4* (17%)	9.9* (38%)
	Treatment B (Supra-therapeutic Dose)							
	Day 1: 15 mg/kg	52	61.0 (19%)	105.2 (21%)	113.6 (21%)	0.45 (39%)	9.8 (17%)	6.2 (37%)

Note: * N=12

2.2.5.3 What are the characteristics of drug absorption?

Not applicable. Defibrotide is given by intravenous infusion for the proposed indication.

2.2.5.4 What are the characteristics of drug distribution?

The plasma protein binding (PPB) characteristics of defibrotide were evaluated in *in vitro* studies using human plasma with a concentration range of 5 to 500 µg/mL. There was no apparent difference in protein binding among species. The average protein binding in human plasma was 93.2%. This level of protein binding is consistent with mean steady-state volume of distribution (Vss) of 9.8 to 10.4 L as determined using non-compartmental analysis in healthy volunteer studies.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

The sponsor did not conduct a human ADME study. In a legacy study IRI-151612, following single intravenous administration of ^{125}I-Defibrotide 400 mg in 3 male healthy volunteers, the major route of excretion was via the urine (~71% of total administered radioactivity) and excretion via the feces accounted for ~19% of total administered radioactivity.

Human urinary excretion of defibrotide was also measured in study R09-1425 and VOD-2012-03-PKRen. The urinary excretion assessment indicated 10-14% of unchanged defibrotide was excreted in the urine in healthy subjects but the amount decreased to 5% in non-VOD subjects with severe renal impairment to ESRD.

Together, the data suggest that defibrotide is mainly eliminated by metabolism followed by renal excretion, and only a small portion of defibrotide is eliminated unchanged by the kidney.

2.2.5.6 What are the characteristics of drug metabolism?

Defibrotide is a mixture of single-stranded polydeoxyribonucleotides, it has no appreciable metabolism in human hepatocytes from donors of different ages. Literature data suggest that defibrotide would predominantly be degraded by exonucleases in the human plasma initially, then be metabolized progressively to the free 2'-deoxyribose sugar moiety, purine and

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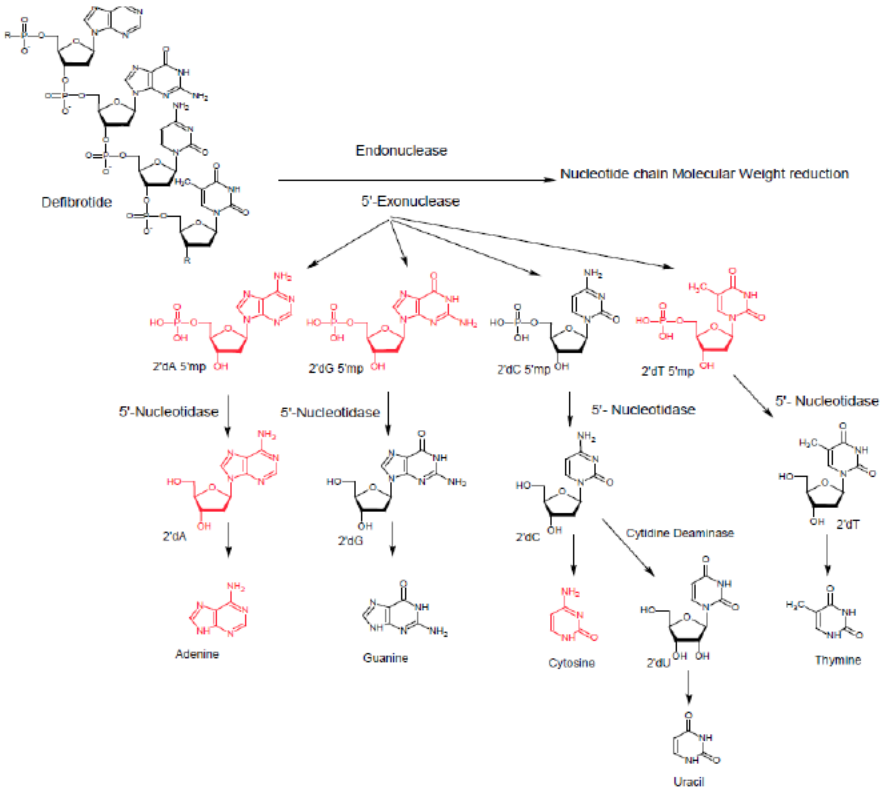
pyrimidine bases by various enzymes involved in DNA degradation. The proposed mechanism of metabolism above was supported by an *in vivo* metabolism study in rabbit, in which the intermediates of DNA degradation were detected in rabbit plasma at various time points after IV infusion. The proposed defibrotide degradation/metabolic pathways are shown in **Figure 7** below.

Figure 7: Proposed major metabolic pathways of defibrotide in vivo (metabolites depicted in black were detected in rabbit plasma by LC-MS/MS)



2.2.5.7 What are the characteristics of drug excretion?

After intravenous administration in healthy subjects, defibrotide is mainly excreted into urine as unchanged form (10-14% of total dose) and degradation metabolites, with approximately 19% of the dose excreted into feces. See section 2.2.5.5 for more detail.

2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

In healthy volunteers, both C_{max} and AUC_{0-t} increased more than dose-proportionally between single dose of 6.25 mg/kg and 15 mg/kg, while the increase of AUC_{0-inf} was dose-proportional.

2.2.5.9 How do the PK parameters change with time following chronic dosing?

As described in section 2.2.5.1 and section 2.2.5.2, because defibrotide has very short half-life, C_{max} and AUC of defibrotide do not change following multiple dosing compared to those

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following single dosing. There is no accumulation following multiple-dose administration.

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

Table 8 below summarizes the population PK parameter estimates and associated inter- and intra-individual PK variability. The inter-individual variabilities for clearance (CL) and volume of distribution (V) were 25% and 44%, respectively. Creatinine clearance and liver ALT levels appeared to be sources of inter-individual variability on clearance.

Table 8: Summary of defibrotide PK parameter estimates with the final model (sponsor's analysis)

Pop PK Parameter	Population Estimates	(RSE%)	BSV%	(RSE%)	IOV%
CL (L/h)	$\left\{CLnr \times \left(\frac{ALTlog}{ALTlog_{Median}}\right)^{-0.530} + CLr \times \left(\frac{CrCL}{CrCL_{70\,kg}}\right)\right\} \times \left(\frac{BW}{70\,kg}\right)^{0.75}$				
where,					
CLr (L/h)	0.913	(23.2)			
CLnr (L/h)	8.15	(4.9)	25.0[a]	(4.9)	18.0
If subjects with RI impairment (DF VOD-2012-03-PKRen)	CLnr x 0.498	(10.6)			
If patient with hepatic VOD (Study DFPK 99-118)	CLnr x 0.405	(8.7)			
V (L)	4.75 x (BW/70 kg)$^{1.0}$	(5.5)	43.6	(9.4)	20.2
Error Model					
Additive (µg/mL)	7.40	(2.1)	N/A	N/A	

[a]Correlation was 100% between BSV of CL and V.
$ALTlog_{median}$=log(17 U/L); BSV: Between-subject variability; CL: Clearance; CrCL: Creatinine clearance (mL/min); $CrCL_{70\,kg}$= 100 mL/min; IOV: Inter-occasion variability; N/A: Not applicable; PK: Pharmacokinetic; RI: renal impairment; RSE: relative standard error; V: volume of distribution; VOD: veno-occlusive disease.

2.3 Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The sponsor conducted a population PK analysis using data from the Phase 2 dose finding study (Study 99-118) and the two clinical pharmacology studies (Study VOD-2012-03-PKRen and Study R09-1425). The influence of intrinsic factors on defibrotide PK was evaluated using the population PK analysis. Those factors included age, weight, height, body surface area, creatinine clearance (CrCL), gender, ethnicity, total bilirubin, liver enzymes, and the demographics. A brief summary is provided in **Table 9**. Available data were insufficient to fully characterize the influence of intrinsic factors on the PK of defibrotide. Renal impairment and liver enzyme ALT

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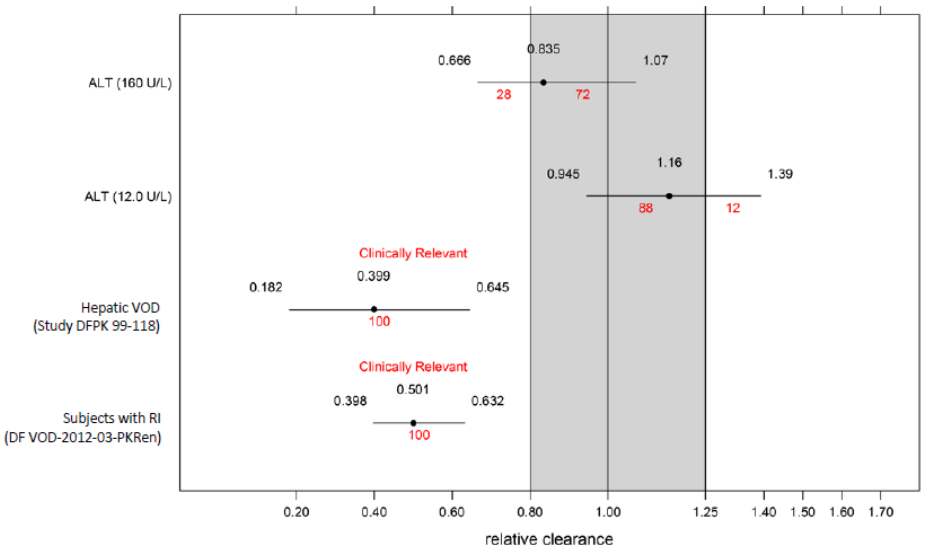
levels were found to be significant covariates on defibrotide clearance. Compared to healthy subjects, subjects with non-VOD renal impairment and those with VOD appear to have similar degree of reduced clearance (**Figure 8).**

Table 9: Summary of demographic characteristics of healthy volunteers, non-VOD subjects with renal impairment and VOD patients

Mean (CV%) Median [Min, Max]	DFVOD-2012-03-PKRen (n=18)		R09-1425 (n=52)	DFPK 99-118 (n=13)	Overall (N=83)	
Characteristics	Healthy Subjects (n=6)	Patients (n=12)	Healthy Subjects	Patients	With Pediatric (N=83)	Without Pediatric (N=81)
Age (years)	65.0 (10.2) 67.0 [52.0, 71.0]	57.8 (19.1) 58.5 [38.0, 76.0]	24.1 (23.4) 22.0 [19.0, 41.0]	34.3 (55.2) 36.0 [4.0 , 58.0]	33.5 (52.2) 24.0 [4.0 , 76.0]	34.2 (49.9) 26.0 [18.0, 76.0]
Weight (kg)	89.0 (15.9) 91.6 [71.2, 108.0]	93.3 (19.0) 88.3 [63.8, 125.6]	71.8 (15.0) 69.7 [55.8, 96.7]	71.7 (43.1) 65.8 [15.8, 110.3]	76.2 (24.1) 74.5 [15.8, 125.6]	77.6 (20.7) 76.0 [52.2, 125.6]
BMI (kg/m^2)	30.5 (15.9) 30.3 [24.6, 38.7]	31.8 (18.5) 31.3 [22.9, 40.0]	23.9 (9.6) 23.6 [19.9, 28.0]	26.0 (25.7) 26.7 [16.1, 37.5]	25.9 (19.4) 24.8 [16.1, 40.0]	26.1 (18.6) 24.8 [19.9, 40.0]
Scr (mg/dL)	0.8 (11.7) 0.7 [0.7, 0.9]	6.3 (64.4) 5.3 [2.2, 15.0]	0.9 (18.7) 0.9 [0.5, 1.2]	1.8 (55.6) 1.7 [0.4, 4.5]	1.8 (136.0) 0.9 [0.4 , 15.0]	1.83 (134.7) 0.9 [0.5 , 15.0]
CrCL (mL/min)	119.1 (22.5) 111.6 [90.2, 160.3]	22.5 (56.8) 19.7 [7.5, 42.9]	129.1 (18.1) 127.2 [78.9,174.5]	60.0 (41.5) 55.5 [19.4, 99.0][a]	102 (45.8) 114 [7.5, 174.5][a]	103.0 (45.7) 114.8 [7.52, 174.5]
ALT (U/L)	20.8 (20.0) 19.0 [17.0, 27.0]	14.3 (42.3) 13.5 [6.0, 29.0]	21.1 (49.7) 18.5 [11.0, 56.0]	25.5 (91.0) 18.0 [7.0, 92.0]	20.8 (61.4) 17.0 [6.0, 92.0]	20.6 (61.7) 17.0 [6.0, 92.0]
AST (U/L)	23.2 (16.7) 22.5 [18.0, 28.0]	16.8 (35.8) 16.0 [10.0, 27.0]	22.8 (28.9) 20.0 [15.0, 43.0]	22.2 (46.5) 20.0 [9.0, 39.0]	21.8 (33.3) 20.0 [9.0, 43.0]	21.8 (32.9) 20.0 [9.0, 43.0]
Bilirubin (mg/dL)	0.5 (41.4) 0.5 [0.3, 0.9]	0.4 (36.9) 0.4 [0.2, 0.7]	1.0 (39.2) 0.9 [0.5, 2.8]	4.7 (71.2) 3.4 [2.0, 11.5]	1.4 (135.2) 0.9 [0.2, 11.5]	1.4 (139.1) 0.9 [0.2, 11.5]

Figure 8: Geometric mean ratios and 90% CI for the effect of disease status and ALT levels on defibrotide clearance (sponsor's analysis)



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2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups?

No dose adjustments are recommended for any specific population (see rationale in section 1.4 and sections below).

2.3.2.1 Elderly

No PK data were collected in patients older than 65 years old.

2.3.2.2 What is the status of pediatric studies and/or any pediatric plan for study?

No dose adjustment is recommended for pediatric patients. The Phase 2 and pivotal Phase 3 studies enrolled approximately 48% of pediatric patients. The observed survival rate was higher in pediatric patients than that in adults at the proposed dose based on subgroup analysis with acceptable safety profile. The sponsor (b) (4) It is not reliable to assess the body weight and clearance relationship with this limited data. Also, since efficacy and safety data in pediatrics at 25 mg/kg/day dose has been established in the clinical trial, (b) (4) doesn't appear to be a critical issue.

2.3.2.3 Gender

Gender effect on PK is not considered clinically relevant based on healthy volunteers data.

2.3.2.4 Race, in particular differences in exposure and/or response in Caucasians, African-Americans, and/or Asians

Not assessed because of limited PK data from other races in patients.

2.3.2.5 Renal impairment

In a renal impairment study VOD-2012-03-PKRen, defibrotide exposure (AUC) in severe renal impairment to ESRD subjects was approximately 50% to 60% higher than that observed in matched healthy subjects. Peak concentration (C_{max}) was about 35% to 37% higher following single and multiple administrations of defibrotide. The amount of defibrotide excreted in urine was approximately 5% in severe renal impairment to ESRD subjects.

In the same study, the exposure (both AUC and C_{max}) of defibrotide in ESRD subjects following a single administration of defibrotide 6.25 mg/kg 2-hour IV infusion on a non-dialysis day were within 10% difference of those observed on a dialysis day (after 4 days of dosing). Therefore, hemodialysis didn't significantly influence the AUC or Cmax of defibrotide.

Because severe VOD patients have multi-organ dysfunction including renal impairment, the efficacy and safety profile of the proposed dose for defibrotide has already been assessed in the Phase 2 and Phase 3 studies, therefore, no dose adjustment is recommended for renal impairment in the target patient population.

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2.3.2.6 Hepatic impairment

The sponsor did not conduct a study in subjects with impaired hepatic function. However, since efficacy and safety data in severe VOD patients at 25 mg/kg/day dose has been established in the clinical trials, lack of PK information in hepatic impairment population in the label doesn't appear to be a critical issue.

2.3.2.7 Pharmacogenetics

The submitted NDA package did not include Pharmacogenetics assessment.

2.3.2.8 What pregnancy and lactation use information is there in the application?

The effect of defibrotide in lactating and pregnant women has not been evaluated. No further pregnancy and lactation information is provided in the proposed label.

2.3.2.9 Other human factors that are important to understanding the drug's efficacy and safety?

There are no other known important human factors that are important to the understanding of defibrotide safety and efficacy.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?

The sponsor did not submit results of specific studies or analyses designed to evaluate the effects of extrinsic factors such as herbal products, diet, smoking or alcohol use on the PK, safety, or efficacy of defibrotide.

2.4.2 Drug-drug interactions

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

No. *In vitro* studies using therapeutic concentrations showed that defibrotide dose not inhibit or induce major human CYP450 enzymes and UGT enzymes. In addition, defibrotide is not a substrate or inhibitor of major human uptake transporters or efflux transporters. Overall, drug-drug interaction potential of defibrotide is not expected at therapeutic concentrations.

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

No. Defibrotide is a mixture of single-stranded polydeoxyribonucleotides, it has no appreciable metabolism in human hepatocytes, suggesting it is not a substrate of CYP enzymes. Therefore, no influence of genetics on metabolism of defibrotide is expected.

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

No. Defibrotide dose not inhibit major human CYP450 enzymes including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 at therapeutic concentrations based on *in*

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vitro evaluations. Defibrotide does not induce CYP1A2, CYP2B6, CYP3A4 and UGT1A1 at therapeutic concentrations either.

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

No. Defibrotide is not a substrate and inhibitor of P-gp based on *in vitro* evaluations.

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

Yes. Literature data and results from *in vitro* study in plasma suggest that defibrotide is predominantly degraded by exonucleases in the human plasma initially, then progressively metabolized to the free 2'-deoxyribose sugar, purine and pyrimidine bases by various enzymes involved in DNA degradation.

2.4.2.6 Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

No. Defibrotide will be used as a monotherapy in the treatment of severe VOD.

2.4.2.7 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

No *in vivo* drug-drug interaction (DDI) studies were conducted for defibrotide.

2.4.2.8 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

There is no known mechanistic basis for PD drug-drug interactions.

2.4.2.9 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

Yes. The actual metabolism enzymes involved in the degradation of defibrotide in human are not fully understood, the proposed mechanism of degradation is based on literature data and animal studies.

2.4.3 What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

No.

2.5 General Biopharmaceutics

Defibrotide is formulated for intravenous administration and it has high aqueous solubility. As such, solubility, permeability and dissolution issues will not influence the exposure to defibrotide.

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2.6 Analytical Section

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Because the drug targets of defibrotide in treatment of severe VOD are unknown, the clinical pharmacology activities of defibrotide that are considered related to the efficacy, i.e. endothelial cell protection and rebalance the thrombo-fibrinolytic activity, were investigated in various *in vitro* assays. However, there were no other active moieties of defibrotide identified from plasma of clinical studies.

2.6.2 Which metabolites have been selected for analysis?

Not applicable. Defibrotide metabolites were not identified and selected for the analysis.

2.6.3 For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

Total defibrotide plasma concentrations were measured in clinical studies.

2.6.4 What bioanalytical methods are used to assess concentrations?

Two bioanalytical methods were used to determine concentrations of defibrotide in clinical studies supporting the NDA application.

The first bioanalytical method was developed and validated at [redacted] (b) (4) for the determination of defibrotide in plasma and urine using ultra-high pressure liquid chromatography with ultraviolet detector (UPLC/UV) detection method. As shown in **Table 10** below, this method demonstrated acceptable sensitivity, linear range and accuracy in determining the concentrations of defibrotide in both human plasma and urine. The method was used for the Phase 2 dose finding study 99-118 and the thorough QT study R09-1425.

Table 10: Summary of bioanalytical methods and their applications in determining defibrotide concentrations in clinical studies

Study	Method	Matrix	Report No.	LLOQ (µg/mL)	Linear Range (µg/mL)	Intra-assay variability (CV%)	Inter-assay variability (CV%)	Accuracy (%)
99-118	UPLC/UV	Plasma	[redacted] (b) (4) 65694	10	10 - 300	<15%	<15%	95.8-115.2
R09-1425	UPLC/UV	Plasma	[redacted] (b) (4) 65694	10	10 - 300	<15%	<15%	95.8-115.2
		Urine	[redacted] (b) (4) 65695	3	3 - 60	<15%	<15%	96.0-102.6
VOD-2012-03-PKRen	Flurometric	Plasma	[redacted] (b) (4) 12-645	0.2	0.2-10	<15%	<15%	95.5-101.9
		Urine	[redacted] (b) (4) 13-408	0.5	0.5-15	<15%	<15%	98.3-101.0
		Dialysate	[redacted] (b) (4) 13-408	0.5	0.5-15	<15%	<15%	96.7-101.9

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To improve sensitivity, a fluorometric method derived from the commercially available kit for measuring oligonucleotides (Quant-iTTM OliGreen ssDNA Reagent) was developed and validated by [redacted] (b) (4) with a much lower LLOQ of 0.2 µg/mL. This bioanalytical method was used to determine the concentrations of defibrotide in plasma, urine and dialysate from the renal impairment study VOD-2012-03-PKRen. **Table 10** above summarized the key characteristics of this fluorometric-based assay and its application in the renal impairment study. Overall, the flurometric bioanalytical method demonstrated improved sensitivity, appropriate precision and accuracy to determine defibrotide concentrations in human plasma, urine and dialysate.

2.6.5 What is the range of the standard curve? How does it relate to the requirements for clinical studies?

The ranges of the standard curves are shown in **Table 10** above in section 2.6.4. The concentrations of the analytes are either within the bounds of the standard curve ranges (Study 99-118 and R09-1425) or require further dilution (VOD-2012-03-PKRen).

2.6.6 What are the lower and upper limits of quantification (LLOQ/ULOQ)?

See **Table 10** in section 2.6.4 above.

2.6.7 What are the accuracy, precision, and selectivity at these limits?

See **Table 10** in section 2.6.4 above.

3 Appendix: Population PK Modeling: Sponsor's Analysis

3.1.1 Introduction

The sponsor conducted a population PK analysis to characterize the PK of defibrotide and to assess sources of variability in drug exposure and to explore the dose-plasma concentration relationship in adult and pediatric patients with hepatic VOD.

3.1.2 Datasets

The population PK analysis was based on PK data collected in the following 3 studies:

Study DF VOD-2012-03-PKRen: An open-label, Phase 1 study, that was divided into 2 parts: 1) Dialysis Study in 6 end-stage renal disease (ESRD) subjects on hemodialysis to investigate the PK profile of single dose of defibrotide in plasma and dialysate during the hemodialysis procedure in ESRD subjects, and 2) Main Study in 6 severe renal impairment to ESRD subjects not on dialysis and 6 healthy matching subjects to investigate the PK profile of single and multiple doses of defibrotide in plasma and urine.

Study R09-1425: A double-blind, Phase 1, randomized crossover trial in healthy volunteers to define the ECG effects of defibrotide using a clinical and a supra-therapeutic dose compared to placebo and moxifloxacin in healthy men and women: a thorough ECG trial

Reference ID: 3865457

Study 99-118: Multi-institutional, Phase 2, randomized dose finding study of defibrotide in patients with hepatic VOD disease and multi-system organ failure post stem cell transplantation.

The subjects included in the population PK analysis dataset are healthy adults (N=6, 52-72 years) and adult subjects with renal impairment (N=12, 38-76 years) from Study DF VOD-2012-03-PKRen, healthy adults (N=52, 19-41 years) from Study R09-1425, and adult patients (N=11, 18-58 years) and pediatrics patients (N=2, 4-5 years) from Study 99-118.

Total number of measurements included in the analysis was 1360 from 83 (with 2 pediatric) subjects. The days of PK sample collection are summarized in **Table A-11**.

Table A-11: PK sample collection in three studies

Study	Day of PK Sample Collection Relative to the First Defibrotide Dose
DF VOD-2012-03-PKRen	Dialysis Study (Week 1, including non-dialysis [Day 1] and dialysis days [Day 4]) Main Study Days 1 & 2 (Week 1)
R09-1425	Day 1 (Week 1), Day 8 (Week 2) and Day 15 (Week 3)*
DFPK 99-118	Week 1 (including Day 1 and Day 2) Day 7 (Week 2)

3.1.3 Methods

Analysis was performed using Phoenix® NLME™ 1.4 with first order conditional estimation-extended least squares (FOCE-ELS) with INTERACTION. Covariates influencing defibrotide CL and V were explored using the stepwise forward addition and backward elimination procedure with a priori statistical significance criteria for the forward and backward search directions. The covariates included age, BMI, baseline body weight, gender, ethnicity, organ function, disease status and dose.
The final model was customized by including a maturation function (MF) on the renal clearance of defibrotide, and simulations were performed to determine the exposure to defibrotide following every 6 hours dosing at doses of 2.5 mg/kg, 6.25 mg/kg, and 10 mg/kg infused over 2 hours for various pediatric age cohorts.

3.1.4 Results

A one-compartment model with an allometric function of body weight on CL and V of defibrotide was chosen as the final model. Renal clearance (CLr) and non-renal clearance (CLnr) were parameterized and alanine transaminase (ALT) and creatinine clearance (CrCL) were identified as significant covariates on CLnr and CLr, respectively. **Table 8** summarizes the final parameter estimates with the final model.

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ALT and CrCL were significant covariates on defibrotide clearance: ALT on CLnr and CrCL on CLr. Considering the smaller contribution of renal clearance to the total clearance, the effect of CrCL would be substantially smaller than the effect of ALT on CL. Disease status was also an important factor on the variability of defibrotide clearance (CLnr): its multiplicative factors are estimated to be 0.498 for subjects with renal impairment and 0.405 for patients with VOD. The geometric mean ratios (90% CI) for the effect of disease status and ALT levels are illustrated in **Figure 8**.

Simulation to predict defibrotide exposure in pediatric patients

The final model was used to derive the predicted defibrotide exposure in a hypothetical population of 1000 pediatric patients (aged 1 day to 17 years). The final model was customized by including a kidney maturation function on the renal clearance of defibrotide in pediatric patients. Body weight values were simulated in each age cohort using a body weight-age matched algorithm. A hypothetical population of 100 pediatric patients (1 day to <17 years) was generated using the body weight distribution provided by the CDC. These pediatric body weights were used in the simulations and assume that the baseline body weight in hepatic VOD pediatric patients (weight at admission to the bone marrow transplant (BMT) unit or prior to conditioning for the HSCT) are similar to the healthy pediatric population that was simulated using a body weight-age matched algorithm.

The sponsor [redacted] (b) (4) Study 99-118 were within 27% of the mean body weights provided by the CDC at each age cohorts.
Then AUC_{ss} of defibrotide in pediatric patients at doses given to adult patients were simulated using the final pop PK model. These simulated AUC_{ss} of defibrotide in pediatric patients following IV dosing of 6.25 mg/kg every 6 hours ranged from 62.9 – 100 μg*h/mL, which were lower than those values (126 – 158 μg*h/mL) in typical adults patients with hepatic VOD.

Reviewer's comments: The simulation was performed based on the observation in 2 pediatric patients (4 and 5 years). These observed data do not appear to be sufficient to provide adequate information for [redacted] (b) (4) defibrotide PK in pediatric patients. [redacted] (b) (4) (b) (4) Sponsor did not propose any other labeling statements [redacted] (b) (4)

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