25 September 2014
EMA/66720/2015
Committee for Medicinal Products for Human Use (CHMP)

Consultation procedure Public Assessment Report (CPAR)

Consultation on an ancillary medicinal substance incorporated in a medical device

Medical device: Hemoblast, haemostatic agent

Ancillary medicinal substance: Human Thrombin

Procedure No. EMEA/H/C/002769/0000

Note

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

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LIST OF ABBREVIATIONS

°C	Degrees Celsius
ACS	American chemical society
B19V	Parvovirus B19
BCT	Behring coagulation timer
CFR	US code of federal regulations
CFU	Colony forming units
DEAE	2-(diethylamino)ethyl
DNA	Deoxyribonucleic acid
e.g.	For example
g	Gram
h	Hours
HBsAg	Hepatitis B virus surface antigen
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
INN	International non-proprietary name
IU	International unit
kg	Kilogram
kDa	Kilodalton
L	Liter
m	Meter
mg	Milligram
min	Minute
mL	Milliliter
NF	National formulary
OD	Optical density
OMCL	Official Medicines Control Authority
OCABR	Official Control Authority Batch Release
Ph. Eur.	European pharmacopoeia
µm	Micrometer
USP	United States pharmacopeia

1. Background information on the procedure

1.1. Submission of the dossier

The Notified Body British Standard Institute Group submitted to the European Medicines Agency (EMA) on 3 April 2013 an application for consultation on Human Thrombin incorporated as ancillary medicinal substance in the medical device Hemoblast, in accordance with the procedure falling within the scope of Directive 93/42/EEC, as amended.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Daniela Melchiorri Co-Rapporteur: Robert James Hemmings

The application was received by the EMA on 3 April 2013.

- The procedure started on 25 May 2013.
- The Rapporteur's first assessment report was circulated to all CHMP members on 7 August 2013 The Co-Rapporteur's first assessment report was circulated to all CHMP members on 7 August 2013.
- During the meeting on 19 September 2013, 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 19 September 2013.
- The applicant submitted the responses to the CHMP consolidated list of questions on 21 February 2014.
- The Rapporteurs circulated the joint assessment report on the applicant's responses to the list of questions to all CHMP members on 31 March 2014.
- During the CHMP meeting on 25 April 2014, 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 consolidated list of questions on 23 May 2014.
- The Rapporteurs circulated the joint assessment report on the applicant's responses to the list of outstanding issues to all CHMP members on 4 June 2014.
- During the CHMP meeting on 26 June 2014, the CHMP agreed on a 2nd list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP consolidated list of questions on 22 August 2014.
- The Rapporteurs circulated the joint assessment report on the applicant's responses to the 2nd list of outstanding issues to all CHMP members on 4 September 2014.
- During the meeting on 22 - 25 September 2014 the CHMP, in the light of the overall data submitted and the scientific discussion within the committee, issued a positive opinion on the quality and safety including the clinical benefit/risk profile of Human Thrombin as ancillary medicinal substance(s) used in Hemoblast on 25 September 2014.
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1.3. Manufacturers

Manufacturer(s) of the active substance used as ancillary medicinal substance

CSL Behring
Emil von Behring Strasse 76
35041 Marburg
Germany

Manufacturer(s) of the finished product used as ancillary medicinal substance

Not applicable

Manufacturer(s) responsible for batch release

CSL Behring
Emil von Behring Strasse 76
35041 Marburg
Germany

Manufacturer responsible for import and batch release in the European Economic Area

Not applicable

Manufacturer(s) of the medical device

Biom'Up
8, allee Irene Joliot-Curie
St-Priest, 69800
France

In accordance with Council Directive 93/42/EEC, as amended, a sample from each batch of bulk and/or finished product of the human blood derivative shall be tested by a state laboratory or a laboratory designated for that purpose by a member state.

1.4. Remarks to the notified body

Quality

The proposed device is intended to be a sterile product. It is anticipated that the device will be sterilised by the manufacturer applying the principles of GMP or ISO standards and that the process has been adequately validated and monitored to verify that an appropriate sterility assurance level is reached. The material incorporated into the finished device should also be of a microbiological quality compatible with the proposed device manufacturing process. It would normally be expected that conformance of the finished product with a specified sterility test at release and at the end of shelf life is routinely demonstrated unless parametric release is proposed. In this respect, the Notified Body statement that documents in support of the parametric release from the manufacturer have been received and that they will be taken into consideration prior to certification is acknowledged.

Clinical

In order to establish how the risk of embolism using the conditions proposed for Hemoblast compares to that suggested by Referral EMA/H/A-31/1337, the Manufacturer used models (experimental and PC simulation) to investigate the impact that various pressures and (spraying) distances would have upon air speed and force. These models aimed to compare the air force (weight), speed and quantity when the haemostatic powder was sprayed at known pressure of 1 bar with different distance (ranging from 2-15 cm). However, information about the permeability of biological tissue to the air/gas mixture is missing and would allow a better quantification of the air/gas absorption and its consequences in terms of risk assessment. This aspect is left to the Notified Body as a remark to be considered when providing the CE mark.

1.5. Recommended measures to the notified body

As discussed at CHMP, it would be highly recommended that the notified body request the following from the medical device manufacturer for device approval:

Area[1]	**Description**
Safety	The Notified Body is advised to ensure that the Medical Device Manufacturer provides Instructions for Use and educational materials with the following key elements: 1. give clear and consistent information to surgeons on the recommended pressure and distance during spray application. 2. ensure that the pressure regulator is always placed between the medical grade air/CO_2 source and the device, and that the device must never be directly attached to a wall medical air grade/CO_2 source. 3. take steps to ensure that the device is used with pressure regulators that do not exceed the maximum pressure required to deliver the content and that the Information to User states the recommended pressure and distance. 4. warning that the risk of gas embolism appears to be substantially higher when Hemoblast is sprayed using medical grade air, as compared to CO_2 due to the high solubility of CO_2 in the blood. 5. ensure that the device is not used in endoscopic and laparoscopic surgery. 6. ensure that patients are closely monitored for signs of possible gas embolism.

[1] Areas: quality, safety, including clinical benefit/risk profile.

2. Scientific overview and discussion

2.1. General information

Medical Device

Hemoblast is classified as a class III medical device according to the relevant Commission Directive 2007/47/EC. Hemoblast incorporates human thrombin as a medicinal substance with ancillary action. The Notified Body, British Standard Institute, is consulting the CHMP regarding the quality and safety including the clinical benefit/risk profile of the incorporation of human thrombin in the device.

Hemoblast is a sprayable medical device containing haemostatic powder that absorbs excess blood; it is indicated for epilesional use in surgical procedures as an adjunct to haemostasis when control of bleeding by conventional procedures is ineffective or impractical. The haemostatic agent can only be used during laparotomy (the Applicant has withdrawn the indication on laparoscopic surgery procedures at Day 180). Hemoblast (HAT-2x) powder consists of 3 components, all in powder form:

1. Collagen from porcine origin,

2. Chondroitin sulfate from porcine origin,

3. Human thrombin.

The final product (15 doses) is composed of mass equivalent 1500 IU/device (mass equivalent 100 IU per dose).

Collagen has been used in haemostatic products for several years. Porcine derived collagen and collagen powder (POW) display the same biological advantages of other collagens without the risks linked to prion transmitted diseases associated with bovine-derived products. The collagen is extracted from pig skins and put into powder form.

Chondroitin Sulfate (CS) from porcine origin is Pharmaceutical grade.

Ancillary substance

Thrombin acts as an ancillary substance because of its small amount in the final device and because it accelerates the haemostatic effect of the collagen powder. The thrombin used is from human origin.

2.2. Quality documentation

2.2.1. For the ancillary medicinal substance or the ancillary human blood derivative itself

Human Thrombin is produced as an active substance for onward manufacturing and has a presentation almost equivalent to a drug product when finished.

It has been stated in the scientific explanation of the device that the human thrombin component of the device is already approved for use in the centrally approved product Tachosil (EU/1/04/277/001-004).

Active substance

Composition and description

The active substance is presented as a non-sterile (total aerobic microbiological count specified limit not more than 10CFU/ml), non-preserved lyophilised powder in glass vials sealed with a bromobutyl rubber stopper and crimp cap. Each vial is filled with 50,000IU human thrombin.

The composition of human thrombin active substance for onward manufacture was provided.

Manufacturing Process and Process Control

The manufacturer responsible for the manufacture of the cryodepleted plasma and active substance, the in-process controls and testing of the active substance is CSL Behring GmbH. A copy of a valid certificate of GMP compliance issued by the German Competent Authority has been submitted.

The active substance manufacturing process has been adequately described. It's main steps are isolation and purification of prothrombin from plasma, virus inactivation, activation of prothrombin to thrombin, concentration, formulation, filling and lyophilisation. Flow charts have been provided.

In summary, the active substance manufacturing process consists of the following steps:

- Separation of plasma into cryoprecipitate and cryo-depleted plasma.
- Adsorption of prothrombin complex on ion exchange chromatography, washing and elution of loaded chromatography gel, stabilization, pasteurization, purification by ammonium sulfate precipitation and calcium phosphate adsorption, elution, activation of prothrombin to thrombin.
- Clarifying filtration, concentration and dialysis.
- Adjustment of bulk solution, clarifying and 0.2 µm filtration, filling (50000 IU thrombin/injection vial), lyophilization and packaging.

Sufficient information on batch size and yield have been provided. The batch numbering system has been described and is acceptable.

Starting materials

The plasma complies with the "Human plasma for fractionation" Ph. Eur. monograph. Collection and testing of individual donations as well as testing of plasma pools for viral markers are documented in the CSL Behring Plasma Master File (PMF; EMEA/H/PMF/000001/04/AU/013G (submitted at time of initial application)). The PMF is annually recertified and the respective PMF certificate and evaluation report are fully applicable to CSL Behring's active substance Human Thrombin.

The manufacturing steps for preparation of the plasma pool and the requirements for individual tests are described in the starting material specification for pooled plasma.

All compendial ingredients used in the active substance manufacturing process conform to their respective monograph which is confirmed by the manufacturer, except ion exchange chromatography gel which is tested according to in house specifications.

Controls of Critical Steps and Intermediates

The critical process control parameters (PCPs) for the manufacture of Human Thrombin Active Substance have been satisfactorily justified in risk assessment reports and the set limits are acceptable. Their effect on the corresponding quality attributes (PQAs) was evaluated.

The IPCs are performed routinely to monitor the product quality attributes of the various intermediate fractions as defined in the relevant production procedures. All IPCs are performed according to approved test methods.

Isolation and purification of the active substance from cryodepleted plasma is a continuous manufacturing process such that an intermediate stage is not defined. However, due to a batch-mode production flow and the need for operational flexibility, holding times are required for intermediate fractions. Process intermediates that can be stored before further processing have been defined and holding times as well as temperatures have been adequately validated.

Process validation

Process validation has been conducted via full scale and down scale studies. Based on the risk assessments, the entire production process was validated at full-scale with a minimum of three consecutive manufacturing batches that were produced with PCPs set to the target values. Both critical PCPs and IPCs were evaluated. Process operation parameters/operations specifically targeted include homogeneity of both the final bulk solution (with regard to the related maximum holding time) and the filled vials (throughout the filling and lyophilisation process) and the lyophilisation process. Full scale studies were also used to confirm the protein and process impurity profile. Small scale studies were also used in range finding for particular unit operations.

The manufacturing process has been shown to be consistent with regard to the impurity removal capacity at each stage of the manufacturing process and for the total process. The main impurities are residual plasma proteins and process related impurities. A consistent impurity profile was demonstrated in all batches of lyophilized product examined in addition to a consistent process yield, total protein content and thrombin specific activity.

Control of active substance

The active substance is adequately controlled by an appropriate set of specifications. Analytical methods have been adequately described and validated. Batch analysis data provided for six batches is consistent.

The potency test is performed by using a clotting assay. The reference standard for routine potency testing is a working standard produced from a routine production batch according to approved production and quality control procedures.

As indicated by the Guideline CPMP/BWP/706271/2010, each batch of Thrombin to be incorporated in the medical device should be subjected to official control authority batch release procedure (in accordance with Articles 111(1), 113, 114(1)-(2) and 115 of Directive 2001/83/EC as amended and section 8 of Annex II to Council Directive 93/42/EEC, as amended). Upon request, the manufacturer of the medical device committed to use only OCABR batches for commercialization. The Paul-Ehrlich-Institute, Germany, has been designated as OMCL for the batch release.

Container closure system

The container closure system has been adequately described. It consists of a 10 mL injection vial (type I glass), a bromobutyl rubber stopper as well as an aluminium/polypropylene combi cap (red/red). Primary packaging complies with the Ph.Eur.

The container closure integrity has been adequately validated using dye ingress method.

Stability

Based on the results of the stability studies, which include data for three batches of active substance filled at 50,000 IU and stored in the proposed packaging configuration at real time conditions (60 months storage at 5°C ± 3°C), accelerated conditions and stress conditions, the claimed shelf-life is supported.

Drug product

The human thrombin, as incorporated into the device Hemoblast, is not manufactured as a finished product but as an active ingredient intended for onward manufacture. See discussion for active substance above.

Adventitious agents' safety

The Manufacturer states that the only biological material of human origin added to the active substance during the production of Human Thrombin is Antithrombin III (Kybernin® P, a high-purity AT III concentrate manufactured by CSL Behring in Marburg, Germany) and the only biological material from animal origin added to the active substance is heparin derived from porcine mucosa. No other materials of human or animal origin are used in the manufacture of Human Thrombin.

Non-viral adventitious agents

With regard to prions, the Manufacturer states that the risk of transmitting prions through Human Thrombin Active Substance is minimized by using plasma sourced exclusively in the USA, which has strict geographic donor deferral guidelines.

The manufacturer provides cross-reference to studies demonstrating that the sanitization of equipment with alkaline solutions effectively reduces prions that may be present, thus limiting batch-to batch contamination. Adequate precautions are in place to avoid cross-contamination in the manufacturing facility.

In the initial documentation the manufacturer did not refer anywhere to specific validation studies aimed at demonstrating that specific steps of the manufacturing process are effective in the reduction of prion transmission risk. On request, the Applicant has provided an Assessment of the Risk of Creutzfeldt-Jakob Disease Transmission by the plasma derived Human Thrombin active substance that is acceptable.

Viral adventitious agents

Complementary to the testing of source plasma donations the first homogenous pools after separation of cryoprecipitate are tested in line with Ph.Eur. monograph Human Plasma for Fractionation Testing requirements for HBsAg, anti-HIV1/2 and HCV RNA. Only pools non-reactive according to analytical sensitivities to HAV RNA, HBV RNA, HIV 1 RNA and that do not show high titres of parvovirus B19 DNA above threshold are released for forward manufacture. Validation of NAT/PCR tests has been performed in line with the Ph.Eur. General chapter 2.6.2.1 and is acceptable.

Five steps of the Human Thrombin active substance manufacturing process were investigated in scaled down spike experiments for their capacity to inactivate and/or remove viruses in accordance with the relevant guidelines (CPMP/BWP/268/95 and CPMP/BWP/269/95 rev. 3)

The effectiveness of virus inactivation / removal during the human thrombin manufacturing process has been validated. The validation investigated the clearance of enveloped (HIV, BVDV, HSV-1, WNV)

and non-enveloped (HAV, CPV, B19V) viruses which are considered most representative of those viruses that present a potential risk to the thrombin manufacturing process.

Overall reduction factors demonstrate the efficacy of the Human Thrombin manufacturing process in removing/inactivating possible viral contaminants. In fact, the results of the virus validation studies show that pasteurization effectively inactivates viruses and the purification and concentration steps reliably contribute to the overall reduction of the model viruses studied, that include enveloped and non-enveloped viruses with a wide range of physicochemical characteristics.

The risk assessment carried out by the manufacturer can be considered satisfactory.

Based on these results, a warning statement has been included in the Instructions for Use as recommended in the remarks to the Notified Body at D120. The text is in accordance with the Guideline on warning on transmissible agents in summary of product characteristics (SmPCs) and package leaflets for plasma-derived medicinal products (EMA/CHMP/BWP/360642/2010 rev. 1).

Other materials used in the manufacture

Antithrombin III and heparin sodium are added to the solution before the pasteurization step.

Antithrombin III

Antithrombin III used in the manufacturing process of Human Thrombin Active Substance is a licensed product marketed in some EU Member States as Kybernin® P. Two steps (ammonium sulfate precipitations and pasteurization) of the Kybernin® P manufacturing process were investigated in scaled down experiments for their capacity to inactivate and/or remove viruses. The virus inactivation and/or elimination at the individual stages of the production process were tested independently at least twice. Mean overall reduction factors demonstrated an adequate removal/inactivation of the model viruses studied.

Heparin

Heparin sodium is prepared from porcine mucosa sourced from China and its production process consists of several manufacturing steps including precipitations with hydroalcoholic solutions and heat treatment in aqueous solution as well as dry heat, elevated pH treatment. It is expected that this production process will effectively remove or inactivate viruses.

The virus validation studies for Human Thrombin Active Substance described above use process material containing heparin from production lots at the various stages. It is accepted that these studies will also demonstrate the removal and inactivation capacity of the manufacturing process for viruses potentially present in heparin sodium.

2.2.2. For the ancillary medicinal substance or the ancillary human blood derivative as incorporated in the medical device

Hemoblast is a medical device comprised of human thrombin incorporated into a collagen-based powder medical device.

Qualitative and Quantitative particular of the constituents

Qualitative and Quantitative particulars of constituents

The mass equivalent to one thousand five hundred units (1500 IU) of thrombin are added to collagen and chondroitin sulphate to constitute a final Hemoblast device. The mass equivalent to one hundred units (100 IU) are homogeneously distributed into each 0,3 mL dose of the carrousel assembly.

One Hemoblast device contains 15 doses of mass equivalent to 100 IU of thrombin each.

The container system is a gamma sterilized Polycarbonate (Poly (bisphenol-A-carbonate, Lexan HPS2) carrousel.

The applicant asserts that potential interactions of the final haemostatic agent containing the ancillary substance are minimized by the use of biocompatible resins (biocompatible according ISO 10993 series or USP Class IV). Biocompatibility of the finished medical device/tamper-sealed containment stored in the dispenser was demonstrated through ISO 10993 biocompatibility studies before and after sterilisation.

Sterility maintenance is ensured by the primary packaging that is the carrousel assembly, the secondary packaging that is a sealed inner blister/tray also used in other CE cleared Biom'Up products and the tertiary packaging that is the outer pouch.

Description of method of manufacture

The manufacturing process for incorporating human thrombin in the device has been adequately described.

The process has been validated for i) homogeneity of thrombin content of the homogenised powder, ii) de-mixing of the powder blend during filling of the carousel and iii) sterilisation.

The activity of thrombin using a standardized assay and expressed as IU had not been measured. Manufacturing process development has established that thrombin activity is reduced by exposure to the proposed sterilisation cycle but sufficient activity is retained according to the manufacturer's own criterion. Upon request, two studies aimed at evaluating the hemostatic potential and the molecular integrity of Thrombin itself or as incorporated in SPR sterile powder (Hemoblast), after the sterilization step have been provided. The data show that the irradiation process does not change the physical structure of thrombin and has negligible effect on quality or potency of thrombin's haemostatic effect.

Control of starting materials

A quality management system is in place to control the starting materials intended for use in the manufacture of the proposed device.

Upon reception of thrombin, Biom'Up performs a documentation verification of conformity of the certificates received, the product and the specifications established with the supplier.

Biom'Up performs a control upon reception to verify that chondroitin sulfate is in conformity with the specifications established with the supplier, which are compliant with Ph. Eur. requirements and performs an identification spectra. Regarding collagen, Biom'Up has introduced a traceability procedure and specification document with the collagen supplier.

Control test carried out at intermediate stages of the manufacturing process of the medical device

No specific in-process testing to control the thrombin content is conducted.

Final control tests of the ancillary medicinal substance or the ancillary human blood derivative in the medical device.

The bioburden test is performed as pre-sterilisation bioburden testing.

Biom'Up makes no label claim for potency in terms of thrombin activity with upper and lower limits. It is intended that the device contains a maximum thrombin potency equivalent to 100 IU per actuation. Thrombin has been incorporated into the device to accelerate the haemostatic effect. The Applicant agreed in reporting in the IFU the following text with respect to thrombin content: "Hemoblast has been manufactured to deliver no more than 100IU thrombin per actuation. Thrombin has been included to accelerate the haemostatic effect of the device".

A more detailed description of the manufacturer's thrombin time assay method (fibrinoformation) and a summary of its validation has been provided. The measurement of product performance in time to clot, as discussed, can be accepted with the conditions described above in place.

Information on the traceability with respect to the contractual relationship between CSLB and Biom'Up has been provided upon request. From this information it is considered that Biom'Up has adequate batch control procedures in place to satisfactorily control and monitor batches of active substance associated with any given device.

Stability (Hemoblast)

Preliminary stability data has been provided for a device in terms of the currently specified thrombin activity assay after 18 months storage at 25°C.

The 9-month shelf-life proposed is supported by the real time temperature stability data provided. The study is ongoing and will be concluded at 18-month time point.

The applicant has confirmed that the first three commercial production batches will enter into real time stability studies with test at specified intervals until the desired expiration date is reached. The tests proposed are water content, clot formation, dispenser functionality and sterility.

The applicant continues that expiration dates may be extended based upon room temperature stability data from a minimum of three production batches. If, in these post-approval stability studies, any lots are found to fall outside the approved specifications, these lots may be withdrawn from the market. The shelf life would also be revised accordingly and the authorities would be informed. Any changes to the expiration date of the device (and storage conditions) require the submission of a variation for approval of the extended shelf life of the ancillary substance incorporated in the device.

The issue of synchronization of expiry dates of the human thrombin incorporated into the medical device has been satisfactorily clarified by the applicant.

2.2.3. Discussion and conclusion on chemical, pharmaceutical and biological aspects

Ancillary substance

Human thrombin, manufactured as active substance, is incorporated as ancillary substance in the medical device Hemoblast. In general, the active substance is of acceptable quality. Adequate details on the manufacturing process, its control and validation have been provided. Specifications chosen are consistent with those of Thrombin used alone or in combination in medicinal products. The information provided in the application demonstrates consistent batch-to-batch production of Thrombin achieving a well-defined quality for the active substance. Safety with regard to transmissible agents, such as

human TSE and enveloped and non-enveloped viruses has been demonstrated in compliance with the relevant CHMP guidelines. In compliance with Directive 2007/47/EC, the Applicant committed to use for production of medical device batches only thrombin batches, which have been released by an Official Medicines Control Laboratory (OMCL).

However it was noted that there were some inconsistencies and dossier deficiencies in this submission (e.g. validation studies on the removal/inactivation capacity of TSE infectivity were not provided). The Manufacturer has updated the relevant section for completeness of the dossier.

All outstanding Quality issues have been solved with one remark to the notified body. The quality related issues in the Instructions for Use have been solved.

2.3. Non-clinical documentation

Pharmacodynamics

The Applicant submitted *in vitro* and *in vivo* studies which aimed to assess the blood clotting time and the haemostatic efficacy of the test articles (comprised of thrombin and collagen) *vs* comparators with a similar qualitative composition but different pharmaceutical form; not in all studies control collagen powder without thrombin was used. All in vivo studies used open surgery models.

Studies were carried out to assess the blood clotting time and haemostatic efficacy of Haemostatic Agent with Thrombin (HAT) powder in two formulations: i) HAT-1X containing a recombinant source thrombin (50 IU/dose) and ii) HAT-2X containing a human source thrombin (100 IU/dose). As a reference, Floseal Haemostatic Matrix, a product with similar qualitative composition but different pharmaceutical form (a gel composed of bovine-derived gelatin matrix component and human-derived thrombin) was used.

In vitro studies

In vitro haemostatic effect on human blood (studies BV-002-32 to 43)
The aim of this series of studies was to evaluate the haemostatic performance of HAT-1X and HAT-2X powders, in comparison to Floseal Haemostatic Matrix, in an in vitro citrated and recalcified human blood model. The objective of the study was to show whether HAT-1X and HAT-2X could reduce the clotting time down to < 2 minutes and whether haemostatic performance of these test articles were similar to that of Floseal (which contains human thrombin at approximately 20 units/50 mg of product).Different sources of thrombin were used (bovine JMI; human recombinant Zymogenetics; Human CSL).

Human whole blood was sampled at the French Blood Establishment from consenting healthy volunteers and treated with 0.014 M citrate. To induce coagulation, a 10 ml aliquot of blood, was added to f 500 µL of CaCl2 solution at 0.3 M.

Table 1: Results from BV-002-32 to 43

	Thrombin source	Amount of repetitions	Mean clotting time (sec)	SD (sec)	Standardised clotting time (taken to the patient's mean clotting time) (%)	Standardised SD (%)
Control blood	-	18	1149	247	100	11
Collagen fibers FB-2007-P004	-	16	327	79	28	5
Floseal Hemostatic Matrix	**Baxter**	**4**	**38**	**15**	3	**1**
HAT-1x (50UI/dose)	ZymoGenetics	12	33	14	3	1
	CSL	6	49	22	6	2
	All thrombins confounded	**18**	**38**	**18**	**4**	**2**
HAT-2X (100UI/dose)	ZymoGenetics	3	27	3	2	0
	JMI	3	28	3	2	0
	CSL	11	29	10	3	1
	All thrombins confounded	**17**	**28**	**8**	3	**1**

Is p<0.05 ?	Control blood	Collagen fibers FB-2007-P004	Floseal Hemostatic Matrix	HAT-1X - all thrombins	HAT-2X – all thrombins	HAT-2X – human thrombin
Control blood						
Collagen fibers FB-2007-P004	No					
Floseal Hemostatic Matrix	Yes	No				
HAT-1X - all thrombins	Yes	Yes	No			
HAT-2X – all thrombins	Yes	Yes	No	No		
HAT-2X – human thrombin	Yes	Yes	No	No	No	

Statistical difference between groups was based on a One Way Analysis of variance on Ranks (alpha=0.05). The control article, collagen fibres, reduced the clotting time of the control blood to a clotting time to 327 ± 79 seconds, which represents 28% ± 5% of the mean control clotting time. The reference haemostatic powder, Floseal, reduced the mean clotting time of the control blood to a clotting time of 38 ± 15 seconds, which represents 3% ± 1% of the mean control clotting time. There was no statistically significant difference between the efficiency of the test articles HAT-1X and HAT-2X whatever the thrombin origin is and the reference article, Floseal ($p>0.05$).

When HAT-2X was compared to the collagen powder (POW-2012-003) used in the final composition of HAT-2X (study BV-002-039), it was noticed that although collagen powder appeared to be less effective as it reduced clotting time to a minimum of 667 ± 42 s than collagen fibers (303 ±23 s), the addition of thrombin to the collagen powder enhanced the clotting efficiency causing a reduction in clotting time down to 42 ± 3 s for HAT-2X. These data are supportive of the usefulness of the addition of thrombin to collagen powder, despite the fact that the observed effects with HAT-1X and HAT-2X were similar.

Results from ET-2007-009-BV-002-039

	Clotting time		Normalized clotting time (taken to the patient's mean clotting time) (%)	
Group (n=3 per article)	**Mean (sec)**	**SD (sec)**	**Mean (%)**	**SD (%)**
Control blood	885	28	100	3
Collagen fibers	303	23	34	3
SPR-2012-P002 (HAT-1x)	68	8	8	1
SPR-2012-P003 (HAT-2x)	42	3	5	0
POW-2012-001	643	15	73	2
POW-2012-002	628	28	71	3
POW-2012-003	667	42	75	5

In vitro haemostatic effect on human blood (study BV-002-47)

The aim of this study was to evaluate the hemostatic potential in terms of clotting time of the powder HAT-2X in comparison to the Floseal since studies *BV-002-32 to 43* did not carry a comparison between the HAT-2X and Floseal. The control collagen powder was missing.

The results showed that the clotting time of whole citrated and recalcified blood is strongly decreased by the presence of HAT-2X or Floseal respect to control blood. The clotting times are 27 ± 4 and 30 ± 5 seconds respectively, which represents 3 to 4% of the clotting time of control blood (815 ± 52). Also, the clotting times of HAT-2X and Floseal products are not statistically significantly different in so demonstrating the comparable clotting activity of the two products.

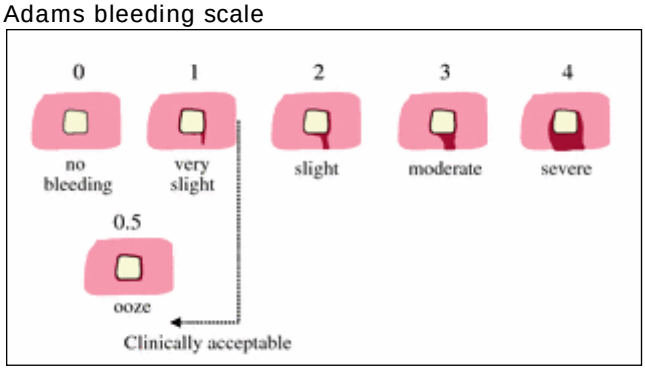
In vivo studies

Only in the two GLP studies (12-34 and 12-27) the final device formulation intended for the licensing (HAT-2X sprayable) was used, while in the majority of the remaining studies the HAT-1X was used. No direct comparison between HAT-2X vs collagen without thrombin nor between Hemoblast in the sprayable form vs a control without thrombin in the same sprayable form, was provided.

Acute haemostatic effect in liver and bone on non-heparinized (HAT-1X) rabbit (study PC-023), pigs (study PC-018), sheep (study PC-019)

The liver (soft tissue) lesion model was employed using a laparotomic surgical approach in the rabbit, pig and sheep, where surface square lesions 1.0cm x 1.0cm x 0.1-0.3 cm were created on the exposed liver with a scalpel. The wounds were then treated either with no product (control) or with the test articles (Floseal or HAT-1X). A slight manual pressure was applied to the wound and the bleeding severity was evaluated (according to the Adams bleeding scale ranging from 0 to 4) by the investigators every minute until 5 minutes (permanent failure) or until the bleeding control was maintained (permanent success).

Adams bleeding scale



At the end of each run, evaluation of the bleeding status was performed according to 2 criteria:

- Considering the Adams scale, haemostasis evaluated with a score of 0, 0.5 or 1 is clinically acceptable (the surgeon could close the wounds at this state). Therefore, success = bleeding score ≤1; failure = bleeding score >1 after 5 minutes of applied pressure.

- Considering a strict definition of haemostasis, a success is defined when no bleeding occurs; therefore, success = bleeding score =0; failure = bleeding score >0 after 5 minutes of applied pressure.

Rabbit (study PC-023) hepatic wound model

The performance of two haemostatic powders, HS0 (HA0 without thrombin) and HS1 (HAT-1X) were observed in four female rabbits. The reference articles were: Avitene (collagen-based) and Surgicel (polysaccharide- based) products without thrombin. The control technique was manual pressure with gauze. After midline laparatomy and liver presentation, liver superficial wounds were carried out. Powder doses or manual pressure was applied and if necessary, supplementary manual pressure, up to 5 runs (5 min/wound) were employed.

Under the success hypothesis set by both Adams score ≤ 1 (clinically acceptable haemostasis) and = 0, (no bleeding) the two test articles HS0 and HAT-1X and the two reference articles Avitene and Surgicel led to haemostasis within the 5 minute protocol. The amount of powder applied for HAT-1x appears overall higher than HS0.

Pig (study PC-018) hepatic wound model

The efficiency of HS0 (HA0 without thrombin) and HS1 (HAT-1x) was evaluated in comparison with the reference article Floseal and with a control manual pressure technique without product in a male swine.

The results showed a tendency for the test article HAT-1X to possess similar haemostatic efficiency as the reference article, Floseal (100% efficiency after 3 minutes of applied pressure).The HS0 test article was efficient but took longer to achieve haemostatic success when compared to HAT-1X and Floseal (100% HS0 efficiency after 4 minutes of applied pressure). Minor secondary bleedings were observed for HS1=HAT-1X (once) and Floseal (once) and were considered as inconsequential. Moreover, in a separate study (study PC-013), the stability of haemostasis after 4.5 hours and one day was determined to be successful for HAT-1X; not tested for Floseal.

In terms of the efficiency criteria of clinically acceptable and complete haemostasis after 5 minutes, the 2 test articles and the reference article were considered to be efficient haemostatic agents.

Sheep (study PC-019) hepatic and vascular wound model

The efficiency of HS0 (HA0 without thrombin) and HS1 (HAT-1x) was evaluated in comparison with the reference article Floseal. The test articles were compared with control articles Surgicel, Avitene, Floseal, Tachosil, and with a control manual pressure technique without product.

Two repetitions on the liver lobes and 1 incision (3 to 5 mm) of the vena cava were performed. The test material was held over the wound with gentle manual pressure using wet gauze (soaked in non-heparinized saline solution and squeezed out) for 1 min. While for the liver model the two success criteria from Adam scale were considered (haemostasis clinically acceptable and no bleeding), for the venous model, due to the critical nature of a vascular bleeding the following criteria were used: success = bleeding score = 0; failure = bleeding score >0.

Using only manual pressure did not lead to bleeding control in 5 minutes. All the test articles and reference articles were considered equally efficient haemostatic agents after 5 minutes of applied manual pressure; while Floseal reached the complete haemostasis after 1 minute. HAT-1X and HS0 were both shown to stop bleeding after 2 minutes; however, given the limited number of repetitions performed, these results should be considered together with the results of the other preclinical tests performed.

Efficiency in study PC-019.

	Amount of tests, n=						
Experimental plan performed	Control manual pressure	HS0	Avitene	Surgicel	HS1=HAT-1x	Floseal	Tachosil
Hepatic bleeding	2	2	2	2	2	2	3
Venous bleeding	1	1	1	1	1	1	1

In the sheep venous explorative bleeding model, HAT-1x, Surgicel and Floseal led to complete haemostasis after applying manual pressure during 1 minute; HS0 (applied in higher amounts) and Tachosil patch led to a complete haemostasis after applying manual pressure during 3 minutes. Avitene test is to be excluded since bleeding was out of scale. Due to only one replicate performed, no conclusive effect of HAT-1X can be claimed for cardiovascular surgery.

Acute haemostatic effect in liver and bone on heparinized: pigs (study PC-027-28): HAT-1X, Floseal

Three haemostatic agents POW (SPR-2011-009 without thrombin), HA0 (SPR-2011-010 without thrombin), and HAT-1X were compared to one of 4 reference products, Surgicel, Floseal, Arista, and Liquid Thrombin (Stago) and to the use of manual pressure only.

For the orthopaedic surgery, a lateral dorsal incision was performed and the underlying iliac crest was exposed. Three iliac crest wounds were performed on each crest, with a dimension of about 12mm x 12mm; with a thickness of approximately 3-5 mm. The bleeding of the created wound (liver and bone) was scored, treated with an adequate quantity of product which covered the wound and scored for haemostasis performance during ten minutes. A modified Adams 2009 scale (0-4 as outlined in Fig. 1) was applied.

Success frequencies for Control, Floseal and SPR-2011-011 (HAT-1X) after 2, 4, 6 and 10 minutes of manual pressure following liver and bone surgery.

		Success Frequency, with success defined by Adams=0				
Indication	Treatment	2 minutes	4 minutes	6 minutes	8 minutes	10 minutes
Abdominal (liver)	Control technique	0/10	0/10	0/10	0/10	0/10
	Floseal Matrix	7/9	9/9	9/9	9/9	9/9
	SPR-2011-011	1/11	6/11	9/11	10/11	11/11
Orthopaedic (bone)	Control technique	0/4	0/4	0/4	0/4	0/4
	Floseal Matrix	3/4	3/4	3/4	3/4	3/4
	SPR-2011-011	0/4	2/4	4/4	4/4	4/4

For liver surgery, considering a success-score defined by Adams = 0 Control technique does not lead to a complete haemostasis after applying manual pressure during 10 minutes.

Floseal led to a complete and stable haemostasis in 100% of the cases after applying manual pressure during 4 minutes. There was no statistically significant difference (Fisher test) between HAT-1X and Floseal when considering a haemostasis success at 6 min or when considering the entire 10 minute time frame.

For orthopaedic surgery, considering a success-score defined by Adams = 0, control technique did not lead to a complete haemostasis after applying manual pressure during 10 minutes. Floseal led to a complete and stable haemostasis in 75% of the cases after applying manual pressure during 10 minutes (1 permanent failure).

There was no statistically significant (Fisher test) difference between HAT-1X and Floseal when considering a haemostasis success at 6 min or when considering the entire 10-minute time frame.

Acute haemostatic effect in liver in non-heparinized pigs and sheep (study PC-033-34): HAT-1X, HAT-2X, Floseal

The haemostatic performance of HAT-1X (SPR-2012-P005) and HAT-2X (SPR-2012-P006) were compared to that of Floseal and the effects of manual pressure with gauze. Three individual studies were carried out PC-033-001, PC-033-002, PC-034, and the results were pooled. The "modified Adams 2009" scale was applied and the product was considered efficient if only the first gauze directly in contact with the product was soaked with blood (the 4 other gauzes are blood-free). Number of doses and manual pressure were at the surgeon's discretion.

Summary of the success frequencies for each group at each end-point:

	ET 2007 009 – PC33 /34	Success Frequency, with success defined by Adams=0		
Indication	Treatment	3 minutes (Run 1)	6 minutes (Run 2)	10 minutes (Run 3)
Abdominal (liver)	Pressure Control	0/11	0/11	0/11
	HAT-1x SPR-2012-P005	2/11	9/11	9/11
	HAT-2x SPR-2012-P006	2/11	7/11	9/11
	Floseal	8/11	10/11	10/11

When considering comparisons among treatments, only following 3 minutes a statistical significant difference (Fischer test) was observed between HAT-1X vs Floseal and HAT-2X vs Floseal; no difference in the haemostatic efficiency was observed between HAT-1X vs HAT-2X during the 10 minute-protocol though showing different performance curve profiles (HAT-1X appearing more efficient following 6 minutes).

Acute haemostatic effect in non-heparinised swine orthopaedic (iliac crest) wound model (study PC-016): HAT-1X, Floseal, Tachosil

The haemostatic performance of HAT-1X was compared to that of Floseal, Tachosil (medicinal product Tachosil containing human fibrinogen+ human thrombin) and collagen powder (HS0)

Considering an efficiency criteria of clinically acceptable hemostasis results obtained 5 minutes after administration with a success criteria of Adam Score ≤ 1, HAT-1x, HS0, Floseal and Tachosil appear as efficient hemostatic agents. Of note, even if less conservative than Adam=0, Adam Score ≤ 1 is considered adequate for the intended use of Hemoblast (as an adjunct to haemostasis).

HAT-1x reached success frequency of 4 (Adam=0) and 0 failures, performing better than HS0, already after 4 minutes. There was a tendency for Floseal to be the most efficient (Adam=0) at the 1,2 and 3 minute time points (see tables below).

Success hypothesis: Adam score ≤ 1

Run	Treatment	Success (score≤1) frequency	Failure (score>1) frequency	Failure (%)
1	HS0	0	3	100.0
	HS1	1	3	75.0
	Floseal	2	2	50.0
	Tachosil	3	0	0.0
2	HS0	1	2	66.7
	HS1	3	1	25.0
	Floseal	4	0	0.0
	Tachosil	3	0	0.0
3	HS0	2	1	33.3
	HS1	4	0	0.0
	Floseal	4	0	0.0
	Tachosil	3	0	0.0
4	HS0	2	1	33.3
	HS1	4	0	0.0
	Floseal	4	0	0.0
	Tachosil	3	0	0.0
5	HS0	3	0	0.0
	HS1	4	0	0.0
	Floseal	4	0	0.0
	Tachosil	3	0	0.0

Efficacy of the haemostatic device Hemoblast and embolism risk in an acute anticoagulated swim model - Biom'up 12-34 and 12-27 (GLP)

Studies were performed using the liver model in heparinized/anti-plateleted pigs in order to compare the haemostatic efficiency of HAT-2X final sprayable device with a short (10 cm) and long (30 cm) nozzle to that of Floseal and manual pressure in open surgery. Since the swine model with anti-platelet treatment led partially to (very) severe (Adams score 4 or ≥4) active bleedings, it was decided to raise the success criterion of the surgical model to Adams≤0.5, which is a clinically acceptable haemostasis outcome.

The pressure and the distance to the wound to be used with Hemoblast in its final formulation were assessed following administration at fixed parameters: <1cm either at 0.8-0.9 bars or at 1.8-1.9 bars (in study PC-12-34); <2cm at 0.8-1.0 bars (in study PC-12-27).

Study Biom'up 12-34

An open acute liver surgery model in pigs under aspirin-treatment was assessed. Three objectives were to determine:

- Feasibility for the air powered delivery dispenser HEMOBLAST for application of the haemostatic powder, especially with the short nozzle adapted to open surgery procedures,
- Haemostatic efficacy of the powder delivered by the device HEMOBLAST to stop hepatic bleedings,
- Risk of Embolism occurrence while using the air-powered device HEMOBLAST.

Adams scores incidences for each group in function of time: Run 0: before treatment, Run 1: 3 min after treatment, Run 2:6 min after treatment, Run 3:10 after treatment in pigs subjected to anti-platelet treatment (aspirin), the haemostasis success at 3 minutes was achieved for 100% (n=24/24) of the wounds treated with Floseal in addition to a moderate two-sided manual pressure and for 50% (n=24/48) of the wounds treated with HEMOBLAST with a slight one-sided manual pressure (n=48). Haemostasis success of wounds treated with moderate two sided manual pressure (Sham) was 0% (n=24/24) at 3 minutes. At 6 minutes haemostasis success was still achieved for 100% of the wounds treated with Floseal and increased to 85% for the wounds treated with HEMOBLAST™, and to 8% among the sham wounds. At 10 minutes the haemostasis success was still of 100% for the wounds treated with Floseal and increased to 98% for the wounds treated with HEMOBLAST™. Haemostasis success remained at 8 % of the sham wounds. P-values (F-test of the linear regression) for testing differences between HEMOBLAST and Floseal at 3, 6 and 10 minutes were <.0001, 0.072, and 0.750, respectively.

Overall, it was feasible to use the HEMOBLAST device in the applied open surgery model.

Group	Manual pressure control (moderate two-sided pressure) (Sham) (n=24)				HEMOBLAST™ (slight one-sided pressure) (Test) (n=48)				Floseal (Reference) (moderate two-sided pressure) (n=24)			
Adams score	Run 0	Run 1	Run 2	Run 3	Run 0	Run 1	Run 2	Run 3	Run 0	Run 1	Run 2	Run 3
4	20	12	5	0	40	4	0	0	19	0	0	0
3	3	5	6	10	8	2	1	0	5	0	0	0
2	1	7	7	9	0	12	2	1	0	0	0	0
1	0	0	4	3	0	6	4	0	0	0	0	0
0.5	0	0	2	1	0	10	9	6	0	0	0	0
0	0	0	0	1	0	14	32	41	0	24	24	24

Study Biom'up 12-27

The efficacy and the safety of the haemostatic device HEMOBLAST device linked to a Short (10 cm) and to a Long (30 cm) applicator tip were evaluated in comparison to that of Floseal in a swine open hepatic surgery model with middle and long-term follow-ups (1 and 4 weeks) to assess product resorption.

Observation of the abdominal cavity was performed to evaluate the occurrence of secondary bleedings. The assessment of adhesions comprised three parameters: the number of adhesions, expressed in percentage (Extent=E); and the density of the adhesions (Index= I).

When HEMOBLAST Short nozzle was applied and maintained on site with a slight pressure on the gauze during three, six and ten minutes, the haemostatic success state achieved was 17%, 67% and 100% of the treated wounds, respectively (n=6).

Using HEMOBLAST linked to a long nozzle, the haemostatic success was equal to or lower than that observed with HEMOBLAST short nozzle, respectively 0%, 67% and 83% after 3, 6 and 10 minutes of gentle padding (n=6). When the reference article Floseal was used and after applying of a moderate manual two-sided pressure on the treated wound during three, six and ten minutes, haemostatic success was achieved in 50%, 100% and 100% of the treated wounds respectively (n=4).

After 3, 6 and 10 minutes, no statistical significant difference was observed among nozzle length and Floseal. The probability of achieving clinically acceptable haemostasis with Short nozzle and Long nozzle was not as high as with Floseal at each endpoint (3, 6 and 10 minutes). The haemostatic performance and significant bleeding reduction efficacy was maintained over time as no secondary bleedings were detected after 1 and 4 weeks post-surgery, especially in the animals that were treated twice with HEMOBLAST.

On week 4 after surgery, no signs of adhesions with the neighbouring tissue were observed overall in all samples, except for one adhesion with the peritoneum at one site with treated HEMOBLAST Short nozzle. The inflammation appeared unchanged for HEMOBLAST Short nozzle and Floseal and decreased from moderate to minimal at the HEMOBLAST Long nozzle treated sites.

Assessment of embolism risk and distance / pressure to be used with Hemoblast
As Hemoblast is intended to be used with pressurized compressed medical air, a risk of embolism may exist.

The Hemoblast device was designed to incorporate pressure release valves and an internal system capable of mitigating the low risk of over pressure.

The data set provided would suggest that application of Hemoblast at <1 cm or <2 cm away from the wound was not associated with the risk of embolism: spraying the product on a large diameter vein and on the nearby present liver parenchyma, did not induce a Trans Esophageal Echocardiography signal modification higher than the one observed during an injection of saline through an ear catheter. In addition, blood gas analysis did not reveal any blood gas composition change that would normally occur at the origin of an embolism.

Evaluation of 1.8-1.9 bars was only performed in a single animal. Evaluation of 0.8-0.9 bars was evaluated in a total of 9 animals (for study 12-27 (n=8); 4 with short applicator (10 cm) and 4 with long applicator (30 cm); for study 12-34, n=1).

While data may be somewhat reassuring, the numbers of animals used was considered too limited to support the pressure (0.8-1.0 bar) and distance (4-8 cm) proposed in the IFU for a safe use of Hemoblast also in reference to the outcome of Referral EMEA/H/A-31/1337 on fibrinogen-containing sealant medicinal products by spray application following concerns over the risk of gas embolism. The Referral procedure concluded that medicinal products containing sprayable fibrin sealant solutions should be used, in open surgery, at 10 to 15 cm distance and at the recommended pressure of 1.5 to 2.0 bar with CO2 only instead of air as a safety precaution, because of the markedly lower risk of gas embolism due to the higher solubility of CO_2 in blood.

In order to support the proposed pressure and spray distances for HEMOBLAST, the Manufacturer provided data from a bench top pressure and air speed model developed by Biom'Up in conjunction with Origin Product Design (Cambridgeshire, UK) aimed to investigate air force (weight), air speed and

air quantity when the haemostatic powder was sprayed at different pressures (1-2 bar) and different distance (ranging from 2-15 cm).

The analysis was divided into 2 phases: one experimental (phase 1) with two different bench top testing configurations, for force measurements and for air speed measurements on a target depending on the pressure and the distance of use for the pressurized air application device; the “theoretical” (phase 2) model comprised of a computer simulation of the analyzed parameters.

In phase 1, for different distances (2, 4, 6, 8, 15 cm) at a known pressure (1 bar) to cover the recommended conditions for Hemoblast), 10 devices were tested (with 6 measurements per device for force and 6 measurements per device for air speed). Statistical analysis was also performed to evaluate the difference between the two worst cases conditions: 4 cm/1 bar (worst condition recommended) and 10 cm/2 bar (worst condition as concluded with the referral procedure EMA/H/A-31/1337).

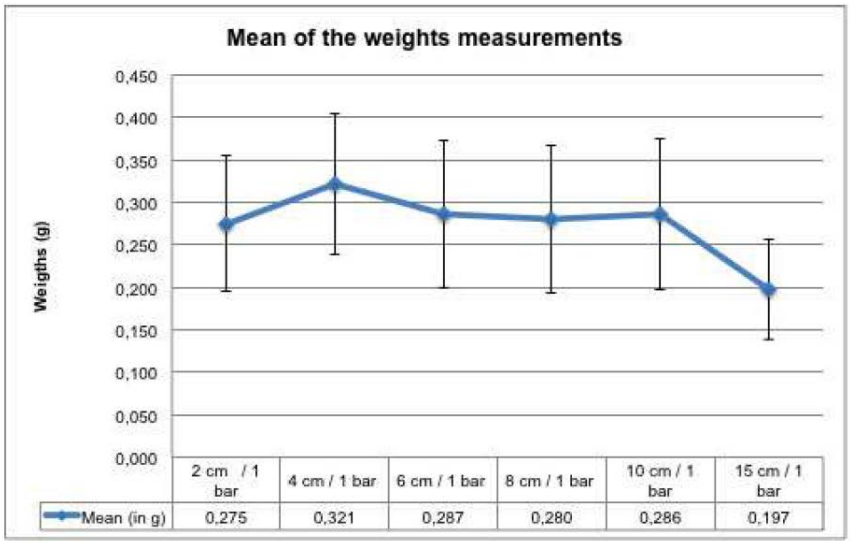
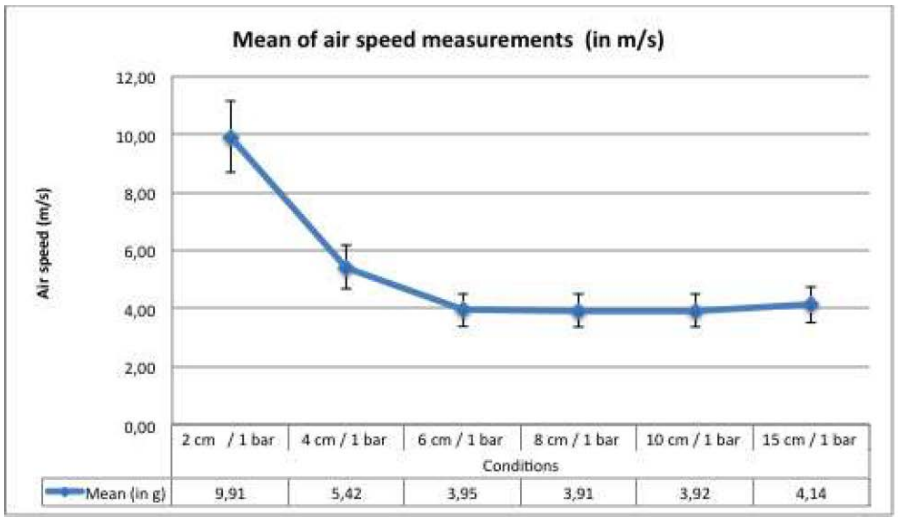


Figure 5: Mean of the weight measurements depending on the distance at 1 bar

Between the 2 recommended distances (4 to 8 cm), the weight (in grams) (force) was considered to be equivalent, which means that whatever the distance chosen by the surgeon to use Hemoblast, at 1 bar, the force applied on the wound would be considered equivalent.



Air speed values (in m/s not in grams as erroneously reported in figure above) decreased when the distances (4 to 8 cm) increased. Between the 2 recommended distances (4 to 8 cm), the air speed was considered as equivalent.

	Conditions						
	2 cm / 1 bar	*4 cm / 1 bar*	*6 cm / 1 bar*	*8 cm / 1 bar*	*10 cm / 1 bar*	*15 cm / 1 bar*	*10 cm / 2 bar*
Mean of the air speed (m/s)	9,912	5,422	3,951	3,910	3,922	4,137	6,962
Air flow (m3/s)	0,0002104	0,0001151	0,0000839	0,0000830	0,0000832	0,0000878	0,0001478
Quantity of air (m3) in 15 s	0,017	0,010	0,007	0,007	0,007	0,007	0,012
Quantity of air (m3) in 30 s							0,024

The quantity of air which may reach the target (wound) has been calculated for each distance in 15-30 seconds, the time assumed to spray the whole product. At 4 cm/1 bar the air quantity was estimated at 0.010 m3 while this quantity was estimated to be between 0.012 to 0.024 m3 for use at 10 cm/2 bar.

These results were confirmed by computer simulation, although pressures estimated were lower than the ones calculated from the actual experiment.

In conclusion, although the recommended pressures and distance from the EMEA/H/A-31/1337 referral (10-15cm; 1.8-2.0 bar) differ from those proposed for Hemoblast, data provided from the models described above support the safety of the Hemoblast device when used at a distance to the wound ranging between 4-8 cm at a pressure of 0.8-1.0 bar. However, information about the permeability of biological tissue to the air/gas mixture is missing and would allow a better quantification of the air/gas absorption and its consequences in terms of risk assessment. This aspect is left to the Notified Body as a remark to be considered when providing the CE mark.

Resorption/absorption evaluation after 1-day in rabbit liver wound model (study PC-013): HAT-1X

The intrinsic absorption/resorption properties (approximately 4.30 hours and 1 day) of HAT-1X was assessed in the rabbit (n=6).

Table 8: Performance of acute haemostasis of the tested products

Rabbit	Approximate size of the wounds (length * width)	Powder dose put on the wound	Appropriate powder mass approximately put (mg)	Duration of compression achieved (sum) in min	Duration of observation before concluding / closing (min)	Success of hemostasis in the acute phase at the end of the observation period?	Notes
L1	0.5 x 0.6 cm (1 mm)	n°1 + 1/2n°2 + 1/2 n°12	185.95	2 min	6 min	yes	
L2	1 x 0.8 cm (2mm)	n°5 + 1/5 n°8	109.3	2min	8 min	yes	
L3	1 x 1cm (2mm)	n°17 + 4/5 n°8	167.8	1min	13min	yes	
L4	1 x 1cm (2mm)	n°16 + n°19	185.8	2min	10min	yes	Prehension hematoma
L5	1.5 x 1.4 cm (3 mm)	n°20 + n°14	188.9	2min	10min	yes	Important bleedings of the skin and muscles layers: hematomas in abdominal cavity observed
L6	1.2 x 1.5 cm (3mm)	n°3 + n°4	186	3min	12 min	yes	

Following hepatic injury, generally, two doses (approximately 180 mg) were able to stop the acute bleeding, along with a compression of three minutes. Following the acute phase, haemostasis was stable during the first minutes after the last product application. In all tests, the powder stopped the bleedings, giving and acute success rate of 100% (6/6 rabbits). During the resorption phase the gel was more smooth and homogeneous after 1 day and shows positive integration of the product in its environment.

In conclusion, the product was in direct contact with the wound and was held in place during the post-surgery period. Haemostasis was maintained in the short term (about 4.5 and 21 hours) afterwards.

Resorption/absorption evaluation after 28-days in rat was also assessed in the toxicity study 131823 (see Toxicity section). Moreover, stability of haemostasis after 1 or 4 weeks was assessed within study Biom'up 12-27.

Tissue integrity evaluation after gas spraying in the intra-peritoneal cavity in rat (study PC-012) HAT-1X

This non-clinical study was performed in order to evaluate possible risks of compressed air drying the surface of organs, formation of adhesions and the risk of lesion formation. The model of hepatic lesions was performed in the rat (n=10). The effect of HAT-1X sprayed (under a pressure of 1-1.5 bars) in the abdominal cavity on the creation of post-surgical adhesions was evaluated in rats. No adhesion between organs or between organs and the peritoneum was observed in any of the rats at 3 weeks post-implantation into the intra- peritoneal cavity. In addition, no abnormalities of the general appearance of the organs were observed.

Pharmacokinetics

Pharmacokinetic investigations were not submitted.

Toxicity

Biocompatibility tests in terms of toxicity and local tolerance were performed in accordance with the ISO 10993 standard that entails a series of studies for evaluating the biocompatibility of a medical device prior to a clinical study.

For most studies, the HAT-2X formulation has been used, while for the 28-day rat study with local tolerance assessment, the old formulation (HAT-1X) was used.

In vitro toxicity (Study 152726)- GLP

The biocompatibility of the test article extract (haemostatic powder composing of Collagen, polysaccharide and thrombin) was evaluated using an *in vitro* mammalian cell culture monolayer system consisting of L-929 mouse fibroblast cells. Cytotoxicity tests were performed with finished sterilized product and two haemostatic extracts were prepared at the following dilutions: pure 100% (full strength) and 50% (v/v). The test article extract showed no cytotoxic potential to L-929 mouse fibroblast cells at the dilutions of 100% (full strength – 0,1 g/mL) and 50% (v/v – 0.05g/mL).

In vivo acute toxicity in mouse (GLP Study 152729)

A single dose of each extract of the haemostatic powder (0.1g/mL, in NaCl 0,9% solution and sesame oil) was injected into mice, using the i.p. route at the dose of 50mL/kg which would be equivalent to 5g drug product. No mortality was observed during the study in mice injected with the test article extracts. All animals appeared clinically normal at the beginning and throughout the study. No abnormal change in body weight data was detected between the control group and the treated group. Toxicity was evaluated according to the following numerical scale:

Table 2: Criteria of toxicity evaluation

0	Normal: no symptoms, mouse exhibits no adverse physical symptoms after injection
1	Slight: mouse exhibits slight but noticeable symptoms of hypokinesia, dyspnoea or abdominal irritation after injection
2	Moderate: mouse exhibits definite evidence of abdominal irritation, hypokinesia, dyspnoea, ptosis or diarrhoea after injection (weight usually drops to between 15 and 17g)
3	Marked: mouse exhibits prostration, cyanosis, tremors or severe symproms of abdominal irritation, diarrhoea, ptosis or dyspnoea after injection
4	Death: mouse dies after injection

Under the conditions of this study, there was no evidence of significant systemic toxicity or mortality after administration of the test article.

28-day rat systemic toxicity study with local tolerance assessment (Study 131823)

A study was performed to investigate both the systemic toxicity and local tolerance of the test article (haemostatic powder composed of Collagen, polysaccharide and thrombin) after 28-day subcutaneous implantation in the rat; 0.9% NaCl solution was used as a negative control rats. Subcutaneous implantation was chosen because the Hemoblast™ will be mainly applied in bleeding soft tissues.

The test groups received in the subcutaneous tissues 183mg of powder moistened with 0.9% NaCl (0.8g/kg i.e. about 10 times the clinical dose for humans). The negative control group similarly received 1mL of 0,9% NaCl. The rats were observed immediately after implantation and each day thereafter to detect mortality, morbidity and any abnormal clinical signs. Body weight and food intake were recorded once a week. No mortality or relevant clinical signs were reported during the study. No significant treatment effects or gender-based treatment effects were evident between the treated

groups and the negative control groups. No relevant variation was observed with regards to the body weight and the food intake. There was no relevant incidence of the treatment on blood parameters and organ weights (*liver, kidneys, spleen, testis, ovaries* and *adrenal glands).* For all main study rats, the *adrenal glands, axillary lymph nodes, heart, kidneys, lateral-aortic lymph nodes, liver, lungs, mesenteric lymph nodes, ovaries, spleen, testes and thymus* were sampled, processed for histology and examined microscopically.

Mutagenic potential (Study 152730)

A genotoxicity test was conducted according to the requirements of ISO 10993 for genotoxicity. This non clinical study was conducted in accordance with GLP.

A bacterial reverse mutation standard plate incorporation study was performed to evaluate whether extracts (0.1g/mL) of the haemostatic powder or their metabolites would cause mutagenic changes in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 or at the tryptophan locus of *Escherichia coli* tester strain *WP2uvrA* in the presence and absence of mammalian metabolic activation. The results showed that Hemoblast was not found mutagenic at the doses tested.

Local tolerance

In vivo irritation toxicity – rabbit (Study 152727)

Rabbits received five injections of 0.2mL of the NaCl 0,9% extract and five injections of the 0,2mL of the sesame oil extracts (0,1g/mL for each extraction vehicle) by intracutaneous route. The sites were examined immediately, 24, 48 and 72 hours after injection, for gross evidence of tissue reaction, such as erythema and oedema (score 0 to 4 for both, 0: no erythema and 4 severe erythema, 0: no oedema and 4 severe oedema). All injections sites appeared normal immediately following injection. The difference between the test and the control mean score was 1.0 or less.

Repeated dose sensitization test - Guinea pig (Study 152728)

The potential of the extracts of the haemostatic agent to cause delayed dermal contact sensitization was analysed. As a preliminary test, animals received a range of concentrations (100%, 50%, 25%, 0%; v/v) of the 0.9% NaCl solution or the sesame oil test article extract (0,1g/mL) via topical administration. The highest concentration that caused no more than mild to moderate erythema but did not otherwise adversely affect the animal after 24 hours was applied on patches during the second induction. The study was divided in 5 phases:

- *Induction I*: intradermal injections of the extracts and positive and negative controls. Three pairs of intradermal injections were administered to the animals.

- *Induction II*: 7 days after the induction I, application of patches soaked with haemostatic article extracts was applied to the previously inflammatory Sodium Lauryl Sulfate (SLS) treated site, and positive and negative controls

- *Rest period*: 14 days with no treatment

- *Challenge*: application of patches soaked with test article extracts and positive and negative controls

- *Evaluation*: observation of sites 24 and 48 hours after patches removal. The topical application of the 0.9% NaCl and the sesame oil extracts evaluated at a concentration of 100% did not induce delayed sensitization in the guinea pig (grade 0). Hemoblast showed no sign of sensitization after repeated applications. Moreover, human thrombin was chosen as an ancillary substance in order to avoid any

safety concerns such as neutralising antibody formation and TSE-related disorders that may occur with the use of thrombin from a bovine source.

The skin reactions were described and graded for erythema and edema according to Magnusson and Kligman scale (According to the ISO 10993-10 Standard) – see table below.

Under the conditions reported, the topical application of the 0.9% NaCI extract evaluated at a concentration of 100% did not induce delayed sensitization in the guinea pig (grade 0). The topical application of the sesame oil extract evaluated at a concentration of 100% did not induce delayed sensitization in the guinea pig (grade 0). Based on these results, the test article was thus not considered a sensitizer in the guinea pig maximization model.

Magnusson and Kligman scale (According to the ISO 10993-10 Standard)

REACTIONS	SCORES
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

The allergenicity of the test material was rated according to the grades presented in table 3 below:

Grades for sensitization (According to the Table II of the ASTM F 720-81 Standard re-approved 2007)

% OF SENSITIZED ANIMALS	GRADES	ALLERGENICITY
0 %	0	No sensitising
1 to 10 %	I	Slight
11 to 30 %	II	Mild
31 to 60 %	III	Moderate
61 to 80 %	IV	Strong
81 to 100 %	V	Extreme

Local tolerance assessment (included in Study 131823)

As part of the rat systemic toxicity study (Study 131823), an assessment of irritation potential was performed. The irritation scores of the test and negative control articles were calculated based on the histological semi-quantitative analysis and corresponded to the tissue damage and cellular inflammatory parameters scores and the repair phase of inflammation and fatty infiltrate parameter scores. When compared with the negative control article, the Irritant Ranking Score (IRS) of the test article was lower than 2.9, which suggests an absence of irritant potential. Under the conditions of the study, there was no evidence of general toxicity. The histological analysis showed that the test haemostatic powder was not locally irritant compared with the negative control article.

In conclusion, HAT-2X has no potential to cause local irritancy as shown in tests in the pig at one and four weeks post application (to stop hepatic bleeding) and similar findings were noted in in the rat at 28 days after subcutaneous implantation of HAT-1X. In addition, HAT-2X has no potential to cause delayed sensitisation in rabbits.

2.3.1. Discussion and conclusion on the non-clinical documentation

Human thrombin was chosen as an ancillary substance in order to avoid any potential concerns such as neutralising antibody formation that may occur with the use of thrombin from a bovine source. Hemoblast is expected to be biocompatible given that Floseal which contains a higher amount of thrombin was found to be biocompatible in accordance with all of the ISO 10993 tests conducted.

The non-clinical documentation provided in support of Hemoblast application aimed – in accordance with MEDDEV 2. 1/3 rev. 3 guideline - to verify the usefulness and safety of the ancillary human blood derivative, thrombin (which completes and accelerates the haemostatic effect of the collagen powder, by cleaving fibrinogen into fibrin). Since thrombin is a well-known medicinal substance for established purposes the abridged non-clinical data package is justified.

In human blood, no significant differences in clotting times (< 2 min) were observed between HAT-1X and HAT-2X, nor between HAT-1X, Floseal and collagen without thrombin; results showed that the clotting performance of HAT-2X was better (even if not statistically different from) than collagen without thrombin (powder form).

The majority of in vivo studies which utilised liver and bone injury models in a number of species including the rabbit, pig and sheep demonstrated that HAT-1X formulation (with half the content of thrombin in the commercial formulation Hemoblast) had a similar haemostatic performance compared to collagen powder without thrombin and Floseal. However, it was noted that in the bone injury model (pig), HAT-1X was shown to be more efficient than collagen powder alone, which lends some support to the proposed combination of thrombin and collagen.

No direct comparison between HAT-2X vs collagen without thrombin, nor between Hemoblast in the sprayable form vs a control without thrombin in the same sprayable form, was provided.

The lack of data on pharmacokinetics of Hemoblast is justified as it is expected that the pharmacokinetic profile of thrombin and collagen is not different when the two components are mixed prior to placing at the surgical site. Moreover, it is accepted that the pharmacokinetics of applied thrombin would not be expected to differ from endogenous thrombin and that the product is applied topically (epilesional use) and thus a negligible amount of systemic distribution is expected with such route of administration.

Regarding the resorption of HAT-2X it was shown to be complete within 1 month of application and stability of the clot was evident as no secondary bleeds were noted at up to 4-weeks post-surgery. No remarkable local or systemic effects were noted and on the basis of these findings and given the intended use, no formal pharmacokinetic investigations were conducted.

The toxicity studies performed suggest that HAT-2X is biocompatible and has no potential to cause sensitisation, genotoxicity, cytotoxicity, acute toxicity and irritation after intracutaneous administration. No mortality or alterations in histopathology were noted during toxicity and local tolerance studies (28-day single dose in rat administered subcutaneously), although these studies were conducted with a dose of thrombin (HAT-1X) that was 50% lower. Nevertheless, given the safety margins for thrombin (5 fold the maximum clinical exposure) and the fact that thrombin is a well-known compound already used in products on the market, data provided do not raise any potential safety concerns for the clinical use of Hemoblast.

As Hemoblast may be indicated for repeated applications over a long period of time, a GLP-compliant repeated dose toxicity study was performed by evaluating the maximization sensitization potential of Hemoblast on guinea pigs (delayed dermal contact sensitization test after repeated doses) and rabbits (intra-cutaneous irritation test) in which no local toxic potential at the administration sites tested were

observed. Although the main predicted clinical use is not intra-cutaneous, dermal or in soft tissue surgery but rather in the context of organ surgery, reassuring data were derived from histopathological evaluations performed in heparinised pigs treated with HAT-2X observed for 4 weeks and in rats treated with HAT-1X and observed for 3 weeks.

Studies on reproductive, developmental toxicity and carcinogenic potential have not been performed. Adverse effects on fertility, postnatal development and reproduction as well as teratogenic effects are not expected in humans, as active ingredients are derived from human plasma. Non-clinical studies with repeated dose applications (chronic toxicity and carcinogenicity) cannot be performed in conventional animal models due to the development of antibodies following the application of heterologous human proteins and are not considered necessary as these are physiological human plasma proteins and potential genotoxicity is not expected. This is in line with ICH S6 (R1) guideline for preclinical safety evaluation of biotechnology-derived pharmaceuticals. Genotoxicity studies have not revealed any findings.

Due to the limited evidence in animal regarding the risk of embolism using Hemoblast sprayable device, data from simulation modelling supported the distance to the wound (4.8 cm) and pressure (0.8-1.0 bar) to be applied with Hemoblast. Moreover, the potential inherent risks due to thrombin (i.e. thromboembolic events) and to the whole device (i.e. gas embolism during spray application) are considered minimised by adequate information on IFU together with an the educational material for the surgeons that include: recommended pressure and distance during spray application, the use of pressure regulator placed between the medical grade air/CO2 source and the device, the device must never be directly attached to a wall medical air grade/CO2 source, the pressure regulators must not exceed the maximum pressure required, the warning on the potential higher risk of gas embolism when Hemoblast is used with medical grade air vs CO2, closely monitor blood pressure, pulse rate, oxygen saturation and end tidal CO2, Moreover, Hemoblast will be not used in laparoscopic procedures.

In conclusion, the non-clinical data provided are considered to be adequate to support that the medicinal product is effective as a haemostatic agent and no potential safety concerns for the clinical use of Hemoblast are raised.

However, information about the permeability of biological tissue to the air/gas mixture is missing and would allow a better quantification of the air/gas absorption and its consequences in terms of risk assessment. This aspect is left to the Notified Body as a remark to be considered when providing the CE mark.

2.4. Clinical evaluation

2.4.1. Usefulness of the ancillary medicinal substance incorporated in the medical device as verified by notified body

Hemoblast is composed of collagen, chondroïtin sulfate and thrombin. The combination of collagen and thrombin as ancillary substance is classified as a Class III medical device. Data from the literature confirm the efficacy of these compounds in haemostasis.

The role of collagen in promoting blood coagulation by way of the interaction with two specific receptors, α2β1 integrin and glycoprotein IV was based on literature review (Jacquelin, 2002; Farndale, 2004). In addition, Balleisen et al (Haemostasis, 1976) showed that the collagen in triple-helical form, which is that contained in Hemoblast, is preferable to achieve platelet aggregation. The

use of collagen and its role in the promotion of the aggregation of platelets and release of their factors are well established.

The usefulness of thrombin inclusion in this new haemostatic device was supported by data derived from *in vitro* and *in vivo* nonclinical studies (see nonclinical section) and by evidence available from other equivalent haemostatic products currently on the market such as Surgiflo which was evaluated in a randomised, phase III, multicentre, prospective, active controlled and double-blind study (Doria et al, Current Medical Research and Opinion, 2008). The Notified Body assessed the usefulness for the inclusion of Human thrombin as an ancillary substance in Hemoblast.

The use of thrombin in haemostasis is therefore well characterised and thrombin from human plasma – as identical to the thrombin component of centrally authorised medical product Tachosil – would avoid a neutralising antibody formation and TSE related safety concerns associated with the use of thrombin from bovine source.

In order to ensure optimum haemostasis, the Hemoblast collagen powder was designed in powder form that can be applied dry with the use of a spraying device. It is ready for use and the spray form makes it easy to cover target tissues properly. The proposed final product will be indicated in open surgical procedures as an adjunct to haemostasis when control of bleeding by conventional procedures is ineffective or impractical. The device should not be used in endoscopic surgery. The dispenser system uses a controlled spray pressure of between 0.5 and 1.0 bar. Once sprayed and deposited onto the area, manual pressure using a humid gauze must be applied to help control bleeding.

The Notified body acknowledged that no clinical studies for Hemoblast are planned and the safety data to support the usefulness of the human thrombin incorporated in Hemoblast are based on the amount of thrombin added to the device which is less than what was assessed by EMA for similar products (1500IU vs. 2000-2500IU).

In conclusion, the Notified body considers that the incorporation of the thrombin as ancillary human blood derivative, is acceptable in terms of usefulness.

2.4.2. Clinical safety of the ancillary medicinal substance incorporated in the medical device

Plasma donations are from US plasma centres only. CSL Behring holds a Plasma Master File certificate from the EMA which covers the plasma used for the manufacture of human thrombin incorporated as ancillary substance in Hemoblast. Plasma donations are screened and tested for HepB surface antigen, anti HIV-1, anti HIV-2 and anti HCV antibodies. The plasma pools are tested and accepted if found to be non-reactive for HBsAg, anti HIV 1/2 antibodies, and as well for HCV RNA, HBV DNA, HIV1 RNA, HAV RNA and B19V DNA as determined by PCR (acceptable limit for B19V is $\leq 10^4$ IU/mL)Viral inactivation steps included in the thrombin manufacturing process mitigate viral transmission risks..

Hemoblast also contains chondroitin sulphate (porcine source) that is not oversulfated. Porcine collagen displays the same biological advantages without the risks linked to prion transmitted diseases associated with bovine-derived products. Indeed, pigs are the only mammals that do not spontaneously present cases of prion diseases (WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies).

Safety issues dealing with collagen alone are mostly related to the placement of a foreign body in the surgical field as well as the swelling of the materials (Spotnitz, 2008). This is particularly the case for products in sponge form whereas porcine gelatins are absorbed by the body in about 4 to 6 weeks

(Spotnitz 2008). Additionally, bovine collagen was shown to be more allergenic than collagen from porcine origin. However, because of their porcine collagen source, patient allergy to porcine proteins is not to be excluded. This is clearly noted in the Hemoblast IFU.

No clinical studies have been carried out to evaluate the safety of the device. The evaluation of safety has been also based on the analysis of data available for each of the components or their combination and from a literature review of similar haemostatic products currently in use. The products considered were Floseal, Gelfoam/Gelfoam Plus (William et al., The journal of bone and joint surgery, 1978) and Surgiflo (D.A. Johns et al, The Journal of the American Association of Gynaecologic laparoscopists, 2003; Mettler et al, Fertility and sterility, 2004; Tjandra et al, Dis.Colon.Rectum, 2008) that are similar to Hemoblast in terms of composition (collagen-based product with thrombin), Arista (Jastin et al, Otolaryngology-head and neck surgery, 2009; Francois-Joseph et al, The Journal of Urology, 2004) similar in terms of form and Sprygel similar in terms of mode of administration (spray). The efficacy and tolerance of Floseal compared to Gelfoam in cardiac, vascular and spinal orthopaedic surgeries was evaluated in a randomised multi-centre study (Mehmet et al, Journal Cardiac Surgery, 2003).

Furthermore, the use of thrombin in haemostasis is well characterised and thrombin from human plasma was chosen because it is identical to the thrombin component of Tachosil (a centrally authorised medical product, EU/1/04/277/001-004) and Evicel (EMEA/H/C/000898) and for its high safety. Human thrombin was chosen to avoid neutralising antibody formation and TSE related disorders that may occur with the use of thrombin from bovine source. In Europe, thrombin is not approved for use as a standalone product, but it is widely used in combination with other haemostatic devices such as collagen or gelatin (Gelfoam / Gelfoam plus, Surgiflo, Floseal).

The conditions of distance / pressure proposed for Hemoblast (4-8 cm at 0.8-1.0 bar) differ to that concluded with EMA/H/A-31/1337 (recommended pressures 1.8-2.0 bar and distance at 10-15cm;). In order to establish the risk of embolism the Manufacturer used models (experimental and PC simulation) aimed to compare the air force (weight), speed and quantity when the hemostatic powder was sprayed at known pressure of 1 bar with different distance (ranging from 2-15 cm). The results support the safety of the Hemoblast device when used at a distance to the wound ranging between 4-8 cm at 0.8-1.0 bar- with respect to the risk of air/gas embolism (see also Non-clinical section).

Based on available non clinical data, the recommendation to use no more than two devices on the same patient should be reported in the IFU. The time interval to achieve haemostasis (< 2 min or 6-10 min) should be clarified and stated in the IFU.

Post-marketing surveillance of the device is governed by medical device regulation and in the remit of the National Control Authority.

2.4.3. Clinical benefit/risk profile of the ancillary medicinal substance incorporated in the medical device

Controlling bleeding during surgery is essential in order to avoid patient morbidity. The addition of thrombin is expected to outweigh the risks associated with the use of this human blood derivative.

Regarding safety, viral transmission is mitigated by viral inactivation methods.

The minimal amount of thrombin, the recommended topical (epilesional) use of Hemoblast, the warning on the potential higher risk of gas embolism when Hemoblast is used with medical grade air vs CO2 and the indication not to exceed 1.0 pressure bars, and the educational materials with the above mentioned key elements are considered adequate measures to minimize the potential risks associated

to thrombin (e.g. thromboembolic events) and to the whole device (i.e. gas embolism during spray application).

Risks and contra-indications are clearly indicated in the instruction for use of the product. In addition, the potential of misuse of the product during preparation is mitigated by the product being ready-to-use with no extra manipulation step.

The proposed final product will be indicated in open surgical procedures as an adjunct to haemostasis when control of bleeding by conventional procedures is ineffective or impractical. The device should not be used in endoscopic or laparoscopic surgery. The dispenser system uses a controlled spray pressure of between 0.5 and 1.0 bar.

2.4.4. Discussion and conclusion on the clinical evaluation

Human thrombin is a well-known substance used in haemostatic medical devices.

The total amount of thrombin in Hemoblast, accounting for the 15 possible doses, is the mass equivalent to 1500 IU, and therefore it is lower than in other devices already approved (2000 IU in Surgiflo and 2500 IU in Floseal). On this basis, data extrapolated from the literature review and related to the single compounds and other devices, are considered sufficient to conclude that the benefit of the addition of the thrombin overcome the possible risks.

It is confirmed that British Standards Institution is a Notified Body that appears on the NANDO website as NB 0086 and is designated to carry out conformity assessments of medical devices according to EU Directive 93/42/EEC, as amended.

The Notified Body has submitted a clinical evaluation that refers to many years' experience of human thrombin in similar products that are already licensed and available on the EU market.

The CHMP completed a review of the safety of medicinal products containing fibrinogen containing solutions for sealant that are sprayed on to a tissue bed using either pressurized air or CO2 in 2012 (referrals EMEA/H/C/000898/A-20/0018 (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000898/WC500143768.pdf) and EMEA/H/A-31/1337 (http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Fibrinogen/WC500141161.pdf).

Recommendations on the need of educational materials and training for healthcare professionals to administer the products correctly (at the recommended distance and pressure for spray application) were agreed. The CHMP advised on use of such spray devices for these medicinal products, monitoring of patients and warnings to be included in patient information texts.

Even though the amount of thrombin is lower in Hemoblast than in these medicinal products, the risk of life-threatening and/or fatal air embolus cannot be excluded. CHMP highly recommends to the Notified Body that adequate information is reflected in the IFU and educational material related to gas pressure and distance from applicator tip to wound surface as well as the warning that the risk of gas embolism appears to be substantially higher when medical grade air is used instead of CO2 .

The recommended pressure and distance proposed by the manufacturer (4-8 cm and 0.8-1.0 bars) are different from parameters agreed in the context of the Referral procedure EMA/H/A-31/1337 (i.e. 10-15cm; 1.8-2.0 bar) on the risk of embolism observed with sprayable medicinal products. However, data from experimental models and PC simulation support the safety of the Hemoblast device with

respect to the risk of air/gas embolism when used at a distance to the wound ranging between 4 to 8 cm and at a pressure of 0.8 to 1.0 bar. These models aimed to compare the air force (weight), speed and quantity when the haemostatic powder was sprayed at known pressure of 1 bar with different distance (ranging from 2-15 cm). However, information about the permeability of biological tissue to the air/gas mixture is missing and would allow a better quantification of the air/gas absorption and its consequences in terms of risk assessment. This aspect is left to the Notified Body as a remark to be considered when providing the CE mark (see section 1.4).

In conclusion, the Notified Body is highly advised to ensure that the Manufacturer provides in the IFU and educational materials the following key elements (see section 1.5):

1. Give clear and consistent information to surgeons on the recommended pressure and distance during spray application.
2. Ensure that the pressure regulator is always placed between the medical grade air/CO_2 source and the device, and that the device must never be directly attached to a wall medical air grade/CO_2 source.
3. Take steps to ensure that the device is used with pressure regulators that do not exceed the maximum pressure required to deliver the content and that the Information to User states the recommended pressure and distance.
4. Warning that the risk of gas embolism appears to be substantially higher when Hemoblast is sprayed using medical grade air, as compared to CO2 due to the high solubility of CO2 in the blood.
5. Ensure that the device is not used in endoscopic and laparoscopic surgery.
6. Ensure that patients are closely monitored for signs of possible gas embolism.

Currently available haemostatic medical devices containing human thrombin are Floseal and Surgiflo - to some extent equivalent to Hemoblast - , and medicinal products containing human thrombin are available as supportive treatment in surgery procedures to improve haemostasis. Hemoblast is the first sprayable device containing ready-to-use powder that could improve the proper use of the product avoiding extra manipulation steps.

In conclusion, the clinical evaluation of usefulness and risk/benefit of the currently proposed human thrombin and clinical safety of the proposed medical device, as described, are acceptable.

2.5. Overall conclusions

Adequate details on the manufacturing process and the quality control of human thrombin incorporated as ancillary substance in the medical device Hemoblast have been submitted to show that the ancillary substance is of acceptable and consistent quality. Safety with regard to transmissible agents, such as human TSE and enveloped and non-enveloped viruses has been demonstrated in compliance with the relevant CHMP guidelines.

The use of human thrombin as ancillary substance included in the device in order to improve the haemostatic activity is considered clinically useful; the clinical safety of the medical device should be carefully monitored in the light of the life-threatening and/or fatal air embolus risk observed with sprayable haemostatic solution.

Taking all together CHMP considers that a positive opinion on the quality and safety, including the clinical benefit/risk profile of the incorporation of the ancillary medicinal substance, human thrombin, in the sprayable medical device, Hemoblast, can be granted.

2.6. Recommendation

Based on the CHMP review of data submitted, the CHMP considered by consensus that the quality and safety including the benefit risk profile of human thrombin incorporated as ancillary medicinal substance in Hemoblast, haemostatic agent, was favourable and therefore granted a positive opinion in the consultation procedure.