

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-278

CROSS DISCIPLINE TEAM LEADER REVIEW

Cross-Discipline Team Leader Review #2

Date	February 20, 2009
From	William M. Boyd, M.D.
Subject	Cross-Discipline Team Leader Review
NDA#	22-278
Applicant	Dutch Ophthalmic Research Center
Date of Submission	August 18, 2008, January 30, 2009, February 17, 2009
PDUFA Goal Date	February 21, 2009
Proprietary Name / Established (USAN) names	MembraneBlue 0.15 % (trypan blue ophthalmic solution)
Dosage forms / Strength	ophthalmic solution
Proposed Indication(s)	for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue
Recommended:	Approval

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• Introduction

MembraneBlue (trypan blue ophthalmic solution) is a sterile solution of trypan blue. MembraneBlue selectively stains membranes of the inner surface of the retina that can lead to visual disturbances from mild distortions and blurred vision due to the presence of metamorphopsia, micropsia and ultimately to reduced vision. These membranes include epiretinal membranes (ERM) and Internal Limiting Membranes (ILM) that can be removed during ophthalmic surgical vitrectomy procedures.

Identification of the membranes can be difficult, as they are transparent or mildly opaque. These membranes are hypocellular, collagenous proliferations (essentially scar tissue) occurring on the inner surface of the retina. Trypan blue, the active drug substance, is a stain widely used to selectively stain dead tissues or cells. Trypan blue is not absorbed in a viable cell, but traverses the membrane in a dead cell. Therefore, only the membranes are stained in contrast to the retina and excess trypan blue is washed out of the eye during the vitrectomy procedure. Staining the membranes improves the contrast between the membranes and the retina and can facilitate their removal; staining also reduces the chance of incomplete peeling of the membranes, which could lead to persistent metamorphopsia.

MembraneBlue is a blue di-azo group dye. It is a symmetrical molecule with three parts connected by two azo bonds. The molecular weight is 960.8. The empirical formula is: $C_{34}H_{24}N_6 Na_4O_{14}S_4$. It is water soluble. During vitrectomy surgery a few drops (0.3 -0.5 mL) are applied directly onto the areas where membranes could be present. Prior to injecting MembraneBlue a "fluid-air exchange," i.e., a filling of the entire vitreous cavity with air, is

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performed to prevent dilution of the MembraneBlue. Excess dye can be washed out of the posterior chamber.

MembraneBlue can also be injected directly into a BSS filled vitreous cavity instead of injecting under air. Sufficient staining is usually achieved after 30 seconds of application under BSS.

• Background

Trypan blue has been marketed as MembraneBlue™ in Europe since 2002 as a medical device Class IIa for the same proposed indication. The applicant reports over ——— units have been used during ophthalmic surgical vitrectomy procedures. No complaints or any other information concerning adverse events have been received during this period. Trypan blue has also been marketed as VisionBlue™ in Europe since 1999 and was approved in the US April 12, 2004 (NDA 21-670) for the indication of staining of the anterior capsule during cataract surgery. The applicant reports over ——— units have been used during cataract surgery.

This is a 505(b)(2) application. The safety and efficacy of MembraneBlue for the proposed indication can be supported from published studies which use the applicant's product. The applicant has marketed the product in Europe without doing any studies on their own. Studies were subsequently done with their product and reported in the literature.

The proposed indication, use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue and ————— is not acceptable and is not supported by the submitted data. A revised indication, for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue, is acceptable.

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Based on the following four adequate and well controlled clinical trials from the literature (reviewed in Section 6.1.4 of the Medical Officer's review):

1. C Haritoglou, et al. Retina 2004; 24(4):582-90,
2. C Haritoglou, et al. Am J Ophthalmol 2004;138(1):1-5,
3. KL Lee, et al. Br J Ophthalmol 2005; 89:420-4, and
4. J Beutel, et al. Arch Ophthalmol 2007; 125:326-332,

and the other literature studies listed in Section 5.1 of the Medical Officer's review, this application for Trypan Blue has demonstrated safety and efficacy in selectively staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

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There are no known safety or effectiveness concerns that have arisen with other members of this pharmacologic class.

• **CMC**

DRUG SUBSTANCE:

Trypan blue, the drug substance in MembraneBlue 0.15%, is _____ by _____
_____ DMF _____ is authorized to be referenced for information regarding the _____ trypan blue. Reviews of DMF _____ were completed and the DMF was noted to be adequate to support the current NDA.

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DRUG PRODUCT:

The formulation of MembraneBlue 0.15% is similar to VisionBlue 0.06%, which was submitted by the same applicant and approved in June 2004. The only difference is an increased trypan blue concentration from 0.06% to 0.15% for MembraneBlue. Each mL of MembraneBlue 0.15% contains 1.5 mg trypan blue, 1.9 mg sodium monohydrogen orthophosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), 0.3 mg sodium di-hydrogen orthophosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), 8.2 mg sodium chloride (NaCl), and water for injection. The pH is 7.3 - 7.6. The osmolality is 257-314 mOsm/kg. MembraneBlue is filled in glass syringes to a volume of 0.5 mL.

The drug product is manufactured by a contract firm, _____ located in _____
_____ The drug product is _____
and a _____ to obtain a 0.15% solution. The sterile _____
_____ is supplied by _____ in _____

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During drug product manufacturing process, the pH of the solution is verified and the solution is _____ through a _____ to _____ any _____. The prepared solution is then filled into a single-use Luer Lok 2.25 ml glass syringe (_____ ml/syringe) and the syringe is closed with a tip cap and stopper. The solution is _____ sterilized. The syringes are then placed into _____ pouches and the outside surface of the syringes is _____ sterilized.

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A two year expiry dating period was proposed for this product when stored at 15-25°C (59-77°F) and protected from direct sunlight. Stability data was available on one batch of MembraneBlue 0.15% up to 3 months at long-term and accelerated conditions. Supporting stability data was available on three batches of VisionBlue 0.06% up to 26 months.

DRUG PRODUCT COMPOSITION:

From the original CMC review, page 21.

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Composition of MembraneBlue 0.15%

Component	Quantity
Purified Trypan Blue	
Total	0.5 mL - mL

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Composition of	(0.5 mL):
Sodium Chloride	4.10 mg
Sodium Phosphate Dibasic Dihydrate	0.95 mg
Sodium Phosphate monobasic dihydrate	0.15 mg
Water for Injection	QS to 0.5 mL

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REGULATORY SPECIFICATIONS:

From the original CMC review, page 11.

The specification of MembraneBlue is listed below, which is same as that for VisionBlue.

Test	Acceptance Criteria	Method (Code#)
Physical Appearance	Dark blue liquid	Visual inspection
Trypan Blue Identity UV/VIS	Identity assay to reference standard	Appendix 4, Amendment dated 12/6/07
Trypan Blue Content UV/VIS	assay to reference standard	Appendix 4, Amendment dated 12/6/07
Trypan Blue Content HPLC-UV nm (stability study)		Appendix 5, Amendment dated 12/6/07
Impurities HPLC-UV nm:		HPLC (Appendix 5 of Amendment date 12/6/07)
	NMT	
Unidentified:		
Rel. RT	NMT	
Rel. RT	NMT	
Rel. RT	NMT	
Any individual unspecified impurity	NMT	
Total impurities	NMT	
pH	7.3 - 7.6	USP <791>
Osmolality	257 - 314 mOsm / kg	Eu. Pharm.
Particulate Matter	NMT	USP <789>
	NMT	
	NMT	
Sterility	Sterile	USP <71>
Bacterial Endotoxins	NM - EU / mL	USP <85>

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ESTABLISHMENT EVALUATION REQUESTS/REPORTS

An Establishment Evaluation Request was made via EES, and an inspection was performed from _____ This inspection revealed multiple GMP deficiencies, and a 483 was issued.

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The observations cited in the 483 included:

- Failure to qualify the maximum amount of sterilizing cycles for which the _____ can be reused without being replaced
- Failure to conduct integrity test (pre and post _____ to the _____ used during the sterilization of all batches of Vision Blue 0.06% trypan blue ophthalmic solution syringes produced at the site and distributed to the U.S.
- Manufacture and distribution to the U.S. batches of Vision Blue 0.06% trypan blue ophthalmic solution syringes without having a validation of the sterilization process for VisionBlue using biological indicators
- Failure to have an _____ conducted during filing and prior to sterilization of Vision Blue 0.06% trypan blue ophthalmic solution syringes
- Failure to establish the manufacturing time for the different critical operations performed during the production of the Vision Blue 0.06% trypan blue ophthalmic solution product.

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The applicant has addressed these issues. Upon re-inspection of _____ the facilities were found acceptable on _____. The Establishment Evaluation Report is attached to the CMC review dated January 7, 2009.

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• **Nonclinical Pharmacology/Toxicology**

Per the original Pharmacology/Toxicology review, page 9:

Trypan blue has been approved for ocular staining in cataract surgery (VisionBlue™ 0.06%) under NDA 21-670. NDA 21,670 is cross-referenced for this NDA. The toxicological profile for carcinogenicity, teratogenicity, and mutagenicity has already been established. It is reported that trypan blue was teratogenic in rats, mice, rabbits, hamsters, dogs, guinea pigs, pigs, and chickens. It caused external, skeletal, and internal malformations. Trypan blue was mutagenic in Ames test. Trypan blue is carcinogenic in rats. Chronic intermittent exposure by subcutaneous injection of trypan blue in Wistar/Lewis rats induced a reticuloendothelial neoplasm, predominantly in the liver. The aforementioned information has been listed in the proposed labeling for MembraneBlue™.

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In this NDA submission, only one cytotoxicity study was included. Trypan blue produced slight cytotoxicity at concentrations of up to 0.15% over 24 hr of exposure in this *in vitro* study using the MTT test system. The sponsor also cited other toxicology information from the literature. Mostly, the papers selected are concentrated on the toxic effects of trypan blue in the retina using *in vitro* cultured human and animal retinal pigment epithelium cells or *in vivo* animal models. Some studies indicated that trypan blue was safe. Other studies showed positive findings of trypan blue including damaged photoreceptors and disorganization, decrease in mitochondrial dehydrogenase activity, morphological changes of the RPE cells, lowered ERG b-wave in bovine retina, and increased p53 and p21 expression. The toxicity was usually seen at higher concentrations and longer treatment duration.

Trypan blue is a vital stain widely used to selectively stain dead cells. The drug is not absorbed in a viable cell. Therefore, only the epiretinal membranes are stained in contrast to the retina. Clinically, only 0.75 mg of trypan blue will be administered to the eye, and almost all dye will be immediately irrigated out from the eye, leaving less than — to mark the membrane that too will be removed from the eye. Therefore the amount of the drug left in the patient after the surgery will be very low. The sponsor indicated that the final dose used in vitreoretinal surgery is less than — mg. It appears that possible systemic and ocular toxic effect of MembraneBlue™ is small. b(4)

Considering the drug history and clinical experience, nonclinical study results, proposed indication, and dosage, the reviewing pharmacologist believes that, from the nonclinical standpoint, the data are adequate for the approval of the drug. The labeling of MembraneBlue™, which is based on the approved labeling of VisionBlue, is considered acceptable.

• Clinical Pharmacology/Biopharmaceutics

Per the original Clinical Pharmacology review, page 1:

MembraneBlue is packaged in a volume of 0.5 mL in a 2.25 mL single-use syringe for application to the retinal membrane by blunt cannula. The actual dosage of MembraneBlue is determined by the ophthalmic surgeon, but is in the range of 0.3 to 0.5 mL. Trypan blue is not absorbed by viable cells, but traverses the membrane of dead cells. Excess dye is washed out of the eye by irrigation, while the stained membranes are removed from the eye, leaving only a minimal amount of trypan blue in the eye following surgery.

No clinical PK studies evaluating the systemic absorption of trypan blue following administration of MembraneBlue have been conducted. A waiver of the *in vivo* bioavailability requirement is granted, based on the expected negligible systemic exposure of trypan blue following use of MembraneBlue (trypan blue ophthalmic solution) during ophthalmic surgical vitrectomy procedures.

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The Clinical Pharmacology and Biopharmaceutics information provided by the Applicant is acceptable.

• Sterility Assurance

Per the original Product Quality Microbiology review, page 4:

The primary package consists of a 2.25 mL BD _____ glass syringe, a _____ tip cap and a _____ plunger stopper. The manufacturing process for MembraneBlue™ is nearly identical to the manufacturing process for the approved product, VisionBlue™. The container closure system is identical for both products. b(4)

The container closure system is identical to that approved for VisionBlue. The container closure integrity test for VisionBlue was evaluated in the product quality microbiology review for NDA 21-670 and approved in November of 2004.

MembraneBlue™ has a bacterial endotoxin limit of NMT – EU/mL. This endotoxin limit was found to be satisfactory by the medical division because excess dye will be washed from the eye and stained tissue will be surgically removed. Endotoxin testing will be conducted according to USP <85> methodology. The results of inhibition/enhancement testing for the drug product were provided in the June 2008 amendment. The results show that inhibition was overcome at a dilution of _____. The applicant has chosen a working dilution of _____ for use with an endotoxin sensitivity of _____ EU/mL. This allows for detection of endotoxin levels of _____ EU/mL. b(4)

Sterility testing will be conducted according to USP <71>. The results of bacteriostasis/fungistasis testing were provided in the June 2008 amendment and found to be satisfactory. Because the product label states that the syringe containing membrane blue is sterile, a sterility test should be conducted on the syringe as well. The applicant provided a commitment to conduct sterility testing on both the contents of the syringe and the contents of the pouch in the June 2008 amendment.

• Clinical/Statistical - Efficacy

Per the original Medical Officer review, page 9:

Clinical studies have been conducted and published in the literature. The applicant has not submitted any additional unpublished clinical studies. The following clinical studies with MembraneBlue for selective staining of epiretinal membranes during ophthalmic surgical vitrectomy procedures have been conducted. The following abbreviations are used in the following tables: Trypan Blue (TB), Indocyanine green (ICG).

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First Author	Journal	Number of patients treated with Test Product	Controls	Results
EJ Feron	Arch Ophthalmol 2002; 120:141-144.	10 TB 0.06%	None	Epiretinal membranes not identified prior to use, identified after supposed complete removal. Pathology confirmation that removed tissues were only epiretinal membranes.
K Li	Br J Ophthalmol 2003; 87:216-9.	14 TB 0.06%	None	Membranes were stained satisfactorily and removed successfully.
M Perrier	Am J Ophthalmol 2003;135(6): 903-5.	18 TB 0.06%	None	Visualization and dissection of the membranes was facilitated by trypan blue staining.
M Perrier	Am J Ophthalmol 2003;135(6): 909-11.	23 TB 0.06%	None	Visualization and dissection of the membranes was facilitated by trypan blue staining without any signs of toxicity.
Francisco A Teba	Ophthalmology 2003; 110: 2409-2412	50 TB 0.2%	None	Epiretinal membranes can be identified.
C Haritoglou	Retina 2004; 24(4):582-90.	10 TB 0.15 %	15: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.
C Haritoglou	Am J Ophthalmol 2004;138(1):1-5.	22 TB 0.06 %	21: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.
BJ Vote	Retina 2004;24(5):736-8.	26 TB 0.15%	None	TB staining of epiretinal membranes is a useful adjunct in vitreoretinal surgery and improves the safety and efficiency of membrane identification and removal.
SY Lesnik Oberstein	Br J Ophthalmol 2007; 91:955-7.	29 TB 0.15%	None	Staining of membranes is a safe method.
F Uno	Retina2006;26(2):237-9.	1 TB 0.15%	None	Case report: Subretinal migration of TB due to either direct trauma due to accidental subretinal injection, direct (chemical) toxicity of TB or solution osmolality; and photodynamic effect of TB, which may absorb wavelengths of light from the endoilluminator.

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Studies using Double Staining Technique - TB and ICG:

First Author	Journal	Number of patients treated with Test Product	Controls	Results
P Stalmans	Br J Ophthalmol 2003; 87:713-6.	30 TB 0.15% and ICG	None	Double staining technique using TB and ICG aided in visualization of membranes during vitreoretinal surgery.
AK Kwok	EYE 2004;18(9):882-8.	16 TB 0.15% and ICG	None	Double staining technique using TB and ICG aided in visualization of membranes during vitreoretinal surgery

Comparable studies of trypan blue to alternatives:

First Author	Journal	Number of patients treated with Test Product	Controls	Results
KL Lee	Br J Ophthalmol 2005; 89:420-4.	5 TB 0.3% 13 TB 0.15%	5 ICG 0.5% 14 ICG 0.05%	The anatomical and visual results in the two groups were comparable.
J Beutel	Arch Ophthalmol 2007; 125:326-332.	20 TB 0.15%	20 ICG 0.05%	No statistical difference was detected between the two groups; though the TB group had better visual recovery.

The applicant did not conduct any clinical studies. The studies published in the literature and submitted by the applicant were reviewed. In addition, a Medline search of the literature for ocular studies using trypan blue in epiretinal surgery was conducted. Articles which could be identified as relevant for the proposed indication were reviewed.

ANALYSIS OF PRIMARY ENDPOINT(S)

Trypan blue is well known as a vital stain which differentially stains different tissues in the body. It is used *in vivo* and *in vitro* as a standard to distinguish between living and dead cells, i.e. living cells do not stain but dead cells do stain. When injected into the posterior region of the vitreous, trypan blue 0.15% selectively stains epiretinal membranes. Dose ranging studies demonstrate that a concentration of 0.06% or higher is effective in selectively staining epiretinal membranes. Concentrations of 0.06% trypan blue sometimes provide only faint staining. Higher concentrations of 0.15% and 0.2% trypan blue improved both visualization scores and ease of removal scores (Trypan blue-assisted vitrectomy. Vote BJ, Russell MK, Joondeph BC. *Retina*. 2004 Oct;24(5):736-8; Trypan blue staining in vitreoretinal surgery. Teba FA, Mohr A, Eckardt C, Wong D, Kusaka S, Joondeph BC, Feron EJ, Stalmans P, Van Overdam K, Melles GR. *Ophthalmology*. 2003 Dec; 110(12):2409-12).

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Nearly 300 eyes were reviewed using trypan blue (TB) at concentrations of 0.06% and 0.15% or greater for selective staining of epiretinal membranes during ophthalmic surgical vitrectomy procedures; of 300 eyes evaluated, 200 eyes were studies using a concentration of TB of 0.15% or greater.

To support the approval of a drug, safety and efficacy is recommended to be demonstrated in at least two adequate and well-controlled trials.

Of the clinical studies referenced, the following four studies used a control during surgery compared with the TB group; the control group included no staining of the epiretinal membrane or staining of the epiretinal membrane with ICG.

Studies comparing TB to a Control (No staining)

First Author	Journal	Number of patients treated with Test Product	Controls	Results
C Haritoglou	Retina 2004; 24(4):582-90.	10 TB 0.15 %	15: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.
C Haritoglou	Am J Ophthalmol 2004;138(1):1-5.	22 TB 0.06 %	21: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.

Studies comparing TB to a Control (ICG):

First Author	Journal	Number of patients treated with Test Product	Controls	Results
KL Lee	Br J Ophthalmol 2005; 89:420-4.	5 TB 0.3% 13 TB 0.15%	5 ICG 0.5% 14 ICG 0.05%	The anatomical and visual results in the two groups were comparable.
J Beutel	Arch Ophthalmol 2007; 125:326-332.	20 TB 0.15%	20 ICG 0.05%	No statistical difference was detected between the two groups; though the TB group had better visual recovery.

1. C Haritoglou, et al. Retina 2004; 24(4):582-90.

In this clinical study 10 eyes of 10 consecutive patients with intraoperative use of TB (0.15%) were analyzed and compared with the functional outcome in a matched group of patients (15 eyes) who had undergone vitrectomy without TB.

The studied demonstrated TB permitted better visualization of the epiretinal membrane which might enable the surgeon to better and more completely remove the epiretinal tissue. No adverse effects of the dye on functional outcome were observed.

2. C Haritoglou, et al. Am J Ophthalmol 2004;138(1):1-5.

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This clinical trial was a prospective, randomized, comparative study. Forty three eyes of 43 consecutive patients were randomized between TB (0.06%) staining or to no staining.

The authors concluded the application of TB may be beneficial since it enables the surgeon to better visualize and therefore remove the epiretinal tissue more completely (though there were no statistically significant differences in functional outcome after a follow-up of up to 6 months - the statistical difference in functional outcome between the groups was $p > 0.5$). The study reported that no apparent dye-related adverse events occurred.

3. KL Lee, et al. Br J Ophthalmol 2005; 89:420-4.

This was a retrospective analysis of 37 eyes from 37 consecutive patients. In 19 patients ICG was used and in 18 patients TB.

There was no significant difference between the preoperative visual acuities in the TB and ICG groups but the postoperative visual acuities were better in the TB than in the ICG group ($p = 0.036$). The TB group also had more lines of improvement than the ICG group (2.94 versus 1.79 lines; $p = 0.046$), follow-up ranged from 1 – 30 months and the median follow-up was 5 months. TB appears to be less toxic than ICG when used in dye assisted removal of epiretinal membranes as reflected by the better visual results in the TB group.

4. J Beutel, et al. Arch Ophthalmol 2007; 125:326-332.

This was a randomized, controlled trial with 20 patients (20 eyes) receiving ICG and 20 patients (20 eyes) receiving TB. Although the primary outcome measurement of visual acuity at 3 months measured by the Early Treatment of Diabetic Retinopathy scale did not demonstrate a significant difference between the TB and ICG study groups, after 6 months the TB group did show significant improvement in visual acuity ($p = 0.002$).

EFFICACY CONCLUSIONS

These four clinical trials with control groups and the other studies listed in the Medical Officer's review support the efficacy of MembraneBlue as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

• Safety

Published literature study results were used to establish the safety of the drug product.

As early as 1967, trypan blue has been used in ophthalmology to achieve vital staining of the cornea and conjunctiva. It has been injected into the anterior chamber since the 1970s to evaluate the corneal endothelium. It is the standard screening agent for organ donor corneas to evaluate the corneal endothelium prior to corneal transplantation. Well over — corneas have been screened and transplanted after administration of trypan blue, 0.3%. This dose is

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approximately 2 times the proposed dose. In donor corneal grafts, there are no documented adverse effects.

Adverse reactions reported following use of MembraneBlue include discoloration of high water content hydrogen intraocular lenses (see Contraindications) and inadvertent staining of the posterior lens capsule and vitreous face. Staining of the posterior lens capsule or staining of the vitreous face is generally self limited, lasting up to one week.

There are no known safety concerns that have arisen with other members of this pharmacologic class.

Of the clinical studies referenced in the application, the following four studies used a control during surgery when compared with the TB group and support the safety of the drug product:

1. C Haritoglou, et al. *Retina* 2004; 24(4):582-90.

In this clinical study of 10 eyes of 10 consecutive patients with intraoperative use of TB (0.15%), no adverse effects of the dye on functional outcome were observed.

2. C Haritoglou, et al. *Am J Ophthalmol* 2004;138(1):1-5.

Forty three eyes of 43 consecutive patients were randomized between TB (0.06%) staining or to no TB staining. There were no dye-related adverse events reported.

3. KL Lee, et al. *Br J Ophthalmol* 2005; 89:420-4.

This was a retrospective analysis of 37 eyes from 37 consecutive patients. The adverse events that were reported were those commonly associated with a vitrectomy procedure.

Postoperatively, in the ICG group one retinal detachment occurred and two small retinal detachments occurred intraoperatively that were repaired at the time of the surgery; in the TB group one patient experienced a choroidal / vitreous hemorrhage.

4. J Beutel, et al. *Arch Ophthalmol* 2007; 125:326-332.

This was a randomized, controlled trial with 20 patients (20 eyes) receiving ICG and 20 patients (20 eyes) receiving TB. Again, the adverse events that were reported were those commonly associated with a vitrectomy procedure. In both groups there was one post-op retinal detachment and in all phakic eyes the progression of nuclear cataracts were noted.

SAFETY CONCLUSIONS

These four clinical trials with control groups and the other studies listed in the Medical Officer's review support the safety of MembraneBlue as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

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• **Advisory Committee Meeting**

No Advisory Committee was necessary or convened for this drug product.

• **Pediatrics**

The drug would not be expected to be used on pediatric patients. The mean age of subjects in the published studies ranged from 60 to 71 years old.

The use of Trypan Blue in lower concentrations in pediatric patients was supported by the literature reports of adequate and well controlled studies in NDA 21-670 for VisionBlue (trypan blue ophthalmic solution) which was approved April 12, 2004.

• **Other Relevant Regulatory Issues**

A Division of Scientific Investigations (DSI) audit was not requested since this was a literature review.

A consult was requested from the Office of Surveillance and Epidemiology regarding a trade name review for the proposed name "MembraneBlue." The results of the Proprietary Name Risk Assessment found that the proposed name, MembraneBlue, appears to be vulnerable to product confusion with VisionBlue. The Division of Medication Error Prevention believes that the risk may be mitigated by differentiating the proposed labels and labeling of MembraneBlue from the labels and labeling of the existing product VisionBlue.

The Division of Drug Marketing, Advertising, and Communications (DDMAC) has reviewed DORC's proposed product labeling (PI) for this application submitted to the Agency on 30 January 2008. Their recommendations regarding the proposed indication, Warnings and Precautions, and Clinical Pharmacology sections of the labeling have been incorporated into the draft label.

• **Labeling**

NDA 22-278 is recommended for approval for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

The labeling submitted by Dutch Ophthalmic Research Center on January 30, 2009, and found in this Cross-Discipline Team Leader Review (see Appendix 1) is acceptable. The carton and container labeling submitted on February 17, 2009 is acceptable.

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• Recommendations/Risk Benefit Assessment

RECOMMENDED REGULATORY ACTION:

NDA 22-278 is recommended for approval for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

The labeling submitted by Dutch Ophthalmic Research Center on January 30, 2009, and found in this Cross-Discipline Team Leader Review (see Appendix 1) is acceptable.

RISK BENEFIT ASSESSMENT:

Based on the following four adequate and well controlled clinical trials from the literature (reviewed in Section 6.1.4 of the Medical Officer's review):

1. C Haritoglou, et al. Retina 2004; 24(4):582-90,
2. C Haritoglou, et al. Am J Ophthalmol 2004;138(1):1-5,
3. KL Lee, et al. Br J Ophthalmol 2005; 89:420-4, and
4. J Beutel, et al. Arch Ophthalmol 2007; 125:326-332,

and the other literature studies listed in Section 5.1 of the Medical Officer's review, this application for Trypan Blue has demonstrated safety and efficacy in selectively staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

The application supports the safety of MembraneBlue (trypan blue ophthalmic solution) 0.15% for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

Pharmacology/Toxicology, Product Quality Microbiology, and Clinical Pharmacology have recommended approval for this application. Clinical and CMC now recommend approval after resolution of the multiple GMP deficiencies after the site reinspection at _____

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RECOMMENDATION FOR POSTMARKETING RISK MANAGEMENT ACTIVITIES:

There are no additional proposed risk management actions except the usual postmarketing collection and reporting of adverse experiences associated with the use of the drug product.

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Appendix 1

The labeling submitted by Dutch Ophthalmic Research Center on January 30, 2009, and found in this Cross-Discipline Team Leader Review is acceptable. The carton and container labeling submitted on February 17, 2009 is acceptable.

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use MembraneBlue™ 0.15% safely and effectively. See full prescribing information for MembraneBlue™ 0.15%.

MembraneBlue™ 0.15% (trypan blue ophthalmic solution)

Initial U.S. Approval: 2004

Indications and Usage

- For use as an aid in ophthalmic posterior surgery;
- Facilitating removal of epiretinal tissue. (1)

Dosage and Administration

- Prior to injection of MembraneBlue™ 0.15% perform a 'fluid-air exchange'; Carefully apply MembraneBlue™ 0.15% to epiretinal membranes using a blunt cannula; Remove all excess dye
- Or
- Inject MembraneBlue™ 0.15% directly in a BSS filled - vitreous cavity; Wait 30 seconds; Remove all excess dye. (2)

Dosage Forms and Strength

- MembraneBlue™ 0.15% (trypan blue ophthalmic solution) in a volume of 0.5 mL. (3)

Contraindications

- Insertion of a non-hydrated (dry state), hydrophilic acrylic intraocular lens (IOL). (4)

Warnings and Precautions

- Excessive staining: Excess MembraneBlue™ 0.15% should be removed from the eye immediately after staining. (5)

Adverse Reactions

- Discoloration of high water content hydrogen intraocular lenses;
- Inadvertent staining of the posterior lens capsule and vitreous face. (6)

To report SUSPECTED ADVERSE REACTIONS contact Dutch Ophthalmic, USA at 1-800-75-DUTCH or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

Use in Specific Populations

- Trypan blue should not be given to a pregnant woman. (8)

Revised 1/2009

FULL PRESCRIBING INFORMATION: CONTENTS*

1. Indications and Usage
 2. Dosage and Administration
 3. Dosage Forms and Strength
 4. Contraindications
 5. Warnings and Precautions
 6. Adverse Reactions
 8. Use in Specific Populations
 - 8.1 Pregnancy
 - 8.3 Nursing mothers
 - 8.4 Pediatric use
 - 8.5 Geriatric use
 11. Description
 12. Clinical Pharmacology
 - 12.1 Mechanism of Action
 13. Nonclinical Toxicology
 - 13.1 Carcinogenesis, mutagenesis, impairment of fertility
 16. How Supplied/Storage and Handling
- *Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION: CONTENTS*

1. Indications and Usage

MembraneBlue™ 0.15% is indicated for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

2. Dosage and Administration

Before injection of MembraneBlue™ 0.15% perform a 'fluid-air exchange', i.e. filling the entire vitreous cavity with air, to prevent aqueous dilution of MembraneBlue™ 0.15%. MembraneBlue™ 0.15% is carefully applied to the retinal membrane using a blunt cannula attached to the MembraneBlue™ 0.15% syringe, without allowing the cannula to contact or damage the retina. Sufficient staining is expected on contact with the membrane. All excess dye should be removed from the vitreous cavity before performing an air-fluid exchange, to prevent unnecessary spreading of the dye. MembraneBlue™ 0.15% can also be injected directly in a BSS filled vitreous cavity (instead of injecting under air). Clinical use demonstrated that, after complete vitreous and posterior hyaloid removal, sufficient staining is achieved after 30 seconds of application under BSS.

MembraneBlue™ 0.15% is intended to be applied directly on the areas where membranes could be present, staining any portion of the membrane which comes in contact with the dye. The dye does not penetrate the membrane.

3. Dosage Forms and Strength

MembraneBlue™ 0.15% (trypan blue ophthalmic solution) is supplied in 2.25 mL syringes filled to a volume of 0.5 mL.

4. Contraindications

MembraneBlue™ 0.15% is contraindicated when a non-hydrated (dry state), hydrophilic acrylic intraocular lens (IOL) is planned to be inserted into the eye. The dye may be absorbed by the IOL and stain it.

5. Warnings and Precautions

Excessive staining

It is recommended that after injection all excess MembraneBlue™ 0.15% be immediately removed from the eye.

6. Adverse Reactions

Adverse reactions reported following use of MembraneBlue™ 0.15% include discoloration of high water content hydrogen intraocular lenses (see Contraindications) and inadvertent staining of the posterior lens capsule and vitreous face. Staining of the posterior lens capsule or staining of the vitreous face is generally self limited, lasting up to one week.

8. Use in Specific Populations

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C. Trypan blue is teratogenic in rats, mice, rabbits, hamsters, dogs, guinea pigs, pigs, and chickens. The majority of teratogenicity studies performed involve intravenous, intraperitoneal, or subcutaneous administration in the rat. The teratogenic dose is 50 mg/kg as a single dose or 25 mg/kg/day during embryogenesis in the rat. These doses are approximately 4,000- and 2,000-fold the maximum recommended human dose of 0.75 mg per injection based in a 60 kg person, assuming that the whole dose is completely absorbed. Characteristic anomalies included neural tube, cardiovascular, vertebral, tail, and eye defects. Trypan blue also caused an increase in post-implantation mortality, and decreased fetal weight. In the monkey, trypan blue caused abortions with single or two daily doses of 50 mg/kg between 20th to 25th days of pregnancy, but no apparent increase in birth defects (approximately 4,000-fold maximum recommended human dose of 0.75 mg per injection, assuming total absorption). There are no adequate and well-controlled studies in pregnant women. Trypan blue should be given to a pregnant woman only if the potential benefit justifies the potential risk to the fetus.

8.3 Nursing mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when trypan blue is administered to a nursing woman.

8.4 Pediatric use

The safety and effectiveness of trypan blue have been established in pediatric patients. Use of trypan blue is supported by evidence from an adequate and well-controlled study in pediatric patients.

8.5 Geriatric use

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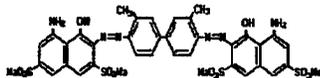
No overall differences in safety and effectiveness have been observed between elderly and younger patients.

11. Description

MembraneBlue™ 0.15% (trypan blue ophthalmic solution) is a sterile solution of trypan blue (an acid di-azo group dye). MembraneBlue™ 0.15% selectively stains epiretinal membranes during ophthalmic surgical vitrectomy procedures.

Each mL of MembraneBlue™ 0.15% contains: 1.5 mg trypan blue; 1.9 mg sodium mono-hydrogen orthophosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$); 0.3 mg sodium di-hydrogen orthophosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$); 8.2 mg sodium chloride (NaCl); and water for injection. The pH is 7.3 - 7.6. The osmolality is 257-314 mOsm/kg.

The drug substance trypan blue has the chemical name 3,3'-[(3,3'-dimethyl-4,4'-biphenylene) bis (azo)] bis(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid) tetra sodium salt, a molecular weight of 960.8, a molecular formula of $\text{C}_{34}\text{H}_{24}\text{N}_6\text{Na}_4\text{O}_{14}\text{S}_4$, and has the following chemical structure:



12. Clinical Pharmacology

12.1 Mechanism of Action

MembraneBlue™ 0.15% selectively stains membranes in the human eye during posterior surgery, such as epiretinal membranes (ERM) and Internal Limiting Membranes (ILM).

13. Nonclinical Toxicology

13.1 Carcinogenesis, mutagenesis, impairment of fertility

Trypan blue is carcinogenic in rats. Wistar/Lewis rats developed lymphomas after receiving subcutaneous injections of 1% trypan blue dosed at 50 mg/kg every other week for 52 weeks (total dose approximately 100,000-fold the maximum recommended human dose of 0.75 mg per injection in a 60 kg person, assuming total absorption).

Trypan blue was mutagenic in the Ames test and caused DNA strand breaks in vitro.

16. How Supplied/Storage and Handling

MembraneBlue™ 0.15% is supplied as follows: 0.5 mL of MembraneBlue™ 0.15% in a sterile single-use Luer Lok, 2.25 mL glass syringe, grey rubber plunger stopper and tip cap with polypropylene plunger rod in a peel pouch. Five pouched products are packed in one distribution box.

MembraneBlue™ 0.15% is stored at 15-25°C (59-77°F). Protect from direct sunlight.

Rx ONLY

Manufactured by
D.O.R.C. International b.v.
Scheijdelveweg 2
3214 VN Zuidland
The Netherlands

Distributed in the United States by
Dutch Ophthalmic, USA
10 Continental Drive, Bldg 1
Exeter, NH 03833, USA
Phone: 800-75-DUTCH or 603-778-6929.

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5 Pouch Distribution Box Label (to be placed on rear of carton)

D672b-1

DORC **STERILE!**

MembraneBlue™ 0.15%
 (trypan blue ophthalmic solution)
 5 Luer Lok Syringes 2.25mL of 0.5mL

Store at 15° to 25°C (59°F to 77°F). Leave in pouch until use. Rx Only



6 8 8 0 3 - 6 7 2

Protect from direct sunlight. Single use only.

Manufactured by:
 D.O.R.C. International b.v.
 Scheijdelweg 2; 3214 VN Zuidland - The Netherlands

Distributed in US by:
 Dutch Ophthalmic, USA
 Exeter, NH 03833
 800-753-8824 or 603-778-6929

LOT 12345

Expiration Date YYYY-MM

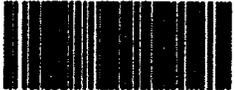
Peel Pouch Label

D672g-1

DORC **STERILE!**

MembraneBlue™ 0.15%
 (trypan blue ophthalmic solution)
 1 Luer Lok Syringe 2.25mL of 0.5mL

Store at 15° to 25°C (59°F to 77°F). Leave in pouch until use. Rx Only



6 8 8 0 3 - 6 7 2

Protect from direct sunlight. Single use only.

Manufactured by:
 D.O.R.C. International b.v.
 Scheijdelweg 2; 3214 VN Zuidland - The Netherlands

Distributed in US by:
 Dutch Ophthalmic, USA
 Exeter, NH 03833
 800-753-8824 or 603-778-6929

LOT 12345

Expiration Date YYYY-MM

Syringe Label

D672b-1

DORC **68803-672**

MembraneBlue™ 0.15%
 (trypan blue ophthalmic solution)
 Single use only 0.5 mL Syringe

STERILE! Rx Only

LOT 12345

See package insert for dosing information. Protect from direct sunlight.

Expiration Date YYYY-MM

Manufactured by:
 D.O.R.C. International b.v.
 Scheijdelweg 2
 3214 VN Zuidland; The Netherlands

Distributed in US by:
 Dutch Ophthalmic USA, Exeter NH 03833
 800-753-8824 or 603-778-6929

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this page is the manifestation of the electronic signature.**

/s/

William Boyd
2/19/2009 10:46:57 AM
MEDICAL OFFICER

Wiley Chambers
2/20/2009 02:24:17 PM
MEDICAL OFFICER

Cross-Discipline Team Leader Review

Date	July 29, 2008
From	William M. Boyd, M.D.
Subject	Cross-Discipline Team Leader Review
NDA#	22-278
Applicant	Dutch Ophthalmic Research Center
Date of Submission	January 30, 2008
PDUFA Goal Date	August 4, 2008
Proprietary Name / Established (USAN) names	MembraneBlue (trypan blue ophthalmic solution)
Dosage forms / Strength	ophthalmic solution
Proposed Indication(s)	for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue
Recommended:	Approvable

b(4)

• Introduction

MembraneBlue (trypan blue ophthalmic solution) is a sterile solution of trypan blue. MembraneBlue selectively stains membranes of the inner surface of the retina that can lead to visual disturbances from mild distortions and blurred vision due to the presence of metamorphopsia, micropsia and ultimately to reduced vision. These membranes include epiretinal membranes (ERM) and Internal Limiting Membranes (ILM) that can be removed during ophthalmic surgical vitrectomy procedures.

Identification of the membranes can be difficult, as they are transparent or mildly opaque. These membranes are hypocellular, collagenous proliferations (essentially scar tissue) occurring on the inner surface of the retina. Trypan blue, the active drug substance, is a stain widely used to selectively stain dead tissues or cells. Trypan blue is not absorbed in a viable cell, but traverses the membrane in a dead cell. Therefore, only the membranes are stained in contrast to the retina and excess trypan blue is washed out of the eye during the vitrectomy procedure. Staining the membranes improves the contrast between the membranes and the retina and can facilitate their removal; staining also reduces the chance of incomplete peeling of the membranes, which could lead to persistent metamorphopsia.

MembraneBlue is a blue di-azo group dye. It is a symmetrical molecule with three parts connected by two azo bonds. The molecular weight is 960.8. The empirical formula is: $C_{34}H_{24}N_6 Na_4O_{14}S_4$. It is water soluble. During vitrectomy surgery a few drops (0.3 -0.5 mL) are applied directly onto the areas where membranes could be present. Prior to injecting MembraneBlue a "fluid-air exchange," i.e., a filling of the entire vitreous cavity with air, is

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performed to prevent dilution of the MembraneBlue. Excess dye can be washed out of the posterior chamber.

MembraneBlue can also be injected directly into a BSS filled vitreous cavity instead of injecting under air. Sufficient staining is usually achieved after 30 seconds of application under BSS.

• Background

Trypan blue has been marketed as MembraneBlue™ in Europe since 2002 as a medical device Class IIa for the same proposed indication. The applicant reports over _____ units have been used during ophthalmic surgical vitrectomy procedures. No complaints or any other information concerning adverse events have been received during this period. Trypan blue has also been marketed as VisionBlue™ in Europe since 1999 and was approved in the US April 12, 2004 (NDA 21-670) for the indication of staining of the anterior capsule during cataract surgery. The applicant reports over _____ units have been used during cataract surgery.

b(4)

This is a 505(b)(2) application. The safety and efficacy of MembraneBlue for the proposed indication can be supported from published studies which use the applicant's product. The applicant has marketed the product in Europe without doing any studies on their own. Studies were subsequently done with their product and reported in the literature.

The proposed indication, use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue _____ is not acceptable and is not supported by the submitted data. A revised indication, for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue, is acceptable.

b(4)

Based on the following four adequate and well controlled clinical trials from the literature (reviewed in Section 6.1.4 of the Medical Officer's review):

1. C Haritoglou, et al. Retina 2004; 24(4):582-90,
2. C Haritoglou, et al. Am J Ophthalmol 2004;138(1):1-5,
3. KL Lee, et al. Br J Ophthalmol 2005; 89:420-4, and
4. J Beutel, et al. Arch Ophthalmol 2007; 125:326-332,

and the other literature studies listed in Section 5.1 of the Medical Officer's review, this application for Trypan Blue has demonstrated safety and efficacy in selectively staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

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There are no known safety or effectiveness concerns that have arisen with other members of this pharmacologic class.

• **CMC**

DRUG SUBSTANCE:

Trypan blue, the drug substance in MembraneBlue 0.15%, is _____ by _____
_____ DMF _____ is authorized to be referenced for information regarding the
_____ trypan blue. Reviews of DMF _____ were completed and the DMF was noted
adequate to support the current NDA.

b(4)

DRUG PRODUCT:

The formulation of MembraneBlue 0.15% is similar to VisionBlue 0.06%, which was submitted by the same applicant and approved in June 2004. The only difference is an increased trypan blue concentration from 0.06% to 0.15% for MembraneBlue. Each mL of MembraneBlue 0.15% contains 1.5 mg trypan blue, 1.9 mg sodium monohydrogen orthophosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), 0.3 mg sodium di-hydrogen orthophosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), 8.2 mg sodium chloride (NaCl), and water for injection. The pH is 7.3 - 7.6. The osmolality is 257-314 mOsm/kg. MembraneBlue is filled in glass syringes to a volume of 0.5 mL.

The drug product is manufactured by a contract firm, _____ located in _____
_____. The drug product is manufactured from _____
_____ to obtain a 0.15% solution. The _____
_____ is supplied by _____ in _____

b(4)

During drug product manufacturing process, the pH of the solution is verified and the solution is _____ any _____. The prepared solution is then filled into a single-use Luer Lok 2.25 ml glass syringe (_____ ml/syringe) and the syringe is closed with a tip cap and stopper. The solution is _____ sterilized. The syringes are then placed into _____ pouches and the outside surface of the syringes is _____ sterilized.

b(4)

A two year expiry dating period was proposed for this product when stored at 15-25°C (59-77°F) and protected from direct sunlight. Stability data was available on one batch of MembraneBlue 0.15% up to 3 months at long-term and accelerated conditions. Supporting stability data was available on three batches of VisionBlue 0.06% up to 26 months.

DRUG PRODUCT COMPOSITION:

From the original CMC review, page 21.

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Composition of MembraneBlue 0.15%

Component	Quantity
Purified Trypan Blue	
Total	0.5 mL

b(4)

Composition of	(0.5 mL):
Sodium Chloride	4.10 mg
Sodium Phosphate Dibasic Dihydrate	0.95 mg
Sodium Phosphate monobasic dihydrate	0.15 mg
Water for Injection	QS to 0.5 mL

b(4)

REGULATORY SPECIFICATIONS:

From the original CMC review, page 11.

The specification of MembraneBlue is listed below, which is same as that for VisionBlue.

Test	Acceptance Criteria	Method (Code#)
Physical Appearance	Dark blue liquid	Visual inspection
Trypan Blue Identity UV/VIS	Identity assay to reference standard	Appendix 4, Amendment dated 12/6/07
Trypan Blue Content UV/VIS	assay to reference standard	Appendix 4, Amendment dated 12/6/07
Trypan Blue Content HPLC-UV — nm (stability study)		Appendix 5, Amendment dated 12/6/07
Impurities HPLC-UV — nm:		HPLC (Appendix 5 of Amendment date 12/6/07)
	NMT	
Unidentified:		
Rel. RT	NMT	
Rel. RT	NMT	
Rel. RT	NMT	
Any individual unspecified impurity	NMT	
Total impurities	NMT	
pH	7.3 - 7.6	USP <791>
Osmolality	257 - 314 mOsm / kg	Eu. Pharm.
Particulate Matter	NMT	USP <789>
	NMT	
	NMT	
Sterility	Sterile	USP <71>
Bacterial Endotoxins	NMT 3 EU / mL	USP <85>

b(4)

b(4)

b(4)

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ESTABLISHMENT EVALUATION REQUEST

An Establishment Evaluation Request was made via EES, and an inspection was performed from _____ This inspection revealed multiple GMP deficiencies, and a 483 was issued.

b(4)

The observations cited in the 483 included:

- Failure to qualify the maximum amount of _____ for which the _____ can be reused without being replaced
- Failure to conduct integrity test (pre and post _____) to the _____ used during the sterilization of all batches of Vision Blue 0.06% trypan blue ophthalmic solution syringes produced at the site and distributed to the U.S.
- Manufacture and distribution to the U.S. batches of Vision Blue 0.06% trypan blue ophthalmic solution syringes without having a validation of the sterilization process for VisionBlue using biological indicators
- Failure to have an _____ conducted during filing and prior to sterilization of Vision Blue 0.06% trypan blue ophthalmic solution syringes
- Failure to establish the manufacturing time for the different critical operations performed during the production of the Vision Blue 0.06% trypan blue ophthalmic solution product.

b(4)

b(4)

• Nonclinical Pharmacology/Toxicology

Per the original Pharmacology/Toxicology review, page 9:

Trypan blue has been approved for ocular staining in cataract surgery (VisionBlue™ 0.06%) under NDA 21-670. NDA 21,670 is cross-referenced for this NDA. The toxicological profile for carcinogenicity, teratogenicity, and mutagenicity has already been established. It is reported that trypan blue was teratogenic in rats, mice, rabbits, hamsters, dogs, guinea pigs, pigs, and chickens. It caused external, skeletal, and internal malformations. Trypan blue was mutagenic in Ames test. Trypan blue is carcinogenic in rats. Chronic intermittent exposure by subcutaneous injection of trypan blue in Wistar/Lewis rats induced a reticuloendothelial neoplasm, predominantly in the liver. The aforementioned information has been listed in the proposed labeling for MembraneBlue™.

In this NDA submission, only one cytotoxicity study was included. Trypan blue produced slight cytotoxicity at concentrations of up to 0.15% over 24 hr of exposure in this *in vitro* study using the MTT test system. The sponsor also cited other toxicology information from the literature. Mostly, the papers selected are concentrated on the toxic effects of trypan blue in the retina

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MembraneBlue (trypan blue ophthalmic solution)

using *in vitro* cultured human and animal retinal pigment epithelium cells or *in vivo* animal models. Some studies indicated that trypan blue was safe. Other studies showed positive findings of trypan blue including damaged photoreceptors and disorganization, decrease in mitochondrial dehydrogenase activity, morphological changes of the RPE cells, lowered ERG b-wave in bovine retina, and increased p53 and p21 expression. The toxicity was usually seen at higher concentrations and longer treatment duration.

Trypan blue is a vital stain widely used to selectively stain dead cells. The drug is not absorbed in a viable cell. Therefore, only the epiretinal membranes are stained in contrast to the retina. Clinically, only 0.75 mg of trypan blue will be administered to the eye, and almost all dye will be immediately irrigated out from the eye, leaving less than — to mark the membrane that too will be removed from the eye. Therefore the amount of the drug left in the patient after the surgery will be very low. The sponsor indicated that the final dose used in vitreoretinal surgery is less than —mg. It appears that possible systemic and ocular toxic effect of MembraneBlue™ is small. b(4)

Considering the drug history and clinical experience, nonclinical study results, proposed indication, and dosage, the reviewing pharmacologist believes that, from the nonclinical standpoint, the data are adequate for the approval of the drug. The labeling of MembraneBlue™, which is based on the approved labeling of VisionBlue, is considered acceptable.

• Clinical Pharmacology/Biopharmaceutics

Per the original Clinical Pharmacology review, page 1:

MembraneBlue is packaged in a volume of 0.5 mL in a 2.25 mL single-use syringe for application to the retinal membrane by blunt cannula. The actual dosage of MembraneBlue is determined by the ophthalmic surgeon, but is in the range of 0.3 to 0.5 mL. Trypan blue is not absorbed by viable cells, but traverses the membrane of dead cells. Excess dye is washed out of the eye by irrigation, while the stained membranes are removed from the eye, leaving only a minimal amount of trypan blue in the eye following surgery.

No clinical PK studies evaluating the systemic absorption of trypan blue following administration of MembraneBlue have been conducted. A waiver of the *in vivo* bioavailability requirement is granted, based on the expected negligible systemic exposure of trypan blue following use of MembraneBlue (trypan blue ophthalmic solution) during ophthalmic surgical vitrectomy procedures.

The Clinical Pharmacology and Biopharmaceutics information provided by the Applicant is acceptable.

• Sterility Assurance

Per the original Product Quality Microbiology review, page 4:

The primary package consists of a 2.25 mL BD _____ glass syringe, a _____ tip cap and a _____ plunger stopper. The manufacturing process for MembraneBlue™ is nearly identical to the manufacturing process for the approved product, VisionBlue™. The container closure system is identical for both products.

b(4)

The container closure system is identical to that approved for VisionBlue. The container closure integrity test for VisionBlue was evaluated in the product quality microbiology review for NDA 21-670 and approved in November of 2004.

MembraneBlue™ has a bacterial endotoxin limit of $NM_1 - EU/mL$. This endotoxin limit was found to be satisfactory by the medical division because excess dye will be washed from the eye and stained tissue will be surgically removed. Endotoxin testing will be conducted according to USP <85> methodology. The results of inhibition/enhancement testing for the drug product were provided in the June 2008 amendment. The results show that inhibition was overcome at a dilution of _____. The applicant has chosen a working dilution of _____ for use with an endotoxin sensitivity of _____ EU/mL. This allows for detection of endotoxin levels of _____ EU/mL.

b(4)

Sterility testing will be conducted according to USP <71>. The results of bacteriostasis/fungistasis testing were provided in the June 2008 amendment and found to be satisfactory. Because the product label states that the syringe containing membrane blue is sterile, a sterility test should be conducted on the syringe as well. The applicant provided a commitment to conduct sterility testing on both the contents of the syringe and the contents of the pouch in the June 2008 amendment.

• Clinical/Statistical - Efficacy

Per the original Medical Officer review, page 9:

Clinical studies have been conducted and published in the literature. The applicant has not submitted any additional unpublished clinical studies. The following clinical studies with MembraneBlue for selective staining of epiretinal membranes during ophthalmic surgical vitrectomy procedures have been conducted. The following abbreviations are used in the following tables: Trypan Blue (TB), Indocyanine green (ICG).

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First Author	Journal	Number of patients treated with Test Product	Controls	Results
EJ Feron	Arch Ophthalmol 2002; 120:141-144.	10 TB 0.06%	None	Epiretinal membranes not identified prior to use, identified after supposed complete removal. Pathology confirmation that removed tissues were only epiretinal membranes.
K Li	Br J Ophthalmol 2003; 87:216-9.	14 TB 0.06%	None	Membranes were stained satisfactorily and removed successfully.
M Perrier	Am J Ophthalmol 2003;135(6): 903-5.	18 TB 0.06%	None	Visualization and dissection of the membranes was facilitated by trypan blue staining.
M Perrier	Am J Ophthalmol 2003;135(6): 909-11.	23 TB 0.06%	None	Visualization and dissection of the membranes was facilitated by trypan blue staining without any signs of toxicity.
Francisco A Teba	Ophthalmology 2003; 110: 2409-2412	50 TB 0.2%	None	Epiretinal membranes can be identified.
C Haritoglou	Retina 2004; 24(4):582-90.	10 TB 0.15 %	15: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.
C Haritoglou	Am J Ophthalmol 2004;138(1):1-5.	22 TB 0.06 %	21: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.
BJ Vote	Retina 2004;24(5):736-8.	26 TB 0.15%	None	TB staining of epiretinal membranes is a useful adjunct in vitreoretinal surgery and improves the safety and efficiency of membrane identification and removal.
SY Lesnik Oberstein	Br J Ophthalmol 2007; 91:955-7.	29 TB 0.15%	None	Staining of membranes is a safe method.
F Uno	Retina2006;26(2):237-9.	1 TB 0.15%	None	Case report: Subretinal migration of TB due to either direct trauma due to accidental subretinal injection, direct (chemical) toxicity of TB or solution osmolarity; and photodynamic effect of TB, which may absorb wavelengths of light from the endoilluminator.

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Studies using Double Staining Technique - TB and ICG:

First Author	Journal	Number of patients treated with Test Product	Controls	Results
P Stalmans	Br J Ophthalmol 2003; 87:713-6.	30 TB 0.15% and ICG	None	Double staining technique using TB and ICG aided in visualization of membranes during vitreoretinal surgery.
AK Kwok	EYE 2004;18(9):882-8.	16 TB 0.15% and ICG	None	Double staining technique using TB and ICG aided in visualization of membranes during vitreoretinal surgery

Comparable studies of trypan blue to alternatives:

First Author	Journal	Number of patients treated with Test Product	Controls	Results
KL Lee	Br J Ophthalmol 2005; 89:420-4.	5 TB 0.3% 13 TB 0.15%	5 ICG 0.5% 14 ICG 0.05%	The anatomical and visual results in the two groups were comparable.
J Beutel	Arch Ophthalmol 2007; 125:326-332.	20 TB 0.15%	20 ICG 0.05%	No statistical difference was detected between the two groups; though the TB group had better visual recovery.

The applicant did not conduct any clinical studies. The studies published in the literature and submitted by the applicant were reviewed. In addition, a Medline search of the literature for ocular studies using trypan blue in epiretinal surgery was conducted. Articles which could be identified as relevant for the proposed indication were reviewed.

ANALYSIS OF PRIMARY ENDPOINT(S)

Trypan blue is well known as a vital stain. It differentially stains different tissues in the body. It is used *in vivo* and *in vitro* as a standard to distinguish between living and dead cells. Living cells do not stain; dead cells stain. Epiretinal membranes stain. Trypan blue 0.15% selectively stains epiretinal membranes. Dose ranging studies demonstrate that a concentration of 0.06% or higher is effective in selectively staining epiretinal membranes.

Nearly 300 eyes using Trypan Blue (TB) at concentrations of 0.06% and 0.15% or greater for selective staining of epiretinal membranes during ophthalmic surgical vitrectomy procedures were reviewed, of 300 eyes enrolled, 200 eyes used a concentration of TB of 0.15% or greater.

To support the approval of a drug, safety and efficacy is recommended to be demonstrated in at least two adequate and well-controlled trials.

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Of the clinical studies referenced, the following four studies used a control during surgery compared with the TB group; the control group included no staining of the epiretinal membrane or staining of the epiretinal membrane with ICG.

Studies comparing TB to a Control (No staining)

First Author	Journal	Number of patients treated with Test Product	Controls	Results
C Haritoglou	Retina 2004; 24(4):582-90.	10 TB 0.15 %	15: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.
C Haritoglou	Am J Ophthalmol 2004;138(1):1-5.	22 TB 0.06 %	21: surgery performed without staining	Epiretinal membranes had better visualization after staining with TB.

Studies comparing TB to a Control (ICG):

First Author	Journal	Number of patients treated with Test Product	Controls	Results
KL Lee	Br J Ophthalmol 2005; 89:420-4.	5 TB 0.3% 13 TB 0.15%	5 ICG 0.5% 14 ICG 0.05%	The anatomical and visual results in the two groups were comparable.
J Beutel	Arch Ophthalmol 2007; 125:326-332.	20 TB 0.15%	20 ICG 0.05%	No statistical difference was detected between the two groups; though the TB group had better visual recovery.

1. C Haritoglou, et al. Retina 2004; 24(4):582-90.

In this clinical study 10 eyes of 10 consecutive patients with intraoperative use of TB (0.15%) were analyzed and compared with the functional outcome in a matched group of patients (15 eyes) who had undergone vitrectomy without TB.

The studied demonstrated TB permitted better visualization of the epiretinal membrane which might enable the surgeon to better and more completely remove the epiretinal tissue. No adverse effects of the dye on functional outcome were observed.

2. C Haritoglou, et al. Am J Ophthalmol 2004;138(1):1-5.

This clinical trial was a prospective, randomized, comparative study. Forty three eyes of 43 consecutive patients were randomized between TB (0.06%) staining or to no staining.

The authors concluded the application of TB may be beneficial since it enables the surgeon to better visualize and therefore remove the epiretinal tissue more completely (though there were no statistically significant differences in functional outcome after a follow-up of up to 6 months - the statistical difference in functional outcome between the groups was $p > 0.5$). The study reported that no apparent dye-related adverse events occurred.

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3. KL Lee, et al. Br J Ophthalmol 2005; 89:420-4.

This was a retrospective analysis of 37 eyes from 37 consecutive patients. In 19 patients ICG was used and in 18 patients TB.

There was no significant difference between the preoperative visual acuities in the TB and ICG groups but the postoperative visual acuities were better in the TB than in the ICG group ($p = 0.036$). The TB group also had more lines of improvement than the ICG group (2.94 versus 1.79 lines; $p = 0.046$), follow-up ranged from 1 – 30 months and the median follow-up was 5 months. TB appears to be less toxic than ICG when used in dye assisted removal of epiretinal membranes as reflected by the better visual results in the TB group.

4. J Beutel, et al. Arch Ophthalmol 2007; 125:326-332.

This was a randomized, controlled trial with 20 patients (20 eyes) receiving ICG and 20 patients (20 eyes) receiving TB. Although the primary outcome measurement of visual acuity at 3 months measured by the Early Treatment of Diabetic Retinopathy scale did not demonstrate a significant difference between the TB and ICG study groups, after 6 months the TB group did show significant improvement in visual acuity ($p = 0.002$).

EFFICACY CONCLUSIONS

These four clinical trials with control groups and the other studies listed in the Medical Officer's review support the efficacy of MembraneBlue as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

• Safety

Published literature study results were used to establish the safety of the drug product.

As early as 1967, trypan blue has been used in ophthalmology to achieve vital staining of the cornea and conjunctiva. It has been injected into the anterior chamber since the 1970s to evaluate the corneal endothelium. It is the standard screening agent for organ donor corneas to evaluate the corneal endothelium prior to corneal transplantation. Well over ——— corneas have been screened and transplanted after administration of trypan blue, 0.3%. This dose is approximately 2 times the proposed dose. In donor corneal grafts, there are no documented adverse effects.

Adverse reactions reported following use of MembraneBlue include discoloration of high water content hydrogen intraocular lenses (see Contraindications) and inadvertent staining of the posterior lens capsule and vitreous face. Staining of the posterior lens capsule or staining of the vitreous face is generally self limited, lasting up to one week.

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There are no known safety concerns that have arisen with other members of this pharmacologic class.

Of the clinical studies referenced in the application, the following four studies used a control during surgery when compared with the TB group and support the safety of the drug product:

1. C Haritoglou, et al. *Retina* 2004; 24(4):582-90.

In this clinical study of 10 eyes of 10 consecutive patients with intraoperative use of TB (0.15%), no adverse effects of the dye on functional outcome were observed.

2. C Haritoglou, et al. *Am J Ophthalmol* 2004;138(1):1-5.

Forty three eyes of 43 consecutive patients were randomized between TB (0.06%) staining or to no TB staining. There were no dye-related adverse events reported.

3. KL Lee, et al. *Br J Ophthalmol* 2005; 89:420-4.

This was a retrospective analysis of 37 eyes from 37 consecutive patients. The adverse events that were reported were those commonly associated with a vitrectomy procedure.

Postoperatively, in the ICG group one retinal detachment occurred and two small retinal detachments occurred intraoperatively that were repaired at the time of the surgery; in the TB group one patient experienced a choroidal / vitreous hemorrhage.

4. J Beutel, et al. *Arch Ophthalmol* 2007; 125:326-332.

This was a randomized, controlled trial with 20 patients (20 eyes) receiving ICG and 20 patients (20 eyes) receiving TB. Again, the adverse events that were reported were those commonly associated with a vitrectomy procedure. In both groups there was one post-op retinal detachment and in all phakic eyes the progression of nuclear cataracts were noted.

SAFETY CONCLUSIONS

These four clinical trials with control groups and the other studies listed in the Medical Officer's review support the safety of MembraneBlue as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

• Advisory Committee Meeting

No Advisory Committee was necessary or convened for this drug product.

• Pediatrics

The drug would not be expected to be used on pediatric patients. The mean age of subjects in the published studies ranged from 60 to 71 years old.

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The use of Trypan Blue in lower concentrations in pediatric patients was supported by the literature reports of adequate and well controlled studies in NDA 21-670 for VisionBlue (trypan blue ophthalmic solution) which was approved April 12, 2004.

• Other Relevant Regulatory Issues

A Division of Scientific Investigations (DSI) audit was not requested since this was a literature review.

A consult was requested from the Office of Surveillance and Epidemiology regarding a trade name review for the proposed name "MembraneBlue." The results of the Proprietary Name Risk Assessment found that the proposed name, MembraneBlue, appears to be vulnerable to product confusion with VisionBlue. The Division of Medication Error Prevention believes that the risk may be mitigated by differentiating the proposed labels and labeling of MembraneBlue from the labels and labeling of the existing product VisionBlue.

The Division of Drug Marketing, Advertising, and Communications (DDMAC) has reviewed _____ proposed product labeling (PI) for this application submitted to the Agency on 30 January 2008. Their recommendations regarding the proposed indication, Warnings and Precautions, and Clinical Pharmacology sections of the labeling have been incorporated into the draft label.

b(4)

• Labeling

NDA 22-278 is not recommended for approval for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue pending the resolution of an unacceptable site inspection at _____ This inspection revealed multiple GMP deficiencies with issuance of a 483.

b(4)

The labeling submitted by Dutch Ophthalmic Research Center on 30 January 2008 and found in this Cross-Discipline Team Leader Review (see Appendix 1) is draft labeling. The Agency will continue to work with DORC regarding the labeling for MembraneBlue.

• Recommendations/Risk Benefit Assessment

RECOMMENDED REGULATORY ACTION:

NDA 22-278 is not recommended for approval for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures,

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facilitating removal of the tissue pending the resolution of an unacceptable site inspection at _____ This inspection revealed multiple GMP deficiencies with issuance of a 483. b(4)

The labeling submitted by Dutch Ophthalmic Research Center on 30 January 2008 and found in this Cross-Discipline Team Leader Review (see Appendix 1) is draft labeling. The Agency will continue to work with DORC regarding the labeling for MembraneBlue.

RISK BENEFIT ASSESSMENT:

Based on the following four adequate and well controlled clinical trials from the literature (reviewed in Section 6.1.4 of the Medical Officer's review):

1. C Haritoglou, et al. Retina 2004; 24(4):582-90,
2. C Haritoglou, et al. Am J Ophthalmol 2004;138(1):1-5,
3. KL Lee, et al. Br J Ophthalmol 2005; 89:420-4, and
4. J Beutel, et al. Arch Ophthalmol 2007; 125:326-332,

and the other literature studies listed in Section 5.1 of the Medical Officer's review, this application for Trypan Blue has demonstrated safety and efficacy in selectively staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

The application supports the safety of MembraneBlue (trypan blue ophthalmic solution) 0.05% for use as an aid in ophthalmic surgery by staining the epiretinal membranes during ophthalmic surgical vitrectomy procedures, facilitating removal of the tissue.

Pharmacology/Toxicology, Product Quality Microbiology, and Clinical Pharmacology have recommended approval for this application. Clinical and CMC have recommended approval after resolution of the multiple GMP deficiencies from the site inspection at _____ b(4)

RECOMMENDATION FOR POSTMARKETING RISK MANAGEMENT ACTIVITIES:

There are no additional proposed risk management actions except the usual postmarketing collection and reporting of adverse experiences associated with the use of the drug product.

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Appendix 1

The labeling submitted by Dutch Ophthalmic Research Center on 30 January 2008 and found in this Cross-Discipline Team Leader Review is draft labeling. The Agency will continue to work with DORC regarding the labeling for MembraneBlue.

4 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

√ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

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/s/

William Boyd
8/1/2008 12:50:33 PM
MEDICAL OFFICER

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