25 July 2013
EMA/CHMP/824715/2012
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Defitelio

International non-proprietary name: Defibrotide

Procedure No. EMEA/H/C/002393

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

A	Adenine
AE	Adverse Event
ALL	Acute Lymphocytic Leukemia
AML	Acute Myelogenous Leukemia
API	active pharmaceutical ingredient
AP1	In 2006-05 study: all enrolled patients who received at least one dose of study drug and reported outcome at the time of interim analysis.
AP2	In 2006-05 study: all enrolled patients who received at least one dose of study drug and who were transplanted AND met study 2005-01 entry criteria
aPTT	activated Partial Thromboplastin Time
ATIII	Antithrombin III
ATG	Anti-Thymocyte Globulin
AUC	Area Under Curve
AV	Atrio-ventricular
BCNU	Carmustine
bpm	Beats per minute
BU	Busulphan
°C	Grade Celsius
C	Cytosine
CD	Circular dichroism
CFR	Code of Federal Regulations
cfu	colony forming units
CGE	capillary gel electrophoresis
CI	Confidence Interval
CL	Clearance
Cmax	Maximum Observed Concentration
CML	Chronic Myeloid Leukemia
CNS	Central Nervous System
CR	Complete Response
CRO	Contract Research Organisation
CrCl	Creatinine Clearance
CUP	Compassionate Use Program
CV	Coefficient of Variation
Cy	Cyclophosphamide
Da	Daltons
Day+21	21 Days Post Stem Cell Transplant
Day+30	30 Days Post Stem Cell Transplant
Day+100	100 Days Post Stem Cell Transplant
Day+180	180 Days Post Stem Cell Transplant
DF	Defibrotide
dL	Deciliter
d.m.	dry material
DNA	deoxyribonucleic acid

Ds	Double stranded
EBMT	European Group for Blood and Marrow Transplantation
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
equiv	Equivalent
ESI-MS	Electron Spray Ionization Mass Spectroscopy
FR	Frosinone (Italian Province)
G	Guanine
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practice
GVHD	Graft Versus Host Disease
HC	Historical Control
HL	Hodgkin's lymphoma
HPLC	High Pressure Liquid Chromatography
HPLC/UV	High-Pressure Liquid Chromatography with Ultraviolet Detector
HSCT	Haematopoietic Stem Cell Transplant
HP-SEC	High-performance size exclusion chromatography
IC50	Half Maximal Inhibitory Concentration
IND	Investigational New Drug
IPC	In-process control
IRB	Institutional Review Board
ITT	Intent-to-treat
IU	International Unit
i.v. /IV	Intravenous
Kel	Elimination rate constant
K.F.	Karl Fischer
Kg	Kilogram
KM	Kaplan-Meier
LBB	Left Bundle Branch Block
LLOQ	Lower Limit of Quantitation
LPS	lipopolysaccharide
mcg/µg	Microgram
mg	Milligram
MI	Myocardial Infarction
mL	Milliliter
mm	Millimeter
MO	Major Objection
MoA	Mechanism of action
MOF	Multi Organ Failure
MRC	Medical Review Committee
ms	millisecond
mw	Molecular weight
N	Number
NA	Not applicable
NHL	non-Hodgkin's lymphoma
NMT	not more than

NLT	not less than
No	Number
OOS	Out of specification
PAGE	Polyacrilamide Gel Electrophoresis
PAI-1	Plasminogen activator inhibitor-1
PF4	Platelet Factor 4
PGE	Prostaglandin E
PGF	Prostaglandin F
PK	Pharmacokinetic
PO	Oral Administration
POAD	Peripheral obstructive arterial disease
PP	Per Protocol
ppm	Parts per million
PR	Partial Response
PrC	Protein C antigen
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
Q6H	Every 6 Hours
QC	Quality Control
QID	Four Times a Day
QTc	Q-T corrected interval
QTcB	Bazett correction
QTcF	Fridericia correction
QTcI	Individual Correction
QTc Q-T	corrected interval
RBB	Right Bundle Branch Block
RNF	Italian national network of pharmacovigilance
RUQ	Right Upper Quadrant
SCT	Stem Cell Transplant
SEC-MALLS	Size exclusion Chromatography- Multiple Angle Laser Light Scattering
SD	Standard Deviation
SE	Standard Error
SmPC	Summary of Product Characteristics
Ss	Single stranded
sVOD	VOD severe
T	Thymidine
TAFI	Thrombin activatable fibrinolysis inhibitor
TAMC	Total aerobic microbial counts
TAM/TTP	Transplant associated microangiopathy/thrombotic thrombocytopenic purpura
TAT	Thrombin-Antithrombin III complex
$T_{1/2}$	Half Life
$t\alpha$	Distribution half life
$t\beta$	Elimination half life
TBI	Total Body Irradiation
TF	Tissue factor
TFPI	Tissue Factor Pathway Inhibitor

TG	Treatment group
Tmax	Time at which the Cmax occurs
t-PA	Tissue Plasminogen Activator
t-PAI	Tissue Plasminogen Activator Inhibitor
TT	Thrombin Time
TYMC	Total combined yeasts/moulds count
Tx	Treatment
TxB2	Thromboxane B2
U	Uracil
UF	Ultrafiltration
UPLC	Ultra High Pressure Liquid Chromatography
UPLC/UV	Ultra High Pressure Liquid Chromatography with Ultraviolet Detector
UV	Ultraviolet
VAD	vascular atherosclerotic disease
Vmax	Maximum Velocity
V_D	Volume of distribution
VOD	Veno-Occlusive Disease
VP-16	Etoposide
WFI	water for injections
WHVPG	Wedged Hepatic Venous Pressure Gradient

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Gentium S.p.A. submitted on 3 May 2011 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Defitelio, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 27 July 2010.

Defitelio was designated as an orphan medicinal product EU/3/04/211 and EU/3/04/212 on 29 July 2004 for the prevention and treatment of hepatic veno-occlusive disease (VOD) respectively in the following indication:

Defitelio is indicated for the prevention of hepatic veno-occlusive disease (VOD) also known as Sinusoidal Obstructive syndrome (SOS) in haematopoietic stem-cell transplantation therapy.

Defitelio is indicated for the treatment of hepatic veno-occlusive disease (VOD) also known as Sinusoidal Obstructive syndrome (SOS) in haematopoietic stem-cell transplantation therapy.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Defitelio as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website ema.europa.eu/Find medicine/Rare disease designations.

The legal basis for this application refers to:

Known active substance (Article 8(3) of Directive No 2001/83/EC)

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data and bibliographic literature substituting/supporting certain test(s) or studies.

Information on Paediatric requirements

Not applicable

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 19 March 2008 (EMEA/H/SA/996/2008/PA/II). The Scientific Advice pertained to clinical aspects of the dossier.

Licensing status

Defibrotide (DF) was nationally approved in Italy in 1986 for “prophylaxis of deep vein thrombosis and treatment of thrombophlebitis. On 21 April 1993, marketing authorizations for defibrotide capsules 400 mg (Prociclide, Noravid and Dinelasi) for the treatment of peripheral obstructive (mild to moderate) arteriopathy, were granted in Italy. On 13 March 1993, the indication for all products was changed by the Italian Ministry of Health to “vascular disease associated with the risk of thrombosis (acute stage)”.

The marketing authorisations for both Noravid and Prociclide were withdrawn by Gentium S.p.A. effective April 2009 for commercial reasons (principally to limit the import of the product in the US and Europe, as this was impacting the investigational use of the product in clinical trials). Dinelasi was never placed on the Italian market and the Marketing Authorization ceased to be valid according to Article 24 of Directive 2001/83/EC as amended (sunset clause) as of 9 July 2010. To date, defibrotide is no longer authorised in any of the EU Member States.

The active substance was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturer of the active substance

Gentium S.p.A.
Piazza XX Settembre, 2
22079 VILLA GUARDIA (Como)
Italy

Manufacturer responsible for batch release

Gentium S.p.A.
Piazza XX Settembre, 2,
22079 Villa Guardia (Como)
Italy

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: Ian Hudson Co-Rapporteur: Kristina Dunder

- The application was received by the EMA on 3 May 2011.
- The procedure started on 25 May 2011.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 12 August 2011. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 12 August 2011.
- During the meeting on 22 September 2011, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the

applicant on 23 September 2011.

- The applicant submitted the responses to the CHMP consolidated List of Questions on 10 February 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 March 2012 and 13 April 2012.
- During the CHMP meeting on 19 April 2012, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 18 June 2012.
- The summary report of the inspection carried out at the following sites Gentium S.P.A., Universitätsklinik für Kinder und Jugendmedizin and University Hospital Hamburg-Eppendorf, Clinics for Stem Cell Transplantation between 10-13 July, 02-06 July and 16-18 July 2012 was issued on 10 September 2012.
- During a meeting of an Expert group on 12 September 2012, experts were convened to address questions raised by the CHMP.
- During the CHMP meeting on 20 September 2012, outstanding issues were addressed by the applicant during an oral explanation at the CHMP.
- During the CHMP meeting on 20 September 2012, the CHMP agreed on a second list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP second List of Outstanding Issues on 17 December 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the second List of Outstanding Issues to all CHMP members on 31 December 2012, 17 January 2013 and 21 February 2013.
- During the CHMP meeting on 21 February 2013, GCP outstanding issues were addressed by the applicant during an oral explanation at the CHMP.
- During the meeting on 21 March 2013, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative scientific opinion for Defitelio.

1.4. Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: Jens Ersbøll Co-Rapporteur: Pierre Demolis

- The applicant submitted written notice to the EMA on 2 April 2013 to request a re-examination of Defitelio CHMP opinion of 21 March 2013.
- During its meeting on 25 April 2013, the CHMP appointed Jens Ersbøll as Rapporteur and Pierre Demolis as Co-Rapporteur.

- The applicant submitted the detailed grounds for the re-examination on 27 May 2013.
- The re-examination procedure started on 02 June 2013.
- The Rapporteur's Assessment Report was circulated to all CHMP members on 05 July 2013. The Co Rapporteur's Assessment Report was circulated to all CHMP members on 21 June 2013.
- During a meeting of an expert group on 08 July 2013, experts were convened to consider the grounds for re-examination.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's detailed grounds for re-examination to all CHMP members on 15 July 2013.
- During the CHMP meeting on 22 July 2013, the grounds for refusal were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting in July 2013, the CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application satisfied the criteria for authorisation and recommended the granting of the marketing authorisation under exceptional circumstances.

2. Scientific discussion

2.1. Introduction

Hepatic veno-occlusive disease (VOD) is a complication of vascular origin, described in patients receiving high-dose myeloablative chemotherapy as conditioning regimens for Haematopoietic Stem Cell Transplant (HSCT).

The pathophysiology of hepatic VOD is complex; the causative event is thought to be injury to sinusoidal endothelial cells and hepatocytes in zone 3 of the hepatic acinus as a result of the high dose chemotherapy preparative regimens used for stem cell transplant procedures. Thus, hepatic VOD is considered a regimen-related toxicity. A pro-coagulant and hypofibrinolytic state is present in hepatic VOD, perhaps representing the essential underlying mechanism of this disorder.

Table 1 Clinical criteria to diagnose VOD

Seattle Criteria [McDonald, 1984 Hepatology]	Baltimore Criteria [Jones, 1987 Transplantation]
Presence before day 20 after haematopoietic stem cell transplantation (HSCT) of two or more of the following: • Bilirubin ≥ 2 mg /dl (≈ 34 µmol / l) • Hepatomegaly, right upper quadrant (RUQ) pain • Ascites +/- unexplained weight gain of >2% baseline	Hyperbilirubinaemia ≥ 2 mg /dl before day 21 after HSCT and at least two of the following: • Hepatomegaly (usually painful) • Ascites • Weight gain ≥5% from baseline

The clinical diagnosis of hepatic VOD usually relies on the application of validated criteria described by Seattle or Baltimore criteria (Table 1). The principal difference in Baltimore criteria is the absolute requirement for bilirubin to rise to a level of >2 mg/dL (>34 µmol/L), in conjunction with ≥2 additional clinical features of hepatic VOD. Baltimore criteria are known to identify the more advanced cases of hepatic VOD.

As a consequence of liver dysfunction, patients may have hepatorenal syndrome with sodium retention and portal hypertension. This frequently progresses to acute oliguric renal failure which occurs in approximately half of the patients with severe disease, often leading to need for dialysis.

Severe hepatic VOD, is defined as VOD in the presence of multi organ failure (MOF); either pulmonary dysfunction (with an oxygen requirement with an oxygen saturation of <90% on room air and/or ventilator dependence), and/or renal dysfunction (defined as doubling of baseline creatinine and/or dialysis dependence), and/or encephalopathy. Severe hepatic VOD is associated with a high risk of severe morbidity and mortality. Rarely do patients with severe hepatic VOD die of their liver failure; progressive MOF with consecutive lethal renal and cardiopulmonary complications are typically the main causes of death.

Well-established risk factors for hepatic VOD include age, liver inflammation, prior abdominal irradiation, hepatic fibrosis or cirrhosis, and repetitive transplants with myeloablative conditioning regimens. The conditioning regimen is also a well-established risk factor in the pathogenesis of hepatic VOD, with cyclophosphamide, total body irradiation, and particularly busulfan being the most commonly associated conditioning agents associated with the onset of hepatic VOD.

No therapy for the treatment or the prophylaxis of hepatic VOD has been approved in the US or in Europe. Investigations with several experimental approaches using anti-thrombotic and thrombolytic agents, including prostaglandin E1 and t-PA with or without concurrent heparin, have not proven successful, leaving the management of hepatic VOD restricted to supportive care alone. The current management of VOD is limited to supportive care, such as diuretics, analgesia, haemodialysis and mechanical ventilation.

About the product

Defibrotide is a sterile, aqueous, concentrate for solution for infusion in clear 2.5 ml glass vials. Defibrotide is a mixture of oligonucleotides obtained from porcine intestinal mucosa and prepared by controlled depolymerisation of deoxyribonucleic acid (DNA). Defibrotide had been approved for thrombotic vascular disease indications in Italy, as both ampoules for injection and oral capsules.

To date, the applicant's development program of defibrotide in the treatment of VOD consists of three studies of defibrotide (Protocol 2005-01 (pivotal), Protocol 99-118 (dose-finding), and Protocol 2006-05, treatment-IND) in patients with severe hepatic VOD that are included in the efficacy database, conducted in the United States, Canada, and Israel. For the prevention indication of hepatic VOD, the study Eudra-CT 2004-000592-33 was performed in Europe and Israel.

Compassionate use programs for the treatment of non-severe and severe VOD were established by the applicant. During the late stage of development, a treatment IND protocol (expanded access program) was initiated in the US to provide defibrotide to patients.

On 21 January 2008, the applicant requested protocol assistance for their product defibrotide pursuant to Article 6(1) of Regulation (EC) No 141/2000. The general subject of the protocol assistance was a planned phase III study for the prevention of VOD in adults and children, which, however, was never initiated.

2.2. Quality aspects

2.2.1. Introduction

Defibrotide (DF) is a polydisperse collection of the sodium salt of predominantly single-stranded oligodeoxyribonucleotides obtained from porcine intestinal mucosa and prepared by controlled depolymerisation of deoxyribonucleic acid (DNA).

The finished product is presented as a sterile, aqueous, concentrate for solution for infusion. The container closure system for Defitelio consists of 2.5 ml type I, neutral, clear glass vials closed with a rubber stopper and an aluminium cap.

2.2.2. Active Substance

Defibrotide is a polydisperse mixture of oligonucleotides produced by random, chemical cleavage (depolymerisation) of porcine DNA. It is predominantly single stranded, of varying base sequence, lengths and conformations; unfolded, folded or combined. The mean oligonucleotide length is 50 bases with a mean molecular weight of 17 ± 4 kDa. No individually defined component is at more than femtomolar concentration. The only meaningful scientific information that can be obtained about the biochemical nature of defibrotide (aside from determination of percentage of each nucleobase) is a measurement of its average length and its average percentage double stranded character. Therefore, it can be established that this active substance is of highly heterogenic nature.

Manufacture

The requirements on the starting material, pigs as well as the organ and tissue collection are well described. The porcine mucosa for defibrotide is provided from three Italian abattoirs used in production of meat for food. The process is detailed adequately. Details on the audit system in place are provided and it is confirmed that all stages of sourcing are subjected to a suitable Quality Management System.

The manufacturing process is overall, well described. The in-process control (IPC) tests are not extensive but the strategy has been justified by the applicant.

Process Validation

Results from three process validation batches were submitted. These are representative of the proposed current commercial process and the results shows consistency across the lots for the parameters and controls measured.

The applicant uses pro-fibrinolytic assays and a cell based viability assay to demonstrate the activity of defibrotide. The CHMP conclusions from the results of these assays is that any *in vitro* activity in both fibrinolysis or endothelial cell protection is weak, and might even be seen as negligible when compared to negative controls.

The claimed cell-protection activities of defibrotide have not been sufficiently demonstrated. Further work to investigate and confirm these properties is needed.

Nonetheless, these are the only activities of defibrotide that could be shown by the applicant. Based on the measure of these biological activities on defibrotide produced for clinical trials, process validation lots, lots produced to support manufacturing process change and stability – there are no discernible differences in activity when samples are compared to appropriate defibrotide reference standards and proposed commercial lots.

Characterisation

The applicant has used a wide panel of methods to try to characterise defibrotide. In view of there being no identifiable discrete moieties, the applicant has characterised the 'bulk' properties. The applicant has shown that it is impossible to separate defibrotide constituents into any meaningful components. Component nucleic base analysis has been undertaken and demonstrates that the results are in line with that expected for DNA from other animal sources.

Impurities

The information presented regarding potential impurities/degradation products is considered sufficient, and the specification limits for impurities/degradation products and residual solvents are considered acceptable. The control strategy for impurities is adequately justified.

Specification

Overall, the assays used for testing in the active substance specification are described and shown to be validated. The validation of the proposed cell protection test has not been adequately demonstrated and completed. Furthermore, if the results support it, the assay should be introduced as a routine quality control test for batch release and stability testing for both defibrotide active substance and finished product.

Stability

Based on the data provided a shelf-life of 36 months for the active substance when stored at 15-30°C is considered acceptable.

2.2.3. Finished Medicinal Product

Defitelio finished product is a sterile, concentrate for solution for infusion containing 80 mg of defibrotide per mL in clear glass type I vials sealed with a rubber stopper and blue aluminium flip off seal.

The excipients of the finished product solution are water for injections (WFI) as solvent, sodium citrate and sodium hydroxide, hydrochloric acid. All excipients comply with Ph. Eur. requirements.

Pharmaceutical Development

The finished product is supplied in single use glass vial of 2.5 mL sealed with a rubber stopper.

Compatibility studies have demonstrated that the finished product is stable when diluted with 0.9% Sodium Chloride Injection and 5% Dextrose in infusion bag and held in sterile and disposable syringes for up to 72 hours (at 15°C -25°C). However, from a microbiological point of view the product should be used immediately as indicated in the SmPC.

Adventitious agents

Virus safety

It can be concluded from the viral safety evaluation, that the three viral clearance steps tested in the studies were robust and effective at clearing virus during the manufacturing process.

Manufacture of the product

The finished product manufacturing steps are adequately described. The manufacturing process can be regarded as a standard process. The in-process controls (IPC) are described and deemed suitable for controlling and monitoring the manufacturing process.

Process Validation

The applicant presents results from three process validation lots and gives IPC and batch analytical data. The clinical studies were conducted using material produced before some changes were made during development and thus not in line with proposed commercial product.

The applicant uses pro-fibrinolytic assays and a cell based viability assay to demonstrate the comparability of defibrotide active substance/product over the years. Though the applicant has provided batch analysis data for the majority of the batches and these show comparability for the parameters measured, due to deficiencies relating to the appropriate characterisation of defibrotide, it is not possible to ascertain whether the changes will impact on relevance of commercial product with respect to clinical trials material and this was raised as a major objection.

Without the ability to measure and compare more significant *in vitro* defibrotide biological activity, which is not likely to be forthcoming for this active substance, then it can be concluded that developmental changes over the years and over stability have not impacted the measured activity attributes. Thus, there appear to be no changes occurring that would affect activity of defibrotide and this might be due to there being negligible ability to detect any changes on account of activity being so close to negative controls. In conclusion, the issue raised relating to the characterisation of defibrotide and comparability is cleared from a quality perspective as no further quality input can be expected to provide useful information.

Appropriately designed media fill studies have been conducted and showed that the results were acceptable. The container-closure has been shown to be resistant to microbial intrusion. All validation batches complied with the established in-process and release specifications as well as additional process monitoring data.

Product specification

Overall, the applicant has detailed the methods, equipment and parameters for the analytical tests. Where appropriate, the tests undertaken to confirm equipment calibration and system suitability have been stated. Methods have been validated.

Stability of the product

Based on the stability data provided the requested shelf-life (24 months, no specified storage condition) is considered acceptable. All studied parameters remained within the specification limits during the stability studies.

For the in-use stability after first opening and dilution the applicant claims that defibrotide is chemically and physically stable at concentration range of 4 mg/mL to 20 mg/mL in sodium chloride 9 mg/mL (0.9%) solution for infusion or 5% glucose solution for infusion at 15-25ºC for 72 hours. However, from a microbiological point of view the product should be used immediately as indicated in the SmPC. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be expected to exceed 24 hours at 2-8°C unless dilution has taken place in controlled and validated aseptic conditions.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

For this polydisperse and difficult to characterise product, a good manufacturing process control strategy plus the use of a discriminatory bioassay capable for showing batch to batch consistency is essential to ensure that commercial product remains representative to material used and qualified in clinical trials.

The applicant has characterised defibrotide with several related profibrinolytic activity assays. The applicant also uses a cell protection assay to determine cell-protective activity *in vitro*. From a quality perspective, the activity of defibrotide in the profibrinolytic and endothelial cell protection assay is weak and possibly confounded by the signal:noise ratio.

The claimed cell-protection activities of defibrotide have not been sufficiently demonstrated. Further work to investigate and confirm these properties is needed. If the results support it, the cell-protection assay should also be included in the finished product tests as well as the finished product testing strategy.

No clear clinical correlation to quality attributes has been demonstrated. In addition to the biological assays described, much reliance will have to be placed on physicochemical tests. The above issues could lead to significant problems in confirming the quality of defibrotide over the lifecycle of this polydisperse and heterogeneous product.

The benefit-risk assessment for authorisation of this product should take into account the limitations listed above.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The manufacturing process is overall, well described. The in-process control (IPC) tests are described and deemed suitable for controlling and monitoring the manufacturing process.

Nevertheless, due to its intrinsic highly heterogenic nature, defibrotide is difficult to characterise using physicochemical tests, and together with the inability to show strong biological activity in an *in vitro* assay, the quality, consistency and control of defibrotide rests significantly on robust control over the manufacturing process. In view of the limitations in defining defibrotide from a quality perspective, and as no clear clinical correlation to quality attributes has been established, this could lead to significant problems in confirming the quality of the product throughout the lifecycle of this biologically sourced and polydisperse product.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended further points for investigation.

2.3. Non-clinical aspects

2.3.1. Introduction

Defibrotide is described as a polydisperse collection of the sodium salt of predominantly single-stranded oligodeoxyribonucleotides derived from porcine intestinal DNA. The mean oligonucleotide length is 50 bases with a mean molecular weight of 17 ± 4 kDa, and at least 40% being >12 kDa. The structural formula is representative only of the composition as the oligonucleotides have varying base sequences, length and conformations; unfolded, folded or combined.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The applicant has presented a review of the pharmacological properties through a mixture of literature articles and non-clinical study data, in order to demonstrate and explain the complex mechanism of action for defibrotide. Three aspects were identified in this review to describe the activity of defibrotide (profibrinolytic/antithrombotic activity, its protection of endothelial cells from injury and its anti-inflammatory ability).

In vitro studies examining the activity of plasmin, which plays an important role in fibrinolysis, have shown that defibrotide can increase its activity and can in turn increase fibrinolytic activity. There was noted that the defibrotide complex composition plays a part in its ability to bind to plasmin, however this has not been confirmed by the applicant. In vitro studies on HMECs and HUVECs have shown that Endothelial Cells (EC) exposed to lipopolysaccharide (LPS), induce increased t-PA and PAI-1 expression, and this mechanism of activation of endothelial procoagulant and fibrinolytic response may be related to transplant complications such as VOD. Defibrotide was able to inhibit LPS-induced PAI-1 expression, and further increased t-PA activity, causing an increase in fibrinolytic activity.

Damage to EC can be caused by an immunosuppressant such as F-Ara via up-regulation of ICAM-1 molecules. Defibrotide is shown to down-regulate ICAM-1 and MHC class II antigen expression on the endothelium as well as a number of other genes, most notably caspase-3, an important pro-apoptotic protein. Defibrotide has also been shown to protect against TNF-α induced cytotoxicity in cultured bovine pulmonary artery endothelial cells but had no effect in protecting L929 mouse tumour cells. The protective action seems to be limited to ECs only.

The third mechanism of defibrotide activity was its anti-inflammatory action. A key part of the inflammatory process appears to be the translocation of leukocytes from the circulation to extravascular compartments.

However, a clear mechanism of action of defibrotide was not convincingly established in the view of the CHMP.

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were conducted by the applicant. A review of the relevant literature has been assessed. This review indicates a possible secondary pharmacodynamic activity of defibrotide of anti-angiogenesis. Angiogenesis is a multi-step process involving the formation of

new blood vessels from pre-existing vasculature and is necessary for primary tumour growth, invasiveness and development of metastasis. It is thought that defibrotide with its endothelium protective properties would stabilise or normalise the endothelium and thus prevent proliferation and/or sprouting of endothelial cells into the malignant tissue. *In vitro* and *in vivo* studies have shown an anti-angiogenic potential of defibrotide (*Koehl 2007, Gottfried 2007*). Other studies have shown that defibrotide may enhance *in vivo* chemosensitivity of multiple myeloma and mammary carcinoma xenografts in animal models. The proposed mechanism is that defibrotide favourably modulates antitumour interactions between bone marrow stromal cells and endothelia in the tumour microenvironment. However, the CHMP concluded that this is not fully demonstrated.

Safety pharmacology programme

Central nervous system(CNS)

CNS effects were studied as part of a 13 week i.v. infusion toxicity study in rats. The study included a Functional Observational Battery (FOB). The rats were given defibrotide at 240, 1200 or 4800 mg/kg/day (multiple of human equivalent dose [HED] is 1.5, 7.7 and 31, respectively - human dose is four 2 hour infusion daily at dose of 6.25 mg/kg [≈25 mg/kg/day]) by continuous i.v.infusion for 9 weeks. There were no test article-related changes in FOB over the course of the study. Some test-article related effects were observed which were not considered to be adverse. Studies performed in rats in the 1970s showed no effect on body temperature, no modification of pentobarbital sleeping time and no antagonism on pentrazole convulsions.

Cardiovascular system (CV)

Defibrotide did not reduce hERG tail current at concentrations up to 500 µg/ml. ECG examinations were performed as part of a 13 week intravenous infusion toxicity study in dogs. Dogs were given defibrotide at 60, 300 or 1600 mg/kg/day (multiple of HED is 1.3, 6.6 and 35, respectively) by 2-hour iv infusions for 13 weeks. There were no effects of defibrotide on quantitative ECG parameters.

Respiratory system

No dedicated study to examine the respiratory effects of defibrotide has been performed. No effects on respiratory functions were observed in 13 week toxicology studies in dogs or rats, and no effect on respiratory function was observed in a QTc study in healthy volunteers.

Pharmacodynamic drug interactions

Defibrotide did not impair the in vitro antitumor effect of F-Ara (*Eissner 2002*) or thalidomide. In a mouse xenograft multiple myeloma model, defibrotide did not blunt the antitumour effect of several cytotoxic chemotherapy agents, such as dexamethasone or the proteosome inhibitor bortezomib (*Mitsiades 2009*).

Since defibrotide has been considered a potential therapeutic agent for use in thrombosis, several studies have been conducted to evaluate its concomitant administration with other antithrombotic drugs. (heparin, molsidomin, acetylsalicylic acid (ASA), Daltroban and the fibrinolytic inhibitor tranexamic acid). In these studies, defibrotide generally enhanced the anticoagulant activity of heparin but did not affect the activity of other antithrombotic drugs.

2.3.3. Pharmacokinetics

Defibrotide is a polydisperse collection of predominantly single-stranded oligodeoxyribonucleotides, as a result, the product is rapidly degraded *in vivo* and posed problems in detecting in ADME studies. Single dose studies were performed in mice, rats and rabbits to assess the pharmacokinetics of defibrotide. Systemic exposure to defibrotide was determined in toxicokinetic studies as part of repeat-dose toxicity studies in rats and dogs.

Methods of Analysis

The older studies conducted with defibrotide and which pre-date the implementation of GLP standards, used a colorimetric assay of the 2'-deoxyribose carbohydrate moiety or measured ^{125}I after administration of labelled defibrotide, to detect sample levels of defibrotide. These methods both lack the ability to distinguish between intact defibrotide and degraded defibrotide. Studies on distribution, metabolism and excretion were performed using ^{125}I-labelled defibrotide. In recent studies a HPLC-UV method, determining intact defibrotide, was used.

Absorption

Defibrotide is intended for intravenous infusion and therefore no specific studies have been performed to study absorption of the product.

Rats were given defibrotide by continuous iv infusion for 9 weeks. Dogs were given defibrotide by i.v. infusion of 2 h duration every 6 hours 4 times daily for 13 weeks. No evidence for accumulation of defibrotide in plasma was observed in either species after multiple dosing, nor was this seen in the extended length studies for rats and dogs. There were short plasma half-lives in all the three species, ranging from 11 to 16 min. This was not seen in juvenile rats, where the half-life was substantially increased to 33 to 51 min.

Distribution

Tissue distribution was measured as total radioactivity and after single i.v. and oral administration of ^{125}I-defibrotide to albino and pigmented rats. Radio-labelled defibrotide showed preferential distribution into highly perfused tissues: kidneys, liver, bone marrow, spleen and small intestine with lower levels in rat testes and low levels to the brain and to fat. There was higher distribution to pigmented skin that may indicate a tendency to bind to melanin. Protein binding assays showed that defibrotide does not bind to plasma proteins.

Metabolism

Limited data on the metabolism of defibrotide have been presented, and it is stated that defibrotide is rapidly degraded to 6-deoxyribose and purine and pyrimidine bases following administration. One *in vitro* metabolism study using human hepatocytes from 5 month, 2 year old and adult donors did not reveal any detectable metabolite products of defibrotide. The extent of data on metabolism is acceptable for this kind of substance. No appreciable *in vitro* metabolism of defibrotide by hepatocytes was observed.

The in vivo metabolism in rabbits was assessed by plasma analysis following defibrotide administration by iv infusion for 2h, 4 times over a period of 24 h at 320 mg/kg/day. The *in vivo*

study demonstrated the occurrence of nucleotides and nucleosides with free bases, resulting from the expected enzymatic degradation of the single-stranded DNA.

Elimination

Defibrotide is likely to be rapidly degraded to its constituent moieties following administration. Excretion in rats is mainly via urine and was very rapid. The majority of administered radioactive defibrotide was excreted by 24 hours post-dosing.

Pharmacokinetic Drug Interactions

One in vitro study in human liver microsomes has been conducted to assess the potential of defibrotide to inhibit common cytochrome P450 enzymes and no inhibitory effect was observed. The lack of in vivo interaction studies is acceptable for this type of substance.

2.3.4. Toxicology

Single dose toxicity

The legacy studies with defibrotide utilise single oral (to mice and rats), i.v. (to mice, rats, rabbits and dogs) or i.p. (to mice, rats and dogs) administrations. When using i.v. bolus, ≤1000 mg/kg dose levels of defibrotide are used. These doses are lower than the doses used in repeat-dose toxicity studies and so their applicability to the safety review for defibrotide is limited.

In a dose range finding study in rats, the dose of 4800mg/kg/day was administered and tolerated for 7 days. This dose was considered the Maximum Tolerated Dose (MTD), even though adverse effects due to defibrotide were not seen. The use of dose levels higher than 5 g/day was generally considered as unethical and the need to run a single dose study was considered as not necessary.

Repeat dose toxicity

A number of studies have been conducted in rats (7 days, 4 weeks and 13 weeks: reduced to 9 weeks), rabbits (21 days: reduced to 17 days) and dogs (7 days, 13 weeks and 26 weeks). There were no apparent treatment related effects for weight gain, food intake or for clinical signs. Animals were dosed intravenously at doses ranging from 80 to 4800 mg/kg/day (rats, equivalent multiple of HED up to 31); 900 mg/kg (rabbits dosed by slow i.v. infusions of 6 hours for 21 days, equivalent multiple of HED of 11); and 15 to 1600 mg/kg/day (dogs, equivalent multiple of HED up to 35).

Of the 8 studies three pre-date implementation of GLP standards: 4 weeks in rats and 13/26 weeks in dogs.

In rats, the 13-week continuous infusion study was however interrupted after 9 weeks due to high mortality. The mortality was mostly related to complications resulting from the infusion procedure. Although mortality was higher in defibrotide treated animals, no firm conclusions on the toxicological properties of defibrotide can be drawn. Liver and kidney were identified as potential target organs. The findings may however to a large extent be related to indirect effects from the infusion procedure, most importantly infections.

In dogs, a 7 day study with 2h infusion four times daily did not reveal any toxicity. Haematological tests in the 13 week study showed decreased erythrocytes, haemoglobin, and haematocrit, and prolonged APTT (activated partial thromboplastin time) and PT (prothrombin time), as seen in the rat study. As was seen in the rat, there was mild Kupffer cell hypertrophy (male and female) –

vacuolated macrophages, and minimal sub-acute inflammation (males). The Kupffer cell related effects are likely due to uptake of defibrotide. There were no adverse effects on body weight, or food intake, urinalysis or clinical chemistry apart from a dose-dependent increase in alanine aminotransferase and the discussed haematological changes. No NOEL could be determined for female dogs, though for male dogs this was 60 mg/kg/day.

TK (toxicokinetic) parameters showed that there was increased exposure to defibrotide with increasing dose, and no difference between gender.

Genotoxicity

In vitro

A GLP compliant study was performed to determine the potential of defibrotide to induce mutations in the required 5 bacterial strains of Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 and the tryptophan operon of Escherichia coli strain WP2 uvrA. The results of the bacterial reverse mutation test with defibrotide, with or without exogenous rat liver S9 fraction, used to induce metabolic activation, demonstrated no dose related and reproducible increases in revertant colony numbers at any dose or in any of the bacterial strains tested.

In vivo

An *in vivo* mouse micronucleus test (GLP-compliant) was performed. The applicant used groups of 5 male and 5 female Swiss Crl: CD1(ICR) mice were treated i.p. on two successive days with defibrotide at doses of 250, 833 and 2500 mg/kg. No significant increase in the incidence of micronucleated PCEs (polychromatic erythrocytes) were found in either males or females at all doses, compared with vehicle treated animals.

Carcinogenicity

Two studies in mice and rats were conducted to review the potential long-term/carcinogenicity effects of defibrotide. The studies were performed to GLP standards and used the oral route of administration. Defibrotide was administered to groups of 50 males and 50 females at doses of 100, 400 and 2000 mg/kg/day to both mice and rats. As this is not the clinical method of administration for the product and the relative defibrotide exposure would be substantially less, there are very limited conclusions to be drawn from these studies. There was no evidence of a carcinogenic potential from these studies.

Reproduction Toxicity

Studies on fertility and general reproductive performance, embryo-fetal development, and peri- and postnatal development were performed in 1975-1976 to support the original anti-thrombosis indication. These studies were performed with i.v. bolus or i.m. (intramuscular) injection.

To support the current indication, studies were performed on embryo-fetal development with i.v. infusion and at higher doses than in previous studies. Pregnant rats were administered with 240, 1200 and 4800 mg/kg/day by continuous infusion over 24 h. Due to an excessive toxicity observed at these dosages, 3 additional groups of 8 mated females were dosed at 60, 120 and 240 mg/kg/day by 2 h infusion followed by 4 h interval 4 times a day. Pregnant rabbits were given 30, 60 and 120 mg/kg/day from gestation Day 6 to Day 18 by 2 h infusion followed by 4 h wash-out periods, 4 times a day. Defibrotide was associated with strong maternal toxicity in both rats and

rabbits when given by infusion. Therefore, effects on embryo-fetal development could not be evaluated. The applicant has stated that the results from 13 week repeat dose studies in the rat and dog at doses of 240, 1200, and 4800 mg/kg/day and 60, 300 and 1600 mg/kg/day, respectively revealed no observed effect on sexual organs for either sex or species.

Local Tolerance

Local tolerance was assessed as part of repeat dose toxicity studies in rats, dogs and rabbits. In infusion studies in rats and rabbits there were reactions at the injection site. These were seen both in control and treated animals and were considered related to the infusion procedure rather than to defibrotide. Local tolerance in patients was reported to be generally good so the relevance of these animal findings is limited.

Other toxicity studies

The antigenicity and the potential of defibrotide to elicit anti-drug antibodies was assessed by testing rat and dog plasma samples generated in the repeat dose 13-week toxicity studies. The applicant has also summarised three further studies conducted in their earlier development package that can also be regarded as supportive.

2.3.5. Ecotoxicity / environmental risk assessment

The applicant has not performed an Environmental Risk Assessment (ERA) in accordance with the "Guideline on the Environmental Risk Assessment of the medicinal products for human use" (EMEA/CHMP/SWP/4447/00)". Defibrotide is argued to be a naturally derived substance and is a characterised polydisperse collection of the sodium salt of predominantly single-stranded oligodeoxyribonucleotides. The substance, similarly to vitamins, electrolytes, amino acids, peptides, proteins, carbohydrates and lipids, is exempted from ERA requirements in the EU Guideline because these substances are unlikely to result in significant risk to the environment. Further discussion on the metabolic fate of defibrotide suggests that the metabolic products are already ubiquitous in the environment.

The drug substance, defibrotide, is designated an orphan drug status by the European Medicines Agency indicated for hepatic veno-occlusive disease (VOD). VOD is almost exclusively associated with the conditioning regimens prior to stem cell transplantation (SCT), with 5 – 60% of recipients going on to develop VOD. The prevalence of SCT per 10000 in the EU was 0.449 and 0.177 for allogeneic SCT, i.e those at highest risk of developing VOD (*Gratwhol 2010*). These prevalence figures have been used to estimate potential environmental exposure in a Phase I ERA.

Assuming 100% market penetration, maximum daily administration of 25 mg/kg of defibrotide to 60 kg, the predicted environmental concentration in surface water (PEC surfacewater) is calculated as 0.03 µg/L for total SCT and 0.01 µg/L for allogeneic SCT (see Table 5). When assuming 60% market penetration (based on maximum number patients undergoing SCT and likely to develop VOD), PEC surfacewater is estimated as 0.02 µg/L for total SCT and 0.007 µg/L for allogeneic SCT. Therefore, due to the medicinal product`s naturally occurring components and the controlled use of defibrotide in transplant units, the risk posed to the environment is negligible and further Phase II studies were not required.

Table 5 Summary of main ERA study results

Substance (INN/Invented Name):			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD 107	≤ - 4.6	Potential PBT (Ɏ/N)
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$	0.03 (total SCT) 0.01 (allogeneic SCT)	µg/L	See assessor's comments
Other concerns (e.g. chemical class)			None

2.3.6. Discussion on non-clinical aspects

A clear mechanism of action has not been convincingly established. No secondary pharmacodynamic studies were conducted by the applicant. Safety Pharmacology studies did not highlight any potential concern. Pharmacodynamic drug interaction studies highlighted a potential to enhance antithrombotic activity of heparin.

The pharmacokinetics of defibrotide was studied *in vivo* in rats, mice, rabbits and dogs and also by *in vitro* cell models. These studies indicate that defibrotide is limited in accumulation and is rapidly degraded by nucleases as would be expected for single-stranded DNA. Elimination is rapid mainly via urine and defibrotide exhibited no inhibitory effects to a range of common cytochrome P450 enzymes.

To support the current application, single-dose and repeat-dose toxicity studies were performed in rats and dogs with intravenous infusion.

In repeat-dose toxicity studies performed by the applicant in rats no clear evidence for toxicity was observed. In dogs, there was an increase in liver weight and some microscopic findings (minimal to mild Kupffer cell hypertrophy, minimal subacute inflammation, mild individual hepatocellular necrosis) with uncertain toxicological significance but probably caused by cellular uptake of defibrotide. Defibrotide was not genotoxic based on a study on gene mutations in bacteria and a micronucleus test in mice. Defibrotide has previously been shown not to be carcinogenic in oral studies in rats and mice.

To support the current indication, studies were performed on embryofoetal development with i.v. infusion in rats and rabbits. Defibrotide was associated with strong maternal toxicity with high mortality in both rats and rabbits when given by infusion, and there were no or very few foetuses. These studies provided very limited information about defibrotide effects on embryo-foetal development as they could not be evaluated; the studies performed were also non GLP-compliant.

Studies were performed in the 1970's to evaluate effects on immune functions. Mice were treated orally or i.p. daily (5 days a week) for 4 weeks and a number of functional assays were performed (NK cell activity, anti-SRBC antibody response, macrophage cytotoxicity, lymphocyte proliferation). No clear effect on immune functions was observed.

The substance, similarly to vitamins, electrolytes, amino acids, peptides, proteins, carbohydrates and lipids, are unlikely to result in significant risk to the environment.

Conclusion on non-clinical aspects

The applicant provided a non-clinical program consisting of studies using intravenous infusion as route of administration, together with bibliographic data.

Based on the provided data, the CHMP concluded that the pharmacological rationale for the use of defibrotide has not been established; therefore, no extrapolations to clinical data can be made based on "pharmacological plausibility". The pharmacokinetic and toxicological aspects of the non-clinical dossier are limited in their relevance to this application and a number of the older studies presented pre-date GLP.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The applicant states that all clinical studies assessing the efficacy and safety of defibrotide were conducted according to GCP and in accord with local and international regulations. Clinical trials carried out outside the European Union are stated to have met the ethical requirements of Directive 2001/20/EC.

In view of the small data set and the open-label nature of the two pivotal trials, a GCP inspection was recommended by CHMP at D180.

The results of the relevant inspection are detailed below in the discussion section in each of the inspected studies.

- Tabular overview of clinical studies

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
PK	Study IRI-151612	5.3.3.1	PK in humans PO and IV	Open	DF 400mg IV DF 800mg PO	3 (IV) 3 (PO)	Healthy adults	Single dose	Completed, full report
PK	Study DFPK88	5.3.3.1	PK in humans PO and IV	Crossover, controlled	DF 800mg IV DF 1600mg PO Deoxyribose 600mg PO	24 (IV, PO)	Healthy adults	Single dose	Completed; full report
PK	Study DFPK 99-118	5.3.3.2	PK in humans	Prospective, open, randomized, uncontrolled Dose finding study	DF IV 10 mg/kg/day (Day 1) followed by DF IV 25mg/kg/day or DF 40mg/kg/day IV. Q6H dosing each over 2 hour infusion	11 total subjects: 5 (25 mg/kg/day) and 6 (40 mg/kg/day)	Adult patients post HSCT with severe veno-occlusive disease (with multi-organ failure)	PK samples drawn on days 1, 2, and 7.	Completed; Full report
PK	Study DFPK91	5.3.3.2	PK in humans PO and IV	Open	DF 223mg IV DF 423mg PO	2 (IV) 2 (PO)	Adult patients with neoplasms	Single dose	Completed; full report
PD	Study HL-12326	5.3.4.1	DF IV pharmacodynamics in single, rising doses	Double-blind, randomized, placebo controlled	DF 200mg IV BID or placebo DF 400mg IV BID or placebo DF 800mg IV BID or placebo	27	Healthy adults	3 days	Completed; full report
PK/PD	Study R09-1425	5.3.4.1	ECG effects of Defibrotide using a clinical and a supratherapeutic dose compared to placebo and moxifloxacin	Double-blind randomized crossover	DF 6.25mg/kg IV single dose DF 15mg/kg IV single dose	52	Healthy adults	Single dose	Completed; full report

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
PD	Study CS001/03	5.3.4.2	DF effect on parameters of blood coagulation, prostaglandin metabolism, claudication distance and platelet activity	Single blind, randomized, placebo-controlled	Day 1: DF 400mg bolus IV + 800mg IV over 2 hours. Day 2-4: 400mg IM BID (or placebo)	24	Adult patients with chronic POAD	5 days	Completed, full report
Safety and Efficacy	Study 2005-01	5.3.5.1	Safety and efficacy of DF vs historical control in the treatment of severe VOD	Prospective treatment arm vs historical control	DF 25mg/kg/day IV (in 4 divided doses, each over 2 hours)	102 DF arm vs 32 historic control	Adult and paediatric patients post HSCT with severe VOD (VOD+MOF)	Median 22 days	Completed; full report
Safety and Efficacy	Study EudraCT:2004-000592-33	5.3.5.1	DF vs control in the prevention of hepatic VOD	Prospective, open, randomized, controlled	DF 25mg/kg/day IV (in 4 divided doses, each over 2 hours) or best supportive care	180 DF arm vs 176 control	Paediatric patients undergoing myeloablative HSCT	Median duration: 35 days (from start of conditioning until 30 days post transplant)	Completed; full report
Safety and Efficacy	Study 99-118 Dose-finding	5.3.5.2	Safety and efficacy, dose-finding 25mg/kg/day or 40mg/kg/day in treatment of severe VOD	Prospective, open, randomized, uncontrolled	DF IV 10 mg/kg/day (day 1) followed by DF 25mg/kg/day IV or DF 40mg/kg/d IV . Regimen for all patients: in 4 divided doses, each over 2 hours	149 total subjects: 75 DF 25mg/kg/day vs 74 DF 40mg/kg/day	Adult and paediatric patients post HSCT with severe VOD (VOD+MOF)	Median 14 days	Completed; full report

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Safety and Efficacy	Study 2006-05	5.3.5.4	Safety and efficacy in patients with severe VOD	Open, uncontrolled (treatment IND Study)	DF 25mg/kg/day IV (in 4 divided doses, each over 2 hours)	224 enrolled; 183 in safety analysis; 104 in efficacy analysis (ongoing)	Adult and pediatric patients post HSCT with severe VOD (VOD+MOF)	Median 20 days	Ongoing; abbreviated report of interim analysis
Safety and Efficacy	Study DF-CUP	5.3.5.4	Safety and efficacy in patients with VOD	Open, uncontrolled	10-80mg/kg/day (median 25mg/kg/d)	711	Adult and pediatric patients post HSCT or post-chemotherapy with VOD	Median 14 days	Completed; abbreviated report
Safety	Study DF-VOD	5.3.5.4	Safety and efficacy in patients with VOD	Prospective, open, randomized, controlled	40mg/kg/day	68	Adult and pediatric patients post HSCT with VOD	Mean 11 days	Closed; abbreviated report.

DF = Defibrotide
IM = intramuscular
IV = intravenous
PO = per os (oral)
POAD= Peripheral Occlusive Arterial Disease
VOD = Veno-occlusive disease

2.4.2. Pharmacokinetics

The main PK study was study R09-1425. This was a double-blind randomized 4-way crossover trial to define PK and the ECG effects of defibrotide using a clinical and a supratherapeutic dose compared to placebo and moxifloxacin (a positive control) in healthy adults. Study RO9-1425 was conducted between May-June 2010. It should be noted that only a single batch of defibrotide was used for this study.

The study included 52 healthy male and female subjects who received each of four treatment regimens separated by a three day washout period. The four treatments were:

(A) therapeutic doses of 6.25 mg/kg defibrotide delivered in a single 2-hour IV infusion,

(B) supratherapeutic doses of 15 mg/kg defibrotide delivered in a single 2-hour IV infusion,
(C) placebo dose of 5% dextrose water and
(D) moxifloxacin delivered in a single oral 400 mg tablet.

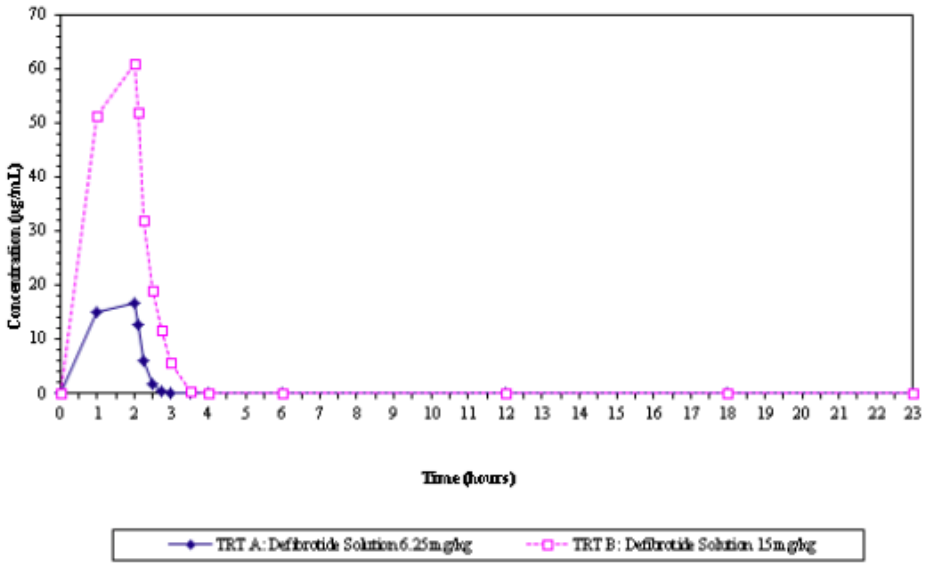
Table 6 Defibrotide Pharmacokinetics: R09-1425

Defibrotide Dose	AUC_{0-inf}/ (µg-hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	Kel (1/hr)	$T_{1/2}$ (hr)	V_D (mL)	CL (L/hr)
6.25 mg/kg (therapeutic dose)	48.14 (± 6.49)	17.27 ±3.83	1.78 ± 0.42	1.2484 ± 0.66	0.71 ± 0.35	9934.07 ±3806.87	10.35 ± 1.77
15 mg/kg (supratherapeutic dose)	113.59 (± 23.54)	60.96 ±11.83	2.00 ±0.01	1.7533 ±0.62	0.45 ±0.17	6188.45 ±2262.34	9.76 ±1.65

For both defibrotide doses, Tmax was at approximately the end of each infusion. Cmax and AUC values were approximately dose related. Terminal half-lives (T1/2) were calculated at 0.71 hours and 0.45 hours for the therapeutic and supratherapeutic doses, respectively.

The time course of defibrotide clearance for the two test doses (Figure 1) illustrates the rapid clearance after the 2-hour infusion is completed. Given this evidence of very rapid clearance of defibrotide, it is anticipated that the standard therapeutic regimen [four times a day (QID) dosing for several consecutive days] would not result in any accumulation in healthy controls with normal renal function.

Figure 1 Time course of Defibrotide Plasma Concentrations During and After Infusion: R09-1425



Distribution

The volume of distribution of 6.1 ±2 L at the high dose of 15mg/kg is consistent with the intravascular compartment. A volume of distribution of 9.9 ±3 L was noted at the lower and proposed therapeutic dose of 6.25mg/kg.

Elimination

The elimination of defibrotide has not been thoroughly elucidated; however, being an oligonucleotide mixture, detailed information was not requested. Defibrotide is likely rapidly degraded to its constituent moieties following administration. From study R09-1425 the urinary clearance of defibrotide was relatively rapid, with the majority of study drug (>98% of that excreted from the therapeutic dose administration and >97% from the supratherapeutic dose) detected in urine during the first collection period of 0-4 hours. Higher exposure and prolonged elimination can be expected in patients with renal impairment. In view of the trend for a dose-related toxicity in terms of increased bleeding events and the increased mortality in children receiving high dose defibrotide in study 99-118, the lack of PK in children and in those with renal impairment remains a major concern.

Limited data on the metabolism of defibrotide have been presented, and it is stated that defibrotide is rapidly degraded to 6-deoxyribose and purine and pyrimidine bases following administration. One *in vitro* metabolism study using human hepatocytes from 5 month, 2 year old and adult donors did not reveal any detectable metabolite products of defibrotide. The extent of data on metabolism is acceptable for this kind of substance.

Pharmacokinetics in the target population

Study DFPK 99-118 (substudy of Study 99-118) was performed in 11 adults with VOD. This was a substudy of the main dose-finding study for defibrotide.

The PK data from DFPK 99-118 cannot be considered to provide reliable information on the PK of defibrotide in adult VOD patients for the following reasons.

The PK was limited to 11 subjects; 5 in arm A and 6 in arm B in adults (18-46 years) only with severe VOD. No data on children or in special populations with VOD was provided. No data on urinary DF concentrations was collected. In addition the requirement for re-analysis of several samples raises doubts about the accuracy of the results. An additional concern with this study was that the samples were 3-10 years old before they were analysed by HPLC for defibrotide levels. From DFPK99-118 overall the number of samples contributing to each data point is between 4 and 5. Although the half-life and volume of distribution were broadly similar to the PK study in healthy adults, high CVs were noted. The exposure at the applied dose of 6.25 mg/kg was higher in VOD patients compared healthy volunteers and it cannot be excluded that the difference is larger, due to stability problems, e.g. if the concentrations in VOD patients are underestimated. The estimated half-lives were somewhat longer in VOD patients.

VOD, if severe, can be associated with multiorgan failure, including hepatorenal syndrome. Thus, the target population may have both impaired hepatic and renal function. No dose adjustment is considered necessary by the applicant for patients with hepatic or renal impairment but no pharmacokinetic data are available to support these recommendations.

Defibrotide is dosed based on weight but the effect of weight on PK parameters has not been assessed. From a pharmacokinetic point of view it is difficult to assess whether the weight-based dosage is adequate, e.g. in obese patients.

Data from healthy volunteers suggest no relevant gender difference in the pharmacokinetics of defibrotide.

In the paediatric population, there is no PK support for the safe use of defibrotide due to the lack of PK data. Hence, the applicant proposes that the posology in this group has to rely upon available clinical efficacy and safety data and that this also applies to elderly subjects.

Far more robust PK data is required for patients with VOD, for those with renal impairment and particularly PK in children is required. The statistically significant elevated mortality in children receiving the high dose of defibrotide in study 99-118 raises the need for PK data in children. Higher exposure is expected in renal impairment due to the renal excretion of defibrotide, and this combined with the observation of increased bleeding events being commoner in those on high dose defibrotide from the compassionate use programme (DF-CUP) further highlights the need for adequate PK data. In view of the lack of PK data to support the dose chosen together with the lack of clearly demonstrated efficacy, the absence of PK data in children and the lack of sufficient PK data in renal failure are considered serious deficiencies. This is particularly important considering the proposed indication which includes children and for those with severe VOD will also include subjects with renal impairment.

Dose proportionality and time dependencies

Only one dose level is recommended for Defitelio, i.e. 25 mg/kg/day divided in four doses per day. Some information about dose proportionality can be obtained from study R09-1425, in which doses of 6.25 and 15 mg/kg were administered. The increases in AUC_{0-t} and C_{max} were greater than dose proportional (3.5 and 3.9-fold, respectively), while AUC_{inf} increased proportional to the increase in dose. AUC_{inf} could not be accurately estimated in several individuals, however. Also in study 99-118 conducted in VOD patients, different dose levels were administered. These data indicate no major deviations from dose proportionality, although the data should be interpreted with caution due to the potential stability problems and small numbers of individuals in each group in study 99-118.

PK data from up to 7 days of treatment with defibrotide were available in the VOD study 99-118. These limited data indicate no obvious changes in the pharmacokinetics of defibrotide over a 7-day period. It can, however, be expected that the pharmacokinetics could change over time to some extent if the organ function and general status of the patient changes over time, due to improvement or worsening of the VOD condition.

In healthy volunteers, the variability in the pharmacokinetic parameters of defibrotide was low (CVs 15-20%). In VOD patients, the CVs were generally in the range 30-60% for Cmax and AUC values, although higher values were observed. The number of subjects in each dose group of VOD was very small, though. No data are presented on intra-individual variability.

Special populations

The number of subjects with pharmacokinetic data in the target population, VOD patients, is small (n=11) and no pharmacokinetic studies have been conducted with defibrotide in special populations.

Data from healthy volunteers suggest no relevant gender difference in the pharmacokinetics of defibrotide. In the paediatric population, there is no pharmacokinetic support for the safe use of defibrotide due to the lack of data. Hence, the proposed posology in this group has to rely upon available clinical efficacy and safety data. This also applies to elderly subjects.

Pharmacokinetic interaction studies

One in vitro study in human liver microsomes has been conducted to assess the potential of defibrotide to inhibit common cytochrome P450 enzymes and no inhibitory effect was observed. The lack of in vivo interaction studies is acceptable for this type of substance. The potency assays provided by the company included several profibrinolytic activity assays which measure profibrinolytic activity. Therefore pharmacodynamic interactions with other drugs with anticoagulant properties may occur.

2.4.3. Pharmacodynamics

Mechanism of action

Multiple proposed mechanisms (MoA) of action for defibrotide were claimed.

One of the MoA describes that defibrotide primarily targets endothelium, particularly of small vessels and appears to modulate endothelial cell injury. Clinical studies have described that defibrotide:

(a) protects endothelial cells against chemotherapy-induced cell death and activation;

(b) promotes fibrinolysis via up-regulation of Tissue Plasminogen Activator (t-PA) and Tissue Factor Pathway Inhibitor (TFPI), and increasing the plasmin activity without activating plasminogen to plasmin;

(c) reduces circulating levels of Plasminogen Activator Inhibitor-1 (PAI-1);

(d) decreases expression of cell surface adhesion molecules (e.g. P-selectin and intercellular adhesion molecule-1) on activated endothelial cells;

(e) down-regulates the gene expression, protein level and activity of heparanase, inflammatory cytokines and chemokines in activated endothelial cells but without modulating the gene expression profile of endothelial cells under normal conditions.

However the review of the literature data provided in support of the various proposed MoA for defibrotide were found not be of adequate quality as the methodology used were not described adequately and many reports had conflicting results. This was further complicated by the fact that the defibrotide used was not always the defibrotide that is the subject of this MAA, namely of porcine intestinal origin - the source of the defibrotide in many papers referred to defibrotide as of mammalian lung/bovine lung origin. This casts further doubt on the relevance of the literature for providing useful information on the PD effects of defibrotide.

Primary and Secondary pharmacology

Primary pharmacology

The applicant provided PD measurement in the dose-finding study in VOD patients (99-118) and

the PD marker measured was PAI-1. PAI-1 levels are known to be raised in VOD and the applicant's position is that the rise in PAI-1 is reduced with defibrotide treatment.

Pharmacodynamic Analyses from study 99-118

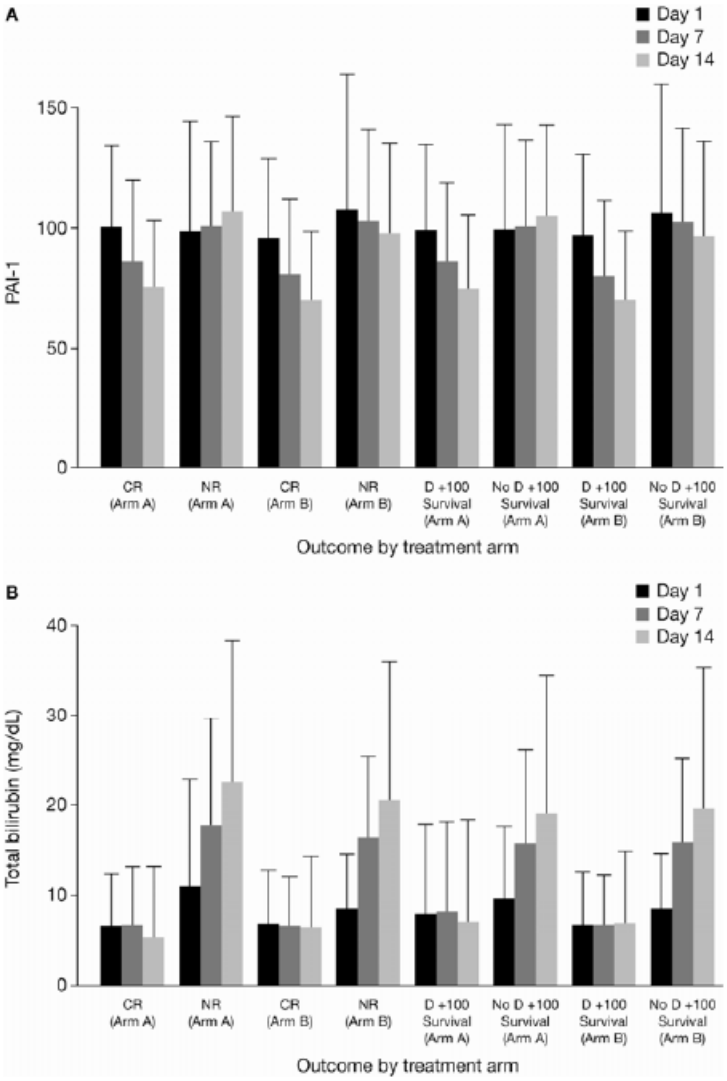
Study 99-118 was an open-label randomised phase II study to determine the effective dose and is the only dose-finding study provided by the applicant.

Study subjects were defined as those with severe VOD (defined as VOD with associated renal, pulmonary and/or CNS dysfunction) or at least a 30% risk for severe VOD based on the Bearman model following HSCT.

For both arms, the starting dose of defibrotide on Day 1 was 2.5 mg/kg every 6 hours (Q6H) for four doses (total dose 10 mg/kg), based on baseline weight. From Day 2, the defibrotide dose was increased to 6.25 mg/kg Q6H (total dose 25 mg/kg/day; Arm A), or 10 mg/kg Q6H (total dose 40 mg/kg/day; Arm B). There were 75 patients in Arm A and 74 patients in Arm B.

Laboratory tests (primarily of coagulation parameters) were serially obtained, starting at study entry and performed twice weekly while on defibrotide. Results of these analyses are considered exploratory and were not provided by the applicant. Both PAI-1 and total bilirubin levels were observed to change with defibrotide treatment (Figure 2). This figure demonstrates PAI-1 levels (A) and total bilirubin levels (B) in those patients with a complete response (CR) compared to patients who were non-responders, patients who survived by Day+100 post-SCT compared to those patients who did not survive, and by treatment arm. PAI-1 and other selected laboratory parameters were summarized at baseline, Day 7, and Day 14 of treatment; changes from baseline to Days 7 and 14 were reported.

Figure 2 **Mean (SD) PAI-1 (A) and total bilirubin (B) by treatment arm and outcome**



There were no statistically significant differences in mean PAI-1 levels by dose group, between patients with or without CR, or between patients alive or dead at Day+100. Mean PAI-1 levels at Days 7 and 14 were lower, although this result did not reach statistical significance when compared to baseline in patients with CR and those who were alive at Day+100, with a similar pattern observed in both treatment arms.

Secondary pharmacology

A QT study was conducted (R09-1425) by the applicant. The moxifloxacin positive control group showed the expected small change in QTc duration. The results of this ECG trial showed no signal of any effect on heart rate, AV conduction or cardiac depolarization as measured by the PR and QRS interval durations. The results from Study R09-1425 demonstrated that defibrotide had no effects on heart rate, PR and QRS interval duration or cardiac morphology.

Pharmacodynamic-pharmacokinetic analysis showed that defibrotide did not affect cardiac repolarization.

2.4.4. Discussion on clinical pharmacology

Only limited human pharmacokinetic data are available for defibrotide. The only reliable PK data is from study R09-1425 in healthy adults, and this was performed with a single batch of defibrotide. The PK data from DFPK 99-118 is very limited in terms of the number (less than 5 subjects contributed to most time points) and the samples were up to 10 years old but validated stability of the samples was only to 325 days, thereby further weakening the obtained results. As defibrotide is renally excreted, a higher exposure is expected in patients with renal failure. As there was a trend for increased bleeding events in the defibrotide compassionate use programmed (DF-CUP) for subjects receiving higher doses this remains an issue.

The absence of any PK data in children is not acceptable in view of the higher mortality for children in study 99-118 in the high dose arm. Pharmacokinetic data can thus not give support for dose recommendations in special populations, e.g. paediatrics and for those with renal impairment (which will be present in many cases of severe VOD).

Regarding secondary PD effects, a QT study was conducted, R09-1425. This study did not detect any effects on heart rate, AV conduction, cardiac depolarisation, or repolarisation, and no clinically relevant changes on heart function.

It can therefore be concluded that no clinical pharmacodynamic effects have been demonstrated for defibrotide.

2.4.5. Conclusions on clinical pharmacology

The PK data provided by the applicant are considered seriously deficient and do not support dose recommendations in special populations, e.g. paediatrics and those with renal impairment. PK data was provided for healthy volunteers with only a single batch of product. Data for PK in children and PK in renal failure are considered by the CHMP as important missing information. This is particularly significant considering the increased mortality in children receiving the high dose in study 99-118. In addition, as defibrotide is renally excreted, higher exposure in those with renal impairment is likely. These concerns are relevant in view of the proposed indication which will include children and renal impairment and constitutes an unresolved PK issue. No PD effects have been demonstrated for defibrotide.

2.5. Clinical efficacy

The core clinical study package for this application consists of the following studies:

* Dose-finding/dose-response 99-118
* Treatment of severe VOD:
  - 2005-01 pivotal study
  - 2006-05 interim analysis of an ongoing treatment-IND study
  - EudraCT 2004-000592-33 VOD treatment cohort

- 99-118

* Prophylactic prevention of paediatric VOD: EudraCT 2004-000592-33

The table below provides a summary of the criteria for hepatic VOD and MOF in each of the three treatment studies.

Table 7 Summary of VOD and MOF Requirements Across Treatment Studies

	Protocol 2005-01	**Protocol 2006-05**	**Protocol 99-118**
Hepatic VOD	Baltimore criteria by Day+21	Baltimore criteria by Day+35 OR biopsy proven hepatic VOD	Baltimore criteria OR biopsy proven hepatic VOD
Severity	MOF by Day+28	MOF by Day+45	MOF by Day+ 35 or Bearman Model (by Day+16)
• Renal criteria	creat > 3x baseline; OR creat clearance or GFR <40% OR dialysis dependence	creat > 3x baseline; OR creat clearance or GFR <40% OR dialysis dependence	creat > 2x baseline; OR creat clearance or GFR <50% OR dialysis dependence
• Pulmonary criteria	O2 sat < 90% on room air; OR oxygen supplementation OR ventilator dependence	O2 sat < 90% on room air; OR oxygen supplementation OR ventilator dependence	O2 sat < 90% on room air; OR ventilator dependence
	Dysfunction must be attributable to fluid overload or mechanical impingement from abdominal distention or hepatic enlargement and not to an infectious cause (e.g., documented pneumonia).		Pulmonary dysfunction must not have been attributable to another cause (e.g. documented infectious pneumonia).
• CNS	None	None	Confusion, lethargy, delirium (not attributable to another cause)

2.5.1. Dose response study(ies)

- **Protocol 99-118**

Defibrotide for hematopoietic SCT Patients with severe VOD: A Randomized Phase II Study to Determine the Effective Dose.

This was a randomised, open label, multicentre, phase 2 clinical trial conducted at 9 medical centres in the US between April 2000-May 2007 to determine the safety and efficacy of two doses of defibrotide (25 and 40 mg/kg/day in 4 divided doses, Arms A (n=75, ITT) and B (n=74, ITT), respectively) for the treatment of severe VOD in hematopoietic SCT patients.

Study Participants

Adult and paediatric patients were defined as those with severe VOD (defined as VOD with associated renal, pulmonary and/or CNS dysfunction) or at least a 30% risk for developing severe VOD based on the Bearman model following HSCT. All of the patients in Study 99-118 had diagnosed VOD at study entry, the majority of which had MOF [96% (72/75) in Arm A, 93% (69/74) in Arm B]. Of the 8 patients without multi organ failure (3 in Arm A, 5 in Arm B) who had Bearman risk of developing severe VOD (Bearman risk 30-60), 8/8 (100%) subsequently developed MOF within 1-11 days (median 4 days).

Endpoints

The primary objective was to determine the complete response (CR) rate of severe VOD (total bilirubin less than 2 mg/dL and resolution of MOF) treated with defibrotide in the two dose groups. CR was defined as bilirubin <2 mg/dL after initiation of defibrotide (regardless of whether treatment was ongoing or completed).

The secondary objectives were:

- To catalogue the possible toxicity of defibrotide used in this setting, including Grade 3-4 toxicities and all grade toxicity;
- To determine the effect of defibrotide on PAI-1;
- To investigate whether there appears to be a dose relationship between defibrotide and/or Day +100 mortality in PAI-1 levels in these patients;
- To determine if one dose has a trend toward a higher therapeutic effect on Day +100 survival post-SCT in the study population;
- To determine the feasibility of pharmacokinetic (PK) analysis across the two dose arms in a subset of patients and generate descriptive data of the PK of defibrotide in the study population.

Numbers analysed

Table 8 Summary of Analysis Sets and Reasons for Exclusion – All Enrolled Subjects

Variable	Arm A (25 mg/kg/day) n (%)	Arm B (40 mg/kg/day) n (%)
Subjects Enrolled (N)	76	75
ITT Analysis Set	75	74
Excluded from ITT Analysis Set [1]	1	1
ITT Population	75 (100%)	74 (100%)
Per Protocol Population	72 (96%)	69 (93%)
Excluded from Per Protocol Analysis Set	3 (4%)	5 (7%)
Received <3 day's therapy of Defibrotide	3 (4%)	4 (5%)
Alternate diagnosis	0 (0%)	1 (1%)
Day+100 Data Available		
CR Analysis	75 (100%)	74 (100%)
Survival Analysis	73 (97%)	72 (97%)
Safety Analysis Set [2]	74	75
Excluded from Safety Analysis Set	0	0

Treatments

Two doses of defibrotide (25 and 40 mg/kg/day, Arms A and B, respectively) for the treatment of severe VOD in hematopoietic SCT patients were administered to patients. Randomization was stratified by whether patients' conditioning regimen included cyclophosphamide and patient age (>18 years versus ≤18 years).

Defibrotide treatment was recommended for a minimum or 14 days or until the time of complete response, progression of hepatic VOD, or unacceptable toxicity.

Results

The results on the primary efficacy variable showed that thirty-two patients (43%) of Arm A and 29 patients (39%) in Arm B were considered to have had a CR. The difference between the two groups was not statistically significant with a p-value of 0.7397 (Fisher exact test). Table 9 provide the overall summary of CR in severe VOD with 95% CI.

Table 9 Summary of Complete Response

		Complete Response	
Variable	Count	%	95.0% CI
Complete Response [n (%)]			
Arm A (N=75)	32	43%	31.3% – 54.6%
Arm B (N=74)	29	39%	28.0% – 51.2%
Difference in Rate (unadjusted) [1]		-3.5%	-19.9% – 12.5%
p-value [1]			0.7397

[1] p-value by the exact Fisher test.
Source: Table 2.1

Comparison of the time to CR demonstrates no difference between the two dose groups. The median time to CR for those patients who achieved a CR equalled 40.5 days and 44.0 days, respectively for Arms A and B.

Secondary explorative analyses showed

- There were no statistically significant findings for any subgroup analysis (ventilator and/or dialysis dependence at study entry; age, type of CST or number of prior SCTs).
- Arm A children (age ≤16) may have demonstrated a slight trend to higher CR rate compared to Arm B children (64% versus 43%; p-value 0.2362 Fisher exact test); a similar trend was observed in children when defined as age ≤18 (60% versus 42%; p-value 0.2668, Fisher exact test)

The difference in CR in those less than 16 years in Arm A compared with Arm B in favour of the lower dose suggests that either this is a chance finding in view of the small absolute numbers or that toxicity is higher at dose B in children only (as a similar trend was not seen for those over 18 years).

In a similar manner to that for the CR analysis, multiple subgroups analyses were performed for Day+100 survival. Key summary points from these secondary explorative analyses include:
For children (whether defined as ≤16 or ≤18 years old) Kaplan-Meier estimates for survival at day 100 were 68.2% in Arm A versus 32.5% in Arm B for the ≤16 years subgroup and 64.0% in Arm A versus 32.5% in Arm B for the ≤18 years subgroup (p-value= 0.0259; p-value= 0.0275 respectively, log-rank test). No other significant trends noted from the subgroup analyses.

From the two doses used in study 99-118, the efficacy results are poorer in the high dose group particularly in children. This raises serious concerns about the efficacy/safety and PK in children particularly for a product where the mechanism of action is poorly defined and can only be considered to be that of a weak profibrinolytic. This is particularly noted in the KM estimates for survival in those under the age of 16 years and also in those less than 18 years.

Conclusion

Given the similar results in terms of CR and D100+ survival and the similar overall (combined adults and children) safety profile in the two treatments arms the applicant concluded that the lower, 25 mg/kg/day dose would be explored further in a controlled phase 3 trial. Although the inclusion criteria and the definition of CR differed from those used in the 2005-01 study, the decision to choose the 25 mg/kg/day level for further investigations is considered appropriate.

2.5.2. Main study(ies)

- **Treatment indication**

Study 2005-01

An open label multicentre study of the effect of defibrotide in patients with severe VOD following hematopoietic stem cell transplant in terms of complete response of severe VOD (total bilirubin less

than 2 mg/dL and resolution of MOF) by Day+100 post-SCT compared with a historical control group. The study was performed between July 2006-November 2008.

Study Participants

The Treatment group (TG) included the following:

Eligible subjects included those who had a clinical diagnosis of VOD by Day+21 post-SCT (defined by jaundice (bilirubin ≥2 mg/dL) and at least 2 of the following clinical findings: ascites, weight gain ≥5% above baseline weight and/or hepatomegaly.

In addition, an eligible subject must have severe VOD, defined as VOD with multi-organ failure (MOF); MOF is defined as the presence of one or both of the following by Day+28 post-SCT:

- Renal dysfunction:

a) serum creatinine ≥3x value on the date of admission to the SCT unit for conditioning or ≥3x lowest value during conditioning prior to SCT (whichever is lowest); or
b) creatinine clearance or GFR ≤40% of admission value; or
c) dialysis dependence;

- Pulmonary dysfunction:

a) documentation of oxygen saturation ≤90% on room air (two consecutive measurements at least one hour apart) or
b) requirement for oxygen supplementation/ventilator dependence.

In this trial 2005-01 both adults and children are included and all had MOF.
The historical control group (HC) were chosen from multiple retrospective case note reviews and the number and characteristics of the patients included in the HC were amended during the trial.

Treatments

The HC group did not receive defibrotide.

All patients enrolled in the treatment group (TG) received 25 mg/kg/day of intravenous defibrotide given in 4 divided doses (approximately every 6 hours) at a maximum concentration of 4 mg/mL, each infused over 2 hours. Defibrotide was recommended to be administered for a minimum of 21 days.

Thereafter treatment was continued, as circumstances allowed, until the patient was discharged from the hospital. Defibrotide administration could be held for toxicity or delayed for necessary medical/surgical interventions. If the patient required re-hospitalization, treatment with defibrotide could be reinitiated.

Objectives

The primary objective was to demonstrate the efficacy of defibrotide in patients with severe VOD in terms of Complete Response of severe VOD (total bilirubin less than 2 mg/dL and resolution of MOF) by Day+100 post-SCT as the primary parameter.

The secondary objectives were:

- To compare survival at 100 and 180 days following SCT in patients receiving Defibrotide to those in a Historical Control who did not receive defibrotide;
- To assess the safety of the selected dose and schedule;
- To collect and bank samples prior to and during therapy for special studies of potential serum and endothelial markers for VOD;
- To collect historical information about the treatment centres, including severe VOD treatment across hospitals and over time and number and type of transplants per year.

It has to be noted that as the HC group notes were not assessable for safety in terms of identifying SAEs it is unclear how the applicant planned to meet the secondary objective of assessing the safety of the selected dose.

Outcomes/endpoints

This was a historically controlled, open label, multicentre, international, Phase III clinical trial to determine the safety and efficacy of 25 mg/kg/day of defibrotide for the treatment of severe VOD in hematopoietic SCT patients. In this study, the term "severe VOD" was applied to patients meeting the Baltimore diagnostic criteria for VOD (hyperbilirubinemia ≥ 2 mg/dL plus two of the following three criteria: ascites, ≥5% weight gain and hepatomegaly), who also have MOF (i.e., pulmonary and/or renal dysfunction). This represents a group of SCT patients in whom mortality at Day+100 has been shown to be 90-100%.

The primary efficacy parameter was CR by Day+100 post-SCT, utilizing historical controls as a comparator. Secondary parameters included survival rate at 100 and 180 days post-SCT, time to CR, concordance of CR with survival, and analysis of special laboratory studies.

Sample size

The original trial planned to have 80 subjects in the TG and 80 in the HC. There were several amendments in the study whereby the HC group were reduced to 32 subjects and the TG was increased to 102. The ITT population were 102 patients in the treatment group and 32 patients in the historical control.

In the Per Protocol population 61 patients were included in the treatment group and 32 patients in the historical control. Safety population 102 patients were included in the treatment group and 32 patients in the historical control.

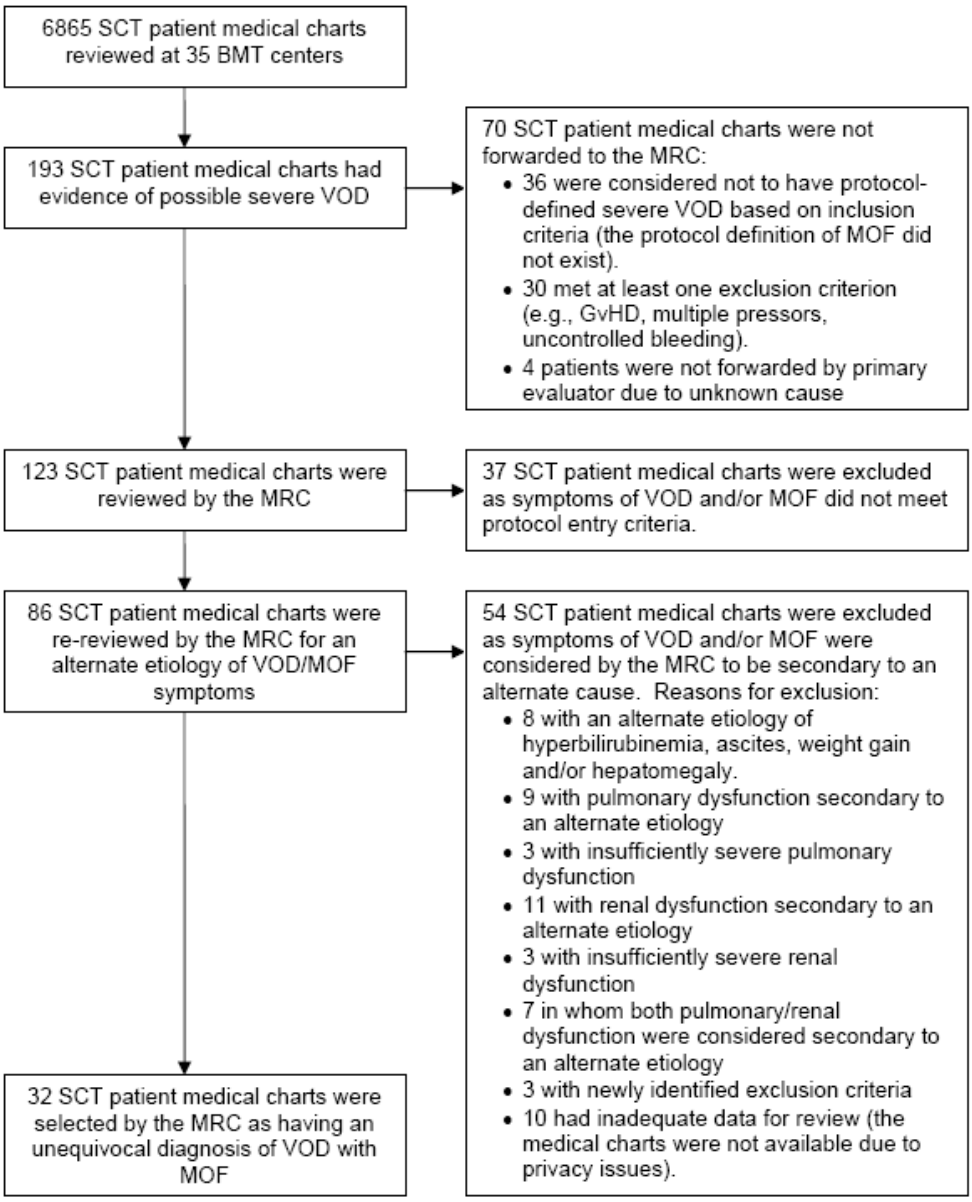
Statistical methods

The final primary endpoint was incidence of Complete Response (CR) at by Day 100 post-SCT regardless of whether defibrotide treatment is ongoing or completed at the time of CR. The initial primary endpoint chosen by the Applicant was Mortality at Day 100. This amendment was made on 3rd December 2007. This was before the first interim analysis formally took place at the DSMB.

Results

Participant flow

Figure 3 Historical Control: Screening



Recruitment

This was a multi-centre (35 sites) open label trial (US 29 sites Canada 4 sites Israel 2 sites).

Changes in Study Conduct

1st DSMB

The DSMB were concerned with the gross imbalance between the 2 arms for the Steinbach site, as well as the high proportion of historical arm patients from this centre. The DSMB was also concerned with the inclusion/exclusion criteria violators by study centre.

2nd DSMB

At the 2nd DSMB, the DSMB recommended that the steering committee confirm the criteria used to select historical controls and the practical application of those criteria to guarantee that the historical patients match the patients in the prospective study arm. At the time of the second DSMB meeting, 3 patients (out of 46) in the defibrotide arm, and 0, out of 86 in the historical control arm were considered to have failed the inclusion/exclusion criteria. Fifty-four SCT patient medical charts were excluded following review by the independent MRC which was blinded to outcome, as symptoms of VOD and/or MOF were considered to be secondary to an alternate cause.

The results of the interim analysis raise serious concerns about the validity of the results. If the enrolled defibrotide population were in general less ill than the historical control arm this would clearly bias the results in favour of defibrotide. The applicant reviewed the patients in the TG group and identified 4 subjects whose symptoms were eventually considered to be attributable to other aetiologies. This large difference in those excluded from the HC as compared with the TG following inclusion in the study highlights the problems with use of a revised HC group.

Changes to the protocol – March 2006

Two entry criteria were amended for the Historical Control:

Hemodynamic instability was added as an exclusion criterion to the historical control group in order to make the treatment and control arms more comparable.

Two additional stratification variables were included: allogeneic transplant versus autologous transplant; prior stem cell transplant (yes/no). These two variables (expected to produce worse patient outcome) were incorporated in order to assure balance between treatment and historical control groups. The protocol therefore incorporated a total of four dichotomous stratification variables.

The Statistical Analysis section was amended.
No change was made to the primary efficacy analysis (comparison of survival at Day 100 between the Treatment Group and Historical Control using a 99% CI). The amendment clarified that a log rank test of the two survival curves over the duration of the study would be a secondary analysis.

Changes to the Protocol - December 2006

The protocol revised the statistical analysis plan to include an alpha penalty to account for the interim analysis to be performed after 40 patients in the prospective group have been followed until Day 100.

Baseline Characteristics

The Applicant presents data for the key baseline characteristics of those enrolled in the defibrotide arm, and the 32 patients finally included in the historical arm.

Final Analysis

Variable		Defibrotide N=102	Historical Control N=32
Age at SCT (Years)	Mean (SD)	26.0 (21.6)	24.9 (20.4)
	Median	21.0	18.0
	Range (Min, Max)	(<1 – 71)	(<1– 57)
Age Category (Years) [n (%)]	≤ 16	44 (43%)	14 (44%)
	> 16 and ≤ 65	57 (56%)	18 (56%)
	>65	1 (1%)	0 (0%)
Gender [n (%)]	Male	64 (63%)	18 (56%)
	Female	38 (37%)	14 (44%)
Weight at Baseline (kg)	Mean (SD)	53.7 (33.7)	52.8 (30.7)
	Median	60.6	58.3
	Range (Min, Max)	(4 – 135)	(6 – 111)
Height (cm) [1]	Mean (SD)	140.6 (42.6)	144.0 (41.9)
	Median	160.0	162.5
	Range (Min, Max)	(55 – 193)	(62 – 191)

The full 86 patients enrolled in the historical arm are also presented in the summary for the DSMB.

Interim Analysis with the full HC of n=86

	Defibrotide N=46	Historical N=86
Age	25.39	27
Male	65.20%	55.80%
Weight(kg)	60.45	57.2
Height(cm)	138.8	150.2
Mean time to VOD diagnosis (days)	13.3	12.5
VOD Diagnosis: Bilirubin>2.0mg AND		
Ascites, weight Gain	36 (78.3%)	58 (67.4%)
Ascites, Hepatomegaly	24 (52.2%)	43 (50.0%)
Weight Gain, Hepatomegaly	27 (58.7%)	63 (73.3%)
All 3	22 (47.8%)	39 (45.3%)

In general, it appears that all 86 patients were in general well matched to the 46 patients on defibrotide in terms of diagnosis and baseline variables. Although the DSMB were surprised that the rate observed in these patients was not what they were expecting from the literature, there does not appear to be substantial evidence for any systematic differences between the populations.

Variable		Defibrotide N=102	Historical Control N=32
Age at SCT (Years)	Mean (SD)	26.0 (21.6)	24.9 (20.4)
	Median	21.0	18.0
	Range (Min, Max)	(<1 – 71)	(<1– 57)
Age Category (Years) [n (%)]	≤ 16	44 (43%)	14 (44%)
	> 16 and ≤ 65	57 (56%)	18 (56%)
	>65	1 (1%)	0 (0%)
Gender [n (%)]	Male	64 (63%)	18 (56%)
	Female	38 (37%)	14 (44%)
Weight at Baseline (kg)	Mean (SD)	53.7 (33.7)	52.8 (30.7)
	Median	60.6	58.3
	Range (Min, Max)	(4 – 135)	(6 – 111)
Height (cm) [1]	Mean (SD)	140.6 (42.6)	144.0 (41.9)
	Median	160.0	162.5
	Range (Min, Max)	(55 – 193)	(62 – 191)
Race [n (%)]	White	76 (75%)	22 (69%)
	Black or African American	5 (5%)	2 (6%)
	Hispanic or Latino	8 (8%)	1 (3%)
	Asian	2 (2%)	2 (6%)
	American Indian or Alaskan Native	2 (2%)	0 (0%)
	Native Hawaiian/Other Pacific Islander	1 (1%)	0 (0%)
	Other [2]	8 (8%)	5 (16%)
Body Mass Index (kg/m²) [1]	Mean (SD)	23.0 (6.3)	22.9 (6.6)
	Median	22.9	21.6
	Range (Min, Max)	(13 – 43)	(14 – 46)
Stratification Factors	≤ 16 Years of Age	44 (43%)	14 (44%)
	Allogeneic Transplant	90 (88%)	27 (84%)
	Prior SCT	13 (13%)	2 (6%)
	Ventilator/Dialysis Dependent [3]	39 (38%)	13 (41%)

[1] Height and BMI available only for 101 TG subjects and 31 HC subjects.
[2] RACE=Other includes "Unknown", "UNK", "Unavailable", mixed race, "Arab", and "UK".
[3] Ventilator/Dialysis Dependent at Study Entry.
Source: Table 1.4

Numbers analysed

Three populations were defined for this study (intent-to-treat, Per Protocol and Safety). The intent-to-treat (ITT) analysis set was the primary analysis set for all efficacy analyses, with secondary efficacy analysis performed on the Per Protocol analysis set.

The ITT set for the Treatment Group consisted of all subjects who were consented to participate in the protocol. The ITT set for the Historical Control consisted of all subjects who were selected by the MRC as having severe VOD without any protocol exclusion criteria. The ITT analysis set was used for as the primary analysis set for the primary and all secondary efficacy variables.

The Per Protocol (PP) analysis set for the Treatment Group consisted of all subjects in the intent-to-treat analysis set who received at least 21 days of defibrotide therapy. The PP analysis set for the Historical Control remained the same as for the ITT group. The PP analysis set was the secondary analysis set for all efficacy analyses. The safety analysis set consisted of all HC subjects and all TG subjects who received at least 1 dose of defibrotide.

Results

Table 10 Primary Analysis for the ITT population as presented by Applicant

Variable	Observed Complete Response			Imputed Complete Response		
	Count	%	99.1% CI	Count	%	99.1% CI
Complete Response by Day+100 [n (%)]						
Defibrotide (N=102)	24	40.0%	24.3% – 60.8%	24	23.5%	12.6% – 34.5%
Historical Control (N=32)	3	28.2%	6.0% – 83.0%	3	9.4%	0.0% – 22.8%
Difference in Rate [1]		17.3%	-0.9% – 35.6%		17.3%	-0.9% – 35.6%
Difference in Rate (unadjusted) [2]		11.7%	-14.5% – 38.0%		14.2%	-3.2% – 31.5%
p-value [1]			0.0131			0.0131
p-value (unadjusted) [2]			0.2076			0.0816

Table 11 Full Historical Control using the 86 HC group

	Observed			Imputed		
	Complete Response			Complete Response		
	Count	%	99.1% CI	Count	%	99.1% CI
Interim Analysis Set						
Complete Response by Day 100						
Defibrotide Arm (N=102)	24	40.0%	24.3%- 60.8%	24	23.5%	12.6%- 34.5%
Historical Arm (N= 86)	17	40.8%	22.7%- 65.5%	17	19.8%	8.6%- 31.0%
Total (N=188)	41	41.5%	28.5%- 57.5%	41	21.8%	13.9%- 29.7%
Difference in Rate [1]		5.9%	-9.8%- 21.7%		5.9%	-9.8%- 21.7%
Difference in Rate (unadjusted)		-0.8%	-20.3%- 18.6%		3.8%	-11.9%- 19.5%
p-values (adjusted) [1]			0.3259			0.3259
p-values (unadjusted) [2]			0.6812			0.5338

GCP inspection findings

The MRC process for selecting subjects into the historical control group was the key to the study 2005-01. The MRC process for selecting subjects in study 2005-01 into the historical control group was not fully GCP compliant. The selection process was not transparent; the rationale and evidence to support the decision was not consistently documented. It was noted that there was no independent documentation of what questions have been asked by the MRC, answers given by the independent physician and rationale for the final decision. The only documentation available was the conclusions recorded in the summary of patient review. In addition, the answers given by the independent physician were not verified. The findings in the selection process cause doubt on the objectiveness of the selection of the historical group against the treatment group.

The failure in identifying suspected cases may have led to under reporting cases of VOD in the prophylaxis arm because only suspected cases in the control arm, given at least one dose of defibrotide were referred. The selection of the historical control group appeared to be more vigorous than the treatment group. The findings in the selection process cause doubt on the objectiveness of the selection of the historical group against the treatment group.

Discussion

The original trial planned to have 80 subjects in the TG and 80 in the HC. The HC group was reduced to 32 subjects and the TG was increased to 102.

As management and treatment improve year on year the use of a HC group is problematic. The problem of using a dated HC group for this trial remains a serious problem in terms of assessment of efficacy in view of the differences in overall clinical management over the last 17 years.
Given this is an open label, historically controlled trial; the results rely heavily on the use of a finally chosen HC group of only 32 subjects.

There is an additional serious concern regarding changing of the HC group from an initial HC group of 86 to the final chosen HC group of 32. The repeated alteration of the composition of the HC group during an ongoing open label study raises the serious risk that the finally chosen subset of HC patients had more severe characteristics than the TG. In addition, the stringency applied to finally ascribing an unequivocal diagnosis of VOD meant that the notes of these HC patients were reviewed over a period of time such that those who fulfilled the inclusion criteria were, in many cases considered finally not to have VOD. This highly stringent selection of VOD cases from historical notes raises the problem that selection was not the same as for the TG. On reviewing the HC notes 54/86 patients (63%) were considered ineligible whereas only 4 (4%) of the enrolled TG were ineligible. The decision to remove 54 patients from the historical control group is not supported. Fifty-one of these 54 patients met the inclusion criteria and thus would have been included in the defibrotide arm. By removing the least ill patients in the historical control group, a large bias in favour of defibrotide will be generated.

The GCP non-compliance of the processes for and documentation of the removal of subjects from the HC group further impact on the acceptability of the small final HC group used.
The GCP inspectors recommended sensitivity analysis of the original 86 HC subjects compared with the TG. This analysis shows no difference between treatment arms and no evidence of efficacy.

Variable	Observed Death Rate			Imputed Death Rate		
	Count	%	95.1% CI	Count	%	95.1% CI
Deaths by Day+100 [n (%)]						
Defibrotide (N=102)	63	61.8%	52.4% – 71.2%	63	61.8%	52.3% – 71.2%
Historical Control (N=32)	24	75.0%	59.2% – 88.3%	24	75.0%	59.9% – 90.1%
Difference in Rate [1]		-15.0%	-32.3% – 2.3%		-15.0%	-32.3% – 2.3%
Difference in Rate		-13.2%	-32.8% – 6.4%		-13.2%	-31.0% – 4.6%

(unadjusted) [2]						
p-value [1]			**0.0341**			0.0341
p-value (unadjusted) [2]			0.0500			0.1710
Deaths by Day+180 [n (%)]						
Defibrotide (N=102)	**69**	**67.6%**	**58.5% – 76.5%**	**69**	67.6%	58.5% – 76.8%
Historical Control (N=32)	**24**	**75.0%**	**59.2% – 88.3%**	**24**	75.0%	59.9% – 90.1%
Difference in Rate [1]		-8.1%	-25.1% – 8.8%		-8.1%	-25.1% – 8.8%
Difference in Rate (unadjusted) [2]		**-7.4%**	**-26.8% - 12.1%**		**-7.4%**	-25.0% - 10.3%
p-value [1]			0.0838			0.0838
p-value (unadjusted) [2]			0.1070			0.4310

[1] Confidence Interval/p-value adjusted for quintile of propensity score for membership in the Treatment Group or Historical Control

[2] p-value for Observed data from Log-rank test; for Imputed data from Chi-Square Test

Source: Table 2.2-ADD2 (95.1% CI).

There are serious methodological flaws in this study that make it extremely difficult to quantify the benefits. This study is severely compromised by the changing of the HC group and therefore the efficacy data as presented by the applicant cannot be considered as robust evidence for an effect in the treatment indication for VOD. The GCP inspection raised a major issue on the process for and documentation of selection of the HC group. These GCP findings further question the utility of the small final HC chosen, as no efficacy was seen when the TG and 86 HC group were compared.

In addition while the diagnosis of VOD was possible from the notes, the safety profile of the patients was not assessable. The absence of such safety data precludes any conclusions and raises concerns about the adequacy of the HC notes. The uncertainty regarding the comprehensiveness of the data from the clinical notes of the HC group raises concern for both diagnosis and safety data collection.

In conclusion the results from the treatment study 2005-01 are not considered to provide adequate evidence for efficacy of defibrotide in the treatment indication.

Additional Study in support of the treatment indication

- **Protocol 2006-05**

Interim analysis of the 2006-05 phase III protocol: Defibrotide for Hematopoietic Stem Cell Transplant (SCT) Subjects with Severe Veno-Occlusive Disease (VOD): A Treatment IND Study (Under 21 CFR 312.34); amended to enrol patients with VOD (without a requirement for associated multi-organ failure).This is a single arm, open label multi-centre study conducted in the US to provide defibrotide (25 mg/kg/day) to subjects diagnosed with VOD. Start date December 2007 and trial is ongoing.

Protocol 2006-05 is a prospective trial of defibrotide 25 mg/kg/day in adult and paediatric patients with a clinical diagnosis of severe hepatic VOD characterized by bilirubin >2 mg/dL and two or more of the following: ascites, weight gain >5% of baseline, hepatomegaly, or biopsy-proven hepatic VOD up to Day+35 after transplantation, and evidence of severe hepatic VOD based on development of pulmonary or renal dysfunction before Day+45.

The design of protocol 2006-05 was similar to that of protocol 2005-01 (study 2005-01 described above), differing mainly by allowing inclusion of patients with late-onset severe hepatic VOD (VOD diagnosed to Day + 35 and MOF diagnosed to day + 45).

Treatments

All subjects treated in the study received 25 mg/kg/day of intravenous defibrotide. Defibrotide was administered in 5% dextrose or saline in water IV given in 4 divided doses (approximately every 6 hours) at a maximum concentration of 4 mg/mL, each infused over 2 hours for a minimum duration of 21 days.

The principle parameters for clinical response were complete resolution of hepatic VOD/MOF and survival at Day+100. The primary efficacy variable, defined as complete response of hepatic VOD according to the definition used for study 2005-01, required that bilirubin improve to less than 2 mg/dL with resolution of MOF.

Objectives

To provide defibrotide under 21 CFR 312.34 “Treatment use of an investigational new drug” to subjects with a diagnosis of severe VOD.

The secondary objectives were:

- To collect additional usage, tolerability and safety data from subjects with VOD.
- To assess the outcome in subjects who met eligibility criteria for Study 2005-01.

Outcomes/endpoints

Efficacy

For all subjects enrolled into Protocol 2006-05, the percentage of subjects surviving to Day+100 and Complete Response rates was calculated.

For subjects enrolled into Protocol 2006-05 who met Protocol 2005-01 entry criteria, mortality at Day +100 and Complete Response (CR) rate at Day+100 was compared to the Historical Control arm of Protocol 2005-01. Complete Response was defined as the percentage of subjects who had a total bilirubin < 2 mg/dL and resolution of MOF (renal and/or pulmonary dysfunction, whether this existed at study entry or anytime on study).

- Resolution of renal dysfunction was defined as a) serum creatinine < 1.5 times baseline or the upper limit of normal for the subjects age; AND CrCl and/or GFR > 80% of admission value (if available); OR For subjects who were dialysis dependent at study entry or became dialysis dependent at any point during the study, resolution of renal dysfunction is defined as independence from dialysis.
- Resolution of pulmonary dysfunction was defined as documentation of oxygen saturation > 90% on room air AND/OR oxygen supplementation no longer required/ resolution of ventilator dependence.

Safety

Safety assessments included clinical assessment of VOD and MOF, review for treatment emergent adverse events, serious adverse events, deaths and withdrawals due to toxicity.

Sample size

Originally planned 100-200 subjects Under Protocol Amendment #3 this was updated to 200-300 subjects.

Results

Efficacy

The protocol planned for a comparison of CR rate and survival by Day +100 for subjects enrolled in the 2006-05 study who met eligibility criteria for Protocol 2005-01 (ITT200605AME1 2005-01) to the 32 Historical Control subjects from Protocol 2005-01. An interim analysis was performed based on a two sided 0.95 confidence interval (CI) of the difference between defibrotide and Historical Control Complete Response rate.

Table 12 Efficacy in Study 2006-05

	ITT population (n=104)	Subgroup 2005-01 (n=68)	Late-onset population (n=30)
Complete Response Day+100	30% (31/104)	34% (23/68)	23% (7/30)
Survival to D+100 post HSCT	32% (33/104)	34% (23/68)	30% (9/30)

The complete response by day 100 is similar to that for Study 2005-01 and for the 86 HC patients used initially for the main treatment trial (2005-1). Further analyses against the 32 HC group finally chosen for study 2005-01 does not add value to this study as the problems with final 32 HC group have been highlighted above on review of study 2005-01 and the GCP inspectorate findings. This open label study 2006-05 is not considered to support the treatment indication.

- **Prevention Indication**

Main study EudraCT 2004-000592-33

Methods

This was a phase III randomised open-labelled study in paediatric patients undergoing SCT at high risk of VOD, conducted to evaluate the efficacy and safety of defibrotide versus no prophylactic treatment on the incidence and outcome of VOD.

Treatments

All patients randomized to the defibrotide Prophylaxis Arm received 25 mg/kg/day of defibrotide. Defibrotide was administered IV in 4 divided doses (approximately every 6 hours), each infused over 2 hours. For patients who did not develop symptoms of VOD, defibrotide was continued until Day+30 post-SCT or upon discharge from inpatient care (with a minimum treatment of 14 days).

Patients randomized to the control arm who were diagnosed with VOD (at any point in the post-SCT course, including after Day+30) crossed-over to defibrotide, receiving the same dose of 25 mg/kg/d as the defibrotide Prophylaxis Arm. For patients with a diagnosis of VOD randomized to either group, defibrotide was continued until complete resolution of symptoms:

- complete resolution of the ascites and
- reversion of the hepatopedal flow (if present) and

- normalisation of the total and direct bilirubin. (This last parameter was omitted if other causes were responsible for additional hepatic injury, i.e. GVHD of the liver)

For patients diagnosed with VOD that subsequently resolved, defibrotide could be tapered by 10mg/kg/d for three to four days and stopped thereafter, with the option to return to therapeutic doses if signs and symptoms of VOD reoccur.

Dose selection

The lower dose of 25 mg/kg/day from the dose-finding study (99-118) was used.

Selection and timing of dose for each patient

The daily dose of defibrotide (6.25 mg/kg every 6 hours) was based on the patient's baseline weight, defined as weight on the first day of conditioning. In the defibrotide prophylaxis arm, patients received their first dose of defibrotide on the day of conditioning (prior to conditioning). In the control arm, patients received their first dose of defibrotide on the day that the patient met criteria for VOD by investigator determination. Although dose delays and omissions could occur, dose escalation and reductions were not planned.

After at least 14 days of therapy, defibrotide could be discontinued for patients who were discharged from the hospital. If VOD recurred, the patient was readmitted and defibrotide therapy reinstituted at the same dose and infusion volume with which he/she was previously treated.

Defibrotide was held for surgical procedures or to accommodate other urgent medication without necessitating dose modification. For surgical procedures, it was recommended that defibrotide administration be completed > 2 hours prior to the procedure. Dosing was recommended to be scheduled around other medications and interventions, such as dialysis. Defibrotide was not held for continuous veno-venous haemofiltration. Interruption of defibrotide prophylactic dosing for more than 4 consecutive days was a protocol violation.

Objectives

The primary objective was to evaluate if prophylactic defibrotide has an impact on the incidence of VOD by Day+30 from SCT in a paediatric patient population at high risk for VOD.

The secondary objectives were:

- To assess the benefit of prophylactic defibrotide on a composite scoring system, taking into account VOD-associated MOF up to Day+100 after SCT and survival through Day+100 after SCT;
- To evaluate if prophylactic defibrotide has an impact on the incidence and severity of graft-versus-host disease (GVHD);
- To evaluate the potential benefit of defibrotide on the incidence of transplant associated microangiopathy (TAM; also known as thrombotic thrombocytopenic purpura or TTP);
- To evaluate the effect of defibrotide on the incidence of MOF up to Day+100 and survival through Day+100 and Day+180.

Outcomes/Endpoints

Primary efficacy variable

Incidence of VOD by Day+30 post-SCT using modified Seattle criteria which required the presence of at least 2 of the following features:

1) bilirubin > 2 mg/dL,

2) hepatomegaly (this may be accompanied by right upper quadrant pain) and

3) ascites and/or unexplained weight gain >5%.

Sample size

An initial enrolment of 270 patients into the defibrotide Prophylaxis Arm and Control Arm was planned with a specified adaptive interim analysis after 120 patients per arm were completed. The sponsor remained blinded to the interim results; results were only available only to an independent statistician and DSMB.

Randomization

Randomisation was centralized with a randomisation sequence list generated by a computer algorithm. Randomisation was 1:1, stratified by centre and the diagnosis of osteopetrosis.

The Control Arm received no VOD prophylaxis with defibrotide. Patients in either arm who developed VOD were allowed to receive treatment with defibrotide at 25mg/kg/day until complete resolution of all symptoms (or death). Two interim assessments were performed by an independent statistician and overseen by an independent Data Safety Monitoring Board (the first after 120 patients total were enrolled, for assessment of patient safety; the second after 120 patients per arm were completed for primary outcome, to determine the final sample size).

Blinding

Due to defibrotide's amber colour, a safe and appropriate placebo could not be provided for the control arm and the study design was therefore open-label. While investigators were not blinded, the independent review committee was blinded in making the determination used in the primary endpoint analyses that VOD was present.

Statistical methods

ITT was defined as all randomized subjects who consented to participate in the protocol. The intent-to-treat analysis set was used as the primary analysis set for the primary and all secondary efficacy variables.

Per protocol: Includes only non-dropouts prior to Day +30 without a serious protocol violation. Serious protocol violations were defined in the protocol as those patients who did not proceed to myeloablative conditioning or SCT, withdrawal prior to Day+30 post-SCT, violation of the defibrotide prophylaxis regimen or daily dose or more than 4 consecutive days without DF Prophylactic therapy or other serious protocol violation (such as no high-risk VOD criteria at entry). The PP analysis set was the secondary analysis set for all efficacy analyses.

Adaptive interim efficacy analysis: Performed when 120 patients in each group were completed for analysis of the primary outcome to determine the final sample size. The purposes of the adaptive interim analysis were to assess the conditional power for achieving a statistically significant result on the primary endpoint, in favor of defibrotide, by end of the study with the protocol-specified

planned final sample size and if such conditional power was less than 30%, to stop the study for futility, or to recalculate the final sample size required to achieve conditional power at least 80%.

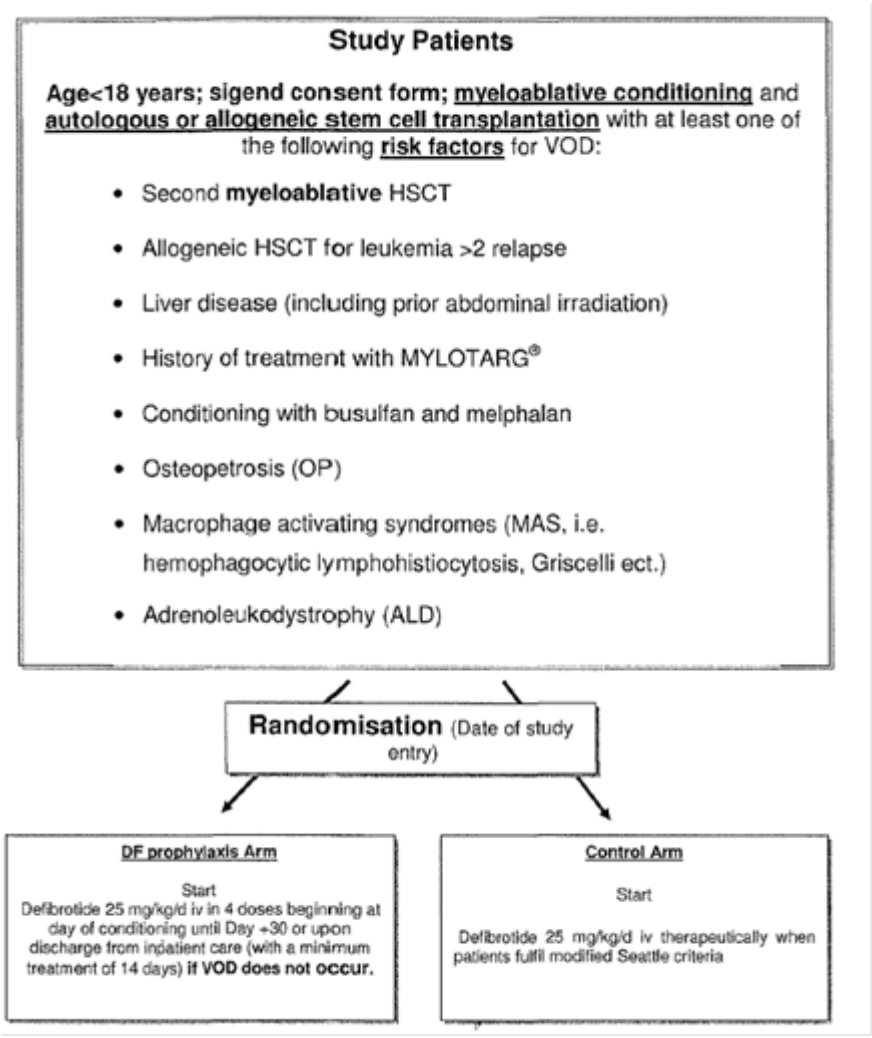
Primary efficacy analysis: Based on the one-sided 0.0001 level of significance (interim stage) and one-sided 0.02498 level of significance (final stage) comparing the defibrotide Prophylactic Group and Control Arm on incidence of VOD. To minimize bias, a blinded Independent Review Committee, comprised of experienced clinicians who were nonparticipating investigators in this study, was appointed to adjudicate the diagnosis of VOD for those patients with investigator-suspected and determined VOD with VOD criteria from SCT through Day+30. The adjudication of this committee provided the primary data used in the analysis of the endpoints.

Treatments were compared on time to VOD, where all randomised patients who did not achieve VOD by Day+30 were censored at Day+30 or date of last known follow-up, whichever was earlier. Death not due to VOD (deaths in patients without a VOD diagnosis), discontinuing the study due to an adverse event, and receipt of a second transplant due to failure of the first transplant were all considered competing risks in this analysis. This method is referred to as Available Data Imputation. The Z-test was used to calculate P-value. For purposes of sensitivity, the analysis of Available Data Imputation was repeated using standard censoring rules by Kaplan Meier analysis (i.e., where premature withdrawals, death not due to VOD, discontinuing the study due to an adverse event, and receipt of a second transplant due to failure of first transplant were censored), with treatment comparisons performed using the log-rank test.

As additional sensitivity analyses, the defibrotide Prophylaxis Arm comparison was carried out the following ways, each with a different method for handling missing data due to patients prematurely withdrawn before achieving VOD and before Day+30 (results were compared across the different methods to assess consistency of results in the presence of missing data): 1) Worst case imputation: All randomized patients who prematurely withdrew before Day+30 were assumed to have VOD by Day+30. Following this imputation, the two sample z-test of proportions using the normal approximation to the binomial distribution was used to compare treatments on incidence of VOD by Day+30. 2) multiple imputation. The logistic regression approach to multiple imputation was carried out to impute missing VOD by Day+30 status. The covariates used in the imputation model were randomized DF Prophylaxis Arm, age, sex, weight and diagnosis of osteopetrosis. Fifty data sets were imputed, and a one-sided test of the significance of the risk difference in VOD between treatments carried out on each data set using the above-mentioned logistic regression of proportions. In the protocol it was planned to impute five data sets but due to stability reasons in the SAP it was decided to impute fifty data sets. SAS PROC MIANALYZE on the results of 50 significance tests were then used to obtain an assessment of the significance of the overall one-sided treatment difference across the five data sets. Each of these methods were used for additional treatment comparisons within subgroups (age category, busulfan, Mylotarg/gemtuzumab, busulfan with melphalan, infants undergoing IV bulsulfan conditioning and allogeneic SCT).

Results

Participants flow



Demographic and Other Baseline Characteristics

Demographic and baseline characteristics are summarized in Table 13. Demographic and baseline characteristics (age, age category, gender, weight at baseline) were similar between the two groups.

Table 13 Demographic and Baseline Characteristics – ITT Analysis Set

Variable		Defibrotide Prophylaxis Arm (n=180) n (%)	Control Arm (n=176) n (%)
Age at Registration (Years)	Mean (SD)	6.5 (5.2)	6.7 (5.4)
	Median	5.1	4.6
	Range (Min, Max)	(<1 – 18)	(<1 – 18)
Age Category (Years) [n (%)]	Infant and toddlers (28 days to 2 years)	46 (26%)	41 (23%)
	Children (> 2 and ≤ 11 years)	91 (51%)	95 (54%)
	Adolescents (>11 years)	43 (24%)	40 (23%)
Gender [n (%)]	Female	70 (39%)	75 (43%)
	Male	110 (61%)	101 (57%)
Weight at Baseline (kg)	Mean (SD)	25.8 (19.5)	25.6 (20.2)
	Median	17.1	18.0
	Range (Min, Max)	(5 – 98)	(4 – 116)
Primary Disease	Acute lymphoblastic leukemia	26 (14%)	22 (13%)
	Acute myelogenous leukemia	31 (17%)	42 (24%)
	Other leukemia	8 (4%)	5 (3%)
	Neuroblastoma	34 (19%)	33 (19%)
	Soft tissue sarcoma [1]	9 (5%)	8 (5%)
	Familial haemophagocytic lymphohistiocytosis	6 (3%)	12 (7%)
	Griscelli syndrome	1 (1%)	1 (1%)
	Other Macrophage Activating Syndrome	1 (1%)	2 (1%)
	Osteopetrosis[2]	7 (4%)	6 (3%)
	Adrenoleukodystrophy	1 (1%)	1 (1%)
	Myelodysplastic syndrome	20 (11%)	11 (6%)
	Other [3]	36 (20%)	33 (19%)

For the type of donor, the numbers were well-matched between the DF Prophylaxis Arm and Control Arm. The two arms were relatively well-matched for type of conditioning and immunosuppressant.

Table 14 provides a summary of baseline criteria that rendered a patient high-risk for VOD and thereby eligible for this protocol. Overall, the two groups were well balanced for high-risk criteria. The most common high-risk category in each arm was conditioning with busulfan and melphalan, followed by pre-existing liver disease, second myeloablative transplantation and allogeneic SCT for leukemia.

Table 14 Summary of Patient Baseline VOD High-Risk Criteria – ITT Analysis Set

VOD High-Risk Criteria	DF Prophylaxis Arm (n=180) n (%)	Control Arm (n=176) n (%)
Second myeloablative transplantation	25 (14%)	23 (13%)
Allogeneic SCT for leukemia	17 (9%)	11 (6%)
Pre-existing liver disease	41 (23%)	54 (31%)
Prior abdominal irradiation	9 (5%)	8 (5%)
Prior treatment with gemtuzumab	11 (6%)	5 (3%)
Conditioning with busulfan and melphalan	106 (59%)	99 (56%)
Osteopetrosis (OP)	7 (4%)	6 (3%)
Haemophagocytic lymphohistiocytosis (MAS)	10 (6%)	15 (9%)
Adrenoleukodystrophy (ALD)	1 (1%)	1 (1%)

Source: ITT Tables 1.7, Listing 1.2.

Data regarding the subjects' medical history are summarized in Table 15 below.

Table 15 Medical History: Baseline Liver Disease, Systemic Infections and KPS – ITT Analysis Set

Parameter	DF Prophylaxis Arm (n=180) n (%)	Control Arm (n=176) n (%)
Liver Disease	44 (24%)	56 (32%)
Coagulopathy	15 (8%)	13 (7%)
Systemic Viral Infection		
CMV	49 (27%)	47 (27%)
Adenovirus	9 (5%)	8 (5%)
EBV	71 (39%)	75 (43%)
HHV6	30 (17%)	36 (20%)
Hepatitis A	30 (17%)	40 (23%)
Hepatitis B	9 (5%)	13 (7%)
Hepatitis C	1 (1%)	2 (1%)
BK-Virus	4 (2%)	1 (1%)
Systemic Fungal Infection	12 (7%)	13 (7%)
Other [1]	50 (28%)	59 (34%)
Performance Status [2]		
Mean (SD)	88.3 (16.0)	91.3 (13.1)
Median	90	100
ALT		
Mean (SD), U/L	51 (63.5)	53 (72.6)
Median, U/L	32	33
AST		
Mean (SD), U/L	49 (53.4)	54 (86.1)
Median, U/L	34	34
Alkaline Phosphatase		
Mean (SD), U/L	187 (136)	173 (114)
Median, U/L	161	156
GGT		
Mean (SD), U/L	73 (185)	58 (174)
Median, U/L	26	19

Parameter	DF Prophylaxis Arm (n=180) n (%)	Control Arm (n=176) n (%)
Bilirubin		
Mean (SD), micromol/L	8.3 (9.2)	8.3 (8.8)
Median, micromol/L	6.8	6.3

Source: ITT Table 1.8; Listings 1.4.1-1.4.3.

[1] For Systemic Viral Infection coding to OTHER, conditions included varicella virus, herpes simplex, parvovirus, norovirus, RSV, rotavirus, influenza virus A, astrovirus, rubella, morbillivirus, praotite virus, parainfluenza, rougeole, callicivirus, poliovirus, and rhinovirus.

[2] Karnofsky or Lansky, as appropriate.

The defibrotide Prophylaxis Arm had a slightly lower incidence of pre-existing liver disease (24% versus 32% in the Control Arm). Overall, the incidence of pre-existing coagulopathy was similar between the groups (8% and 7%, respectively), as were systemic viral infections.

Numbers analysed

Three populations were defined for this study (ITT, Per Protocol and Safety). The ITT analysis set was the primary analysis set for all efficacy analyses. Analysis sets and primary reasons for exclusion from the Per Protocol population are summarized in Table 16 below.

Table 16 Summary of Analysis Sets– All Randomized Subjects

	DF Prophylaxis Arm n (%)	Control Arm n (%)
Subjects Randomised	181	179
Subjects Providing Informed Consent (ITT)	180	176
Subjects Treated (Safety)	177	176
Per Protocol Analysis Set	159 (88%)	166 (94%)
Patients without SCT [1]	5 (3%)	4 (2%)
Patients withdrawn before Day+30 [2]	8 (4%)	4 (2%)
Registration after Conditioning [3]	2 (1%)	0 (0%)
Violation of DF Prophylaxis Treatment Schedule [4] or Interruption of DF Prophylaxis Treatment Schedule [5]	1 (1%)	0 (0%)
Disallowed medication [6]	2 (1%)	1 (1%)
Other Protocol Violation 6 [7]	3 (2%)	1 (1%)

Source: ITT Tables 1.2, 1.2.add and 1.2.1; Listing 1.6.

Note: Percentages are based on number of randomised subjects providing informed consent in each treatment group.

[1] This also includes patients who withdrew consent prior to SCT. DF Prophylaxis Arm Subjects: AL09JUL2004M196, AA01JUL2002F204, GW10APR2005M239, AC10DEC2006M528 and FK09OCT2004M750). Control Arm Subjects: PB06OCT2005M298, AR31JUL1991M503, LK04SEP1989F513 and AH01JUL1997F528.

[2] Patients prematurely withdrawn before Day+30 (see Listing 1.6: 'Drop-out'). DF Prophylaxis Arm Subjects: FF10APR1994F279, RC12MAR2007M521, SK13MAR1992M212, DL12APR1993M212, DL07FEB1997M274, VS22AUG2002M295, ED05JUN1993M285, LE14JUN1995F750). Control Arm Subjects: DB20SEP2003M274, FS23SEP1995M279, JM20MAR1996F274, LH25MAY2003M750).

[3] Registration of a patient after the start of conditioning. DF Prophylaxis Arm Subjects: KA10JUN1997M204 and GB05APR2003F279.

[4] Violation of the treatment schedule (less than three infusions of Defibrotide per day). DF Prophylaxis Arm Subject: LL10APR2000M295.

[5] Interruptions of Defibrotide prophylaxis of more than 4 days (10% of the duration of prophylactic Defibrotide). DF Prophylaxis Arm Subject: LL10APR2000M295 (the same subject is also included under [4]).

[6] Addition of any other drug besides heparin for maintenance of venous access for the prophylaxis or treatment of VOD. This includes DF Prophylaxis Arm Subjects DR12FEB2004M295 (ATIII, enoxaparin, ceprotin); and SA03JUN1994F295 (enoxaparin); Control Arm Subject HB10AUG1991M295 (enoxaparin)

[7] Other reasons for exclusion (protocol entry criteria violation). DF Prophylaxis Arm Subjects GB01OCT2004F274, AS12APR2007F279, and HL17OCT1998M285; Control Arm Subject JH28SEP2004M535.

GCP inspection findings

The variability in the diagnosis of VOD in the VOD-DF study (2004-000592-33) was a critical concern due to the fact that each site applied its own criteria for diagnosing VOD. The differences in methods calculating the 5% weight gain, the availability and timing of the baseline ultrasound, processes for performing the ultrasound, interpreting the thickening of the gallbladder wall and flow in para-umbilical vein, the precedence of clinical judgment over ultrasound result and the need for ultrasound result to confirm VOD diagnosis have all contributed to the inter-site variability.

The small difference in VOD at day 30 of 8% from this open label trial; the finding that the absolute difference falls to 6% when missing data is taken as failure and the concerns about the very restricted IRC input raise serious doubt as to the reliability and reproducibility of the results. The GCP findings of the critical and major non-compliances included the observation that the two centres inspected used different approaches for reaching a diagnosis of VOD.

In addition, the failure in identifying suspected cases may have led to under reporting cases of VOD in the prophylaxis arm because only suspected cases in the control arm, given at least one dose of defibrotide were referred.

The inspection team was of the opinion that the lack of consistency in the processes has contributed to the data quality issues identified in the inspection report. The GCP findings are relevant to the interpretation of the study results. As the integrity of the data is compromised, the results from the prevention study cannot be accepted as providing reliable evidence of efficacy. Only with a second blinded prospective trial in adults and children would there be a sufficient level of evidence available on which to conclude on the efficacy of defibrotide.

Discussion on prevention trial results

Primary efficacy analysis results

For the final analysis, the primary efficacy variable was VOD by modified Seattle criteria up to Day+30 post-SCT (at least two of the following: total bilirubin > 2.0 mg/dL, hepatomegaly with or without RUQ pain, ascites and/or weight gain >5%) using an overall one-sided 2.5% level of significance across the interim and final analysis. The primary analysis compares the ITT population of the DF Prophylaxis Arm (180 patients) to the ITT population of the Control Arm (176 patients). The hepatic VOD incidence in the defibrotide prophylaxis arm was 12% (22/180), and the hepatic VOD incidence in the control arm was 20% (35/176) representing an absolute difference of 8%.

Table 17 **Hepatic VOD to Day+30 post-HSCT (ITT Analysis: Competing Risks): EudraCT 2004-000592-33**

	DF Prophylaxis Arm (n=180)	Control Arm (n=176)	**P-value**
	n (%)	n (%)	
Analysis A1: Available Data, Competing Risks			
VOD by Day+30	22 (12%)	35 (20%)	
Competing Risk by Day+30	3 (2%)	2 (1%)	
No VOD / Competing Risk by Day+30 [1]	149 (83%)	135 (77%)	
Censored [2]	6 (3%)	4 (2%)	
Cumulative Incidence Competing Risks (95% Confidence Interval) [5]	0.13 (0.08 – 0.19)	0.20 (0.15 – 0.27)	
p-value of Z-test			0.0488
Analysis A2: Available Data, Kaplan Meier			
VOD by Day+30	22 (12%)	35 (20%)	
No VOD by Day+30	149 (83%)	136 (77%)	
Censored [2]	9 (5%)	5 (3%)	
Hazard Ratio (95% Confidence Interval) [3]			1.69 (0.99 – 2.88)
p-value of log-rank [4]			0.0507
Analysis B, Multiple Imputation			
p-value of logistic regression of 50 significance tests			0.0644
Analysis C, Worst Case			
VOD by Day+30 (missing = VOD)	31 (17%)	40 (23%)	
No VOD by Day+30	149 (83%)	136 (77%)	
Difference in rates (95% Confidence Interval)			-0.06 (-0.14 – 0.03)
p-value of two-sample Z-test of proportions			0.1937

[1] Patients are censored at Day+30 post-SCT for analysis.

[2] Censored by Day+30

[3] Hazard Ratio from Cox Proportional Hazards Model

[4] P-value from the Kaplan-Meier estimator

[5] Confidence Interval Estimation by log transformation

The 2 pre-specified time-to-event analyses provide very similar results, with p-values of 0.0488 and 0.0507. Although formally one of these analyses failed at the 5% level, the difference between them is negligible. The Multiple Imputation (MI) approach yielded broadly similar results, and the difference between active (20%) and control (12%) is approximately 8%.

Due to 5% of the data being missing in the defibrotide arm and 3% being missing in the control arm, missing as failure led to a non-significant result, with the point estimate of efficacy being smaller at 6%. With this imputation for missing as failure it is relevant that the number of subjects that accounts for this 6% difference is n=9. Therefore even though the amount of missing data is extremely small, the small treatment effect and the small imbalance between arms has led to a key sensitivity analysis yielding a non-significant result, with the non-significance being driven by a smaller point estimate. It is entirely plausible that this imbalance between arms in missing data is due to chance, as the numbers are so small.

Secondary endpoints

Several secondary efficacy endpoints were defined for this study. Composite score for hepatic VOD-associated multi-organ failure and death (incorporating respiratory failure, renal failure, encephalopathy, and survival through Day+100), showed for all patients in the ITT population that there was a significant reduction in the composite score for patients randomized to the defibrotide prophylaxis arm (p=0.0340).

The incidence of multi-organ failure through Day +100 showed a significant reduction in the incidence of hepatic VOD-associated renal failure in patients receiving defibrotide prophylaxis, 1% vs. 6% (p=0.0169) for defibrotide and control arms respectively. Other multiorgan failure incidence comparisons did not show treatment differences.

Composite scores for those patients in either group who were diagnosed with hepatic VOD did not show a difference between groups; however this was likely due to the small sample size.

This study was not powered to demonstrate a reduction of hepatic VOD-associated mortality and for ethical reasons (in view of defibrotide usage at the centres) did not incorporate an arm of patients who were not treated for hepatic VOD. VOD-associated mortality at Day+100 was lower in the defibrotide arm than the control arm (2% and 6%, respectively), but these results were not statistically significant. Survival through Day+100 after HSCT and through Day+180 (data not shown) did not show a significant difference in survival between the two treatment groups (90% of patients in each arm survived to Day+100 post-HSCT; 87% and 86% of the defibrotide Prophylaxis and Control patients, respectively, survived to Day+180).

The mean time to death also was similar between groups (55.4 + 31 days and 49.5 + 29 days for the DF Prophylaxis and Control patients, respectively). Overall, patients from both arms with severe hepatic VOD had a mortality rate of 32%. Patients with mild to moderate disease had a mortality rate of 12%. Below is the table for the secondary outcome of mortality:

		DF Prophylaxis Arm N = 180	Control Arm N = 176	Total N = 356
Survival by Day +100	Yes	162 (90%)	159 (90%)	321 (90%)
	No	18 (10%)	17 (10%)	35 (10%)
Time to death	N	18	17	35
	Mean	55.4	49.5	52.5
	Std	31.22	26.29	28.67
	Median	55.5	41.0	54.0
	Q1 - Q3	28 - 86	30 - 73	28 - 78
	Range	6 - 99	11 - 92	6 - 99
Survival (KM-Estimate)		89.9	90.3	90.1
Log-Rank Test (p-value)				0.9186

Mortality analysed by Day +100 was significantly higher in patients with hepatic VOD (24.6%, compared to 6% in those without hepatic VOD; p<0.0001). Of 28 patients from both treatment arms with hepatic VOD and MOF, 19 (68%) survived to Day +100 and 9 (32%) died. Recovery of hepatic VOD in those with MOF is 71% in the control arm versus 64% in the defibrotide arm. However in those with hepatic VOD without MOF, the recovery rates were 83% and 100% in the control and defibrotide groups respectively.

With a large number of analyses, the relevance of these small differences, which are not all in favour of the defibrotide arm, are not clear. This is particularly important when considering the lack of reliability of the data as highlighted by the GCP inspection.

For a secondary efficacy parameter, the incidence of graft vs. host disease through Days+30, +100 and +180 was significantly lower in the defibrotide prophylaxis arm on Day+30 and Day+100 compared to the control arm. In addition, the severity (grade) of graft vs. host disease was significantly lower in the defibrotide prophylaxis arm at both these timepoints.

Table 18 Summary of Graft vs. Host Disease, ITT analysis EudraCT 2004-000592-33

Variable	Defibrotide Prophylaxis Arm (n=180) n (%)	Control Arm (n=176) n (%)	P-value
Incidence of aGVHD by D+30	42 (23%)	61 (35%)	
Grade of GVHD by D+30			
0	138 (77%)	115 (65%)	
1	21 (12%)	26 (15%)	
2	13 (7%)	26 (15%)	
3	4 (2%)	5 (3%)	
4	4 (2%)	4 (2%)	
Chi-Square for Incidence by Day+30			0.0185
Wilcoxon Test for severity by Day+30			0.0175
Incidence of GVHD by D+100	57 (32%)	76 (43%)	
Grade of GVHD by Day+100			
0	123 (68%)	100 (57%)	
1	30 (17%)	33 (19%)	
2	18 (10%)	30 (17%)	
3	5 (3%)	9 (5%)	
4	4 (2%)	4 (2%)	
Chi-Square for Incidence by Day+100			0.0247
Wilcoxon Test for severity by Day+100			0.0172
Incidence of Chronic GVHD by Day+180	16 (9%)	17 (10%)	
Chi-Square for Incidence by Day+180			0.8022

Note: Percentages are based on the number of patients in the ITT Analysis set. Premature withdrawals are assumed not to have GVHD.

There is weak statistical evidence for the efficacy in the prevention of VOD, with a p-value of approximately 0.05 using the absolute number of VOD as diagnosed by the IRC. One reason the p-value is large, given the size of the trial, is that the effect size is modest (missing as failure analysis yields 23% VOD in control, 17% VOD in defibrotide). Other analyses provide slightly more favourable point estimates for the difference of approximately 8%.

The small differences seen in this open label trial for VOD at Day 30 (12% versus 20% in the active and control arms respectively) cannot be confidently ascribed to defibrotide. The finding that the absolute difference falls to 6% when missing data is taken as failure and the concerns about the very restricted IRC input all raise serious doubt as to the reliability of the results.

With no effect on mortality and the fact that this was a single adaptive trial which was open-label, the results are not considered robust enough to conclude that defibrotide has efficacy in prevention of VOD in children at high risk of developing VOD. With a large number of secondary endpoint and additional analyses, the relevance of these small differences, which are not all in favour of the DF arm, are not clear. This is particularly important when considering the lack of reliability of the data as highlighted by the GCP inspection.

The small difference in VOD at day 30 of 8% from this open label trial; the finding that the absolute difference falls to 6% when missing data is taken as failure and the concerns about the very restricted IRC input, together with uncertainty as to whether the number of VOD cases in the control arm was 35 or 32, all raise serious doubts as to the reliability of the results. This study is not considered sufficient to clearly demonstrate a beneficial effect from prophylactic defibrotide. The

critical and major non GCP-compliance findings identified by the GCP inspection led to the conclusion that data from the prevention trial are not reliable. Only with a second blinded prospective trial in adults and children would there be a sufficient level of evidence available on which to conclude on the efficacy of defibrotide in the prevention of VOD.

Summary of main study(ies)

The following tables summarise the efficacy results from the main studies supporting the present application.

Table 19 Summary of Efficacy for trial 2005-01 (treatment indication)

Title: Defibrotide for the Treatment of Severe Hepatic Veno-Occlusive Disease in Hematopoietic Stem Cell Transplant Patients: A Historically-Controlled, Multi-Center Phase 3 Study to Determine Safety and Efficacy				
Study identifier	Study 2005-01			
Design	Historically-controlled, multicentre, open label Phase 3 study			
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Jul 2006 – Nov 2008 not applicable not applicable	
Hypothesis	Superiority vs. historical control (HC) group			
Treatment groups	Defibrotide (DF) group		Defibrotide 25mg/kg/day in 4 divided doses i.v., for a minimum of 21days (mean duration 23.3 days) N =102 patients with severe VOD post-SCT (i.e. VOD and Multi-Organ Failure)	
	HC group		Standard supportive care Full historical control, N=86, reduced historical control, N=32 patients (from 6,865 screened medical charts) with severe VOD post-SCT (i.e. VOD and Multi-Organ Failure)	
Endpoints and definitions	Primary endpoint	CR Day+100	Complete Resolution (CR) defined as a decrease of bilirubin to <2.0 mg/dL and resolution of Multi Organ Failure	
	Secondary endpoint	Survival by Day+100	Survival by Day+100	
		Survival by Day+180	Survival by Day+180	
Database lock	13 July 2009			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	ITT population versus historical control groups Time point: Day+100 post-transplant			
Descriptive statistics and estimate variability	Treatment group	DF group	Reduced HC group	Full HC Group N=86 17 (19.8%)
	Number of subject	N=102	N=32	

	Day+100 CR rates	24 (23.5%)	3 (9.4%)	
	95% CI	15.3%, 31.8%	0.0%, 19.5%[1]	
	99.1% CI	12.6%, 34.5%	0.0%, 22.8%[2]	8.6%, 31.0%
Effect estimate per comparison	Primary endpoint: Day+100 CR	Comparison groups	Difference in rate: DF vs. *reduced* HC group	
		Chi-Square test	16.7% 17.3%	
		95% CI	3.3%, 30.2%[3]	
		99.1% CI	-0.9%, 35.6%[4]	
		P-value	P=0.0131	
		P-value	P=0.0131	
Effect estimate per comparison	Primary endpoint: Day+100 CR	Comparison groups	Difference in rate: DF vs. *full* HC group	
		Chi-Square test	5.9% 3.8%	
		99.1% CI	-9.8%, 21.7%	
		P-value	P=0.3259	
Analysis description	**Secondary analysis**			
Analysis population and time point description	ITT population versus historical control groups Time point: Day+100 and Day+180 post-transplant			
Descriptive statistics and estimate variability	Treatment group	DF group	Reduced HC group	
	Number of subject	N=102	N=32	
	Day+100 mortality	63 (61.8%)	24 (75.0%)	
	95.1% CI	52.4%, 71.2%	59.2%, 88.3%	
	Day+180 mortality	69 (67.6%)	24 (75.0%)	
	95.1% CI	58.5%, 76.5%	59.2%, 88.3%	
Effect estimate per comparison	Day+100 mortality	Comparison groups	Difference in rate: DF vs. HC group	
		Log rank test	-15.0%	
		95.1% CI	-32.3%, 2.3%	
		P-value	P=0.0341	
	Day+180 mortality	Comparison groups	Difference in rate: DF vs. HC group	
		Log rank test	-8.1%	
		95.1% CI	-25.1%, 8.8%	
		P-value	P=0.0838	

[1] New primary analysis data reported into the CSR addendum 1
[2] New primary analysis data reported into the CSR addendum 1
[3] New primary analysis data reported into the CSR addendum 1
[4] New primary analysis data reported into the CSR addendum 1

Table 20 Summary of Efficacy for trial 2004-000592-33 (prevention indication)

Title: Prospective Study of the Incidence and Outcome of Veno-Occlusive Disease (VOD) with the Prophylactic Use of Defibrotide in Pediatric Stem Cell Transplantation.				
Study identifier	EudraCT 2004-000592-33			
Design	Prospective, open, randomized, controlled			
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Jan 2006 – July 2009 not applicable not applicable	
Hypothesis	Superiority			
Treatments groups	Defibrotide Arm		Defibrotide 25mg/kg/day, from start of pre-transplant conditioning until Day+30 post Hematopoietic Stem Cell Transplantation (HSCT). 180 randomised.	
	Control Arm		Best supportive care, from start of pre-transplant conditioning until Day+30 post-HSCT. 176 randomised.	
Endpoints and definitions	Primary endpoint	Incidence of VOD	Incidence of VOD (using modified Seattle criteria) by Day+30 post-HSCT	
	Secondary endpoint	VOD-associated multi-organ failure (MOF) and death	Composite score for VOD-associated multi-organ failure (MOF) and death Incidence of MOF (respiratory failure, renal failure and/or encephalopathy) by Day+100 post HSCT	
		Graft vs Host Disease (GVHD)	Incidence and severity of GVHD	
		Survival Day+100 & Day+180	Survival at Day+100 and D+180 post HSCT	
Database lock	11 November 2009.			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	ITT population DF Prophylaxis Group N=180 Control Group N=176 Time point: Day+30 post-HSCT			
Descriptive statistics and estimate variability	Treatment group	DF Group	Control Group	
	Number of subject	N=180	N=176	

	Incidence of VOD at Day+30 [Available data imputation under Cumulative Incidence Competing Risk (CICR)]	22 (12%)	35 (20%)	
	CICR 95% CI	0.13 (0.08, 0.19)	0.20 (0.15, 0.27)	
Effect estimate per comparison	Primary endpoint: Incidence of VOD at Day+30 Competing risk analysis	Comparison groups	DF vs Control	
		Z-test P-value	0.0488	
Notes	Confidence interval estimate by log transformation			
Analysis description	**Secondary analyses**			
Analysis population and time point description	ITT population ITT DF Prophylaxis Group N=180 patients ITT Control Group N=176 patients Time points: Day+100, Day+180 post HSCT			
Descriptive statistics and estimate variability	Treatment group	DF Group	Control Group	
	Number of subject	N=180	N=176	
	VOD associated MOF and death (composite score)[1] ---0 points ---1 point ---2 points ---3 points ---5 points	 169 (94%) 6 (3%) 1 (1%) 0 (0%) 4 (2%)	 154 (88%) 7 (4%) 4 (2%) 1 (1%) 10 (6%)	
	Acute Graft vs Host Disease (GVHD) at Day+30 post-HSCT -Incidence -Severity ---Grade 1 ---Grade 2 ---Grade 3 ---Grade 4	 42 (23%) 21 (12%) 13 (7%) 4 (2%) 4 (2%)	 61 (35%) 26 (15%) 26 (15%) 5 (3%) 4 (2%)	

	Acute Graft vs Host Disease (GVHD) at Day+100 post-HSCT -Incidence -Severity ---Grade 1 ---Grade 2 ---Grade 3 ---Grade 4	 57 (32%) 30 (17%) 18 (10%) 5 (3%) 4 (2%)	 76 (43%) 33 (19%) 30 (17%) 9 (5%) 4 (2%)
	Chronic Graft vs Host Disease (GVHD) at Day+180 -Incidence	 16 (9%)	 17 (10%)
	Survival Day+100 post-HSCT	162 (90%)	159 (90%)
	Survival Day+180 post-HSCT	156 (87%)	152 (86%)
Effect estimate per comparison	VOD Associated MOF and death (Composite score)	Comparison groups	DF vs Control
		Wilcoxon-test P-value	0.0340
	Acute Graft vs Host Disease (GVHD) at Day+30 post-HSCT -Incidence -Severity	Comparison groups	DF vs Control
		Chi-square test P-value	0.0185
		Wilcoxon test P-value	0.0175
	Acute Graft vs Host Disease (GVHD) at Day+100 post-HSCT -Incidence -Severity	Comparison groups	DF vs Control
		Chi-square test P-value	0.0247
		Wilcoxon test P-value	0.0172
	Chronic Graft vs Host Disease (GVHD) at Day+180 post-HSCT -Incidence	Comparison groups	DF vs Control
		Chi-square test P-value	0.8022
	Survival Day+100 post-HSCT	Comparison groups	DF vs Control
		Log-rank test P-value	0.9186
	Survival Day+180 post-HSCT	Comparison groups	DF vs Control
		Log-rank test P-value	0.9433
Notes	[1] Composite Score on Day+100 is computed for patients with VOD by Day +30. Score is calculated as follows: Respiratory failure yes +1 Renal failure yes +1 Encephalopathy yes +1 Survival until day +100 no 5 (independent of MOF)		

Analysis performed across trials (pooled analyses and meta-analysis)

Although the applicant has provided information on pooled efficacy analysis from the treatment trials, this analysis relies heavily on the 32 historical control patients finally chosen for study 2005-01. A brief overview is presented below but no conclusions can be drawn in view of the outstanding major objections relating to the final 32 HC patients.

An Integrated summary of efficacy was presented in the dossier. In this document, efficacy summaries are presented for 2 analysis populations:

- ITT5: patients enrolled in Protocol 2005-01, plus those patients enrolled in Protocols 99-118 and 2006-05 who would have met eligibility criteria for Protocol 2005-01. This combined group includes 201 patients with severe VOD enrolled in three clinical trials who received a dose of 25 mg/kg/day.
- HC: the historical control created for Protocol 2005-01 (n=32).

A patient enrolled in Protocol 99-118 or 2006-05 was considered to be eligible for Protocol 2005-01 if the patient had a Baltimore diagnosis of VOD by Day+21 post-SCT with renal and/or pulmonary dysfunction as defined by Protocol 2005-01, together with a diagnosis of MOF up to and including Day +28 post-SCT. The ITT5 group was analyzed and compared to the Historical Control.

- Comparison of ITT5 did not show any statistically significant differences between any of the baseline demographics.
- Comparison of ITT5 to the HC did not show any major difference in renal or pulmonary dysfunction at study entry.

Table 21

Complete Response, Pooled Analysis

	Number (%) of Patients		
	ITT5 (N = 201)	HC (N = 32)	
Complete Response			
Yes	61 (30%)	3 (9%)	
No	140 (70%)	29 (91%)	
95% Confidence Interval	(24.1, 37.2)	(2.0, 25.0)	
Difference in Rate (unadjusted) [1]			21.0 (2.3, 39.1)
Difference in Rate (adjusted) [2]			20.7 (7.9, 33.4)
p-value [1]			0.0174
p-value [2]			0.0015

[1] The exact Fisher test was used for unadjusted analysis.
[2] Confidence Interval was calculated by propensity score for membership in ITT5 or HC

CR in the treatment population was 30% as compared to 9% in the historical control group, a statistically significant finding for the propensity-adjusted analysis (p-value = 0.0015).
Also survival by Day+100 (40.2% vs 25.0%) was significantly better in the treatment arm (p=0.0294).

It remains unclear whether patients included retrospectively from the 99-118 and 2006-05 studies were subjected to the same rigorous selection process as for the final HC or how a biased selection was actually and practically avoided.

Currently, the pooled analysis is associated with the major uncertainties relating to the selection of the original and final HC cohorts and is not considered supportive.

Clinical studies in special populations

The prevention trial was performed in children at high risk of VOD. The treatment trial was performed in adults and children with severe VOD.

Supportive study(ies)

DF-VOD Trial was a multicentre, randomized, controlled, open-label clinical trial to determine the safety and efficacy of defibrotide iv (40 mg/kg/day) for the treatment of hepatic VOD in hematopoietic SCT patients. Although 340 patients were planned only 68 were analysed due to slow recruitment and the study was terminated.

Efficacy

Only primary endpoints were analysed in the interim analysis. Eleven patients (32.4%) of the defibrotide group and 15 patients (44.1%) of the Control group were considered to have had a Complete Remission (Complete Response) by Day +100. The difference between the two groups was not statistically significant (at a p-value of 0.456 by Log-Rank test). Twenty-one patients (61.8%) of the defibrotide group and 20 patients (58.8%) of the control died by Day+100. The difference between the two groups was not statistically significant (at a p-value of 0.829 by Log-Rank test).

The interim analysis data from defibrotide-VOD show a higher CR in the control arm. Although this was only an interim analysis and the number were limited (68) it needs to be considered that each arm in the DF-VOD was similar in size to the HC group.

The results from this study DF-VOD Trial (September 2001 – October 2005) are not supportive of efficacy and the widespread off-label use of DF led to slow recruitment and early termination. Although limited in size and open label, the DF-VOD trial has the advantage over the pivotal treatment trial in that a concurrent control group (n=34), which was numerically larger than the final HC group (n=32) in the pivotal treatment trial has been used. The results from this study further highlight the lack of a clear demonstration of efficacy in the treatment of VOD.

Such additional information from the DF-VOD-Trial needs to be taken into account when viewing the entire data-set provided in support of efficacy. Although it is also noted that there were baseline imbalances in this trial favouring the control arm in the DF-VOD trial it could be argued that the data from DF-VOD-Trial, being the only study with a concurrent control group, are relevant. Supportive studies from the literature were also provided by the applicant for treatment and prevention indications.

Compassionate Use Program Experience: Treatment of Hepatic VOD

In 1998, the applicant established a prospective, open-label, non-comparative compassionate-use program to monitor the safety and effectiveness of defibrotide for the treatment of hepatic VOD (Study DF-CUP). To receive compassionate supply, treating physicians were asked to complete an 'Eligibility form' which included patient initials, age, gender, weight, transplant details, hepatic VOD criteria, exclusion criteria and documentation of informed consent. Physicians were also requested

to comply with applicable legal and regulatory requirements specific to their Member State, and to obtain any necessary authorisations (Ethical Committee) for the compassionate use of a defibrotide, an unapproved drug for the treatment of hepatic VOD. After review of the eligibility form, the applicant's Medical Department shipped the defibrotide to the site address provided by treating physicians.

In response to requests from treating physicians from 311 sites worldwide, defibrotide intravenous solution was supplied for 1,129 patients for the treatment of hepatic VOD post HSCT or following chemotherapy from December 1998 until March 2009. Physicians from 230 sites provided voluntary safety and outcome information via standardised forms for 711 patients who had received at least one dose of drug. Patients eligible to receive defibrotide treatment included those who had a clinical diagnosis of hepatic VOD by Seattle criteria or those who developed hepatic VOD post chemotherapy (without HSCT). Patients who did not meet the Seattle criteria but whom had other criteria (ultrasound or histological criteria) were also eligible to receive drug. Severe hepatic VOD was classified when the criteria for hepatic VOD were met and MOF in at least one of the following organ systems was documented: renal (and or dialysis-dependence), respiratory and/or central nervous system; ventilator dependence was also documented.

The 711 patients with safety outcome data and the 1,129 patients who were originally requested for defibrotide are similar for demographic data, baseline and primary diseases characteristics. Conditioning regimens were found to be similarly represented between Europe, Asia and America, with the most frequently received regimens including cyclophosphamide, busulfan and total body irradiation Of the 711 patients documented to have hepatic VOD, 676 (95%) had at least two hepatic VOD criteria (elevated bilirubin >2mg/dL, hepatomegaly, ascites, or weight gain >5%) and were included in the ITT (treated) hepatic VOD Population. Severe hepatic VOD was documented for 42% (285/676) of these patients. The summary of complete response is presented in Table 22.

Table 22 **Summary of Complete Response (CR) by Population, Age Group and Severity**

		Hepatic VOD N=676	Hepatic VOD Chemo N=61	Hepatic VOD HSCT Seattle N=611	Hepatic VOD HSCT Balt N=453
Non-severe hepatic VOD (N=397)	CR Non-severe hepatic VOD	236 (59%)	20 (59%)	214 (59%)	131 (54%)
	CR Non-severe hepatic VOD, Paediatric	133 (75%)	13 (72%)	120 (75%)	71 (70%)
	CR Non-severe hepatic VOD, Adult	103 (47%)	7 (44%)	94 (47%)	60 (43%)
	PR Non-severe hepatic VOD	41 (10%)	2 (6%)	39 (11%)	27 (11%)
	PR Non-severe hepatic VOD, Paediatric	13 (7%)	-	13 (8%)	10 (10%)
	PR Non-severe hepatic VOD, Adult	28 (13%)	2 (13%)	26 (13%)	17 (12%)
Severe hepatic VOD (N=279)	CR Severe hepatic VOD	82 (29%)	15 (56%)	67 (27%)	51 (24%)
	CR Severe hepaticVOD, Paediatric	45 (40%)	12 (63%)	33 (36%)	24 (31%)
	CR Severe hepaticVOD, Adult	37 (22%)	3 (38%)	34 (21%)	27 (20%)
	PR Severe hepatic VOD	35 (13%)	4 (15%)	30 (12%)	25 (12%)
	PR Severe hepatic VOD, Paediatric	16 (14%)	4 (21%)	11 (12%)	9 (12%)
	PR Severe hepatic VOD, Adult	19 (11%)	-	19 (12%)	16 (12%)

Table 23 **Summary of Day+100 Survival by Population, Age Group and Severity**

	Hepatic VOD N=676	Hepatic VOD Chemo N=61	Hepatic VOD HSCT Seattle N=611	Hepatic VOD HSCT Balt N=453
Survival	379 (56%) KM 52.5%	42 (69%) KM 68.3%	333 (55%) KM 51.0%	222 (49%) KM 44.7
Survival Paediatric	197 (68%) KM 63.3%	29 (78%) KM 73.5%	166 (66%) KM 61.6 %	104 (58%) KM 52.4
Survival Adult	182 (47%) KM 45.0%	13 (54%) KM 60.8 %	167 (46%) KM 44.2%	118 (43%) KM 40.1
Survival severe hepatic VOD	120 (43%) KM 40.1%	19 (70%) KM 72.0%	99 (40%) KM 36.5%	78 (37%) KM 33.2%
Survival non-severe hepatic VOD	259 (65%) KM 61.6%	23 (68%) KM 64.6%	234 (65%) KM 61.3%	144 (60%) KM 55.2

Kaplan-Meier Estimates for Time-to-Event analysis by Day+100.

The CR and survival data are variable and better in those with VOD associated with chemotherapy without HSCT. For those with severe hepatic VOD survival was lower and at ~40% at day 100, similar to the results from 2005-01. However the efficacy collection was voluntary and not standardised. Only a subset of patients who received defibrotide in the DF-CUP had efficacy data provided. The results therefore can only be considered as supportive for safety.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

For the main trial provided in support of the treatment indication (Study 2005-01) there are major problems with the conduct of this multicentre open label historically controlled trial. The historical control group were reduced from 86 to 32 subjects following an interim analysis. The baseline characteristics of the original 86 HC subjects were similar to the treatment group. However 54 cases were identified as not being eligible due to exclusion criteria over a period of days following inclusion. The removal of these subjects altered the results markedly. The conduct of this trial and the requirement to re-review the notes of the originally agreed HC group over a period of time such that an "unequivocal" diagnosis of severe VOD could be assigned raises the major concerns that this stringency was not applied to the treatment group.

The GCP inspection raised a major issue on the process for and documentation of selection of the HC group. These GCP findings further question the reliability of the small final HC chosen, as no efficacy was seen when the TG and 86 HC group were compared.

In conclusion, the results from the treatment study 2005-01 are not considered to provide adequate evidence for efficacy of DF in the treatment indication.

The main trial in support of the prevention of VOD (Study 2004-000592-33) was in children only and was an open label multi-centre study with one group receiving defibrotide prophylaxis and the other as a control. This is termed the prophylaxis phase. On development of VOD subjects in either arm received defibrotide at the same dose as for the prophylaxis and entered the treatment phase. The lack of blinding is a serious limitation for a single pivotal multi-centre trial where the endpoint (VOD) is not as clearly objective as a single centralised laboratory parameter or mortality would be. The contribution of the independent review panel (IRC) was limited to review of cases only in whom a diagnosis of VOD was made by the investigator. This restriction weakens the IRC contribution to the study. Further to the triggered GCP inspection further problems have been highlighted with the design and conduct of the prevention study. It is of note that in 3/35 cases in the control arm that were diagnosed as having VOD by the IRC, these 3 subjects subsequently had their diagnosis rejected by the principal investigator.

Efficacy data and additional analyses

Treatment indication

For study 2005-01 for the treatment of VOD, the primary endpoint was complete response by Day 100 and The Imputed Analysis was the primary method for analysis. For the imputed complete response rates there was a difference when the treatment group was compared to the 32 HC group (23.5% versus 9.4%) whereas no significant difference was seen when the treatment group was compared with the original HC group of 86 (23.5% versus 19.8%).

The results from study 2005-01 cannot be regarded as providing evidence of efficacy as the 32 HC group is not considered an acceptable control. The results from study 2006-5 were similar to those from 2005-01 in terms of CR, and the comparison to the finally chosen HC group is considered to be problematic as for study 2005-01.

Study DF-VOD was a standard of care-controlled trial for the treatment of VOD. This study was terminated and data for only 34 patients in each arm was available. There was no statistical difference between the two arms in CR or Day 100 survival. CR was 32.4% in the defibrotide arm versus 44.1% in the control arm. Death at day 100 was 61.8% in the defibrotide arm compared with 58.8% of the control arm. This trial cannot be considered as supportive for the treatment indication.

Prevention indication

For the final analysis of the prevention trial (2004-000592-33), the primary efficacy variable was VOD by modified Seattle criteria up to Day+30 post-SCT using an overall one-sided 2.5% level of significance across the interim and final analysis. The primary analysis compares the ITT population of the defibrotide Prophylaxis Arm (180 patients) to the ITT population of the Control Arm (176 patients).

The Multiple Imputation (MI) approach yielded broadly similar results, and the difference between active (20%) and control (12%) is approximately 8% (weakly statistically significant).

Due to 5% of the data being missing in the defibrotide arm and 3% being missing in the control arm, missing as failure led to a non-significant result, with the point estimate of efficacy being smaller at 6%. Therefore even though the amount of missing data is extremely small, the small treatment effect and the small imbalance between arms has led to a key sensitivity analysis yielding a non-significant result, with the non-significance being driven by a smaller point estimate. It is entirely plausible that this imbalance between arms in missing data is due to chance, as the numbers are so small.

From a clinical perspective the small difference in VOD at day 30 of 8% from this open label trial is not considered sufficient to clearly demonstrate a beneficial effect from defibrotide in view of the problems associated with the trial design and conduct.

The small difference in VOD at day 30 of 8% from this open label trial; the finding that the absolute difference falls to 6% when missing data is taken as failure and the concerns about IRC input all raise serious doubt as to the reliability and reproducibility of the results. This study is not considered sufficient to clearly demonstrate a beneficial effect from prophylactic defibrotide. The GCP findings raise serious doubt about the integrity of the data.

Only with a second blinded prospective trial in adults and children would there be a sufficient level of evidence available on which to conclude on a beneficial effect from defibrotide.

Additional expert consultation

An ad hoc expert group meeting was held on 12.09.2012 to provide advice to the CHMP on specific questions.

The expert panel made the following recommendations:

- The experts agreed that the mechanism of action (MoA) is not yet understood from the data submitted by the applicant. The expert panel agreed that limitations in defining defibrotide from a quality perspective, could lead to significant problems in confirming the quality of the product in the management of the lifecycle for this biologically sourced and polydisperse product. It was also mentioned that since the pharmacological rationale for

the use of DF has not been established, no extrapolation of clinical data can be made based on “pharmacological plausibility”.

- The experts were of the view that the HC group could not be accepted as a good and reliable control group in the clinical trial setting. The HC group was altered during the clinical trial. Although there were 86 patients initially included in the clinical trial, the HC group was reduced to 32 cases during an interim analysis. A total of 54 patients in the historical control group were removed although 51 out of these 54 patients met the inclusion criteria. The experts’ view is that the removal of these subjects could have altered the study results in favour of DF. In addition the experts noted that the data on complete response rates in each arm showed no difference when the original 86 HC group were compared with the treatment group.

- For the VOD prevention indication the experts noted that the applicant performed a single open label multicentre trial in children at high risk of developing VOD. The study was planned to evaluate the efficacy and safety of defibrotide (intravenously at 25 mg/kg/day) versus no prophylactic treatment on the incidence and outcome of VOD. The experts agreed that the results are not sufficiently robust to conclude that defibrotide can be used for prophylaxis of VOD.

- From the safety point of view, the expert group noted that the death rate was higher in the prevention study when children who did not develop VOD and received DF were compared with subjects in the control arm who did not develop VOD. This fact still remains unexplained and in their views could be associated with defibrotide exposure. This according to the experts is particularly important considering that the proposed indication by the applicant includes both children and those with renal impairment who would have higher exposure to defibrotide. it was agreed that the lack of clear safety data results from the lack of a placebo controlled group available for comparison to those with or without VOD.

- The expert group agreed that whereas for the treatment indication there is a lack of an effective alternative therapy for patients suffering from severe hepatic VOD, for the prophylactic indication, management may include general supportive therapy and treatment or prophylaxis of, for instance, infection or metabolic derangements. They are of the opinion that current data do not support the prevention indication for defibrotide.

2.5.4. Conclusions on the clinical efficacy

Efficacy has not been clearly demonstrated for the treatment of VOD due to concerns about the conduct of the treatment study 2005-01, particularly the adjustment of the HC group, such that 32 cases with very poor outcome were ultimately chosen. The GCP inspection raised a major issue on the process for and documentation of selection of the HC group. These GCP findings further question the utility of the small final HC chosen, as no efficacy was seen when the TG and 86 HC group were compared. In conclusion, the results from the treatment study 2005-01 are not considered to provide adequate evidence for efficacy of DF in the treatment indication.

The prevention indication trial has methodological weaknesses and with a point estimate of 8%,

reduced to 6% for a worse case analysis is not considered robust evidence for efficacy from an open label study. No difference in mortality was seen between the 2 arms although the trial was not powered to demonstrate such an effect. Therefore the data to support efficacy for the prophylactic indication from a single pivotal open label trial is not considered robust.

The critical and major non GCP-compliance findings identified by the GCP inspection lead to the conclusion that the data for the prevention trial are not reliable. Therefore although the data and multiple analyses provided by the applicant have been presented and discussed above, based on the data integrity issues highlighted by the GCP inspection the validity of the results are in serious doubt.

2.6. Clinical safety

Patient exposure

The four principal studies in severe VOD treatment and VOD prophylaxis included a total of 647 HSCT subjects who received at least one dose of defibrotide. In addition, 52 healthy volunteers were dosed with defibrotide in the R09-1425 cardiac safety study. Nearly all subjects in the treatment and prophylaxis studies received a daily dose of 25 mg/kg/day (in 4 divided doses) with the exception of 75 subjects in 99-118 who received 40 mg/kg/day. The duration of treatment in the post-HSCT trials was dependent upon the patient's condition and ranged from a mean of 21.6 days in 2006-05 to a mean of 28.7 days in patients who were switched from prophylaxis to treatment in EudraCT 2004-000592-33. Subjects in R09- 1425 received single exposures from each of two defibrotide doses: 6.25 mg/kg and 15 mg/kg.

Table 24 **Study Subject Drug Exposure by Mean Daily Dose and Duration of Exposure: severe VOD treatment and VOD prevention studies**

Study ID	99-118		2005-01		2006-05	EudraCT 2004-000592-33				R09-1425	
	DF	DF	DF	HC		Defibrotide Prophylaxis Arm		Control Arm		DF	DF
						Prophylaxis	VOD Tx	Standard care	VOD Tx		
Dose (mg/kg/day)	25[a]	40[a]	25	-	25	25	25	-	25	6.25 (mg/kg single dose)	15 (mg/kg single dose)
No. of subjects	74	75	102	32	183	177	24	176		52	52
								140	36		
No. of doses											
Mean (SD)	85.6 (62.2)	73.2 (42.1)	65	NA	86.5	-	-	-	-	1	1
Median	74.5	71.0	63	NA	NA	-	-	-	-	1	1
Range	6-325	5-239		NA	NA	-	-	-	-	-	-
Length of treatment (days)											
Mean (SD)	23.0 (±15.9)	20.2 (±10.8)	23.3	NA	21.6	32.4 (± 9.2)	28.7 (± 19.6)	-	21.7 (± 12.6)	1	1
Median	20.0	20.0	22.0	NA	NA	35.0	23.0	-	18.5	1	1
Range	3-83	2-62	1-60	NA	1-103	4-71	9-96	-	7-79	-	-

[a] All subjects received 10 mg/kg/day on Day 1
DF= Defibrotide, HC= Historical Control, Tx= Treatment

Adverse events

The demographic profiles of subjects in defibrotide's VOD treatment protocols 99-118, 2005-01 and 2005-06 were similar in most demographic variables. The major difference between the various studies was that EudraCT 2004-000592-33 was conducted in a paediatric population exclusively, whereas the severe VOD treatment studies spanned an age range from infants to the elderly. Accordingly, the patients in EudraCT 2004-000592-33 were younger, weighed less and were shorter than the patients in the other three studies. All patient populations were predominantly white (69%-81%). The principal studies in support of defibrotide are 99-118, 2005-01, and 2006-05 for treatment of severe VOD, and EudraCT:2004-000592-33 for prevention of VOD in paediatric patients. All were multiple-dose studies in very ill patients following stem cell transplantation. The cardiac safety trial R09-1425 was limited exposure (two single dose administrations) in healthy volunteers, and is not included in this adverse event analysis across studies.

In each of the four VOD studies, subjects were undergoing stem cell transplantation for significant underlying disease (e.g., cancer in most cases). The paediatric prevention study EudraCT:2004-000592-33 was designed to provide randomized efficacy and safety data in comparison to a "best care" cohort which did not receive prophylactic defibrotide. Study 2005- 01, which was historically controlled for ethical and practical considerations, provided a controlled evaluation of defibrotide safety in a patient population generally sicker, on the basis of severe VOD with organ system involvement beyond the liver as an inclusion criterion, than in the paediatric prevention study. Study 99-118 provided a randomized evaluation of safety at two different defibrotide doses to potentially detect dose-limiting toxicities. Study 2006-05 was not controlled, but the key inclusion and exclusion criteria matched those of study 2005-01 such that the latter study's historical control group can provide context for the observed safety findings in 2006-05.

There was high risk of clinically important adverse events in the populations studied in these defibrotide trials based on the clinical condition of subjects at entry. Based on the known clinical courses of patients undergoing stem cell transplantation, it was anticipated there would be substantial risk of serious adverse events including fatality during the time period of evaluation in

these trials (follow-up to as long as 100-180 days after transplantation). We believe these risks were not likely equivalent between the four studies on the basis of some differences in inclusion/exclusion criteria and in some key demographic variables relative to the potential impact of such differences on efficacy endpoints. In particular, factors such as extent of dialysis or ventilator dependence at entry, which were not equivalent for these studies, would be expected to meaningfully affect a patient's clinical course and safety assessment following HSCT. For a relative assessment of safety across the studies, we rank the patients entered in the 2005-01 treatment arm as the sickest in terms of underlying multi-system dysfunctions at entry. At the other end of the risk spectrum, the EudraCT:2004-000592-33 paediatric prevention trial clearly represented a healthier patient population than those of the severe VOD trials since VOD had not developed at study entry, and high-risk conditions at initiation of prophylaxis such as renal failure and use of assisted ventilation were very unusual.

Adverse events were very common across these studies. Nearly all subjects experienced at least one adverse event, and the majority also experienced at least one serious or severe (grade 3-5) event. Haemorrhage was noted in half of more of subjects in each study. Adverse events leading to discontinuation from the studies was more variable, as were events associated with death. In general, the relative adverse events profiles across studies appear to correlate with the variable clinical status of subjects in these studies at entry, prior to receiving defibrotide. The subjects at greatest risk for clinical complications in the post-HSCT period due to their poor medical status, those in study 2005-01 as discussed above, demonstrated the worst overall adverse events profile. Subjects in EudraCT:2004-000592-33, who did not have VOD or advanced multi-organ failure at study entry, demonstrated the least severe adverse events profile.

Investigators in each of these studies were very experienced with stem cell transplantation and its complications, and the majority of recorded adverse events were considered related to clinical factors other than administration of defibrotide. Defibrotide-related adverse events were greatest in study 2005-01 (occurring in 45% of subjects) and study 2006-05 (22% of subjects), and very infrequent in the dose-ranging 99-118 and EudraCT:2004-000592-33 prophylaxis trials (4-9%).

The three severe VOD trials, which primarily involved the major U.S. transplant centres, had extensive overlap in the participating investigators, and relatedness classification decisions would be expected to be relatively consistent across these studies. However, there were protocol specified differences in causality assessment which clearly would influence related/unrelated determinations despite the investigator continuity. In study 99-118 related events were fairly stringently defined as probably or definitely related to defibrotide, and therefore their contribution to all adverse events is logically lower than in studies 2005-01 and 2006-05 which each also included possibly-related within this category. Related events in EudraCT 2004- 000592-33 were defined as possibly, probably or definitely related to defibrotide, and their relative paucity compared to studies 2005-01 and 2006-05 is not clear. This may be a function of the participating investigators, who in comparison to most US investigators had extensive prior experience with defibrotide, or it may reflect different philosophies for relatedness determinations. It is also conceivable that the nature of events occurring during prophylaxis in EudraCT 2004-000592-33 were more readily attributable to the HSCT experience itself. The explanations for the variable reporting and causality association given by the applicant above are acknowledged. This variability in reporting and of assigning levels of causality which differ by trial and by investigator further complicates identification of DF-related toxicity.

In studies 2005-01, 2006-05 and EudraCT 2004-000592-33, events considered by investigators to be related to defibrotide administration were mostly haemorrhagic in nature (especially epistaxis, gastrointestinal haemorrhage, pulmonary haemorrhage, cerebral haemorrhage, or catheter site haemorrhage) or episodes of hypotension.

Serious adverse event / deaths / other significant events

Deaths

The patient population in the three severe VOD treatment studies were subjects undergoing stem cell transplantation for significant underlying disease (e.g., cancer in most cases) with VOD and multiple organ dysfunctions. Based on an extensive clinical experience in these patient populations, the subjects in all studies were very sick at entry and had substantial risks to develop serious complications during the course of the study due to the transplantation procedure. Accordingly, the risk of death was high in these patients independent of any potential complication from addition of defibrotide to a complicated therapeutic milieu. In the paediatric VOD prevention study, subjects did not have VOD with organ failure at study entry, and their risk of death would be anticipated to be much lower.

The frequencies of patients receiving defibrotide in the three severe VOD treatment studies with adverse events leading to death were substantial. Events associated with fatal outcomes in these studies were 65% in the 2005-01 study, 47% in 2006-05, and 16-19% in the two treatment arms of 99-118. As discussed in the overall analysis of adverse events, the relative severity of a patient's clinical status when entered into these studies was not equivalent between the studies. In general, the relative distributions of events associated with fatal outcomes parallels the overall clinical status at entry in these severe VOD treatment trials. The incidence of events leading to death in the paediatric prophylactic study EudraCT:2004-000592-33 was relatively low (16-18% in the two arms); however, due to the large size of the trial this represented a substantial number of fatalities.

Adverse events associated with fatal outcomes were varied, and distributed across many of the body systems.

The most common events associated with fatalities (arbitrarily chosen at >2% for convenience of discussion) were generally attributable to the clinical condition under investigation of stem cell transplantation for cancer, with the further complications of VOD and multi-organ system dysfunction in the severe VOD treatment trials. Accordingly, most events associated with fatalities were in the hepatobiliary, renal, respiratory, and neoplastic disorders systems.

The general categories of events that are reproducibly distributed across these studies are related to underling cancers, VOD, organ dysfunctions, infectious complications, and graft vs. host disease. In the 2005-01 and EudraCT:2004-000592-33 trials for which control groups were evaluated, fatalities were overall equivalent between treatment and control groups and there are no apparent associations between specific events and use of defibrotide. In the uncontrolled studies, similarities in the general distribution of fatalities to events associated with death from both treatment and no-treatment arms in the controlled studies further suggest that events associated with fatal outcomes are inherent to the study populations.

In study 99-118, none of the events associated with fatal outcomes were related to defibrotide according to the investigators. In 2005-01, all drug-related deaths in the treatment group (10) had some component of haemorrhage (pulmonary, gastrointestinal, or involving the central nervous

system) involved in the death. However, a similar distribution of haemorrhagic events was associated with death in the historical control group, suggesting that these related events may have been components of the underlying clinical state. In 2006-05, defibrotide-related events associated with death in 3 subjects similarly had either a component of haemorrhage (gastrointestinal, involving the central nervous system, mouth or epistaxis) or hypotension. In the paediatric study EudraCT 2004-000592-33, investigators considered only one case involving gastrointestinal haemorrhage to be potentially related to defibrotide. Within the high background risk of fatal outcomes in both treatment and control arms of these studies, no signal is apparent to suggest a contribution from defibrotide-related toxicity.

Other Serious Adverse Events

Non-fatal serious adverse events were presented by the applicant (note that serious adverse events could not be evaluated from the historical control group of study 2005-01). Serious adverse events were reported from all the major body systems. From EudraCT:2004-000592-33 the distribution of serious adverse events was similar between the active defibrotide arm and the untreated control arm. From 99-118, serious adverse events are similarly distributed between the high-dose 40 mg/kg/day arm and the low-dose 25 mg/kg/day arm. The adverse events that are reproducibly distributed across these studies are consistent with the patients' underling cancers, VOD, renal, pulmonary or central nervous system dysfunctions, infectious complications, graft vs. host disease, and high frequencies of fatal outcome. No adverse events have been identified that are suggestive of defibrotide toxicity.

Laboratory findings

Standard laboratory evaluations, including determinations at baseline, during and/or at the end of defibrotide dosing, and during long-term follow-up, were conducted in studies 99-118, 2005-001, and EudraCT: 2004-000592-33. Treatment IND study 2006-05 did not collect laboratory results.

As expected for very ill patients in the post-stem cell transplant period, laboratory results were highly variable. This variability reflected very heterogeneous clinical courses as well as frequent support with blood products and coagulation factors, large fluid shifts over time, and development and resolution of concomitant disease processes such as coagulopathy, bleeding, infection, and metabolic complications. Renal function abnormalities, and elevated bilirubin and other liver analytes, were found by definition in all subjects who met entry criteria of VOD with multi-organ failure. Analytes such as total bilirubin, fibrinogen concentrations, and renal function tests often improved with response to defibrotide and worsened in subjects with progressive VOD. Otherwise, it is difficult to identify laboratory trends that reflect more than the effects of subject variability in clinical course.

In controlled studies 2005-001 and EudraCT: 2004-000592-33, there were no obvious differences in laboratory parameters between cohorts with active defibrotide treatment and untreated control groups. In dose-ranging study 99-118, there were no apparent differences between low-dose and high-dose defibrotide therapy. There also were no obvious differences in laboratory abnormalities that were identified as adverse events.

Safety in special populations

Intrinsic Factors

Study EudraCT:2004-000592-33 was conducted in paediatric patients in the age range from infants (28 days) to 18 years. Defibrotide was well tolerated in this VOD prevention study, with no apparent differences in safety profile to the control group (randomized to no defibrotide prophylaxis). Few elderly were studied in the other defibrotide trials because stem cell transplantation is uncommon in this age subgroup. However, occasional subjects in the age group > 65 years in 2005-01 (1 subject) and 2006-05 (1 subject) did not appear to have a different safety profile from younger subjects. Our intrinsic facts such as gender, height, weight, body mass and composition, other illness, and organ dysfunction did not appear to impact defibrotide tolerability.

Extrinsic Factors

There is no available information related to interactions of defibrotide with alcohol, tobacco, food habits or other extrinsic factors.

Use in Pregnancy and Lactation

Defibrotide has not been studied in pregnant or lactating females.

Overdose

There have been no overdoses in clinical trials of defibrotide.

Drug Abuse

No information is available about potential for drug abuse.

Withdrawal and Rebound

No information is available about potential with withdrawal effects or rebound.

Effects on Ability to Drive or Operate Machinery or Impairment of Mental Ability

No information is available about potential for impairment of mentation or coordination.

Immunological events

No antibody assays were developed for detection of an immune response to defibrotide. No reports of anaphylactic reactions were reported in the clinical trials.

Safety related to drug-drug interactions and other interactions

There is no available information related to interactions of defibrotide with other drugs.

Discontinuation due to adverse events

Events Leading to Discontinuation

Defibrotide was generally well tolerated in the clinical trials, although adverse events leading to discontinuation in these studies were relatively common. Six subjects in 99-118 discontinued due to adverse events; however, only 3 were considered related to defibrotide. Thirty-six subjects in the active treatment arm of 2005-01 discontinued due to adverse events; in 22 cases the events leading to discontinuation were considered related to defibrotide. In 2006- 05, 69 subjects in discontinued due to adverse events; in 26 cases the events were considered related to defibrotide.

In EudraCT: 2004-000592-33, 17 subjects in the defibrotide prophylaxis arm discontinued for adverse events, and 21 subjects in the control arm.

The common adverse events associated with discontinuation from the studies were varied, and often related to organ dysfunction, underlying neoplastic processes, or bleeding complications.

In general, the adverse events that led to discontinuation were severe toxicities that are frequently part of the post-HSCT course (infection, recurrent malignancy, transplant rejection, etc.). Such events are unlikely to be considered potentially related to study drug. Those events leading to discontinuation that were considered by investigators to be related to defibrotide treatment were relatively few, and consist predominantly of haemorrhagic events and hypotension. Given the likelihood that haemorrhagic events and hypotension are part of underlying disease processes in these patients, supported by the generally similar component of such events in the control groups when all events leading to discontinuation are viewed, these events do not appear to represent a signal of defibrotide intolerability or limiting toxicity.

Post marketing experience

Twelve PSURs were received from the time defibrotide was available both as an oral and IV formulation on the Italian market from 1995 to 2008. Three reports were spontaneous and 9 were received from the Italian authorities.

Although defibrotide has been used for over 20 years, it is clear from the literature reports that defibrotide was not from the same animal and tissue source as the defibrotide that in the current applicant. As such the data from PSURs (which include oral usage) cannot be considered as supportive evidence for safety.

2.6.1. Discussion on clinical safety

Although the size of the total safety database in total is considered to be reasonable for the proposed indications, the safety evaluation is hampered by the following:

1) In the historical control group it was not possible to retrospectively classify SAEs and AEs separately. This hampers the utility of the safety data from the HC group to act as a control.

2) Signs and symptoms related to SCT or pre-existing conditions/VOD/MOF were generally not reported, unless worsened, representing an SAE or presentation at a higher seriousness or intensity than expected. But in the absence of a control group possible relatedness to defibrotide cannot be established.

3) Decisions regarding the relation of a sign or symptom specifically to a study drug in such a complicated clinical situation as the setting of post SCT, especially when VOD/MOF is present, must be considered to be associated with a degree of uncertainty.

In the total safety database for VOD treatment (n=1986), MedDRA PTs reported as related to defibrotide in ≥1% of patients are gastrointestinal haemorrhage (2.17%), hypotension (1.56%), coagulopathy (1.16%), epistaxis (1.01%), and pulmonary haemorrhage (1.46%); none is reported in ≥1% of patients receiving defibrotide for prophylaxis (n=772).

In clinical studies, 568 patients (88%) who received any dose of defibrotide experienced AEs, with the most frequent being Hypotension (20%), Diarrhoea (13%), and Vomiting (10%), Gastrointestinal haemorrhage, Nausea and Pyrexia (8%), Epistaxis (7%), Pulmonary haemorrhage, Pleural effusion, Exfoliative rash, Hypertension, Haemorrhage (site not specified), Abdominal pain (5%). The most frequent defibrotide-related adverse events were Haemorrhages (104/135 patients 77%); Gastrointestinal haemorrhage, Pulmonary haemorrhage and epistaxis. Fatal AEs that were considered defibrotide-related by the investigator were reported in 7 patients in the treatment population and 1 patient in the prevention population. All of these included a component of bleeding and occurred at the 25 mg/kg/day level. This number does not include the 18 deaths predominantly due to haemorrhage in DF-CUP.

For reasons given above, as well as the lack of a PD marker and uncertainties regarding the MoA, interpretation of laboratory findings potentially related to defibrotide is difficult and laboratory abnormalities potentially related to defibrotide therefore may remain to be detected.

There are no major safety signals that can be clearly attributed to defibrotide although an increased risk for bleeding which is higher in higher dose groups has been noted and the concern about an increased mortality in children receiving high dose defibrotide have been raised. The lack of clear safety data is in part a result of the lack of a placebo controlled group available for comparison in those with VOD.

Data from study DF-VOD which included a concurrent control VOD group was limited to 34 subjects in the DF and control arm. Major bleeding events were present for 9 patients (26.5%) in the Control group and 11 (32.4%) in the defibrotide group, but SAEs considered as possibly related to treatment were similar in both arms. Haemorrhage is being identified as a risk by the applicant.

The safety of defibrotide is provided for healthy volunteers, for those at risk of developing VOD and for those with VOD. As those undergoing HSCT and those with VOD have serious concurrent morbidity, it is difficult to distinguish between disease-associated and treatment related AEs. This is problematic also because of the protracted duration of infusion and treatment (4 daily IV infusions each of 2 hours for a total duration of at least 14 days). Another complication in assessing the safety relates to the unclear MoA for defibrotide and the absence of an identified PD effect.

Assessment of paediatric data on clinical safety

The assessment of paediatric safety showed an excess of mortality in the high dose arm (group B) in the dose-finding study 99-118 for those under 18 year. This issue raises questions about the safety of defibrotide in children.

For assessing the safety of DF in those without VOD, a comparison of safety in the prevention study comparing the defibrotide arm with the control arm for only those subjects who never developed VOD was provided by the applicant. The safety profile was less favourable in the defibrotide arm and a higher death rate of 25 (17%) in the defibrotide arm compared with 18 (13%) in the control arm was noted. With the exception of one case of haemorrhage for which

some contribution of defibrotide cannot be excluded it is not possible to ascribe these deaths to DF with any degree of certainty. While the absolute number of excess deaths was small (n=7), there remains doubt as to whether defibrotide contributed to the excess deaths in the prophylaxis arm.

2.6.2. Conclusions on the clinical safety

The CHMP noted that the data are hampered by the absence of sufficient concurrent controlled safety data, however no major safety signals that can be definitely attributed to defibrotide were observed. A dose-response in toxicity (increased bleeding at higher doses in DF-CUP and increased mortality in children on high dose in the dose finding study) cannot be excluded from the data provided.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to conclude on pharmacovigilance and risk minimisation activities at this time

Risk Management Plan

The applicant submitted a risk management plan, which included a risk minimisation plan.

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to consider risk minimisation activities at this time.

3. Benefit-Risk Balance

Benefits

Beneficial effects

Hepatic Veno-Occlusive Disease (VOD) is a serious pathology resulting from endothelial damage most commonly seen in the setting of haematopoietic stem cell transplantation (SCT). The predisposing factors include agents causing endothelial injury such as chemotherapy for SCT conditioning and total body irradiation. The precise pathogenesis of VOD is not fully elucidated but damage to the hepatic sinusoidal endothelium followed by thrombosis in the sinusoids leads to hepatic dysfunction, fluid retention and, if severe, can be associated with renal impairment and multi-organ failure. The diagnosis of VOD is very difficult as it requires exclusion of other pathologies seen often in the Haematopoietic Stem Cell Transplant (HSCT) setting such as infection and Graft Versus Host Disease (GVHD). In addition the diagnosis may take a few days to make and is dependent upon the criteria used. The Baltimore criteria are more stringent than the Seattle criteria. In addition the more recently modified Seattle criteria allow for the diagnosis of VOD up to day 30 post-transplant.

Another difficulty in the management of cases with VOD is when to give potentially toxic therapies (e.g. recombinant tissue plasminogen activator) in an attempt to stop the microthrombosis in the liver. For aiding clinical decisions in these cases the Bearman model has been employed. This model

takes into account the timing, degree and rate of rise in body weight and serum bilirubin levels. This prospectively validated prediction model applies to patients treated with cyclophosphamide and may not be applicable to patients treated with less toxic conditioning regimens. Using this model has been shown to have a high specificity, but only moderate sensitivity, for predicting those who will go on to develop severe VOD, which is defined as VOD with multi-organ failure.

Currently the literature reports on both the incidence of and prognosis for VOD support the fact that for severe VOD the prognosis is poor with a high mortality rate. With less toxic conditioning regimens, lower doses of TBI and overall better HLA matching available over the last decade, it is possible that the incidence of VOD may be declining but on the other hand the increasing use of SCT in more patients and in older patients may offset this. Currently there is no licensed therapy for this indication and the availability of an effective treatment for this patient group would be of great benefit.

This application is for both the treatment of VOD and the prevention of VOD. Both proposed indications were supported by open label clinical trials.

For the treatment indication trial (2005-01) a historical control (HC) group was used, and patients in both arms were defined as having severe VOD. The multiple amendments during the on-going open label study to the HC group led to a change from the originally chosen 86 HC subjects to a finally chosen 32 cases. When the primary endpoint of complete response was compared between the treatment group receiving DF (TG) and the original HC group no differences were seen. When the comparison was made between the TG group and the final HC group of 32 cases the results were significant with 23% versus 9% in complete response (CR) at Day 100.

For the prevention indication paediatric patients at high risk of developing VOD were assigned to an active prophylaxis arm and a placebo arm in Study 2004-000532. Once subjects in either the control or prophylaxis arm developed VOD they received treatment with defibrotide. The results showed a very small benefit for the active group in terms of the primary endpoint: development of VOD at day 30; with an 8% difference in the incidence of VOD between the 2 arms with borderline statistical significance. This difference between the arms was reduced to a non-statistically significant difference of 6% when missing data was treated as failure.

Uncertainty in the knowledge about the beneficial effects

The underlying MoA of defibrotide was claimed initially by the applicant as having multiple endothelial protective properties. Much of the older literature cites this as the MoA for defibrotide.

The applicant has provided information on an endothelial cell protection assay and this remains to be confirmed in validation studies. The data are preliminary and it cannot be ascertained at this stage whether any activity seen is due to a pharmacological or physicochemical effect. The claimed cell-protection activities have not been sufficiently demonstrated. Further work to investigate and confirm these properties is needed.

An updated expert statement provided by the applicant also claims a MoA for plasmin activation. The CHMP noted that a weak profibrinolytic effect has been shown and the MoA for Defibrotide is likely to be that of a weak fibrinolytic agent. The applicant has characterised defibrotide with several related profibrinolytic activity assays.

The CHMP' conclusions from the results of these assays is that the *in vitro* activity for fibrinolysis is weak and might even be seen as negligible when compared to negative controls and that the endothelial cell protection needs to be confirmed with more adequately designed studies.
For both indications of treatment and prevention the absence of well characterised pharmacology and justified dose and posology highlights the need for clear evidence of efficacy. There is also uncertainty about the relevant dose to use and the optimal posology and optimal duration of infusions and number of days treatment required. This is because the dose-finding study did not demonstrate a dose-response either for the PD marker chosen of PAI-1 or for efficacy. In the treatment indication, because the original HC group fulfilled the eligibility criteria for inclusion as pre-defined, the changes to the HC constitute a key confounding factor.

The data from the additional open label interim analysis of study 2006-05, which compared the results with the final 32 HC subjects from study 2005-01, is not considered to provide reliable efficacy data either, considering that these 32 HC subjects had a particularly poor outcome and do not constitute an acceptable control group. Indeed the stringent criteria applied by the Medical Review Committee (MRC) in choosing these 32 cases from the originally agreed HC group of 86 subjects was to follow the patient progress over time in order to reach an "unequivocal" diagnosis of severe VOD. However, the notes from the TG were not subject to a similar MRC stringent blinded review. When the applicant then reviewed the TG with these criteria 4 (4%) of the TG were considered ineligible. It is noted that removal of these 4 cases would not impact on the overall data on CR from the trial but the differences highlight the difficulties with the amended HC group. A major GCP finding was that the processes for and documentation of the reduction in the HC group from 86 to 32 was unclear. This raises further concerns about the reliability of the 32 HC group.

There is also uncertainty regarding the adequacy of the documentation available for the accurate diagnosis of VOD and also for the assignment of complete response. These combined uncertainties raise serious doubts as to the level of evidence provided in support of efficacy in the treatment indication.

The only data where concomitant controls were used for a treatment indication in VOD was the

DF-VOD-Trial. In view of the widespread off-label use this trial failed to recruit and was terminated. Nonetheless there is data on complete response rates in each arm (n=34 in each arm) and no difference was found between the active and a concurrent control arm.

For the single pivotal trial for prevention the weak efficacy seen is not considered to provide sufficient evidence for a clear conclusion that defibrotide is effective in preventing VOD. The small percentage difference (8%, 12 patients) between the two arms with borderline statistical significance is further reduced to a non-significant 6% (n=9) in the key sensitivity analysis of treating missing data as failure. Although the study was not powered for an effect on mortality, a trend in favour of the treatment arm would have provided additional support for a beneficial effect from treatment.

The use of an Independent Review Committee can improve the reliability of data from an open label study; however, the independent review committee for the prevention trial did not have access to all of the subjects CRFs nor to all cases of "suspected VOD" and their input was restricted to cases where the investigator diagnosed VOD or administered DF. This limits the strength of the data from this open label trial.

The small difference in VOD at day 30 of 8% from this open label trial; the finding that the absolute difference falls to 6% when missing data is taken as failure and the concerns about the limited IRC input raises serious doubt as to the reliability of the results. This study is not considered sufficient to clearly demonstrate a beneficial effect from prophylactic defibrotide.

The critical and major non GCP-compliance findings identified by the GCP inspection have resulted in the conclusion that it is not possible to rely on the integrity of the data and therefore the validity of the results is in serious doubt. Only with a second blinded prospective trial in adults and children would there be a sufficient level of evidence available on which to conclude on a beneficial effect from defibrotide.

Risks

Unfavourable effects

Although haemorrhagic complications were anticipated with defibrotide, it is difficult to ascribe with any degree of certainty AEs to the treatment itself in post-SCT patients, particularly those with VOD. The lack of a clear dose-response in AEs from the dose-finding study in the overall population also suggests that the unfavourable effects of DF cannot be characterised with certainty from the clinical data provided. An exception is the statistically significant differences in Kaplan-Meier estimates for survival (68.2% Arm A 25mg/kg/day, versus 32.5% arm B 40mg/kg/day, p= 0.0259) in those under 16 years, and this remains unexplained but does raise concerns regarding safety in children at doses higher that the proposed posology (25 mg/kg/day).

In terms of trying to assess the safety of defibrotide in VOD in the absence of a control group with adequate safety data, the largest safety database where different doses were administered to VOD patients is from DF-CUP and these results suggest a dose-related increase in bleeding. There were 18 deaths in DF-CUP considered possibly, probably or definitely related to defibrotide – all these deaths were as a result of haemorrhage or in one case coagulopathy. 9 children and 9 adults died

and of the 18 subjects only one was on the low dose of 10mg/kg/day, 4 subjects received between 20-40mg/kg/day, 12 received 40mg/kg/day or higher, and for one subject the dose was not provided. Also of note is that in one case where defibrotide was stopped due to bleeding and then re-started again eleven days later, a further bleeding episode occurred within 5 days of re-commencement and DF was discontinued. Although the applicant states that there was no increase in efficacy with the 40mg/kg/day dose it does seem that for fatal bleeding events there was an association with higher doses.

Although no clear major signals for defibrotide related bleeding AEs across the clinical programme have been found, the relatedness of defibrotide to the signals seen cannot be clearly concluded in view of the lack of a concurrent control with VOD.

The prevention trial does allow some comparison between arms. When comparing those in each arm who never developed VOD the safety profile of those on DF prophylaxis was worse than the placebo arm. Overall in the prevention trial a higher death rate of 25 (17%) in the defibrotide arm compared with 18 (13%) in the control arm was noted. Although most deaths occurred more than 1 month after cessation of defibrotide, in 7 cases AEs related to the death occurred during treatment with defibrotide or within one week of stopping defibrotide. Only one case of haemorrhage was considered as possibly related to defibrotide. Treatment discontinuation occurred in 5 (3%) in the defibrotide prophylaxis arm and these discontinuations were predominantly because of bleeding-related AEs during the prophylaxis phase. The reasons for discontinuation in the defibrotide prophylaxis arm included epistaxis, haemorrhagic cystitis, prolonged PT and GI haemorrhage. In the treatment phase 1 subject (4%) in the defibrotide prophylaxis arm and 4 subjects (11%) in the control arm discontinued. The discontinuations in the treatment phase were predominantly for haemorrhage.

The data from defibrotide use in other indications does raise a signal for occasional allergic reactions, but no such signal was seen in the clinical trials provided in support of VOD. Interpreting the significance of the safety data is difficult as it is not clear when the applicant changed the animal and tissue source of defibrotide as the older literature reports refer to defibrotide as being of “mammalian origin” or derived from “bovine lung”. As the defibrotide in this application for the pivotal trials is from porcine intestine, this is not identical to the product previously marketed by the applicant.

In summary the unfavourable effects at present appear to be dose-related haemorrhage and increased toxicity (death) in children with the higher dose.

Uncertainty in the knowledge about the unfavourable effects

From a quality perspective, the activity of defibrotide in the profibrinolytic and endothelial cell protection assay is weak and possibly confounded by the signal: noise ratio. Defibrotide is polydisperse and difficult to characterise using physicochemical tests, and together with the inability to show strong biological activity in an *in vitro* assay, the quality, consistency and control of defibrotide rests significantly on robust control over the manufacturing process. In view of the limitations in defining defibrotide from a quality perspective, and as no clear clinical correlation to quality attributes has been demonstrated, this could lead to significant challenges in confirming the quality of the product over the lifecycle of this polydisperse product.

The finding of a dose-related increase in bleeding from DF-CUP is noted but in view of the open label nature of DF-CUP and the voluntary data provision by clinicians which is incomplete, firm conclusions cannot be drawn.

There is only very limited data on PK of defibrotide in VOD. The lack of PK data in children and in those with renal failure, while both of these groups are included in the proposed indication of VOD, raises the level of uncertainly regarding the posology for children, in view of the safety signals at high doses, and in those with renal failure, as defibrotide is renally excreted and increased exposure will be expected in those with renal impairment.

Importance of favourable and unfavourable effects

Favourable effects from the treatment trial are not considered to have been demonstrated in view of the small and amended HC group chosen, where the documentation of the decision making process of the MRC to remove 54 from the original 86 cases was inadequate and a major GCP non-compliance.

The favourable effect for prevention of VOD is based on a small difference of 6-8% in the prevention trial. Such a small difference is not accepted as robust evidence of efficacy particularly when the data is from an open label, adaptive design trial. The critical and multiple major issues identified in the GCP inspection further add doubts to the reliability and integrity of the data from the prevention trial.

The unfavourable effects of defibrotide have not been clearly elucidated, although a dose-related increase in bleeding and increased mortality with higher exposure in children was seen. In addition effects such as those associated with prolonged infusions and the administration of an animal product carry additional risks including administration-associated AEs and allergic reactions to the product.

Discussion on the benefit-risk balance

The benefits demonstrated from the treatment trial are considered unreliable in view of the multiple amendments and changes to the HC group during the conduct of the study. For a single pivotal open label trial the use of a historical control group and the choice of that group needs to be transparent and the process GCP compliant. The GCP findings that the processes for and documentation of the revision of the HC group were not transparent add to the concern about this HC group as a valid and reliable comparator group.

The only data where concomitant controls were used for the treatment indication was DF-VOD-Trial, which was terminated early due to inadequate recruitment. No difference was seen between concurrent control (n=34) and treatment arms (n=34) in terms of complete response. The lack of a dose-response results from study 99-118 also raises doubts about the effect of defibrotide.

The benefits demonstrated are considered very weak for the prevention indication and the level of difference between the two arms for prevention cannot be considered to provide sufficient evidence to support efficacy from a single open label trial. In the absence of a well-characterised pharmacology profile the requirements of a single pivotal study are higher. The small difference in

VOD at day 30 of 8% between the defibrotide and control arm is not considered sufficient to clearly demonstrate a beneficial effect from defibrotide and may be due to chance. Further compounding the acceptance of the reliability of the results is the very restricted IRC input which was limited to cases where the investigator has diagnosed VOD or administered defibrotide. The GCP inspection identified critical and multiple major non-compliances which undermine the integrity of the data from this study. It is considered that the data provided in support of the prevention of VOD indication lacks the level of reliability and robustness which are required from a single pivotal trial.

The risks are those associated with administration of prolonged frequent IV treatments and in addition the administration of an animal-derived product. In terms of clarification of the safety profile of DF, one of the proposed MoAs is a weak profibrinolytic effect and this raises concern about increased bleeding in a group where haemorrhage occurs frequently due to concomitant factors. Without a comparison between a defibrotide group and a placebo/physician's choice group, AEs cannot be attributed clearly to the defibrotide in the setting of post-SCT patients with VOD.

Additional risks are the lack of information on PK in children and in those with renal failure where in view of the trend for increased haemorrhages in higher dose patients in DF-CUP, together with increased deaths in children in the high dose group raise the need for further PK data in these groups.

4. Recommendations

Based on the CHMP review of data on quality, safety and efficacy of Defitelio in the following indications:

> *Defitelio is indicated for the prevention of hepatic veno-occlusive disease (VOD) also known as Sinusoidal Obstructive (SOS) in haematopoietic stem-cell transplantation therapy.*
>
> *Defitelio is indicated for the treatment of hepatic veno-occlusive disease (VOD) also known as Sinusoidal Obstructive (SOS) in haematopoietic stem-cell transplantation therapy.*

The CHMP considers by consensus that the efficacy of the above mentioned medicinal product is not sufficiently demonstrated, and therefore, pursuant to Article 12(1) of Regulation (EC) No 726/2004, recommends the refusal of Marketing Authorisation for the above mentioned medicinal product.

The CHMP considers that:
One open label pivotal trial was provided in support of the prevention indication. The statistical significance of the results was not sufficiently convincing in the context of a single pivotal trial. Furthermore critical and major GCP non-compliances relating to the documentation and reporting of efficacy, the lack of clarity in the protocol and the differences in interpretation of the protocol at the two sites inspected raise doubts as to the reliability and generalizability of the data. It was therefore not possible to conclude that efficacy had been demonstrated in the prevention of hepatic veno-occlusive disease.

One open label pivotal trial was provided in support of the treatment indication. The historical control group was not considered a suitable control arm in view of the small size, the timeframe of recruitment and the amendment to the historical control group where 54 subjects were removed. In addition, the lack of transparency in the processes and decision making relating to the amendment of the historical control group raise further uncertainty as to the reliability of the final 32 subjects chosen. It was therefore not possible to conclude that efficacy has been demonstrated in the treatment of hepatic veno-occlusive disease.

5. Re-examination of the CHMP opinion of 21 March 2013

Following the CHMP conclusion that Defitelio was not approvable for the treatment of:

> *Defitelio is indicated for the prevention of hepatic veno-occlusive disease (VOD) also known as Sinusoidal Obstructive syndrome (SOS) in haematopoietic stem-cell transplantation therapy.*
>
> *Defitelio is indicated for the treatment of hepatic veno-occlusive disease (VOD) also known as Sinusoidal Obstructive syndrome (SOS) in haematopoietic stem-cell transplantation therapy.*

as the efficacy of the above mentioned medicinal product was not sufficiently demonstrated, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

5.1. Detailed grounds for re-examination submitted by the applicant

The applicant presented in writing and at an oral explanation their grounds that the adopted CHMP opinion may not have considered the data fully and also provided further information. The applicant presented the revised indication:

- *Prevention of hepatic veno-occlusive disease (VOD), also known as sinusoidal obstructive syndrome (SOS), in high risk patients undergoing haematopoietic stem-cell transplantation therapy (HSCT)*
- *Treatment of severe hepatic veno-occlusive disease (VOD), also known as sinusoidal obstructive syndrome (SOS), in haematopoietic stem-cell transplantation therapy (HSCT)*

During the oral explanation held on the 22 July 2013, the applicant explained that the prophylactic indication for defibrotide had been withdrawn. The applicant presented the following grounds for re-examination:

The applicant emphasises that for the treatment indication, efficacy data from controlled trials show positive effects of defibrotide and this is supported by data from other sources. The safety profile of defibrotide has been well characterised using a large database given the orphan indication. The identified risk of bleeding can be very effectively managed in the specialist centres where defibrotide will be prescribed and the clinicians are very confident in their ability to administer the drug safely.

In summary, for defibrotide, there is a large database of clinical experience and the applicant has provided the CHMP with as full information as can be obtained not only from formal controlled

clinical trials but also from an on-going treatment IND (2006-05), an independent US registry, additional studies, the literature, and the compassionate use program. Overall the balance of Benefit/Risk is favourable in these very small populations.

The applicant addresses specifically the CHMP's grounds for refusal as follows:

Ground 1

One open label pivotal trial was provided in support of the prevention indication.

1.a The statistical significance of the results was not sufficiently convincing in the context of a single pivotal trial.

1.b Critical and major GCP non-compliances relating to the documentation and reporting of efficacy

1.c the lack of clarity in the protocol and the differences in interpretation of the protocol at the two sites inspected raise doubts as to the reliability and generalizability of the data.

1.a Statistical significance of the results was not sufficiently convincing in the context of a single pivotal trial

Consistent with the submitted evidence, the Applicant points out that the prevention study (2004-000592-33) was conducted exclusively in the paediatric population as these children are considered to be at higher risk of developing VOD than the adult population. This study is the largest study ever performed for this orphan disease in the paediatric HSCT population. The study protocol was jointly developed with the Paediatric Working Party of the European Group for Blood and Marrow Transplantation (EBMT) and the EBMT co-sponsored the trial. The study is a controlled, multicentre, prospective, randomised 1:1 study, comparing prophylaxis with defibrotide versus no treatment (control). Rescue medication was provided only after the primary endpoint (development of VOD) was assessed so this did not impact the interpretation of the study primary results. However, in the Applicants' view, the analysis of secondary and exploratory endpoints evaluating differences between the subsets of patients who developed VOD in the different treatment groups must be interpreted with caution because secondary endpoints compared two strategies, defibrotide prophylaxis plus defibrotide treatment versus no defibrotide prophylaxis plus defibrotide treatment.

An Independent Review Committee (IRC) comprising independent clinical experts, who were not involved with the conduct of the study, was tasked with adjudicating diagnoses, thus ensuring independence and reliability of the results. The IRC conducted its review of patient data in a blinded fashion without knowledge of which treatment group the patients were allocated to. The study enrolled 356 patients from 28 paediatric HSCT centres in 11 countries and took nearly four years to complete.

The applicant reiterates that the study met its pre-specified primary efficacy endpoint in the intention-to-treat (ITT) population demonstrating that defibrotide prophylaxis significantly reduced the incidence of VOD with a conventionally accepted two-sided alpha p-value <0.05. The size of reduction from 20% in the control arm (35/176) to 12% (22/180) in the defibrotide arm, represents a relative reduction of 40% in the incidence of VOD. In addition to the primary analysis of VOD incidence by Day+30, secondary analyses from this study are consistent and support the conclusion of the primary endpoint. The percentage of patients developing severe VOD was 6% in the

defibrotide prophylaxis arm and 10% in the control arm and the pattern of organ failure shows a benefit for defibrotide prophylaxis.

1.b Critical and major GCP non-compliances relating to the documentation and reporting of efficacy

The Applicant's view is that many of the GCP findings reflect some confusion about how the diagnosis of VOD is made. The diagnosis of VOD is in fact well-understood and clearly described. The sites involved with this study were all registered by the EBMT and this group has issued clear guidance for the 2-stage differential diagnosis of VOD.

Regarding the GCP findings related to VOD diagnosis (clinical judgment versus ultrasound results), the Applicants' view is that there is a misunderstanding of how the diagnosis is made and the role of ultrasound in the diagnostic process. Ultrasonography assessments are not considered the basis of diagnosis of VOD but in some cases supportive and useful for confirmatory purposes.

Regarding the lack of unified set of objective rules in diagnosing VOD, the Applicant accepts the fact that ideally some of these aspects (e.g., baseline time point from which the weight change was to be calculated) could have been defined in the study protocol, but the Applicant understood that there are internationally accepted guidelines to diagnose VOD as reflected in the EBMT Handbook.

Other GCP concern relating to the open label nature of the study included the possibility that investigators were more inclined to start defibrotide treatment at the first sign of VOD for the control group because of the fear of disease progression and that investigators could be more sanguine with the prophylaxis arm because subjects were on defibrotide and as a result be less likely to report mild or suspect VOD cases. The Applicant's position was that these concerns were not based on evidence that subjects in the control group who were referred to the Independent Review Committee for adjudication of the VOD diagnosis did not have a higher proportion of 'mild VOD' reported and that VOD cases in the control group were more severe than those who received defibrotide prophylaxis.

The applicant has provided information as to whether the GCP findings had an impact on the study results. The Independent Review Committees (IRC), a group of specialists blinded to treatment group and the investigator's final view of whether VOD was present or not, added a layer of independent adjudication to the primary endpoint of VOD diagnosis. In addition to all cases where the investigator diagnosed VOD, the IRC was sent all cases where the patient received 'treatment defibrotide' as that implied a provisional diagnosis of VOD, and so all these cases were also fully reviewed by the IRC.

Since some concerns remain about the IRC adjudication step, the Applicant has performed various sensitivity analyses. The sensitivity analyses set higher thresholds than the modified Seattle criteria as used in the protocol, so only the more serious cases of VOD would be included i.e., Seattle criteria with hyperbilirubinemia (comparable with Baltimore criteria), Seattle criteria with MOF, or Seattle criteria with both hyperbilirubinemia and MOF. The results are consistent whether the Seattle criteria are used, or alternative criteria that set a higher threshold of disease severity. Whichever criteria are used, a consistent relationship between defibrotide prophylaxis and control is observed, with defibrotide use consistently conferring at least a 40% reduction in the risk of developing VOD at any given threshold of severity.

In addition to these sensitivity analyses, a new Expert Panel was convened by the Applicant to review data from this study. This new group of experts reviewed a sample of approximately 10% of

the patient's data from the study. The new Expert Panel concluded that the issues identified in the study would not have affected the reliability of the results.

1.c the lack of clarity in the protocol and the differences in interpretation of the protocol at the two sites inspected raise doubts as to the reliability and generalizability of the data.

Since the GCP inspectors observed the possible differences between sites and raised concerns about the 'reliability and generalisability' of the data, the applicant generated Forest plots with 95% CIs (by centre, by country and by baseline prognostic factors). There is a considerable overlap across the centres and the observed centre differences are consistent with chance. The Forest plots have been based on differences in the incidence rates for VOD (not odds ratios) since in some sites there was no VOD event in the treatment group.

Forest plots include CIs have also been generated grouping the sites by country to investigate country differences (Austria, Czech Republic, France, Germany, Great Britain, Ireland, Israel, Italy, Netherlands, and Sweden). No events were observed for any of the sites in Switzerland and that country has therefore been omitted from this analysis. In addition, Forest plots have been generated by presence of background risk factors together with p-values for interaction. These analyses support the presence of a consistent treatment effect across the various subgroups defined by baseline factors. There is no evidence from these analyses that the treatment effect lacks consistency.

Taken together these analyses comparing the treatment effect across centres, across countries and for different levels of the key baseline prognostic factors strongly support a consistent treatment difference for the population as a whole and the external validity of the trial.

An apparent survival benefit was seen for patients developing VOD following defibrotide prophylaxis and for patients given immediate treatment with defibrotide upon the diagnosis of VOD, compared with waiting until VOD is established.

Regarding the 'generalisability' of the data, it is acknowledged that the initial indication statement, 'prevention of VOD', was inclusive of all patients and this study was performed only in those at high risk of developing VOD. The Applicant proposed to modify the indication statement "*… in high risk patients in haematopoietic stem-cell transplantation therapy (HSCT)*".

Ground 2

One open label pivotal trial was provided in support of the treatment indication.

2.a The historical control group was not considered a suitable control arm in view of the small size.
2.b The timeframe of recruitment and the amendment to the historical control group where 54 subjects were removed.
2.c The lack of transparency in the processes and decision making relating to the amendment of the historical control group raise further uncertainty as to the reliability of the final 32 subjects chosen.
2.a The historical control group was not considered a suitable control arm in view of the small size.

This orphan indication of severe VOD associated with multi organ failure is applicable to fewer than 1000 patients per year in the EU. There was an ethical problem with conducting a placebo-controlled study in this indication because of the risk of death if severe VOD is left untreated. This pivotal study was conducted in adult and paediatric patients suffering from severe

VOD with multi organ failure. The applicant accepts that the historical control group was not ideal but it was considered the best option conducting a study in this life-threatening disease. The primary efficacy endpoint was the complete response of severe VOD (defined as total bilirubin below 2 mg/dL (34 µmol/L) and resolution of all multi organ failure). A key secondary endpoint was Day+100 mortality. Mortality rate in the 2005-01 HC group was 75.0% at Day+100 post HSCT. This was consistent with the available literature. One hundred and two patients suffering from severe VOD with multi organ failure were recruited to the Treatment Group (TG) and 32 to the Historical Control (HC) group. The study showed a significant difference in the primary efficacy endpoint (Complete Response, CR) in favour of the defibrotide-treated patients. A total of 23.5% of patients in the defibrotide treatment group achieved CR compared with only 9.4% in the HC group (p=0.0131). Mortality in the defibrotide TG compared with the HC group was decreased; 38% of the TG were alive at Day+100 *vs* 25% of the HC group.

Whilst the use of an historical control in this indication has been accepted, the validity of the actual HC group used has been questioned. The patients included in the HC arm were selected by an independent Medical Review Committee (MRC) who were kept blinded to the outcome. The finally agreed HC group was comprised of 32 patients. Although small, this percentage of the total HSCT patients finally included in the HC (0.5%) was similar to the percentage of patients screened and finally included in the defibrotide treatment group (0.9%).

2.b The timeframe of recruitment and the amendment to the historical control group where 54 subjects were removed.

Regarding the timeframe of recruitment there is a concern related to the fact that the HC arm included patients transplanted between 1995 and 2006 and the dataset was considered rather old. The Applicant has pointed out that the majority of the HC patients (21/32) were recruited from the year 2000 onwards. Some concern was raised that in the refinement of the HC group from 86 to 32 patients, the more historic patients data may have been included preferentially and that these patients might have been expected to have a worse outcome. The mortality rate of the HC used in the 2005-01 study (75.0% at Day+100 post HSCT) is consistent with the literature, which provides an additional external validation for its use as a control in this study.

Regarding the amendment to the historical control group, the Applicant has provided further information on the fact that the 54 patients not included in the HC group were removed because the MRC (blinded to patient outcome), upon review, considered that the signs/symptoms evident in these patients were more likely due to an alternate aetiology, and not due to VOD-associated MOF, or because they met an exclusion criterion for the study. The stringency applied by the MRC in reaching an unequivocal diagnosis of severe VOD was to ensure they underwent a similar level of stringency to that applied to the TG patients in the clinic. It became apparent during the study that once potential TG patients were identified, the TG investigators performed differential diagnosis in line with internationally accepted standard practices in order to confirm the diagnosis of VOD with associated MOF. This standard clinical approach was in line with the EBMT guideline. These guidelines require the exclusion of alternate aetiologies that might have caused the patients' signs and symptoms. Therefore it was necessary to request that the independent MRC follow the same approach for the identification of the HC patients as well.

In summary, the approach taken to include patients into the TG and HC groups with VOD was equally stringent in both arms.

In the HC group, similarly the MRC were allowed to request information from the patient notes for a few days after they met the initial inclusion criteria for the study so that the MRC could review the possibility that other aetiologies were the cause of the signs and symptoms.

It is of crucial importance to note that the MRC were blinded to patient outcome when they performed their decision making to include/not include patients in the HC Group.

2.c The lack of transparency in the processes and decision making relating to the amendment of the historical control group raise further uncertainty as to the reliability of the final 32 subjects chosen.

This concern relates to the GCP findings on the process for inclusion of the patients into the HC group. The physician's clinical diagnoses involve a 2-step process with inclusion criteria which must be met followed by exclusion of signs and symptoms caused by alternative aetiologies prior to diagnosing VOD.

It is worth reiterating that the independent MRC, composed of leading haematologists, was blinded to patient outcome throughout the entire process when they conducted their deliberations about whether patients should be included in the HC group. During the initial reviews by MRC, the committee gathered all patients who met the inclusion criteria for the diagnosis of VOD. The second step was the exclusion, by differential diagnosis, of those patients with other conditions. Within the clinics treating the VOD patients in the TG, the second step of the exclusion criteria for VOD and/or MOF associated with VOD was applied extremely thoroughly and excluded patients unless the diagnosis was unequivocal i.e. VOD with MOF associated with the VOD as opposed to any other possible aetiologies. Equal rigor was applied in selecting the HC patients.

In addition, the reasons for non-inclusion of every patient were clearly listed in the MRC documentation. The Applicant acknowledged that not every diagnostic consideration was recorded, however the MRC document provide details on non-inclusion patients. In order to know the Impact of GCP Findings on Results, two approaches have been taken by the Applicant. The first one is a comparison of the baseline characteristics of the HC group *vs* the TG. The second approach was the performance of sensitivity analyses to test the robustness of the results when different inclusion criteria are applied.

Regarding the comparison of the baseline characteristics of the HC group *vs* the TG, the results show no clinically meaningful differences in the distribution of these factors between the 32 HC patients, and the TG, and support that the HC and the TG were well-balanced and comparable.

In connection with the second approach to know the impact of the GCP findings on the results, the Applicant has performed three sensitivity analyses to evaluate the robustness of the data. These sensitivity analyses address concerns (a) about the amount of time taken for diagnosis of VOD with MOF in the 2 groups and (b) to evaluate whether applying a greater level of stringency to the TG would have made a material difference to the results.

Regarding the time taken to make a diagnosis, concerns were raised about the time taken to determine the diagnosis of VOD and the question was asked whether the time taken in the HC meant that there was a greater stringency applied to the HC than the TG.

In fact, for both the HC and the TG, there was a very similar pattern in the period of time taken to determine a diagnosis of VOD. In general, a diagnosis of VOD can be made for the majority of patients within 2 days; this was the case for 81% of the patients in the HC and 78% of patients in the TG. Because the assessor raised doubts about the accuracy of the diagnosis of patients for

whom determination of VOD took longer than 2 days, the applicant has performed a sensitivity analysis including only those patients in whom a diagnosis of VOD was confirmed within 2 days. In this population, 27.5% (22/80) TG patients were complete responders compared with 11.5% (3/26) in the HC group, with a p value of 0.0076. Regarding survival, 38.8% (31/80) of TG patients survived compared with 23.1% (6/26) of patients in the HC Group, with a p value of 0.0345. Therefore, if the applicant had excluded patients for whom definitive diagnosis took more than 2 days, this would have made no difference qualitatively to the conclusions.

Applying a greater level of stringency to the TG, two sensitivity analyses have been undertaken. The first excludes patients who were, after recruitment, considered to be protocol violators and the second excludes patients in the TG that according to the GCP inspectors may not have met an unequivocal diagnosis of VOD with associated MOF.

There were 4 patients in the TG who were eventually considered to be violators. Since none of these 4 TG patients were complete responders, if the applicant had excluded these patients from the TG, this would only have led to a greater difference in favour of defibrotide in CR rate between the TG and the HC group; instead of 23.5% (24/102) TG patients being complete responders compared with 9.4% (3/32) in the HC group, the revised figures would be 24.5%(24/98) *versus* 9.4%, with a p-value of 0.0117 compared with the p-value for the whole group of 0.0131. Regarding mortality, instead of 61.8% (63/102) TG patients dying compared with 75.0% (24/32) in HC Group, the revised figures would be 60.2% (59/98) *versus* 75.0% with a p-value of 0.0268 compared with a p-value for the ITT group of 0.0341.

There were 8 patients in the TG that the GCP inspectors quoted as in their view, not meeting an unequivocal diagnosis of VOD with associated MOF (6 who were taking nephrotoxic drugs where the renal failure might possibly have been considered due to the drugs and not the VOD and 2 who had pulmonary infections that were considered by the GCP inspectors to possibly have been the underlying cause of the pulmonary failure). Notwithstanding that the applicant would strongly argue that the treating investigators are best placed to evaluate a diagnosis of VOD, especially since the investigators would have been able to amend the use of the drugs and review other aspects of infection and take an overall view of the condition of the patient, the applicant has evaluated the impact of exclusion of these patients. Once again, the results were unaffected. For Complete Response, instead of 23.5% (24/102) TG patients being complete responders compared with 9.4% (3/32) in the HC group, the revised figure would be 24.5% (23/94) compared with 9.4%, with a p-value of 0.0110 compared with the p-value for the whole group of 0.0131. Regarding survival, instead of 38.2% (39/102) TG patients alive at Day+100 compared with 25.0% (8/32) in HC Group, the revised figures would be 38.3% (36/94) *vs* 25.0% (8/32) with a p-value of 0.0432 compared with a p-value for the whole group of 0.0341.

In summary, there is no evidence that the HC patients were not well balanced and comparable with the TG patients. The mortality of the HC group (75.0%) is in fact slightly lower than predicted by the literature for this life-threatening disease (>84.0%; Coppell, 2010). The stringent review process ensured that only patients with an unequivocal diagnosis of severe VOD with multi organ failure were recruited to the HC group to allow appropriate comparison with the TG.

5.2. Additional expert consultation- Report from the Ad hoc expert group meeting

Following a request from the applicant at the time of the re-examination, the CHMP convened an Ad hoc Expert Group inviting the experts, including a patient representative, to provide their views on the questions posed by the CHMP, taking into account the applicant's response to the grounds for refusal.

5.2.1. Prevention indication

1.a. Considering the methodological and GCP weaknesses as pointed out by the inspectors (single pivotal study, open-label, allowance of defibrotide as rescue for control patients, potential differences in the work-up for establishing the VOD diagnosis among centres etc.) do you feel that the fact that the study met its pre-specified primary efficacy endpoint in the intention-to-treat (ITT) population demonstrating that defibrotide prophylaxis significantly reduced the incidence of VOD with a conventionally accepted two-sided alpha p-value <0.05, represents a sufficient demonstration of clinical efficacy?

b. Considering the methodological and GCP weaknesses, is the demonstration of benefit convincing by itself or of any support to the treatment study?

c. An incidence of 20% in the control arm (35/176) was reduced to 12% (22/180) in the defibrotide arm (p=0.0488). Is the difference clinically meaningful?

The experts were of the opinion that, although a 40% reduction in incidence of VOD is considered clinically meaningful, the results are not sufficiently robust to demonstrate clinical efficacy in the prevention indication and should rather be viewed as a signal. The experts noted that there is a significant number of study confounders and that the methodological issues remain the main weaknesses of the study and do not allow for a positive conclusion. Furthermore, the pivotal study did not demonstrate any survival advantage for the defibrotide group. There was also concern regarding a safety signal of increased death in patients who had been given defibrotide as prophylaxis and not developed VOD when compared with patients not given prophylaxis who did not develop VOD.

The group agreed that in general, the GCP findings are considered less of a concern regarding the interpretation of the outcome of the study; nevertheless the study results are on their own not convincing enough for the prophylaxis indication. However, the group agreed that the results of those who were treated for VOD were consistent with the results of the treatment study. The experts were of the view that only by performing further studies would there be a sufficient level of evidence available on which to conclude on a beneficial effect of DF in the prophylaxis of VOD.

5.2.2. Treatment indication

2. Considering the issues raised against the screening for identification of an adequate historical control (HC) group that ended with 32 patients with unequivocal VOD diagnosis would it be possible to look at the data as the results of a simple phase II study and assess clinical benefit on a VOD CR rate at D100 of 24%.

The experts agreed that looking at the data as a simple phase II trial would not be adequate. The VOD CR rate at D100 is not an appropriate endpoint for this indication and should be considered a surrogate one as it does not allow for an assessment of overall clinical benefit. In their view, the applicant could have chosen a more appropriate end point (i.e: overall survival).

3. If the answer to question 1 is no, do you think that the applicant has applied sufficient meticulous diagnostic criteria (blinded MRC, strict application of the Seattle Criteria as first step, and strict exclusion of other aetiologies) to convince you that the Historical Control group albeit small represents a sufficient external control.

The experts noted the fact that a high number of patients (54 out of 86) had been excluded from the control group due to alternative aetiologies, which resulted in a very small final HC group comprising 32 patients in total. It was further noted that the trial did not meet the pre-specified significance level of 1% due to the small patient numbers included, and that only a trend in favour of DF had been shown. However, the experts were of the view that the HC group, although small, was appropriately chosen and could be accepted as comparable to the treatment group and the best available control group in the clinical trial setting. Thus, it was considered that the historical control group chosen represents an appropriate external control. Based on this, the experts supported the rapporteur's view on the treatment indication.

4. Do you consider peer-reviewed literature and the independent US REGISTRY data supportive of treatment benefit or should such data be dismissed in the drug approval procedure.

The expert group agreed with the fact that independent registry data can be considered supportive of a benefit in the treatment of VOD. Overall, the expert group agreed that independent registry data can be useful if the study endpoints are clearly defined.

In the experts' views, recent registry data indicate that patients who do not receive DF still have a high mortality rate. In addition the US REGISTRY data support the fact that DF increases the VOD resolution rate and reduces the mortality rate.

Furthermore, the group agreed that peer review literature is also considered generally supportive of the treatment indication for DF, although less so than independent registry data in view of the frequent methodological limitations observed.

5.3. Additional information provided by the applicant

During the Oral Explanation on 22 July 2013, the applicant provided additional clarification on the following issues:

1. The 'treatment study' is formally a failed study if starting pre-planned rules are considered: the risk was set at a value of 0.01 to take into account the relatively low robustness of the study design. Alternative and simple statistical analyses further decrease the global robustness of an efficacy conclusion (24/102 vs 3/32, hi-square test p = 0.0816; Fisher's test p = 0.1275). Please discuss (i) the use and validity of a stratified analysis on a propensity score, as opposed to a simpler analysis, (ii) the discrepancies between the possible statistical analyses and (iii) the overall statistical reliability of the efficacy conclusion in the treatment indication.
2. Please provide further justification for the use of other external (historical) control data in support of the small HC group (CIBMT data, US treatment-IND study, others).

5.4. Discussion on grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant and considered the views of the ad hoc expert group meeting held on 8 July 2013.

Ground 1

The CHMP noted that the difference between the active arm (20%) and control arm (12%), i.e., 8%, for the primary efficacy endpoint is only of borderline statistical significance: p = 0.0488 and thus cannot be considered as providing the compelling scientific evidence that would be required in the context of a single pivotal trial for the prevention indication.

The lack of robustness of the results is illustrated by the results of the sensitivity analyses which fail to confirm significance: p = 0.0507 for Analysis A2 (Kaplan Meier, available data), p = 0.0644 for Analysis B (Multiple imputation) and p = 0.1937 for Analysis C (Worst case).

Moreover, this study presents several methodological weaknesses:

- Open label study: this is a serious limitation for a single pivotal study where the primary endpoint is not as clearly objective as a single centralised laboratory parameter or mortality would be.

- The contribution of the independent review panel is limited to review cases only for whom a diagnosis of VOD was made by the investigator. This restriction weakens the IRC contribution to the study.

- No clear advantage in overall survival at Day 180.

Regarding the problems associated with the trial design and conduct, the results on VOD at day 30 cannot be considered as sufficient to clearly demonstrate a beneficial effect from defibrotide.

The CHMP is of the opinion that although the study has met its pre-specified primary endpoint, despite the size of the study the results are not considered sufficiently convincing because of methodological and GCP wweaknesses (single pivotal study, open-label, allowance of defibrotide as rescue for control patients, potential differences in the work-up for establishing the VOD diagnosis among centers).

The treatment difference is small in absolute terms and may be of borderline significance. The overall borderline result for the primary endpoint makes any subgroup or sensitivity analysis difficult to interpret. The results for the secondary endpoints give little support because of the allowance of defibrotide as rescue in control patients. The CHMP therefore maintain the view that efficacy of defibrotide in the prevention of VOD is not established.

The proposed restriction of indication to high risk patients would not lift the doubts about generalisability of the data to real life even though a smaller target population may be more representative of the inclusion population of the study (i.e., high risk patients).

During the re-examination procedure, at the oral explanation held on the 22 July 2013, the applicant withdrew the prevention indication, limiting the claimed indication of defibrotide only to the treatment population.

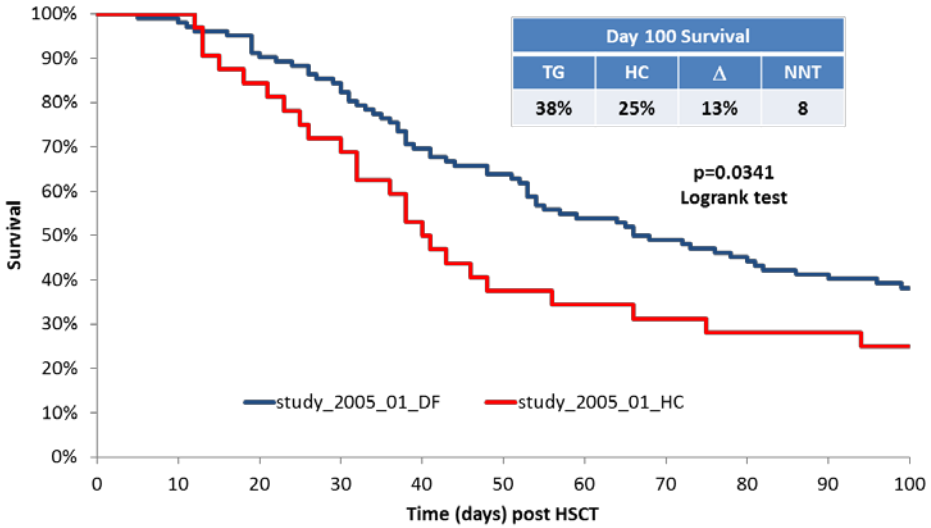
Ground 2

For the treatment indication the applicant provided data from study 2005-01, a phase II study including 102 patients with severe VOD/MOF showing a CR rate by Day 100 of 24% and a mortality rate by Day 100 of 62%. Due to the uncontrolled nature of the study some type of external control is necessary as a control group of only 32 patients is too limited and cannot stand alone. The controversial issue was the removal of 54/86 patients due to alternative aetiologies resulting in a very small final HC group of 32 patients. The applicant has provided the reasons given by the MRC for patients' exclusions and have compared the reasons with exclusion/inclusion criteria of the treatment protocol. The CHMP's view is that the MRC did not preferentially select patients with a particular poor outcome for the HC group. In addition, the reasons for not including the 54 patients appear to be sufficiently documented. The exclusion of 10/86 patients due to insufficient record data is not an issue considering the inherent problems with the use of retrospective clinical data.

It is acknowledged that a placebo-controlled study is not feasible. Therefore, the CHMP's view is that the results in the treatment group in terms of percentage of patients who have complete resolution of severe VOD by Day 100 post SCT and complete resolution of multi organ failure (MOF) as well as survival rate Day 100 should either be evaluated on their own merits or compared with an adequate external historical control. In other words, since there are concerns about the nature of the HC group and running a controlled study in this indication is not possible, it is appropriate to consider additional sources of data that could support study control data provided by the Applicant.

Registry data have been the major driver in the advances of allogeneic HSCT in the absence of randomised clinical trials (conditioning regimens, anti-GVHD prophylaxis and therapies, infection prophylaxis). The CHMP's view is that there appears to be a treatment effect in severe VOD and the high mortality for severe VOD from the literature and the finally chosen historical control group suggest that DF is of use. The mortality of sVOD is reported to be in excess of 85% without DF treatment.

Figure 1: Study 2005-01: Kaplan-Meier Survival Curves for Defibrotide TG and HC Patients



The CHMP also agrees that the mortality of patients with severe VOD/MOF using supportive therapy without defibrotide has remained quite constant over two decades despite other therapeutic improvements for patients undergoing HSCT and that the mortality rate of 62% compares favourably with the expected mortality rate of 75-85% .

The US treatment-IND study (2006-05) is an open study treating patients with VOD in US and the results were presented at ASH 2012. These results also indicate a reduction in Day 100 mortality as compared to the expected 75-85%.

The Applicant also provided additional data analysis from a US Registry as an external comparator (2008-2011 timeframe). All data analyses were performed by the US Registry statistical group in Wisconsin directly from the Registry dataset. The applicant provided analyses of sVOD patients (Baltimore criteria + MOF) treated and not treated with defibrotide.

The number of patients with comprehensive records in the transplant registry was 8341, and of these, 275 were reported to have VOD (3.2%), with 101 [*Note: Of the 101 patients, 2 were excluded from further analysis because it is unknown whether they received any treatment; 3 patients were excluded because it is unknown whether they received defibrotide or standard of care, so in the sVOD group 96 records could be evaluated for outcomes*] patients meeting the definition of severe VOD (1.2%). This confirms the rarity of the condition and is also a similarly small proportion to that included in the TG and HC groups of the 2005-01 study.

In patients who were reported not to have received any specific interventions for their VOD, a mortality rate of 78% showed that despite any improvements in supportive care, the mortality rate remains very high with this disease. This US Registry data could provide an estimate of the effect size that can be expected in the clinic under real life conditions. Day+100 survival of patients treated with standard of care (SOC) plus defibrotide was 39% compared with 31% for patients treated with SOC alone. VOD resolution occurred in 51% of the patients treated with SOC plus defibrotide compared with 29% of patients treated with SOC alone.

The following endpoints of relevance in the treatment of sVOD were presented in 2 sub-groups; those receiving/not receiving defibrotide with no other treatments, and those receiving/not receiving defibrotide with standard of care treatments (such as diuretics, steroids, Ursodiol, etc.):

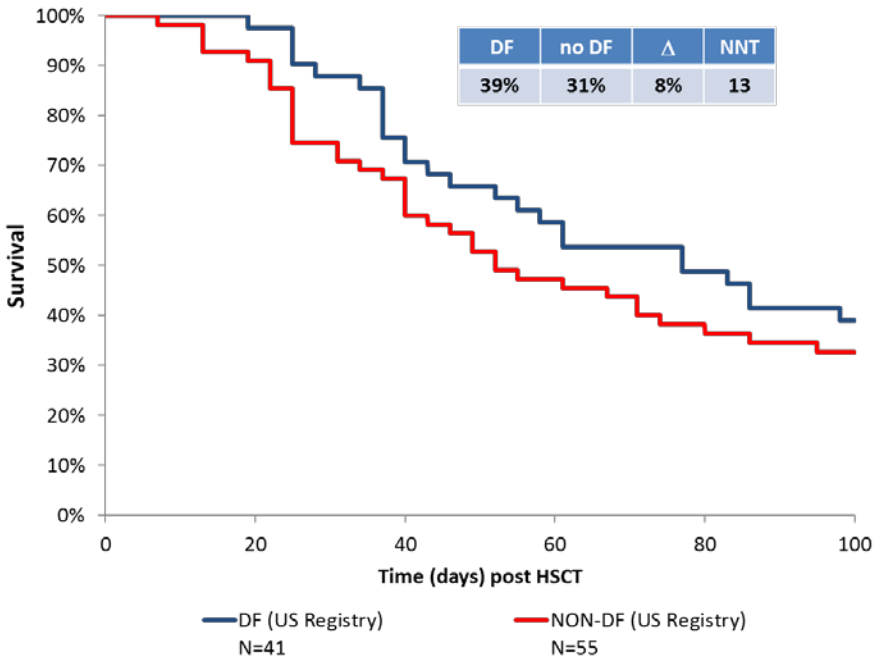
Survival by Day +100 post HSCT;

VOD resolution by Day +100 post HSCT (yes/no).

Table 1 Sub-group with standard of care (US REGISTRY data)

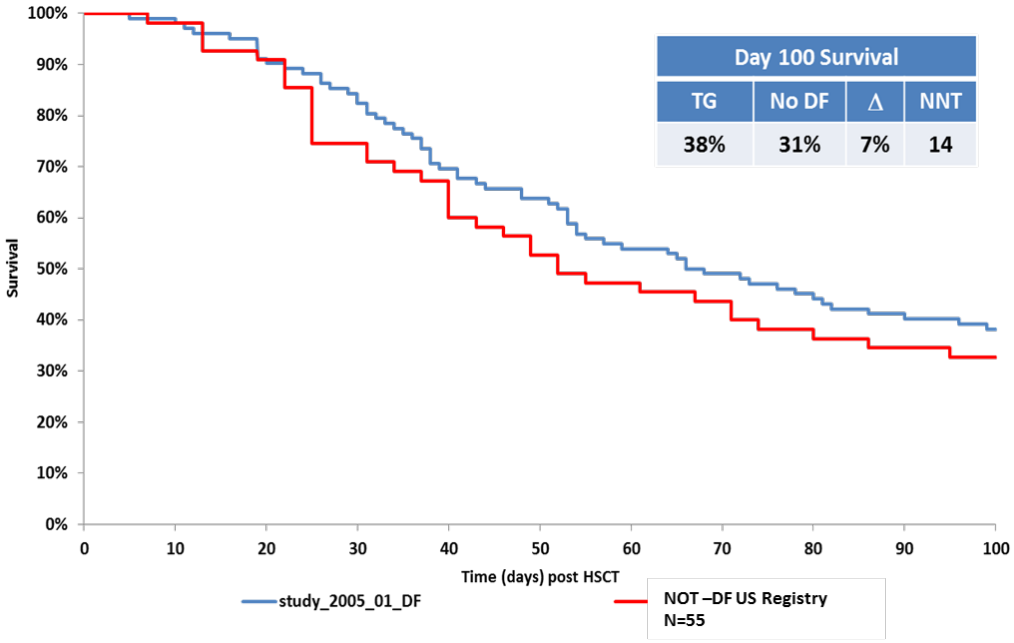
Preliminary Analyses of endpoints	**non-DF treated**	**DF treated**
	55	**41**
Alive at Day +100	17 (31%)	16 (39%)
Dead at Day +100	38 (69%)	25 (61%)
VOD resolved by Day +100	16 (29%)	21 (51%)
VOD not resolved by Day +100	39 (71%)	20 (49%)
Patients alive at last contact	4 (7%)	10 (24%)
Dead	51 (93%)	31 (76%)

Figure 2: US REGISTRY Data: Defibrotide Treatment Effect on Survival At Day+100 in Severe VOD.



Taking the most conservative comparison i.e., between the TG from study 2005-01 and the US REGISTRY non-defibrotide treated group, there is an absolute 7% benefit in favour of defibrotide, giving a Number Needed to Treat (NNT) of 14 to save one life (Figure 3). The data from the US REGISTRY registry substantiate the clinical benefit seen for defibrotide in the 2005-01 study. Taking all the data into consideration, the weight of the evidence suggest a survival benefit for defibrotide in the treatment of severe VOD.

Figure 3: 2005-01 Treatment Group vs US REGISTRY no Defibrotide



The only treatment trial where there was a concurrent control group for VOD (mixed VOD ~50% severe) was DF-VOD. Although the trial was terminated due to slow enrolment and as a result only 34 subjects were included in each arm, the interim analysis results were on randomised patients only (twelve patients were included in the observational registry at time of the interim analysis but were not included in the results).

Only primary endpoints were analysed in the interim analysis. Eleven patients (32.4%) of the Defibrotide group and 15 patients (44.1%) of the Control group were considered to have had a Complete Remission (Complete Response) by Day +100. Twenty-one patients (61.8%) of the Defibrotide group and 20 patients (58.8%) of the Control died by Day+100. No trend for a beneficial effect from defibrotide in either CR or mortality was seen. While this randomised study failed to show a benefit, it has limited numbers of subjects and based on the totality of the data, including the ongoing IND and the US REGISTRY data, CHMP considered that sufficient supportive evidence has been provided in support of the revised treatment indication.

In conclusion, following the assessment of the detailed grounds for refusal provided by the applicant, and in view of the revised indication proposed, the CHMP is of the view that the benefit risk balance of the above mentioned medicinal product can be considered positive in the treatment indication on the following grounds:

- The HC group cannot stand alone but it is accepted that even recent registry data indicate that patients who do not receive defibrotide still have a very high mortality rate;
- The US treatment IND- study (2006-05) although uncontrolled indicate a beneficial effect on mortality of defibrotide;
- The US REGISTRY data support that defibrotide increases the VOD resolution rate and reduces the mortality rate;
- External control data is the only possible comparison.

5.5. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

5.6. Risk Management Plan

Based on the review of the Risk Management Plan version 1.0, the CHMP considers by majority decision that the risk management system for defibrotide (Defitelio) in the treatment of severe hepatic veno-occlusive disease in haematopoietic stem-cell transplantation therapy in adults, adolescents, children and infants over 1 month of age is acceptable. The following points should be taken into account in the next update.

1. The MAA decided to submit the RMP in the new format; however, not all aspects of the new format are entirely compliant with the guidelines. For instance, references are missing, description of the epidemiology is incomplete and Table V.3 is not in the correct format. This needs to be corrected.
2. Throughout the document, there are instances where frequency measures are provided as percentages and referred to as incidence rates. This is incorrect and needs to be amended.
3. Reference throughout the RMP to measuring the impact of risk minimisation through '*monitoring of incidence reported from the market*' is insufficiently clear and this needs to be made more specific.

Advice on conditions of the marketing authorisation

The CHMP advises that the following should be conditions of the Marketing Authorisation:

Risk management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;

- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

The CHMP considers that no additional risk minimisation measures will be necessary for the safe and effective use of the medicinal product.

Obligation to conduct post-authorisation measures

The CHMP recommends that a study to investigate all safety concerns identified in the RMP should be a condition of the MA.

The CHMP recommends that this should take the form of a non-interventional (observational) study, namely a patient registry.

6. Assessment Overview of the content of the RMP

6.1. Safety concerns

The applicant identified the following safety concerns in the RMP:

Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	Haemorrhage (including, but not limited to, gastrointestinal haemorrhage, pulmonary haemorrhage and epistaxis)
	Hypotension
	Coagulopathy
	Immunogenicity (Allergic/Hypersensitivity reactions)
Important potential risks	Injection site reactions/infections/septicaemia
	Thromboembolic events
	Immunogenicity (Generation of Anti-Nuclear Antibodies)
	Reproductive toxicity
Important missing information	Pregnant or lactating women
	Patients treated concomitantly with defibrotide and medications that increase the risk of haemorrhage (including the newer oral anti-coagulants direct thrombin and factor Xa

Summary of safety concerns	
	inhibitors)
	Patients with grade B-D GVHD
	Patients with pre-existing liver or severe renal insufficiency (aetiologies other than VOD)
	Patients with intrinsic lung disease
	Patients with ethnic background other than Caucasian
	Patients over the age of 65 years
	Off-label use

The CHMP agreed.

Table of on-going and planned studies in the Post-authorisation Pharmacovigilance Development Plan

Study / activity Type, title and category (1-3)	**Objectives**	**Safety concerns addressed**	**Status (planned, started)**	**Date for submission of interim or final reports (planned or actual)**
Renal PK Study: Dialysis Study: A Phase 1, Open-label Study to Investigate the Effect of Haemodialysis on Plasma Defibrotide Pharmacokinetics in End-stage Renal Disease Patients Main Study: A Phase 1, Open-label Study to Investigate the Pharmacokinetics of Defibrotide Administered to Severe and	To investigate the pharmacokinetic profile of defibrotide in renal-impaired patients	Use of defibrotide in patients with renal impairment	Planned	Q1 2015 final report

Study / activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
End-stage Renal Disease Patients not on Dialysis Compared to Healthy Matching Subjects Category 3				
A multi-centre, multinational, prospective observational registry to collect safety and outcome data in patients diagnosed severe hepatic VOD following hematopoietic stem cell transplantation (HSCT) and treated with Defitelio or supportive care (control group). Category 2	To investigate the safety and outcome of patients with severe VOD treated with defibrotide during normal marketing use	All known and potential risks and data for missing information	Planning	Annual reports submitted within the annual reassessment. Registry to remain throughout the product's life cycle
Defibrotide for patients with hepatic veno-occlusive disease (VOD); A Treatment IND study (under CFR 312.34). Category 3	Collection of safety data	All safety concerns	Ongoing	Interim report: yearly in the PSUR. Final report: one month after NDA approval.

VI.1.3 Summary of Post authorisation efficacy development plan

Not applicable.

VI.1.4 Summary table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Identified Risks		
Haemorrhage (including, but not limited to, gastrointestinal haemorrhage, pulmonary haemorrhage and epistaxis)	Routine risk minimisation SmPC: **4.3 Contraindications** Concomitant use of thrombolytic therapy (e.g. t-PA) (see section 4.5) **4.4 Special warnings and precautions for use** Use of medicinal products that increase the risk of haemorrhage within 24 hours of Defitelio administration (within 12 hours in the case of unfractionated heparin) is not recommended. Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors) (see section 4.5), except for routine maintenance or reopening of central venous line, requires careful monitoring. Consideration should be given to discontinuation of Defitelio during use of such therapy. Medicinal products that affect platelet aggregation (e.g. non–steroidal anti-inflammatory agents) should be administered with care, under close medical supervision, during Defitelio administration. In patients who have or develop clinically significant acute bleeding requiring blood	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	transfusion, Defitelio is not recommended or should be discontinued. Temporary discontinuation of Defitelio is recommended in patients who undergo surgery or invasive procedures at significant risk of major bleeding. **4.5 Interaction with other Medicinal Products and Other Forms of Interaction** In a mouse model of thromboembolism, recombinant t-PA potentiated the antithrombotic effect of defibrotide when given intravenously and thus co-administration may present an increased risk of haemorrhage and is contraindicated (see section 4.3). **Section 4.8 Undesirable Effects** Mentions haemorrhage as well as specific organ haemorrhages as ADRs.	
Hypotension	Routine risk minimisation measures SmPC: **4.4 Special warnings and precautions for use** Administration of Defitelio to patients who have haemodynamic instability, defined as inability to maintain mean arterial pressure with single pressor support, is not recommended. **Section 4.8 Undesirable Effects**	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	Hypotension is included as an ADR	
Coagulopathy	Routine risk minimisation measures SmPC: **4.3 Contraindications** Concomitant use of thrombolytic therapy (e.g. t-PA) (see section 4.5) **4.4 Special warnings and precautions for use** Use of medicinal products that increase the risk of haemorrhage within 24 hours of Defitelio administration (within 12 hours in the case of unfractionated heparin) is not recommended. Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors) (see section 4.5), except for routine maintenance or reopening of central venous line, requires careful monitoring. Consideration should be given to discontinuation of Defitelio during use of such therapy. Medicinal products that affect platelet aggregation (e.g. non–steroidal anti-inflammatory agents) should be administered with care, under close medical supervision, during Defitelio administration. In patients who have or develop clinically significant acute bleeding requiring blood	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	transfusion, Defitelio is not recommended or should be discontinued. Temporary discontinuation of Defitelio is recommended in patients who undergo surgery or invasive procedures at significant risk of major bleeding. **4.5 Interaction with other Medicinal Products and Other Forms of Interaction** In a mouse model of thromboembolism, recombinant t-PA potentiated the antithrombotic effect of defibrotide when given intravenously and thus co-administration may present an increased risk of haemorrhage and is contraindicated (see section 4.3). Defibrotide has a profibrinolytic effect (see section 5.1) and this may potentially enhance the activity of antithrombotic/fibrinolytic medicinal products. At this time there is no reported experience in patients on the concomitant treatment with Low Molecular Weight Heparins (LMWHs), warfarin or the concomitant treatment with direct thrombin inhibitors (e.g., dabigatran) or direct Factor Xa inhibitors (e.g., rivaroxaban and apixaban). Therefore, the use of defibrotide with antithrombotic/fibrinolytic medicinal products is not recommended. However, if used, in exceptional circumstances, caution should be exercised by	

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	closely monitoring the coagulation parameters (see section 4.4). **Section 4.8 Undesirable Effects** Coagulopathy is included as an ADR.	
Immunogenicity (Allergic/Hypersensitivity reactions)	Routine risk minimisation measures SmPC: **4.3 Contraindications** Hypersensitivity to defibrotide or to any of the excipients listed in section 6.1. **Section 4.8 Undesirable Effects** Mentions hypersensitivity and anaphylactic reaction as ADRs.	Not warranted
Potential Risks		
Reproductive toxicity	Routine risk minimisation measures SmPC: **4.6 Fertility, pregnancy and lactation** <u>Pregnancy</u> There are no studies using defibrotide in pregnant women. Embryo-foetal developmental toxicology studies in pregnant rats and rabbits of defibrotide doses close to the recommended therapeutic human dose, revealed a high rate of haemorrhagic abortion (see section 5.3). Defitelio should not be used during pregnancy unless the	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	clinical condition of the woman requires treatment with Defitelio. <u>Contraception in males and females</u> Effective contraception is required for patients and partners of patients during exposure to Defitelio and for one week subsequent to discontinuation	
Important missing information		
Safety in Pregnant or lactating women	Routine risk minimisation measures SmPC: **4.6 Fertility, pregnancy and lactation** <u>Pregnancy</u> There are no studies using defibrotide in pregnant women. Embryo-foetal developmental toxicology studies in pregnant rats and rabbits of defibrotide doses close to the recommended therapeutic human dose, revealed a high rate of haemorrhagic abortion (see section 5.3). Defitelio should not be used during pregnancy unless the clinical condition of the woman requires treatment with Defitelio. <u>Contraception in males and females</u> Effective contraception is required for patients and	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	partners of patients during exposure to Defitelio and for one week subsequent to discontinuation. Breast-feeding It is not known whether defibrotide is excreted in human milk. Considering the nature of the product, a risk to the newborns/infants is not expected. Defitelio may be used during breast-feeding Fertility There are no studies investigating the effects of defibrotide on human fertility.	
Patients treated concomitantly with defibrotide and medications that increase the risk of haemorrhage (including the newer oral anti-coagulants direct thrombin and factor Xa inhibitors)	Routine risk minimisation measures SmPC: **4.3 Contraindications** Concomitant use of thrombolytic therapy (e.g. t-PA) (see section 4.5) **4.4 Special warnings and precautions for use** Use of medicinal products that increase the risk of haemorrhage within 24 hours of Defitelio administration (within 12 hours in the case of unfractionated heparin) is not recommended. Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors) (see	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	section 4.5), except for routine maintenance or reopening of central venous line, requires careful monitoring. Consideration should be given to discontinuation of Defitelio during use of such therapy. Medicinal products that affect platelet aggregation (e.g. non–steroidal anti-inflammatory agents) should be administered with care, under close medical supervision, during Defitelio administration. In patients who have or develop clinically significant acute bleeding requiring blood transfusion, Defitelio is not recommended or should be discontinued. Temporary discontinuation of Defitelio is recommended in patients who undergo surgery or invasive procedures at significant risk of major bleeding. **4.5 Interaction with other Medicinal Products and Other Forms of Interaction** In a mouse model of thromboembolism, recombinant t-PA potentiated the antithrombotic effect of defibrotide when given intravenously and thus co-administration may present an increased risk of haemorrhage and is contraindicated (see section 4.3). Defibrotide has a profibrinolytic effect (see section 5.1) and this may potentially enhance the activity of	

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	antithrombotic/fibrinolytic medicinal products. At this time there is no reported experience in patients on the concomitant treatment with Low Molecular Weight Heparins (LMWHs), warfarin or the concomitant treatment with direct thrombin inhibitors (e.g., dabigatran) or direct Factor Xa inhibitors (e.g., rivaroxaban and apixaban). Therefore, the use of defibrotide with antithrombotic/fibrinolytic medicinal products is not recommended. However, if used, in exceptional circumstances, caution should be exercised by closely monitoring the coagulation parameters (see section 4.4)	
Off-label use	Routine risk minimisation SmPC: **4.1 Therapeutic indication** Defitelio is indicated for the treatment of severe hepatic veno-occlusive disease (VOD) also known as sinusoidal obstructive syndrome (SOS) in haematopoietic stem-cell transplantation (HSCT) therapy It is indicated in adults and in adolescents, children and infants over 1 month of age.	Not warranted

The CHMP, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

The CHMP also considered that routine PhV is sufficient to monitor the effectiveness of the risk minimisation measures.

6.2. Risk minimisation measures for Defitelio

Summary table of Risk Minimisation Measures

Safety concern	**Routine risk minimisation measures**	**Additional risk minimisation measures**
Identified Risks		
Haemorrhage (including, but not limited to, gastrointestinal haemorrhage, pulmonary haemorrhage and epistaxis)	Routine risk minimisation SmPC: **4.3 Contraindications** Concomitant use of thrombolytic therapy (e.g. t-PA) (see section 4.5) **4.4 Special warnings and precautions for use** Use of medicinal products that increase the risk of haemorrhage within 24 hours of Defitelio administration (within 12 hours in the case of unfractionated heparin) is not recommended. Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors) (see section 4.5), except for routine maintenance or reopening of central venous line, requires careful monitoring. Consideration should be given to discontinuation of Defitelio during use of such therapy. Medicinal products that affect platelet aggregation (e.g. non–steroidal anti-inflammatory agents) should be administered with care, under close medical supervision, during Defitelio administration. In patients who have or	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	develop clinically significant acute bleeding requiring blood transfusion, Defitelio is not recommended or should be discontinued. Temporary discontinuation of Defitelio is recommended in patients who undergo surgery or invasive procedures at significant risk of major bleeding. **4.5 Interaction with other Medicinal Products and Other Forms of Interaction** In a mouse model of thromboembolism, recombinant t-PA potentiated the antithrombotic effect of defibrotide when given intravenously and thus co-administration may present an increased risk of haemorrhage and is contraindicated (see section 4.3). **Section 4.8 Undesirable Effects** Mentions haemorrhage as well as specific organ haemorrhages as ADRs.	
Hypotension	Routine risk minimisation measures SmPC: **4.4 Special warnings and precautions for use** Administration of Defitelio to patients who have haemodynamic instability, defined as inability to maintain mean arterial pressure with single pressor support, is not recommended.	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	Section 4.8 Undesirable Effects Hypotension is included as an ADR	
Coagulopathy	Routine risk minimisation measures SmPC: **4.3 Contraindications** Concomitant use of thrombolytic therapy (e.g. t-PA) (see section 4.5) **4.4 Special warnings and precautions for use** Use of medicinal products that increase the risk of haemorrhage within 24 hours of Defitelio administration (within 12 hours in the case of unfractionated heparin) is not recommended. Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors) (see section 4.5), except for routine maintenance or reopening of central venous line, requires careful monitoring. Consideration should be given to discontinuation of Defitelio during use of such therapy. Medicinal products that affect platelet aggregation (e.g. non–steroidal anti-inflammatory agents) should be administered with care, under close medical supervision, during Defitelio administration.	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	In patients who have or develop clinically significant acute bleeding requiring blood transfusion, Defitelio is not recommended or should be discontinued. Temporary discontinuation of Defitelio is recommended in patients who undergo surgery or invasive procedures at significant risk of major bleeding. **4.5 Interaction with other Medicinal Products and Other Forms of Interaction** In a mouse model of thromboembolism, recombinant t-PA potentiated the antithrombotic effect of defibrotide when given intravenously and thus co-administration may present an increased risk of haemorrhage and is contraindicated (see section 4.3). Defibrotide has a profibrinolytic effect (see section 5.1) and this may potentially enhance the activity of antithrombotic/fibrinolytic medicinal products. At this time there is no reported experience in patients on the concomitant treatment with Low Molecular Weight Heparins (LMWHs), warfarin or the concomitant treatment with direct thrombin inhibitors (e.g., dabigatran) or direct Factor Xa inhibitors (e.g., rivaroxaban and apixaban). Therefore, the use of defibrotide with antithrombotic/fibrinolytic medicinal products is not recommended.	

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	However, if used, in exceptional circumstances, caution should be exercised by closely monitoring the coagulation parameters (see section 4.4). **Section 4.8 Undesirable Effects** Coagulopathy is included as an ADR.	
Immunogenicity (Allergic/Hypersensitivity reactions)	Routine risk minimisation measures SmPC: **4.3 Contraindications** Hypersensitivity to defibrotide or to any of the excipients listed in section 6.1. **Section 4.8 Undesirable Effects** Mentions hypersensitivity and anaphylactic reaction as ADRs.	Not warranted
Potential Risks		
Reproductive toxicity	Routine risk minimisation measures SmPC: **4.6 Fertility, pregnancy and lactation** Pregnancy There are no studies using defibrotide in pregnant women. Embryo-foetal developmental toxicology studies in pregnant rats and rabbits of defibrotide doses close to the recommended therapeutic human dose, revealed a high rate of haemorrhagic abortion	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	(see section 5.3). Defitelio should not be used during pregnancy unless the clinical condition of the woman requires treatment with Defitelio. <u>Contraception in males and females</u> Effective contraception is required for patients and partners of patients during exposure to Defitelio and for one week subsequent to discontinuation	
Important missing information		
Safety in Pregnant or lactating women	Routine risk minimisation measures SmPC: **4.6 Fertility, pregnancy and lactation** <u>Pregnancy</u> There are no studies using defibrotide in pregnant women. Embryo-foetal developmental toxicology studies in pregnant rats and rabbits of defibrotide doses close to the recommended therapeutic human dose, revealed a high rate of haemorrhagic abortion (see section 5.3). Defitelio should not be used during pregnancy unless the clinical condition of the woman requires treatment with Defitelio. <u>Contraception in males and females</u>	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	Effective contraception is required for patients and partners of patients during exposure to Defitelio and for one week subsequent to discontinuation. Breast-feeding It is not known whether defibrotide is excreted in human milk. Considering the nature of the product, a risk to the newborns/infants is not expected. Defitelio may be use during breast-feeding. Fertility There are no studies investigating the effects of defibrotide on human fertility.	
Patients treated concomitantly with defibrotide and medications that increase the risk of haemorrhage (including the newer oral anti-coagulants direct thrombin and factor Xa inhibitors)	Routine risk minimisation measures SmPC: **4.3 Contraindications** Concomitant use of thrombolytic therapy (e.g. t-PA) (see section 4.5) **4.4 Special warnings and precautions for use** Use of medicinal products that increase the risk of haemorrhage within 24 hours of Defitelio administration (within 12 hours in the case of unfractionated heparin) is not recommended. Concomitant systemic anticoagulant therapy (e.g. heparin, warfarin, direct thrombin inhibitors and direct factor Xa inhibitors) (see	Not warranted

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	section 4.5), except for routine maintenance or reopening of central venous line, requires careful monitoring. Consideration should be given to discontinuation of Defitelio during use of such therapy. Medicinal products that affect platelet aggregation (e.g. non–steroidal anti-inflammatory agents) should be administered with care, under close medical supervision, during Defitelio administration. In patients who have or develop clinically significant acute bleeding requiring blood transfusion, Defitelio is not recommended or should be discontinued. Temporary discontinuation of Defitelio is recommended in patients who undergo surgery or invasive procedures at significant risk of major bleeding. **4.5 Interaction with other Medicinal Products and Other Forms of Interaction** In a mouse model of thromboembolism, recombinant t-PA potentiated the antithrombotic effect of defibrotide when given intravenously and thus co-administration may present an increased risk of haemorrhage and is contraindicated (see section 4.3). Defibrotide has a profibrinolytic effect (see section 5.1) and this may potentially enhance the activity of	

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
	antithrombotic/fibrinolytic medicinal products. At this time there is no reported experience in patients on the concomitant treatment with Low Molecular Weight Heparins (LMWHs), warfarin or the concomitant treatment with direct thrombin inhibitors (e.g., dabigatran) or direct Factor Xa inhibitors (e.g., rivaroxaban and apixaban). Therefore, the use of defibrotide with antithrombotic/fibrinolytic medicinal products is not recommended. However, if used, in exceptional circumstances, caution should be exercised by closely monitoring the coagulation parameters (see section 4.4)	
Off-label use	Routine risk minimisation SmPC: **4.1 Therapeutic indication** Defitelio is indicated for the treatment of severe hepatic veno-occlusive disease (VOD) also known as sinusoidal obstructive syndrome (SOS) in haematopoietic stem-cell transplantation (HSCT) therapy It is indicated in adults and in adolescents, children and infants over 1 month of age.	Not warranted

The CHMP, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

7. Other considerations by the CHMP on the RMP

7.1. Plans for post-authorisation efficacy studies

The CHMP notes that the applicant did not propose an efficacy study.

7.2. Part VI of the RMP (Summary of activities in the risk management plan)

The content of Part VI of the RMP is considered acceptable.

7.3. Published public summary of the RMP

The public summary of the RMP may require revision following the conclusion of the procedure.

8. Benefit-Risk Balance

Benefits

Beneficial effects

Hepatic Veno-Occlusive Disease (VOD) is a serious pathology resulting from endothelial damage most commonly seen in the setting of haematopoietic stem cell transplantation (SCT). The precise pathogenesis of VOD is not fully elucidated but damage to the hepatic sinusoidal endothelium followed by thrombosis in the sinusoids leads to hepatic dysfunction, fluid retention and, if severe, can be associated with renal impairment and multi-organ failure. The diagnosis of VOD is very difficult as it requires exclusion of other pathologies seen often in the Haematopoietic Stem Cell Transplant (HSCT) setting such as infection and Graft Versus Host Disease (GVHD). Another difficulty in the management of cases with VOD is when to give potentially toxic therapies (e.g. recombinant tissue plasminogen activator) in an attempt to stop the microthrombosis in the liver. For aiding clinical decisions in these cases the Bearman model has been employed. This model takes into account the timing, degree and rate of rise in body weight and serum bilirubin levels. This prospectively validated prediction model applies to patients treated with cyclophosphamide and may not be applicable to patients treated with less toxic conditioning regimens. Using this model has been shown to have a high specificity, but only moderate sensitivity, for predicting those who will go on to develop severe VOD, which is defined as VOD with multi-organ failure.

Currently the literature reports on both the incidence of and prognosis for VOD support the fact that for severe VOD the prognosis is poor with a high mortality rate. Currently there is no licensed therapy for this indication and the availability of an effective treatment for this patient group would be of great benefit.

This application is for the treatment of severe hepatic VOD. In support of the claimed indication the applicant provided data from study 2005-01, an open-label phase II study including 102 patients with severe VOD/MOF showing a CR rate by Day 100 of 24% and a mortality rate by Day 100 of 62%. Due to the uncontrolled nature of the study some type of external control is necessary as a control group of only 32 patients is too limited and cannot stand alone.

It is acknowledged that a placebo-controlled study is not feasible. Therefore, the CHMP's view is that the results in the treatment group in terms of percentage of patients who have completed resolution of severe VOD by Day 100 post SCT and complete resolution of multi organ failure as well as survival rate Day 100 should either be evaluated on their own merits or compared with an adequate external historical control. In other words, since there are concerns about the nature of the HC group and running a controlled study in this indication is not possible, it is appropriate to consider additional sources of data that could support study control data provided by the Applicant.

Registry data have been the major driver in the advances of allogeneic HSCT in the absence of randomised clinical trials (conditioning regimens, anti-GVHD prophylaxis and therapies, infection prophylaxis). There appears to be a treatment effect in severe VOD and the high mortality for severe VOD from the literature and the finally chosen historical control group suggest that DF is of use. The mortality of sVOD is reported to be in excess of 85% without DF treatment.

The mortality of patients with severe VOD/MOF using supportive therapy without defibrotide has remained quite constant over two decades despite other therapeutic improvements for patients undergoing HSCT and the mortality rate of 62% compares favourably with the expected mortality rate of 75-85%.

The US treatment-IND study (2006-05) is an open study treating patients with VOD. Results from this study also indicate a reduction in Day 100 mortality as compared to the expected 75-85%.

The Applicant also provided additional data analysis from the US REGISTRY as an external comparator. In patients who were reported not to have received any specific interventions for their VOD, a mortality rate of 78% showed that despite any improvements in supportive care, the mortality rate remains very high with this disease. This US REGISTRY data could provide an estimate of the effect size that can be expected in the clinic under real life conditions. Day+100 survival of patients treated with standard of care (SOC) plus defibrotide was 39% compared with 31% for patients treated with SOC alone. VOD resolution occurred in 51% of the patients treated with SOC plus defibrotide compared with 29% of patients treated with SOC alone.

Taking the most conservative comparison i.e., between the TG from study 2005-01 and the US REGISTRY non-defibrotide treated group, there is an absolute 7% benefit in favour of defibrotide, giving a Number Needed to Treat (NNT) of 14 to save one life. The data from the US REGISTRY substantiate the clinical benefit seen for defibrotide in the 2005-01 study. Taking all the data into consideration, the weight of the evidence suggest a survival benefit for defibrotide in the treatment of severe VOD.

Uncertainty in the knowledge about the beneficial effects

The underlying MoA of defibrotide was claimed initially by the applicant as having multiple endothelial protective properties. Much of the older literature cites this as the MoA for defibrotide.

The applicant has provided information on a cell protection assay and this remains to be confirmed in validation studies. The data are preliminary and it cannot be ascertained at this stage whether any activity seen is due to a pharmacological or physicochemical effect. The claimed cell-protection activities have not been sufficiently demonstrated. Further work to investigate and confirm these properties is needed.

An updated expert statement provided by the applicant also claims a MoA for plasmin activation. The CHMP noted that a weak profibrinolytic effect has been shown and the MoA for Defibrotide is likely to be that of a weak fibrinolytic agent. The applicant has characterised defibrotide with several related profibrinolytic activity assays.

The CHMP' conclusions from the results of these assays is that the *in vitro* activity for fibrinolysis is weak and might even be seen as negligible when compared to negative controls and that the endothelial cell protection needs to be confirmed with more adequately designed studies.

From a quality perspective, the activity of defibrotide in the profibrinolytic and endothelial cell protection assay is weak and possibly confounded by the signal: noise ratio. Defibrotide is polydisperse and difficult to characterise using physicochemical tests, and together with the inability to show strong biological activity in an *in vitro* assay, the quality, consistency and control of defibrotide rests significantly on robust control over the manufacturing process. In view of the limitations in defining defibrotide from a quality perspective, and as no clear clinical correlation to quality attributes has been demonstrated, this could lead to significant challenges in confirming the quality of the product over the lifecycle of this polydisperse product. In this regard, the Applicant has the obligation to provide the results of the validation of the SK-HEP-1 cell based assay as a a quality post-authorisation measures. Together with the results a proposal to include the assay as an additional routine Quality Control test for batch release and stability testing for both defibrotide active substance and finished product should be provided.

Risks

Unfavourable effects

Although haemorrhagic complications were anticipated with defibrotide, it is difficult to ascribe with any degree of certainty AEs to the treatment itself in post-SCT patients, particularly those with VOD. The lack of a clear dose-response in AEs from the dose-finding study in the overall population also suggests that the unfavourable effects of DF cannot be characterised with certainty from the clinical data provided. An exception is the statistically significant differences in Kaplan-Meier estimates for survival (68.2% Arm A 25mg/kg/day, versus 32.5% arm B 40mg/kg/day, p= 0.0259) in those under 16 years, and this remains unexplained but does raise concerns regarding safety in children at doses higher than the proposed posology (25 mg/kg/day).

In terms of trying to assess the safety of defibrotide in VOD in the absence of a control group with adequate safety data, the largest safety database where different doses were administered to VOD patients is from DF-CUP and these results suggest a dose-related increase in bleeding. There were 18 deaths in DF-CUP considered possibly, probably or definitely related to defibrotide – all these deaths were as a result of haemorrhage or in one case coagulopathy. 9 children and 9 adults died and of the 18 subjects only one was on the low dose of 10mg/kg/day, 4 subjects received between 20-40mg/kg/day, 12 received 40mg/kg/day or higher, and for one subject the dose was not provided. Also of note is that in one case where defibrotide was stopped due to bleeding and then re-started again eleven days later, a further bleeding episode occurred within 5 days of re-commencement and DF was discontinued. Although the applicant states that there was no increase in efficacy with the 40mg/kg/day dose it does seem that for fatal bleeding events there was an association with higher doses.

Although no clear major signals for defibrotide related bleeding AEs across the clinical programme have been found, the relatedness of defibrotide to the signals seen cannot be clearly concluded in view of the lack of a concurrent control with VOD.

The data from defibrotide use in the treatment indication does raise a signal for occasional allergic reactions, but no such signal was seen in the clinical trials provided in support of VOD. Interpreting the significance of the safety data is difficult as it is not clear when the applicant changed the animal and tissue source of defibrotide as the older literature reports refer to defibrotide as being of "mammalian origin" or derived from "bovine lung". As the defibrotide in this application for the pivotal trial is from porcine intestine, this is not identical to the product previously marketed by the applicant.

In summary, the unfavourable effects at present appear to be dose-related haemorrhage and increased toxicity (death) in children with the higher dose.

Uncertainty in the knowledge about the unfavourable effects

The finding of a dose-related increase in bleeding from DF-CUP is noted but in view of the open label nature of DF-CUP and the voluntary data provision by clinicians which is incomplete, firm conclusions cannot be drawn.

There is only very limited data on PK of defibrotide in VOD. The lack of PK data in children and in those with renal failure, while both of these groups are included in the proposed indication of VOD, raises the level of uncertainly regarding the posology for children, in view of the safety signals at high doses, and in those with renal failure, as defibrotide is renally excreted and increased exposure will be expected in those with renal impairment.

Importance of favourable and unfavourable effects

Favourable effects from the uncontrolled treatment study have demonstrated an increase of the VOD resolution rate and reduction of the mortality rate. Taking all the data into consideration, the weight of the evidence suggest a survival benefit for defibrotide in the treatment of severe VOD.

The unfavourable effects of defibrotide have not been clearly elucidated, although a dose-related increase in bleeding and increased mortality with higher exposure in children was seen. In addition effects such as those associated with prolonged infusions and the administration of an animal product carry additional risks including administration-associated AEs and allergic reactions to the product.

Discussion on the benefit-risk balance

For the treatment indication the applicant has provided data from a Phase III study including 102 patients with severe VOD/MOF showing a CR rate by Day 100 of 24% and a mortality rate by Day 100 of 62%. Due to the uncontrolled nature of the study some type of external control is necessary. However, a control group of only 32 patients is too limited and cannot stand alone.

The benefits demonstrated from the treatment trial are considered reliable. For a single pivotal open label trial the use of a historical control group and the choice of that group have been clarified

by the applicant and the GCP findings do not seem to have an impact on the study results. The CHMP is of the opinion that the selection of the control group is sufficiently transparent and well explained by the Applicant.

It is acknowledged that a placebo-controlled study is not feasible. Therefore, the CHMP's view is that the results in the treatment group in terms of percentage of patients who have completed resolution of severe VOD by Day 100 post SCT and complete resolution of multi organ failure as well as survival rate Day 100 should either be evaluated on their own merits or compared with an adequate external historical control. In other words, since there are concerns about the nature of the HC group and running a controlled study in this indication is not possible, it is appropriate to consider additional sources of data that could support study control data provided by the Applicant.

Registry data have been the major driver in the advances of allogeneic HSCT in the absence of randomised clinical trials (conditioning regimens, anti-GVHD prophylaxis and therapies, infection prophylaxis). There appears to be a treatment effect in severe VOD and the high mortality for severe VOD from the literature and the finally chosen historical control group suggest that DF is of use. The mortality of sVOD is reported to be in excess of 85% without DF treatment.

The mortality of patients with severe VOD/MOF using supportive therapy without defibrotide has remained quite constant over two decades despite other therapeutic improvements for patients undergoing HSCT and that mortality rate of 62% compares favourably with the expected mortality rate of 75-85%.

Considering all available information, the CHMP is of the opinion to grant Marketing Authorisation under exceptional circumstances and that in order to have additional data, prior to launch, the Marketing Authorisation Holder (MAH) shall set up a patient registry to investigate the long term safety, health outcomes and patterns of utilisation of defibrotide during normal use. It shall be a multi-centre, multinational and prospective observational disease registry of patients diagnosed with severe hepatic VOD following haematopoietic stem cell plantation (HSCT) and enroll patients treated with defibrotide, other treatments or supportive care. The MAH shall ensure that information regarding all safety concerns identified in the most recent version of the Risk Management Plan is being collected. The MAH shall also ensure that all health care professionals who might prescribe defibrotide are provided with information on the importance of, and how to enter patients in, the registry.

The US treatment-IND study (2006-05) is an open study treating patients with VOD in the US. Results from this study also indicate a reduction in Day 100 mortality as compared to the expected 75-85%.

The Applicant also provided additional data analysis from the Centre for International Blood and Marrow Transplant Research (US REGISTRY) as an external comparator. In patients who were reported not to have received any specific interventions for their VOD, a mortality rate of 78% showed that despite any improvements in supportive care, the mortality rate remains very high with this disease. This US REGISTRY data could provide an estimate of the effect size that can be expected in the clinic under real life conditions. Day+100 survival of patients treated with standard of care (SOC) plus defibrotide was 39% compared with 31% for patients treated with SOC alone. VOD resolution occurred in 51% of the patients treated with SOC plus defibrotide compared with 29% of patients treated with SOC alone.

Taking the most conservative comparison, i.e. between the TG from study 2005-01 and the US REGISTRY non-defibrotide treated group, there is an absolute 7% benefit in favour of defibrotide, giving a Number Needed to Treat (NNT) of 14 to save one life. The data from the US REGISTRY substantiate the clinical benefit seen for defibrotide in the 2005-01 study. Taking all the data into consideration, the weight of the evidence suggest a survival benefit for defibrotide in the treatment of severe VOD.

The risks of defibrotide treatment are those associated with administration of prolonged frequent IV treatments and in addition the administration of an animal-derived product. In terms of clarification of the safety profile of DF, one of the proposed MoAs is a weak profibrinolytic effect and this raises concern about increased bleeding in a group where haemorrhage occurs frequently due to concomitant factors. Without a comparison between a defibrotide group and a placebo/physician's choice group, AEs cannot be attributed clearly to the defibrotide in the setting of post-SCT patients with VOD.

Additional risks are the lack of information on PK in children and in those with renal failure where in view of the trend for increased haemorrhages in higher dose patients in DF-CUP, together with increased deaths in children in the high dose group raise the need for further PK data in these groups.

In conclusion, based on the totality of the data including the ongoing IND and the US REGISTRY data, the CHMP considered that sufficient evidence has been provided in support of the proposed revised treatment indication.

Following the assessment of the detailed grounds for refusal provided by the applicant, the CHMP is of the view that the benefit risk balance of the above mentioned medicinal product can be considered positive in the proposed treatment indication on the following grounds:

- The HC group cannot stand alone but it is accepted that even recent registry data indicate that patients who do not receive defibrotide still have a very high mortality rate;
- The US treatment IND- study (2006-05) although uncontrolled indicate a beneficial effect on mortality of defibrotide;
- The US REGISTRY data support that defibrotide increases the VOD resolution rate and reduces the mortality rate;
- External control data is the only possible comparison.

Furthermore, the CHMP is of the opinion that a comprehensive clinical data package in the above indication cannot reasonably be expected to be provided, as the disease is very rare and a placebo controlled designed study impossible to conduct. Therefore, the CHMP recommends the granting of marketing authorisation under exceptional circumstances, with the condition that a registry is put in place to continuously collect safety and efficacy data to be reported annually.

The CHMP is of the view that the risk management system for Defitelio in the treatment of severe hepatic veno-occlusive disease is acceptable. Routine risk minimisation measures are considered sufficient for the safe and effective use of the medicinal product. Further, the CHMP is of the opinion that the proposed post-authorisation PhV development plan is sufficient to further identify and characterise the risks of the product.

9. Recommendations following re-examination

Based on the arguments of the applicant and all the supporting data on quality, safety and efficacy, the CHMP re-examined its initial opinion and in its final opinion concluded by majority decision that the risk-benefit balance of Defitelio in the *treatment of severe hepatic veno-occlusive disease (VOD), also known as sinusoidal obstructive syndrome (SOS), in haematopoietic stem-cell transplantation therapy (HSCT)* is favourable and therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal products on "restricted" medical prescription, reserved for use in certain specialised areas (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

Periodic Safety Update Reports

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

In addition, an updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Obligation to complete post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
Results of the validation of the SK-HEP-1 cell based assay should be provided. Together with the results a proposal to include the assay as an additional routine	March 2014

Quality Control test for batch release and stability testing for both defibrotide active substance and finished product should be provided.	
Defibrotide for the treatment of hematopoietic stem-cell transplants (HSCT) patients with severe hepatic veno-occlusive disease (VOD); data from the US REGISTRY database - additional information from the US REGISTRY database such as baseline characteristics, risk factors in terms of conditioning regimen, type of HSCT, etc. to be provided.	31 January 2014

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measure:

Description	Due date
Prior to launch, the Marketing Authorisation Holder (MAH) shall set up a patient registry to investigate the long-term safety, health outcomes and patterns of utilisation of defibrotide during normal use. It shall be a multi-centre, multinational and prospective observational disease registry of patients diagnosed with severe hepatic VOD following haematopoietic stem cell plantation (HSCT) and enroll patients treated with defibrotide, other treatments or supportive care. The MAH shall ensure that information regarding all safety concerns identified in the most recent version of the Risk Management Plan is being collected. The MAH shall also ensure that all health care professionals who might prescribe defibrotide are provided with information on the importance of, and how to enter patients in, the registry.	Annual reports within the annual re-assessment

Divergent positions to the majority recommendation are appended to this report.

APPENDIX

DIVERGENT POSITIONS

Divergent Positions

The undersigned members of CHMP did not agree with the CHMP's opinion recommending the granting of a Marketing Authorisation for Defitelio.

The reasons for divergent opinion were as follows:

The pivotal study supporting the indication consists in a comparison between 102 patients treated with Defitelio for VOD with an historical control arm composed by 32 patients suffering from VOD. The study is formally a failure as it did not meet its pre-planned level of significance, which was set at 1%. The control arm has been repetitively altered leading to the exclusion of 54 patients initially included. It cannot be excluded that has led to bias. Moreover the robustness of the results is questioned.

Finally, external support obtained from ongoing studies such as the US REGISTRY is of limited value as individual patient based data is not yet available to the MAA for a critical review and in-depth assessment by the CHMP.

Taking into account these elements, the benefit/risk of defibrotide in the treatment and prevention of VOD is not established.

London, 25 July 2013

……	……
Piotr Fiedor (Poland)	Ivana Mikačić (Croatia)
……	……
Concepcion Prieto Yerro (Spain)	Pierre Demolis (France)
……	……
Hubert Leufkens (co-opted member)	Barbara van Zwieten-Boot (The Netherlands)
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Sol Ruiz (co-opted member)	Romaldas Mačiulaitis (Lithuania)