



European Medicines Agency
Pre-authorisation Evaluation of Medicines for Human Use

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**WITHDRAWAL ASSESSMENT REPORT
FOR**

**Vitragan
(Hyaluronidase, ovine)**

EMEA/H/C/696

Day 120 Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

This should be read in conjunction with the "Question and Answer" document on the withdrawal of the application: the Assessment Report may not include all available information on the product if the CHMP assessment of the latest submitted information was still ongoing at the time of the withdrawal of the application.

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LIST OF ABBREVIATIONS

AE	adverse event
API	active pharmaceutical ingredient
AQH	aqueous humour
BCVA	best corrected visual acuity
BDV	border disease virus
BRP	biological reference preparation
BRVO	branch retinal vein occlusion
CNS	central nervous system
CRVO	central retinal vein occlusion
EMD-CNM	exudative macular degeneration with choroidal neovascular membrane
ERG	electroretinogram
HA	hyaluronic acid
IOP	intraocular pressure
ITT	intention to treat
IU	international units
ivt	intravitreal
MA	macroaneurysm
MTD	maximum tolerated dose
NFU	national formulary unit
NLP	no light perception (blindness)
NOAEL	no adverse effect level
PP	per protocol
PDR	proliferative diabetic retinopathy
PVD	posterior vitreous detachment
RD	retinal detachment
RPE	retinal pigment epithelium
SAE	serious adverse event
TU	therapeutic utility (per protocol)
VA	visual acuity

I. RECOMMENDATION

Based on the review of the data on quality, safety and efficacy, the CHMP considered that the application for Vitragan, in the treatment of vitreous haemorrhage was not approvable since major objections were identified, which precluded a recommendation for marketing authorisation at the present time.

The major objections precluding a recommendation of marketing authorisation, pertained to the following principal deficiencies:

At the quality level:

- Further data to support the consistency need to be submitted using more informative technology. It is of particular importance to verify consistency with the material used in the clinical studies for the proposed indication and to take also the remaining proteins analysed today into account. The control of the product quality should be further analyzed by inclusion of additional tests and specifications. Limits need to be reconsidered based on what has been qualified and what is found in batch analysis. The Applicant should also provide a thorough justification for not using a drug substance of higher purity.
- The specification for the drug product allows for an increase in the molecular mass due to glycation. The applicant should discuss how it has been qualified that levels at the maximum limit do not impair the clinical safety or the efficacy of the drug product and further justify his choice of lactose as a bulking agent.
- Important aspects of validation of the production process are missing.
- Regarding viral safety a risk assessment of the overall capacity of the production process to remove/inactivate viruses should be performed

At the non-clinical level:

- The retinal safety has not been satisfactorily addressed. This includes the concern regarding retinal detachment that was observed following the 2nd and 3rd intravitreal injection of 75 IU of Vitragan in rabbits.

At the clinical level:

- In relation to the unfavourable ocular safety profile of Vitragan, a clinically relevant efficacy has not been demonstrated. At 2 months post-injection, some 60 – 70 % of patients treated with the intended 55 IU Vitragan dose still do not have enough media clarity to allow for a proper diagnosis and treatment. Since the vast majority of participants were diabetics with proliferative disease, which may need to be addressed with immediate echography, vitrectomy or laser, the CHMP are of the opinion that the clinical effect is too modest and too slow, even though it is statistically significant vs. saline, and that necessary secondary procedures are deferred for unnecessarily long periods of time. Data that identifies a patient population in whom a clinically meaningful treatment benefit may be gained in comparison with the clinically relevant alternative “watchful waiting” is needed. Benefits of the treatment translating into a reduced need for vitrectomy should have been demonstrated.
- The rationale of developing hyaluronidase for the treatment of vitreous haemorrhage is unclear since hyaluronidase will induce vitreous liquefaction without a simultaneous decrease in vitreous adherence, and this combination of factors is likely to cause retinal traction (bleeding) and retinal tears formation (leading to retinal detachment), events that have indeed been observed in clinical trials following Vitragan injections.

In addition, there are a number of other concerns that have also been identified.

II. EXECUTIVE SUMMARY

II.1 Problem statement

Vitreous haemorrhage is an important cause of painless sudden loss of vision. The yearly incidence of spontaneous vitreous haemorrhage is approximately 7 cases per 100,000 population. Besides being a phenomenon following ocular trauma, the most common causes of spontaneous vitreous haemorrhage are:

- proliferative diabetic retinopathy (32%),
- retinal tear (30%),
- proliferative retinopathy after retinal vein occlusion (11%), and
- posterior vitreous detachment (PVD) without retinal tear (8%).

Vitreous haemorrhage can be caused by the pathologic mechanisms of disruption of normal retinal vessels, bleeding from diseased retinal vessels or abnormal new vessels, and extension of haemorrhage through the retina from other sources. Haemorrhage into the vitreous gel results in rapid clot formation and is followed by a slow clearance of approximately 1% per day, i.e. depending on the severity of the haemorrhage, it may take several months for the patient to regain vision. The alternatives for managing vitreous haemorrhages are laser photocoagulation, vitrectomy and no treatment (“watchful waiting”) since even severe haemorrhages may clear spontaneously. Vitreous haemorrhage is characterised by decreased visual acuity, obscuration of important and potentially treatable ocular pathology, and complications related to the vitreous haemorrhage and its resolution. Ultrasound investigation is indispensable in cases where the vitreous haemorrhage is so dense as to prevent physicians from visualising the retina, and if a harmful condition such as retinal detachment or tears are identified, a prompt vitrectomy is performed before the underlying cause is addressed with the proper procedure. Investigators have considered therapeutic approaches using drugs to lyse or dissipate vitreous haemorrhage or to modify the structural characteristics of the vitreous to allow the relocation of blood out of the visual axis.

II.2 About the product

Vitragan (hyaluronidase) is a lyophilized (freeze-dried) formulation of hyaluronidase extracted from the testes of sheep. Hyaluronidase is monographed in the European Pharmacopoeia, and is an enzyme that breaks down hyaluronic acid (HA, hyaluronan) by cleaving glycosidic bonds of HA and, to some extent, other acid mucopolysaccharides of the connective tissue. HA is a major constituent of the intercellular matrix (ICM) of connective tissue, and is found at high concentrations in the vitreous, under the skin, in the umbilical cord and in the synovial fluid in joints. HA is also implicated to have a role in biological processes such as cell adhesion, migration and proliferation. For this Marketing Authorisation Application (MAA), the enzyme is extracted from the testes of sheep and it contains an α - and a β -form of hyaluronidase. It also contains two major impurities – a protein and a fragment of ovine IgG.

In cases of vitreous haemorrhage, a clot (or dispersed blood elements) of densely packed red cells with interlaced fibrin fibrils can remain trapped in the vitreous gelatinous HA/ collagen fibril network for months. The slow resolution of the intravitreal clot is partly due to the gel state of the vitreous, hindering diffusion of chemotactic factors and the movement of phagocytic cells. Published data indicate that HA as such may decrease the clotting time of fibrinogen, increase the rate of fibrin polymer formation and inhibit clot lysis. The failure of the clot, a foreign body in the eye, to stimulate a polymorphonuclear cell response has been documented histologically in a rabbit model¹.

In the present indication, hyaluronidase is aimed to cleave HA into smaller fragments to create liquefaction of the vitreous, to allow diffusion of and movement of cells within the vitreous to phagocytise the clot. In addition, the xenobiotic hyaluronidase in Vitragan is intended to produce a limited, acute inflammatory reaction to increase the recruitment of phagocytic cells and to further increase the rate of clearance of the residual clot.

II.3 The development programme/Compliance with CHMP Guidance/Scientific Advice

Hyaluronidase was developed as a spreading or diffusing substance to increase the permeability of connective tissue through the hydrolysis of HA. It is approved in the United States of America and certain European countries under the name of Vitrase. As such, hyaluronidase has been injected by subcutaneous (s.c.) and intravenous (i.v.) administration and its systemic effects at high doses has been characterised. For example, in one set of publications, hyaluronidase was investigated for use following myocardial infarction at doses up to 220,000 IU in over 1100 patients. In these studies, occasional transient allergic reactions that resolved with treatment were the major side effects. Other previously approved hyaluronidase products have different inactive ingredients and, while being demonstrated as safe systemically at very high doses, are toxic when injected into the vitreous humour because of the preservatives used. Vitragan is sterile and non-preserved and thus can be injected intravitreally. Consequently, the non-clinical and clinical testing has been focused the use of hyaluronidase in the eye.

There is a need for less invasive approaches than vitrectomy to treat vitreo-retinal disorders. However, physiological considerations raise doubts on the validity of this rationale. The vitreous is a viscoelastic gel made of 98% water, 1% macromolecules (collagen II, hyaluronic acid) and low molecular weight materials. The structure of the gel is formed by fibrils of collagen II. HA binds to non-fibrillar collagen IX, which acts (through several non collagenous domains) as a proteoglycan, to bind the glycosaminoglycans to the collagen II fibrils. Two important points should be considered when referring to vitreous aging or pathology: vitreous liquefaction (decrease in the size of HA leading to more aqueous properties and aggregation of fibrils giving the symptoms of floaters) and vitreous adherence/separation from the surface of the retina giving rise to posterior vitreous detachment (PVD). Vitreous liquefaction without simultaneous decrease in vitreous adherence is the major cause of retinal traction (bleeding), and retinal tears formation (leading to retinal detachment). Therefore, an ideal drug or combination of drugs should first separate, and then liquefy the gel (Sebag, 2004; Graefe's Arch Clin Exp Ophthalmol, 242:690–698). However, hyaluronidase is purely a liquefying agent. If it has a beneficial effect, this will not only occur through vitreous liquefaction, but also, and maybe mostly, through increased intravitreal diffusion rate of erythrocytes and exudates along with phagocytes, which will facilitate blood cell lysis and phagocytosis. Vitragan also induces a strong inflammatory reaction which, in itself, promotes the clearance of red blood cells.

As a general comment, many references on animal models provided by the applicant are from seventies (or even sixties). More recent literature shows that hyaluronidase is not able to induce PVD in the animal (Hikichi T et al., 2000 Retina, 20:195-8).

During the development, the drug product manufacturing site was changed due to scale-up demands and slight manufacturing changes were implemented.

There is no paediatric development. No CHMP Scientific Advice was sought.

II.4 General comments on compliance with GMP, GLP, GCP

GMP-certificates issued by an EU authority have neither been submitted for the manufacturer of the Drug substance nor for the Drug product.

GMP-inspections are recommended for both the manufacturers. For the Drug Substance manufacturer, particular emphasis should put to the documentation of the starting material. The Drug Product manufacturer is used for many different products and the particulars of this product should be addressed in a product specific inspection.

A valid GMP certificate has been provided for the site responsible for batch release in the EU.

The pivotal repeat dose toxicity study in rabbits was stated to be performed according to GLP, but without any quality audits. Consequently, the quality of this study is not confirmed. The pivotal study in non-human primates was performed according to GLP, in Canada. A recent inspection was performed by an EU Competent Authority to clarify the GLP status of the study conducted in this laboratory.

All 6 double-masked Phase II and III clinical studies except one (Phase II) were conducted according to GCP.

II.5 Type of application and other comments on the submitted dossier

This is a complete and independent application according to Article 8.3 of Directive 2001/83/EC, as amended, for a previously known active substance according to Council regulation No 2309/93. The active substance in Vitragan is hyaluronidase of ovine origin, which is supplied as a lyophilisate with an activity of 5500 IU. The freeze-dried powder should be reconstituted in 0.9% saline before use.

The indication sought is “*for the treatment of vitreous haemorrhage to improve visual acuity and to facilitate the physician’s ability to diagnose the underlying retinal pathology*”. The recommended dose is a single intravitreal (ivt) injection of 55 IU, in a volume of 50 µl. Hyaluronidase is monographed in the European Pharmacopoeia. There are no new excipients.

The quality part of the dossier was of poor quality, both in form and in content. The organisation of the non-clinical and clinical parts of the dossier were considered to be acceptable. However, the overviews did not critically assess the data and there were several issues detailed in the study reports that should have been highlighted.

III. SCIENTIFIC OVERVIEW AND DISCUSSION

III.1 Quality aspects

Drug substance

The drug substance is extracted from ovine testes and collected in bags. The ovine testes are sourced and processed in New Zealand. The bags are blast frozen and transported to the Drug Substance manufacturer under contract for the applicant.

The drug substance, is extracted and purified from the frozen ovine testes in four major stages including differential ammonium salting out precipitants and ion exchange chromatography. To the greatest extent the steps of the manufacturing process and its control are sufficiently described, however for the virus filtration step more details on conditions should be given. Some issues on the testing during manufacture have been identified as well.

Data to support stability during storage of the intermediates have been submitted, but did not allow a good interpretation of the data. During storage of the early intermediates a decrease in activity is seen and the applicant should discuss the reason for this and furthermore expand the analysis of the down stream material to verify if the instability seen in activity also is affecting other characteristics. Further questions related to these studies have also been identified.

The starting material, ovine testes, its origin and handling, are well described. New Zealand has strict control of animals and animal imports and has not had any BSE case at all and no scrapie since 1954. The classification of the starting material are deemed sufficient to assure the freedom of TSE .

Manufacturing improvements were made after the pivotal Phase 3 clinical lot, 224C, and a lot used in other phases of clinical trials, 224B, were manufactured. The changes introduced has been acceptably described and justified and when looking at them, they are not expected to negatively influence the quality of the substance. However as seen in the characterization data and batch analysis, there is lacking information and differences seen between the clinically used batch and the commercial production. This is further expanded on below.

The applicant has performed a variety of experiments designed to identify and characterize the API and major protein impurities. The experiments and studies compare the primary structure of the individual proteins, the distribution of the enzymatically active species, the secondary structure of the proteins in the drug substance, the size distribution of the components of the drug substance, and average internal forces in the drug substance. It is shown that ovine hyaluronidase occurs in two natural forms: α and β hyaluronidase. Hyaluronidase is the only active ingredient in the drug product Vitragan but only accounts for less than 20% of the total protein content. From these data two major objections arise. The most important is that the batch used in clinical trials has not been used consistently throughout the characterisation studies and that the batch analyses of the product indicate differences compared to commercial batches. Since showing comparability between the clinically used batches and the commercial product to be put on the market is vital, the applicant should further justify how it can be ascertained that the commercial batches will be representative for the batch used in the phase III studies. The applicant needs to expand his analytical arsenal to verify that the product is consistent.

There exists a monograph of the European pharmacopoeia and the tests of this should be followed which is not the case today. Certain additional tests are added to the test specification. A number of questions have arisen relating to the methods of analysis as well as on the validations, where the latter in particular have been very difficult to follow since a lot of addenda etc. have been added to the reports as well as reports submitted which are established for previous revisions without a clear overview of the reason behind it. As mentioned above the applicant should expand his testing of the API and furthermore reconsider the limits based on what has been qualified and what is found in routine production. A final assessment of the proposed specification is awaited.

Results from the hyaluronidase assay using the Ph. Eur. method should be submitted to verify reproducibility and fulfilment of specification also using this method. The applicant should also use the hyaluronidase BRP.

The applicant proposes a retest period of 18 months for the active ingredient when stored at $-20 \pm 5^\circ\text{C}$. The data support a storage period of 18 months under these conditions, however a retest period is not allowed for biological products like these. Instead a strict shelf life should be established. However such a shelf life cannot be established before the questions in relation to the testing and characterisation of the product has been solved.

One single and definitive protocol should be provided and followed for future stability studies.

Viral safety

The Applicant has performed virus inactivation and removal studies in support of the safety of the drug substance and drug product. In these studies, four model viruses were used to evaluate the virus clearing ability of selected production steps to remove or inactivate spiked virus.

In addition to the validation of the process for virus clearance described above, the applicant has tested several lots of product, in an *in vitro* assay for adventitious viruses performed according to 9 CFR requirements. No viruses were detected in either the Phase III clinical lot or four lots representative of the commercial manufacturing process.

The CHMP considers that the Applicant should furthermore address the virus risk, especially for non-enveloped viruses, taking into account that the eye is a so-called immune-privileged site and also being part of the CNS. Based on the risk assessment batch wise testing of adventitious agents should be considered.

Drug Product

Vitragan is a sterile lyophilisate for solution for injection, supplied in a single-use glass vial closed with a rubber stopper and aluminium seal. Although the formulation chosen for Vitragan is not considered optimal (only 50 µL of 5.4 mL reconstituted solution is used, i.e. 1 %), no risk is seen with the large excess of product and the stability reasons claimed by the applicant, for not using a lower fill size, can be accepted.

During the development a number of formulations and overfills have been used. Some questions regarding these development batches in terms of their relation to commercial and clinically used lot are still outstanding at the time of this report.

One concern with lactose as excipient is that it is a reducing sugar, which may undergo glycation (Maillard reaction) with proteins during storage. The main degradation products due to glycation lead to a structurally heterogeneous group of adducts that remain bound irreversibly to the protein components of the drug product. It has been demonstrated that changes in the mobility of all protein components occur at elevated temperatures and that the protein shift assay is suitable to monitor these changes. Also at storage indications of an increase in molecular mass is observed. It is only the size of the molecular mass shift that has been discussed, the fraction of protein molecules, which are glycated is not commented on. The applicant has been asked to verify the mechanism of the glycation for the product. The applicant should discuss how it has been qualified that levels at the maximum limit do not impair the clinical safety or the efficacy of the drug product and that a test for extent of glycation is not needed. Since it cannot be verified for the time being, that the proposed specification is qualified in terms of safety and efficacy, this is considered a major deficiency. The applicant should also comment on the choice of lactose as an excipient.

The validation of the production was presented as an assembly of not organised documents, protocols, reports and amended reports, and as a consequence difficult to understand. A number of deficiencies regarding unexplained out of specification results, failing of release requirements, bulk hold times, validation of sterile filtration, validation of lyophilisation, validation of filling uniformity has been identified raising concerns on the control and consistency of manufacture.

The drug product is approved in the USA, however for a different indication and route of administration. Several commercial batches have been manufactured for the US market, however a number of clarifications are needed to confirm that Ph Eur requirements are met. No batch data is available on batches tested according to the EU specification, and it is not clear if the EU re-testing is a full re-testing of all parameters in drug product specification. Further questions on the methods of analyses used in batch testing have been identified as well.

At the accelerated storage condition decreases in activity and increases in protein shift data are observed. From the stability data presented, the molecular mass (protein shift data) is increasing also during storage, especially taking into account the data from accelerated studies, which demonstrate a clear increase in molecular mass. The applicant was asked to justify why it was not deemed necessary to apply a more stringent release requirement to cover the increase during storage. No shelf life can be approved at this stage.

III.2 Non clinical aspects

Pharmacology

The primary mechanism of action of hyaluronidase, an enzyme that cleaves the glycosidic bonds of HA, is well characterised and no basal studies have been performed.

Primary pharmacodynamic studies were conducted in a rabbit model, using intravitreal injection of autologous blood to evaluate the effect of hyaluronidase on clot clearance. Even though the effect was quite modest, the studies generally showed that clot clearance was accelerated after hyaluronidase injection resulting in an increased clarity of the optic nerve and retina. In these studies, a number of mistakes and changes have been seen to be made during the course of the studies. These pertained to randomisation of animals, which lots of active drug product had been used, and there were also changes in evaluation parameters and statistical methods during the studies. However, the changes were documented, and therefore possible to track. Still, the effect of Vitragan when the vitreous haemorrhage is more or less extensive is unclear.

The injection produced a transient inflammatory response with invading macrophages and T- and B-lymphocytes. The cellular response peaked at the first two weeks and returned to early phase levels by the end of the 4th week. The findings of these studies support part of the proposed rationale behind hyaluronidase, i.e. that the recruitment of phagocytosing cells is improved even though there are no studies that actually confirm that hyaluronidase liquefies the vitreous. On the other hand, signs of vitreous liquification were evident in some of the toxicity studies in rabbits. A second injection of hyaluronidase improved efficacy in non-clearing and poor responders. Addition of the major impurity, did not affect clearance of the clot, nor did removal of IgG fragments (another major impurity).

In the primary pharmacodynamic studies, rabbits were used. The relevance of the selected species was not discussed by the applicant. There are large differences between the vitreous of different species. Rabbits have a low concentration of vitreous HA compared to humans, and their vitreous is almost fluid, while in humans it is in a gel-state. These differences may have an impact on the choice of the clinical dose. Higher doses were more effective; the highest activity tested was 150 IU and no plateau was reached. In humans, the intended dose was 55 IU. Besides, its higher HA content, the volume of the human vitreous is approximately 4 ml, while in the rabbit the vitreous volume is 1.5 ml, and no correction for volume differences has been made. Consequently, the intended clinical dose is not justified and it is not clear how rabbit data have been extrapolated to the clinical situation. In addition, the rabbit model of vitreous haemorrhage does not reflect the diabetic disease states of the majority of patients. In diabetes ocular disease, an inflammatory component is present and it is not clear how this component is affected by hyaluronidase (type and time-course). Finally, the type and time course of the inflammatory response in the presence of a blood clot was not studied.

There were no studies addressing potential secondary effects of hyaluronidase. From a systemic point of view, this could be considered to be acceptable. However, potential ocular effects of the major protein component in hyaluronidase, which happens to be an impurity, needs to be addressed since this impurity may activate plasminogen, decrease cell-cell adhesion and increase cell migration. Hyaluronidase also contains the amino acid tyrosine (~ 18 µg/clinical dose). Retinal levels of tyrosine are reported to be in the region of 0.5 µg/g protein (rat) and the potential effects of tyrosine need to be further addressed since tyrosine is the rate limiting compound in the synthesis of dopamine, which in turn is utilized as a neurotransmitter by a subpopulation of retinal interneurons.

There are no safety pharmacology studies. There is a vast amount of non-clinical, as well as clinical published material. For example, in the publications regarding its use following myocardial infarction, doses of up to 220,000 IU actually improved ECG parameters (less change in QRS complexes) for the hyaluronidase-treated patients compared to control subjects. In addition, in the context of the low, single

dose of hyaluronidase together with a rapid inactivation of the compound in serum, it is highly unlikely that there will be any adverse effects on the major organ systems.

Pharmacokinetics

Since hyaluronidase is an enzyme with a reasonably characterised systemic pharmacokinetic profile, the main focus was to determine the fate of intravitreally injected hyaluronidase. Hyaluronidase absorption and disposition kinetics following the ivt route of administration was evaluated in rabbits by using ¹²⁵I-labelled and biotinylated hyaluronidase. As previously, the choice of species was poorly justified and considering the liquid state of the rabbit vitreous compared to humans, the pharmacokinetic data obtained from these studies are probably not fully relevant for humans. On the other hand, the general behaviour of hyaluronidase in the vitreous is probably sufficiently representative.

A study was performed in Canada and the GLP status of this study cannot be assured. There are no formal requirements to adhere to GLP while characterising the general pharmacokinetics; these requirements relate to the toxicokinetic (TK) part, but no TK studies were performed. The pharmacokinetics of hyaluronidase is considered to have a limited impact on the safety evaluation.

Intravitreally injected hyaluronidase resides in the vitreous, with a half-life of approximately three days. The vitreous acts as a depot for release of hyaluronidase to the circulation, which is reflected by the long plasma half-life (~ 2 days). Intact hyaluronidase (as measured by using biotinylated hyaluronidase as a standard), as well as radioactivity, were distributed to the vitreous and aqueous humour (AQH). The radiolabelled material was also found in ocular tissues with the highest levels in the retina. Plasma and extra-ocular tissue levels of radioactivity were found to be ~ 1000-fold below vitreous levels. There seemed to be some degradation of hyaluronidase in the eye, but the metabolism and excretion of ivt administered hyaluronidase is poorly characterised. However, in view of the low anticipated systemic exposure after a single ocular dose of hyaluronidase, it is unlikely that the lack of characterisation of hyaluronidase metabolism and excretion will have an impact on the overall safety of Vitragan.

Toxicology

Only the ocular effects of intravitreally injected hyaluronidase were studied. The lack of standard systemic single and repeat dose toxicity studies was however considered to be acceptable considering the well characterised systemic action of hyaluronidase together with the anticipated low systemic exposure. By the same reasons, it was also considered to be acceptable to essentially restrict the evaluation to ocular tissues in these studies, even though formally, a summary of previous systemic toxicity studies should have been submitted.

The ocular toxicity studies were performed in rabbits and non-human primates, - most of them with a single dose administration, although in some studies up to three injections were given. Investigations included slit lamp biomicroscopy, funduscopy, tonometry, electroretinography (ERG) and histopathology (eyes only). The clinical evaluations were performed at repeated intervals during the studies. Besides analysis of body weights (several studies), haematology, clinical biochemistry or urine analysis and gross pathology (organs preserved, but not sectioned) in the pivotal non-human primates study, there was no assessment of systemic safety. In this limited evaluation, there were no signs of systemic effects in any studies. In the non-human primates study, immunochemical analyses for hyaluronidase-specific antibodies were performed.

There were a number of weaknesses in the submitted studies. There were no GLP-study allowing assessment of dose-response in any species, which precludes the identification of NOAEL and MTD. Consequently, an accurate assessment of potential risks to humans could not be performed and this was not considered to be acceptable. Furthermore, in two of the pivotal single dose rabbit studies, it was not clear how ocular inflammation was scored, and the severity of inflammation was not possible to determine

from the submitted data. In these studies, grading of cells and haze should have been performed on a scale from 0 to 4, but the results included grades above 4 despite the inflammation being classified as mild. In contrast, there was a total absence of ocular inflammation at 4-fold higher doses in some studies. These discrepancies needed to be further addressed.

The first pivotal repeat dose toxicity study in rabbits was stated to have been performed according to GLP, but without any quality audits. Although the quality of the study seemed to be acceptable, it has not been confirmed.

The other pivotal non-human primate study was performed according to GLP in Canada. A recent inspection was performed by an EU competent authority, to clarify the GLP status of the pivotal study conducted in this laboratory.

Overall, in rabbits, the clinical examinations revealed an early (~ days 1 - 7) anterior segment inflammation of varying severity, with flare and cells infiltrating the AQH. In some cases this resulted in iris synechia, which could be resolved with mydriatic treatment. The anterior inflammation was followed by a posterior ditto (cells and haze sometimes with precipitates). The degree of inflammation usually declined over the course of the studies. In some studies however, vitreous cells were persistent and full recovery was not achieved. High doses of hyaluronidase (750 IU) to the rabbit eye were retinotoxic. The persistency of inflammation was evident especially in the repeat dose studies, in which the severity and duration of the inflammatory response tended to increase with the number of injections. It is likely that the ocular immune response is further activated after repeated injections of a foreign protein and there is concern regarding the safety of a second injection of hyaluronidase to the human eye. It is also possible that there will be an antibody response in humans, as observed in two other non-human primate studies after the second injection. If repeated injection should ever be considered, the impact of such antibodies on ocular and systemic safety must be further addressed. In most studies there were few or no signs of vascular leakage, but in the severely inflamed eyes, the evaluation was hampered by vitreous haze. It is however likely that with pronounced inflammation, there would be vascular leakage due to the increased permeability of retinal vessels.

In a number of eyes in the pivotal non-human primate study, the inflammatory response seemed more pronounced than in the rabbits, and it was still seen to be severe and persistent 90 days after injection. Even though one of the aims with this compound was to induce a mild, transient inflammation, in some cases, the vitreous inflammation was so severe that it precluded inspection of retinal structures. Therefore it was not clear how a mild, transient inflammation is defined.

Retinal detachment is observed following the 2nd and 3rd intravitreal injection of 75 IU of hyaluronidase in rabbits. As hyaluronidase causes liquefaction of the vitreous, traction on the retina with ensuing retinal detachment could be expected if vitreous liquefaction occurs in the absence of or in the presence of incomplete posterior vitreous detachment (PVD). Retinal detachment is observed in humans following a single administration of 55 and 75 IU Vitragan. The occurrence of retinal detachment in the rabbit model only following 2 or 3 intravitreal injections of 75 IU of Vitragan (and the total absence of retinal detachment following a single dose administration of hyaluronidase in non-human primates) versus the occurrence of retinal detachment following a single dose of 55 or 75 IU hyaluronidase in humans, raises questions about the sensitivity of the animal model used to assess the safety of intravitreal hyaluronidase. Moreover the mechanism underlying hyaluronidase-induced retinal detachment is not clear.

Also of major concern is the retinal changes observed mainly in one study (supportive non-GLP study). Therefore, the applicant performed two peer-reviews, comparing these eyes with sections from naïve animals. The results of these reviews were conflicting. The first review indicated that the changes were antemortem, while in the second review they were believed to be the result of the fixation procedure (neurosensory retina/retinal pigment epithelium [RPE] separation), and similar to those seen in the naïve eyes. It is well known that an improper fixation of ocular tissues results in a separation of the

neurosensory retina from the RPE and it is possible that part of the retinal changes were due to a suboptimal fixation. However, minimal peripheral retinal changes as well as not fully reversible effects on ERG were also evident in the non-human primate studies. In addition, reactive RPE were observed occasionally in exploratory studies and in two eyes in the rabbit repeat dose toxicity study, but its clinical relevance was not clear. In humans, the condition of the retina was not possible to examine due to the intraocular haemorrhage and no ERG measurements were made. Consequently, the retinal safety could not be considered as being satisfactorily addressed. The applicant is therefore requested to perform a GLP, (at least a single-dose) dose-response study, preferentially in non-human primates (where the vitreous is more similar to humans), with ERG-measurements, ophthalmological examinations and histology of eye and optic nerve (adequately processed) to establish a NOAEL (where a mild transient inflammatory reaction (to be defined) is included), from which a safety margin for human use could be calculated.

There were no genotoxicity and carcinogenicity studies. This is acceptable due to the nature of the compound. Considering the limited systemic exposure and rapid elimination of circulating hyaluronidase, the lack of studies addressing potential toxicity to reproduction is also regarded as being acceptable, since it is considered unlikely that relevant amounts of hyaluronidase would reach the foetal circulation. However, if antibodies towards hyaluronidase are formed, a risk toward the developing foetus can not be excluded.

There were no studies assessing the immunotoxic potential of hyaluronidase. One of the aims with this compound was to generate an ocular immune response and it is considered that this response is sufficiently characterised even though there are questions regarding the severity of the response. Presently, there is no concern regarding a systemic immunotoxic effect of the compound.

The major impurity is the main protein component in Vitragan, as well as a heavy chain IgG fragment. Representative amounts of the major impurity and the IgG fragment have been present in the batches used in the non-clinical toxicity studies (as well in the clinical studies). Although ocular histology was included, the studies addressing the impurities did not adhere to GLP. It has not been ascertained whether the commercial batches are representative of those used in the toxicology testing programme. This has been addressed in the Quality part of the assessment.

No environmental concern has been identified.

III.3 Clinical aspects

Pharmacokinetics

Hyaluronidase is intended for administration as a single intravitreal injection of 55 IU in 50 µL for the treatment of vitreous haemorrhage. This dose is equivalent to 68 USP units.

No human pharmacokinetic studies have been performed with hyaluronidase. The applicant has submitted one publication with pharmacokinetic data after intravenous administration from one man and one woman (67 and 70 years of age respectively) with acute myocardial infarction. The patients received bovine testicular hyaluronidase 500 NFU/kg every 6 hours. The half-life in plasma after intravenous administration was reported to be 2.4 and 4.1 minutes with no detectable levels after 20 minutes.

Although one can assume that most of the drug dose (55 IU) that is delivered in 50 microlitres into the vitreous will be bioavailable, some reflux with the withdrawal of the needle could be possible. For ethical reasons it would have been inappropriate to require that intravitreal samples are taken for the purpose of concentration measurements, but some interindividual variability in administered doses and rate of elimination is expected. Even though the animal data cannot be fully extrapolated to man, it is likely that the half-life in plasma after intravitreal administration would be limited by the rate of release from the eye, and that the total systemic exposure would be minimal. The low systemic exposure is not deemed relevant

from an efficacy or safety point of view. Safety data after intravascular administrations of >3500 times higher doses (200 000 IU) in other indications are available from different publications.

No dose-response relation has been established.

Pharmacodynamics

The proposed activity of hyaluronidase is to clear intravitreal bleeding through a mechanism of cleaving hyaluronic acid and other matrix components, thereby breaking the entrapment of the blood. No human pharmacodynamic studies were performed. It is thus not certain that hyaluronidase actually cleaves HA into smaller fragments and liquefies the vitreous. This may be partly explained by the fact that there is no convenient clinical model in which the pharmacodynamic property of hyaluronidase could be studied. In theory however, vitreous samples from man could have been studied *in vitro*. Thus, the putative pharmacodynamic effects of hyaluronidase were investigated directly in phases II and III of the clinical development. Consequently, with the lack of pharmacodynamic data *in vivo*, justification of the posology as claimed in the Summary of Product Characteristics (SPC) is merely based on two Phase III studies. In one of these studies, 7.5 IU showed a statistically significant effect, compared with saline. Only the fact that the chosen 55 IU dosing is deemed to be unsatisfactorily effective prevents from a further question on a possible clinical efficacy of doses lower than 55 IU. It is possible however, that some of the modest clinical effects documented may have been a result of an increased phagocytic activity elicited by the injection itself or by the foreign proteins contained in the compound. In the Phase IIb program, increased vitreous clearance was indeed related to the highest inflammatory score in the anterior chamber for the highest dose, 75 IU.

Clinical efficacy

The phase IIb and phase III double-masked studies are displayed in this table.

Study ID	No. of study centres / locations	Design	Study Posology	Study Objective	Subjs by arm entered, ITT/safety	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
ACS202-HYA-001A	21 centres, USA	Double-masked 3 arms	Single ivt inj. of hyal. In different doses (no placebo)	Efficacy/safety	53/53 (7.5IU) 50/50 (37.5IU) 50/50 (75IU)	1 year	52%/48% Mean range: 61 - 64 years	Vitr. haemorr. > 1 mo.	Clearance of vitreous
Phase IIb									
ACS203-HYA-001MEX	3 centres, Mexico	Double-masked 3 arms	Single ivt inj. of hyal. (no placebo)	Efficacy/safety	76/76 (7.5IU) 74/74 (37.5IU) 75/75 (75IU)	≥ 8 weeks	44%/56% 57 years	Vitr. haemorr. > 1 mo.	Clearance of vitreous
Phase IIb									
VIT-02-08961X	9 centres, Canada 3 centres, Mexico 61 centres, USA	Double-masked 4 arms	Single ivt inj. of hyal. In 3 doses or saline	Efficacy/safety	181/180 (7.5IU) 179/175 (55IU) 197/194 (75IU) 193/191 (saline)	3 months	52%/48% 62 years	Vitr. haemorr. > 1 mo.	Clearance of vitreous
Phase III									
VIT-03-08961X	64 centres Worldwide (Europe, Australia, Africa)	Double-masked 3 arms	Single ivt inj. of hyal. In 2 doses or saline	Efficacy/safety	186/184 (55IU) 180/180 (75IU) 190/187 (saline)	3 months	50%/50% 62 years	Severe vitr. haemorr. > 1 mo.	Clearance of vitreous
Phase III									

In the Phase II program, there were indications of an effect with the 75 IU dose, although a dose-response pattern was not evident. The applicant selected 7.5 IU (only in one of the two studies), 55 IU, an intermediate dose not hitherto tried and 75 IU for the pivotal Phase III program. In addition, a saline injection was given as a control treatment. Studies were randomised, double-masked and multi-centred, and recruited patients with a dense vitreous haemorrhage (duration > 1 month) precluding visualisation of the retina (and BCVA < 20/200). Exclusion criteria were underlying conditions such as retinal detachments or retinal tears (confirmed with ultrasound B-scan), previous vitrectomy, previous recent vitreous haemorrhage.

The overview of efficacy is based on pooled data from two Phase IIb Studies (ITT n = 153 and n = 225), which are compared with pooled data from two Phase III studies (ITT n = 750 and 556). These studies were selected because of similarity in study designs in each Phase of the clinical investigation.

The primary endpoint in the Phase III program was improvement in best corrected visual acuity (BCVA) by three lines on the ETDRS chart. This was in fact a redefinition of an originally designated primary endpoint named “surrogate success evaluation,” encompassing BCVA improvement and clearing of the vitreous according to three criteria (sufficient clearing allowing for a) laser or b) surgical intervention targeting the underlying condition or c) a diagnosis of the underlying condition not warranting any additional treatment).

Secondary endpoints were clearance of haemorrhage defined according to the amount of visible retina; vitreous clearing as judged by the investigator based on recordings of the CRF (labelled “surrogate success variable”) reflecting the visibility of the retina in relation to the treatment initiated to address the

underlying condition, the response to treatment of diabetics stratified into type 1 and 2, and the use of vitrectomy. The endpoint follow-up was 3 months post-injection.

Amendments to the protocol included change to the above designated primary endpoint from the surrogate success variable and the replacement of a non-treatment “watchful waiting” group by the control group receiving saline. Study conduct appears to have been acceptable. The change of the primary endpoint was not entirely appropriate since the indication wording includes both improvement in patient’s vision and the physician’s ability to diagnose and treat. Also, the 3-month time-point being the endpoint signifies an inconveniently protracted follow-up in this incapacitating condition and the applicant has indeed emphasised the 2-month data.

Results

Pooled Phase III Studies - Summary of Vitreous Haemorrhage Causality (Study Eye) and Duration at Study Entry. (7.5 IU dose not displayed)

	Saline Control	HYALURONIDASE DOSE		
		55 IU	75 IU	All hyaluronidase
Included in ITT ^a	383	365	377	742
Vitreous Haemorrhage Causality				
PDR	278	250	281	531
CRVO	20	17	17	34
BRVO	18	25	10	35
EMD-CNM	20	14	15	29
MA	1	2	1	3
PVD	6	11	8	19
Other	6	10	11	21
Missing or NA	34	36	34	70
Duration at Entry (days)				
≤ 28	2	0	2	2
29-90	195	193	182	375
> 90	179	168	187	355
Missing or NA	7	4	6	10

Abbreviations: PDR – proliferative diabetic retinopathy, CRVO – central retinal vein occlusion, BRVO – branch retinal vein occlusion, EMD-CNM - Exudative Macular Degeneration with Choroidal Neovascular Membrane, MA - Macroaneurysm, PVD - Haemorrhagic Posterior Vitreous Detachment

Patient compliance and discontinuations before Month 12, which was the safety endpoint, was around 15% in the 55 IU dose group, which was a matter of concern. There was an even higher discontinuation rate in VIT-02 (~ 20 %), where the main reason was patient withdrawing consent. Considering that treatment was given only on one occasion, the reasons not to continue the study need to be further addressed.

Patient disposition in VIT-02: In the ITT (randomised) population, 750 patients were analysed. In the modified ITT (randomised, treated and at least 1 follow-up completed), 740 patients were analysed. And in the therapeutic utility (TU) subset (a PP subpopulation including all modified ITT compliant with inclusion/exclusion criteria), 655 patients were analysed.

Patient disposition in VIT-03: In the ITT population, 556 patients were analysed. In the modified ITT 551 patients were evaluable. And in the TU subset 504 patients were analysed.

Pooled phase III Studies - Cumulative Percentage of BCVA Improvement

		HYALURONIDASE DOSE			All hyaluronidase N=742
Visit	Saline Control n=383	55 IU n=365	75 IU n=377	Overall p-value	
Month 1	77 (20.1%)	112 (30.7%)	105 (27.9%)		217 (29.2%)
p-value, pairwise against saline		0.0009	0.0127	0.0029	
Month 2	105 (27.4%)	150 (41.1%)	144 (38.2%)		294 (39.6%)
p-value, pairwise against saline		0.0001	0.0016	0.0002 b	
Month 3	132 (34.5%)	164 (44.9%)	164 (43.5%)		328 (44.2%)
p-value,, pairwise against saline		0.0035	0.0113	0.0065	

The corresponding values for the 7.5 IU were 29.8% at Month 1, 33.7% at Month 2 and 37% at Month 3. Only at Month 1, the results of the data from VIT-02 only, show that 7.5 IU was statistically significant vs. saline (p = 0.008),.

Pooled Phase III Studies – Proportion of Patients with Efficacy Outcome Based on Recorded Monthly Assessment by Month 2, Surrogate success variable (clearance of haemorrhage only, secondary efficacy endpoint as stated in the CRFs)

		HYALURONIDASE DOSE		
	Saline Control	55 IU	75 IU	All hyaluronidase
Included in ITT ^a	N=383	N=365	N=377	N=742
Success (%) ^b	106 (27.7%)	143 (39.2%)	144 (38.2%)	287 (38.7%)
p-value ^c		0.0008	0.0023	

The so called surrogate success evaluation that incorporated the BCVA results in addition to clearance of haemorrhage yielded less favourable results; 25% success for the 55 IU dose and 16% for the saline group at the 2-month evaluation time point.

Reduction in vitreous density

Pooled Phase III Studies - Summary of ‘Improvement’ in Vitreous Haemorrhage Density Improvement from Baseline on or prior to Month 2

		HYALURONIDASE DOSE		
	Saline Control	55 IU	75 IU	All hyaluronidase
Included in ITT	383	365	377	742
Marked Improvement				
≥6 clock hours (%)	80 (20.9%)	119 (32.6%)	114 (30.2%)	233 (31.4%)
<6 clock hours (%)	297 (77.5%)	240 (65.8%)	260 (69.0%)	500
p-value ^a		0.0007		
Improvement				
≥6 clock hours (%)	137 (35.8%)	176 (48.2%)	169 (44.8%)	345 (46.5%)
<6 clock hours (%)	240 (62.7%)	183 (50.1%)	205 (54.4%)	388
p-value ^a		0.0016		

There was little doubt that hyaluronidase 55 IU was statistically superior to saline injection in achieving haemorrhage clearing of the vitreous, translating into a gain in BCVA and a potential to establish the diagnosis and to initiate further treatment if indicated, of the underlying cause at 2 months post-injection. However, the treatment effect was moderate, both in absolute numbers and in relative terms compared

with saline, which is a major objection to this application. The effect was also slow in coming, which may adversely affect the retinopathy of the diabetic population who in general, needs timely additional laser therapy to halt neovascularisation.

A high proportion of patients still had a dense vitreous at Month 2 post-injection (i.e at least 3 months after symptom onset) with 55 IU hyaluronidase; 59% had not had a BCVA improvement, 75% - 60% were not deemed as treatment successes, and 65% had not had marked improvement of the vitreous clouding. Thus there was doubt that hyaluronidase could compete with standard clinical management of dense vitreous haemorrhage, which is early vitrectomy, both in terms of short-term and long-term risk/benefit assessments. Moreover, the superiority to saline is marginal, translating into 1 out of 9 – 10 subjects benefiting from the active treatment in a number-to-treat analysis. The lack of a clinically relevant non-treatment control group makes conclusions even more difficult to draw, both from an efficacy and safety point of view. The saline injection may have had true treatment effects by stimulating inflammation, which may be of some advantage in clearing the vitreous, but in all probability also caused adverse effects in excess of a “watchful waiting group”. Inflammation, addressed in the safety assessment, could thus be instrumental in the treatment effect also with hyaluronidase, and the actual mechanism of action was considered to be a matter for discussion.

A number of other efficacy issues were also raised.

Clinical safety

The Phase I population had an intended minimum follow-up of 2 months. The Phase II population had an intended follow-up ranging from 2 months to 1 year.

The main body of the safety data was generated in the placebo-controlled Phase III program, which recruited the vast majority of hyaluronidase-treated and saline-treated patients, with a planned follow-up of 12 months and onward. The exposure is deemed sufficient given the single administration of the active drug.

Patients exposed to saline or hyaluronidase in the intravitreal program (Phase I – III):

Doses	Saline	7.5 IU	37.5 IU	55 IU	75 IU
Number of patients	417	327	130	377	609*
Mean days of follow-up	312	361	380	327	316
Median days of follow-up	346	364	455	352	343
Range	5 - 784	1 - 964	2 - 811	7 - 972	6 - 1412

* follow-up of 4 patients from study ACS201-HYA-002A could not be determined since the day of injection was not recorded.

A safety update information as of August 2005 has been provided by the applicant including safety data beyond the cut-off (September 2001) for clinical documentation. This describes patients who have been followed for up to 4 years. No new safety signals emerged. The total follow-up time is also deemed sufficient.

The safety evaluation program targeted incidence of ocular events occurring shortly after the injection and during the follow-up. Primary safety variables of the Phase III program were events of No Light Perception (blindness), visual acuity (VA) reduction, retinal detachment (RD), recurrent vitreous haemorrhage, iritis, hypopyon, hyperaemia of the conjunctiva, and eye pain. Secondary variables were changes in BCVA, intraocular pressure (IOP), lid, conjunctiva, cornea, anterior chamber, lens, vitreous density signs and the occurrence of interventions (e.g. cataract surgery and vitrectomy).

Serious adverse events in the Phase III program included corneal oedema (grade 3), various lens changes indicating cataract formation, iris synechia or rubeosis, blindness (NLP), IOP elevation > 10 mm Hg.

This assessment focussed on the pivotal Phase III studies, where 1291 patients had been recruited - or 68.8% of the safety population.

A high incidence of relatively severe or severe AEs were documented for: iritis (30 – 60%), hypopyon = severe iritis (2 – 30%), eye pain (20 – 40%), vitreous haemorrhage (roughly 20%), reduced VA (18 – 28%), RD (4 – 10%), various forms of cataract (1 – 10%), corneal oedema (0 – 6%), rubeosis of the iris (0 – 6%) and blindness (0 – 3%).

There was a 30% increase in the rate of macular oedema in treated eyes. However, it should be noted that the overall rate of macular oedema was low in the ITT population. In fact it was too low considering the baseline diabetic retinopathy. This probably reflected the absence of objective measurements for the presence of macular oedema, such as fluorescein angiography or OCT. No case of endophthalmitis was recorded. Whereas the investigator judged retinal detachment and recurrence of ocular haemorrhage to be unrelated to treatment in many cases, the opposite was stated about eye pain and iritis. RD and recurrence of ocular haemorrhage occurred mainly late in the follow-up period as did cataract; for the latter there was an increased incidence beyond 6 months.

Ocular adverse events (AEs) that were statistically significantly more common for all three hyaluronidase treatment groups as compared with saline in the pivotal studies were: abnormal sensation in the eye, eye pain, iritis, increased lacrimation, ocular hyperaemia, photophobia, and photopsia. More severe AEs such as recurrence of bleeding, increased IOP (>10 mm Hg in 9% and 2.5% of patients in studies VIT-02 and VIT-03, respectively) or cataract did not differ between placebo and active. However, there was an apparent 50% increase in the rate of retinal detachment, which was borderline significant ($p=0.06$) in the 55 IU group and statistically significant ($p<0.05$) in the 75 UI group compared to saline. This raised a serious concern.

Selected Ophthalmologically Important Events in VIT-02 and VIT-03: Analysis of Numbers Needed to Harm (to have one patient sustaining an AE)^a Compared to Number Needed to Treat^b(to have one patient benefiting from the treatment).

Event	Saline (n=378)	Hyaluronidase Treatment Group			NNH ^a	NNT ^b
		55 IU (n=377)	75 IU (n=391)	55 + 75 IU (n=768)		
≥1 Eye Disorder ^c	0.788	0.857	0.893	0.875	11.5	9 or 10
Retinal Detachment	0.069	0.093	0.115	0.104	28.6	9 or 10
Recurrent Vitreous Haemorrhage	0.175	0.241	0.230	0.236	16.4	9 or 10
Blindness NEC	0.011	0.016	0.023	0.020	111	9 or 10
Increased IOP ^f	0.111	0.119	0.118	0.118	143	9 or 10
Total Cataracts	0.257	0.281	0.274	0.277	50	9 or 10
Total Corneal ^e	0.148	0.220	0.220	0.220	13.9	9 or 10
Visual Acuity Reduced	0.196	0.268	0.251	0.259	15.9	9 or 10
Macular Oedema	0.029	0.029	0.049	0.039	100	9 or 10
Eye Pain	0.222	0.369	0.412	0.391	5.9	9 or 10
Iritis	0.333	0.589	0.621	0.605	3.7	9 or 10
Hypopyon	0.000	0.016	0.054	0.035	28.6	9 or 10
Uveitis NOS	0.005	0.019	0.010	0.014	111	9 or 10

^a NNH is the reciprocal of the absolute safety risk and indicates the number of eyes (patients) the clinician needs to treat to find one with the specific safety concern as compared to saline control treatment. For NNH presented in this table, the proportion of patients in the combined 55 and 75 IU Vitrase groups was used to calculate the absolute safety risk associated with Vitrase treatment. For example, the NNH for retinal detachment is calculated as follows: $[1/(0.104)-(0.069)]$.

^b NNT = 9 for vitreous haemorrhage density improvement; NNT = 10 for BCVA improvement of at least three lines (0.3 LogMAR units)

^c Proportion of patients with one or more ocular adverse events.

^d Total Cataracts included the following preferred terms: cataract nuclear; cataract subcapsular; cataract cortical; and cataract NEC.

^e Total Corneal included the following preferred terms: corneal erosion; corneal oedema; corneal disorders NOS; corneal epithelium defect; corneal abrasion; corneal opacity; keratitis and staining investigations).

^f Adverse events of IOP code to both the “Eye Disorder” and “Investigations” System Organ Classes.

^g Blindness was a delayed event and occurred following vitrectomy.

Issues of duration of AEs relate in particular to eye pain, hypopyon, iritis, and increased IOP. It is important to point out that these adverse events did not seem to lead to discontinuation of patients from the study.

The mean durations of eye pain for the saline control, 55 IU and 75 IU treatment groups ranged from 33 to 40 days. Approximately 70-80% of the adverse event eye pain was considered to be related to the test agent. Severity of eye pain correlated to the dose of hyaluronidase.

The duration of iritis was a clinically important issue. Iritis was normally present already at Day 1. The mean durations by treatment groups for all reported adverse events of iritis were 39.5, 52.1, 31.0, and 26.8

days for saline, 7.5 IU, 55 IU, and 75 IU hyaluronidase treatment groups, respectively, although 12 – 19% of actively treated eyes exhibited some degree of iritis between Month 3 and 12. Approximately 80% of the various hyaluronidase treatment group patients were considered to have iritis that related to test agent. About half of the iritis incidents resolved without treatment (i.e. topical steroids). There was a pattern of increased frequency of moderate to severe iritis with higher doses of hyaluronidase. The applicant attributes this phenomenon to the increased presence of fragments of hyaluronic acid. The high incidence of iritis in saline-treated eyes needed to be further addressed.

Across treatment groups, 50 to 60% of the adverse events of increased IOP were considered to be related to the test agent. For these related events, the mean durations ranged from 30 to 45 days across treatment groups. The general incidence was low however.

As for systemic serious AEs, very few were reported and deemed unlikely to be related or not related to test treatment. Renal failure, other renal or urinary disorders, cerebrovascular incidents, vascular incidents and infections were all reported in > 10 patients in the entire program.

Death occurred in 3.3% (48/1443) of hyaluronidase-treated patients, and in 4% in the saline injection group of the pivotal studies. In the safety update of VIT-02 and VIT-03 including 40 – 60% of patients having data up to 24 months, the rate of death increased to 9%.

The safety profile in terms of systemic AEs and death did not give rise to any concern.

Across all studies, for each treatment group, the percentage of patients discontinued from the studies due to an SAE/AE or death was low. No more than 1.5% of patients in a treatment group discontinued because of an adverse event. The result is based on a follow-up of at least 3 months in the majority of cases.

Summary of AEs or SAEs Causing Discontinuation: All Studies by Association to Treatment

Event	Associated with Treatment	Not Associated with Treatment	Total Events
Systemic Adverse Events	0	5	5
Death	0	2	2
Retinal Detachment	4	0	4
Vitreous Haemorrhage	0	3	3
Cataract	0	1	1
Increased IOP	1	0	1

Source: **Section 2.7.4.7, Listings 3 and 9**

Note: Discontinuations for serious adverse events were collected for the Primary Phase III Studies (VIT-02-08961X and VIT-03-08961X). All other studies collected discontinuations due to adverse events.

In summary, the alarming or distressful ocular adverse events were retinal detachment, vitreous haemorrhage, blindness, reduced visual acuity, macular oedema, iritis, hypopyon, cataract and to some extent corneal untoward effects and eye pain. The relation between active and injected control is reassuring in most cases, but since watchful waiting was not included as a control (other than in 18 cases, which is too limited for an appropriate analysis), there is no information of the ocular incidents in the natural course of the condition. Conversely, there are no current studies describing the safety profile of the generally used aggressive approach of initial vitrectomy.

From the present data, it can be stated that an intravitreal injection of hyaluronidase causes iritis and eye pain in excess of an injection with saline. Furthermore, control and hyaluronidase treatment is associated with retinal detachment, vitreous haemorrhage, and reduced visual acuity but the impact of treatment on these incidents, which may very well be secondary to the underlying condition, cannot be determined. In

this respect, the safety profile of hyaluronidase 55 IU is debatable, and with reference to the major objections regarding efficacy it contributes to a negative risk/benefit balance.

As things appear, according to the calculations of numbers-to-treat and numbers-to-harm, in injecting 55 IU of hyaluronidase in roughly 30 individuals suffering from dense vitreous haemorrhage, 3 would benefit from the treatment, but 7 patients would get iritis, almost 2 subjects would get a recurrence of the bleeding and 1 subject would get a retinal detachment.

IV. ORPHAN MEDICINAL PRODUCTS

N/A

V. BENEFIT RISK ASSESSMENT

As summarised in the assessment, major objections and a number of other concerns need to be addressed before the quality documentation could be considered acceptable.

From a non-clinical viewpoint, the retinal safety could not be considered satisfactorily addressed since retinal detachment, retinal changes and not fully reversible effects on ERG were observed in the toxicity studies.

From a clinical view-point the risk/benefit balance of hyaluronidase treatment is judged negative given the modest efficacy and a debatable safety profile.