

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**209569Orig1s000**

**NON-CLINICAL REVIEW(S)**

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH**

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 209569  
Supporting document/s: 3  
Applicant's letter date: 4-29-2019  
CDER stamp date: 4-29-2019  
Product: TissueBlue (Brilliant Blue G Ophthalmic Solution)  
0.025%  
Indication: For use as an aid in ophthalmic surgery by  
selectively staining the (b) (4) internal limiting  
membrane (ILM) (b) (4)  
Applicant: Dutch Ophthalmic Research Center (International)  
B.V. (DORC)  
Review Division: Transplant and Ophthalmology Products  
Reviewer: María I Rivera, PhD  
Supervisor/Team Leader: Lori E. Kotch, PhD, DABT  
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*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

Brilliant Blue G 0.025% Solution is a single-dose ophthalmic solution formulation containing 0.025% Brilliant Blue G (BBG). The formulation is intended for use as an aid in ophthalmic surgery by selectively staining the (b) (4) internal limiting membrane (ILM) (b) (4). The dye is used to stain and/or visualize the ILM, facilitating the removal of this tissue, reducing the risk of retinal damage.

Brilliant Blue G 0.025% Solution is a new chemical entity in the USA. However, the (b) (4) has been approved in Europe and has been marketed by the Applicant as ILMBlue® in the European Union since August 2010. The sponsor claims that over (b) (4) units of European Conformance-approved ILM-Blue® have been distributed in the European Union and no reports of adverse effects or complaints related to the use of the product have been received.

Brilliant Blue G Ophthalmic Solution, 0.025% was granted an orphan designation by the FDA in July 2012. The Applicant is seeking approval via the 505(b)(2) regulatory pathway. The nonclinical support comes from Applicant-owned studies and scientific publications evaluating the toxicity of BBG in various cell and animal models. There is no listed drug for this application.

## 1.2 Brief Discussion of Nonclinical Findings

The ocular toxicity was evaluated after a single intravitreal (IVT) or subretinal injection in rabbits of a BBG formulation (not the clinical formulation) with an observation period of 14 days. Key findings included:

- In eyes treated by subretinal administration, anterior segment inflammation, vitreous opacity and/or presence of vitreal hemorrhage was reported in saline control and BBG-treated groups. However, the increased incidence and/or severity in BBG-treated groups suggests a contribution by the test article.
- Reductions in scotopic a-wave and/or b-wave amplitude at both test-article routes of administration and at both dose levels. The reduction of the ERG amplitudes could be in part related to absorption of the applied light by the dye before the dye reaches the retina. Similar findings were observed in in vitro studies from the published literature and found to be reversible after a washout period.
- In eyes treated by subretinal administration, minimal to marked microscopic retinal changes (detachment and degeneration) were observed. These changes were present in both control and treated groups, but with increased severity in test article-treated groups. Therefore, a contribution by the test article cannot be ruled out.



- Plasma concentrations of BBG from 24 hours postdose through Day 14 were below the limit of quantitation (< 10 ng/mL) for both intravitreal and subretinal route of administration. At the same timepoint, high levels of BBG were still measured in the eye, particularly in the retina and choroid.

At the intended clinical dose regimen, the total dose to be administered to the human eye is 0.125 mg. Therefore, there is no exposure margin at any of the doses used in the animal ocular toxicity study (≤50 µg/eye). Based on the fact that most of the dye is removed from the eye during the ILM peeling procedure, the design of the ocular toxicity study maximizes exposure to the dye, and is considered adequate for risk assessment.

A limitation of the ocular toxicity study is that it was conducted with a purified BBG preparation (DYME) [redacted] (b) (4). The composition of the DYME preparation is different than that of the intended clinical product. However, there is marketing experience with the proposed formulation to support its safe use in humans.

The Applicant provided a review of scientific publications of in vitro as well as in vivo studies with BBG. Overall, these studies support the safety of the intended clinical concentration (0.025% or 0.25 mg/mL) and short-term ocular exposure.

The reviewer believes that the nonclinical study reports provided, the nonclinical literature cited, together with the existent marketing experience for Brilliant Blue G 0.025% Solution (over [redacted] (b) (4) units distributed in Europe; marketed as ILM-Blue® outside of the United States), provide adequate safety support for the approval of Brilliant Blue G 0.025% Solution for the intended indication.

### 1.3 Recommendations

#### 1.3.1 Approvability

Approval is recommended.

#### 1.3.2 Additional Nonclinical Recommendations

#### 1.3.3 Labeling

Applicant's proposed text	Reviewer's recommendations
<p><b>HIGHLIGHTS OF PRESCRIBING INFORMATION</b></p> <p><b>INDICATIONS AND USAGE</b> TissueBlue (Brilliant Blue G Ophthalmic Solution) 0.025% is indicated to selectively stain the [redacted] (b) (4) internal limiting membrane (ILM) [redacted] (b) (4) [redacted] (1)</p>	<p><b>HIGHLIGHTS OF PRESCRIBING INFORMATION</b></p> <p><b>INDICATIONS AND USAGE</b> TissueBlue (Brilliant Blue G Ophthalmic Solution) 0.025% <i>is a disclosing agent</i> indicated to selectively stain the [redacted] (b) (4) internal limiting membrane (ILM) [redacted] (b) (4) [redacted] (1)</p>



<p><b>8.1 Pregnancy</b></p> <p><b>Risk Summary</b></p> <p>There are no available data on the use of TissueBlue 0.025% in pregnant women to inform a drug associated risk. Systemic absorption of TissueBlue 0.025% in humans is negligible following intravitreal injection (b) (4) removal of the drug at the completion of surgical procedures. Due to the negligible systemic exposure, it is not expected that maternal use of TissueBlue 0.025% will result in fetal exposure to the drug.</p> <p>Animal reproduction studies were not conducted with TissueBlue 0.025%.</p>	<p><b>8.1 Pregnancy</b></p> <p><b>Risk Summary</b></p> <p>There are no available data on the use of TissueBlue 0.025% in pregnant women to inform a drug associated risk. Systemic absorption of TissueBlue 0.025% in humans <i>is expected to be</i> negligible following intravitreal injection (b) (4) <i>and subsequent</i> removal of the drug at the completion of surgical procedures. Due to the negligible systemic exposure, it is not expected that maternal use of TissueBlue 0.025% will result in fetal exposure to the drug.</p> <p><i>Adequate</i> animal reproduction studies were not conducted with TissueBlue 0.025%.</p>
<p><b>8.2 Lactation</b></p> <p><b>Risk Summary</b></p> <p>No data are available regarding the presence of BBG in human milk, (b) (4) the effects of BBG on the breastfed infant or on milk production. However, breastfeeding is not expected to result in exposure of the child to BBG due to the negligible systemic (b) (4) of BBG in humans following intravitreal injection (b) (4) y removal of the drug at the completion of surgical procedures.</p>	<p><b>8.2 Lactation</b></p> <p><b>Risk Summary</b></p> <p>No data are available regarding the presence of BBG <i>Brilliant Blue G</i> in human milk <i>after ocular administration of TissueBlue 0.025%</i>, (b) (4) the effects of BBG on the breastfed infant, or <i>the effects</i> on milk production. However, breastfeeding is not expected to result in exposure of the child to BBG <i>Brilliant Blue G</i> due to the <i>expected</i> negligible systemic (b) (4) <i>exposure</i> of BBG in humans following intravitreal injection of <i>TissueBlue 0.025%</i> (b) (4) <i>and subsequent</i> removal of the drug at the completion of surgical procedures.</p>
<p><b>12.1 Mechanism of Action</b></p> <p>BBG has been shown to selectively stain the ILM but not the epiretinal membrane nor the retina, making it (b) (4) easier to visualize for removal, although the exact mechanism of this selectivity is not elucidated.</p>	<p>No edits</p>
<p><b>13 NONCLINICAL TOXICOLOGY</b></p> <p>(b) (4)</p>	<p><b>13 NONCLINICAL TOXICOLOGY</b></p> <p><i>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</i></p> <p><i>Studies to evaluate the potential for carcinogenicity or impairment of fertility of TissueBlue 0.025% have not been conducted.</i></p> <p><i>Brilliant Blue G was not mutagenic in the Ames assay, the in vitro mouse lymphoma assay, or the in vivo rat micronucleus assay.</i></p>

## 2 Drug Information

### 2.1 Drug

CAS Registry Number  
6104-58-1

Generic Name  
Brilliant Blue G-250, 42655, Acid Blue 90, Acid Blue G, Coomassie Brilliant Blue G-250, Brilliant Indocyanine G, Brillantindocyanin G, Xylene Brilliant Cyanine G, Serva Blue G

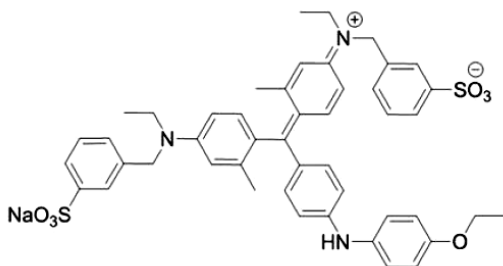
Code Name  
BBG

Chemical Name

- ECHA (European Chemicals Agency): Hydrogen [4-[4-(p-ethoxyanilino)-4'-[ethyl(m-sulphonatobenzyl)amino]-2'-methylbenzhydrylene]-3-methylcyclohexa-2,5-dien-1-ylidene](ethyl)(m-sulphonatobenzyl)ammonium, monosodium salt
- IUPAC (International Union of Pure and Applied Chemistry): Sodium;3-[[4-[(Z)-[4-(4-ethoxyanilino)phenyl]-4-[ethyl-[(3-sulfonatophenyl)methyl]azaniumylidene]-2-methylcyclohexa-2,5-dien-1-ylidene]methyl]-N-ethyl-3-methylanilino]methyl]benzenesulfonate

Molecular Formula/Molecular Weight  
 $C_{47}H_{48}N_3NaO_7S_2/854.02$  g/mol

Structure



Pharmacologic Class: Disclosing agent

### 2.2 Relevant INDs, NDAs, BLAs and DMFs

- Pre-IND 117753 (ILM-Blue, 0.025% Brilliant Blue G [BBG] ophthalmic solution)



### 2.3 Drug Formulation

Brilliant Blue G 0.025% Solution is a sterile, stable, single-dose ophthalmic solution formulation containing 0.025% Brilliant Blue G (BBG). The components of Brilliant Blue G 0.025% Solution, their concentration, function and compendial status are given in Table 1.

**Table 1: Composition of Brilliant Blue G 0.025% Solution**

Component	% w/w	Function	Compendial Status
Brilliant Blue G (BBG)	0.025	Active Ingredient	Noncompendial
Polyethylene Glycol (PEG) 3350	[REDACTED]	(b) (4)	USP/NF
Buffered Sodium Chloride solution			Noncompendial

### 2.4 Comments on Novel Excipients

None of the excipients are novel for ocular use. Per FDA Inactive Ingredient Database, PEG 3350 is approved for ocular topical use at concentrations of 4.6% (cream) and 40% (ointment). The ocular toxicity study was conducted with a formulation that does not contain PEG3350. [REDACTED] (b) (4)

### 2.5 Comments on Impurities/Degradants of Concern

Pending CMC review

### 2.6 Proposed Clinical Population and Dosing Regimen

- TissueBlue (Brilliant Blue G Ophthalmic Solution) 0.025% is indicated to selectively stain the [REDACTED] (b) (4) internal limiting membrane (ILM) [REDACTED] (b) (4).
- TissueBlue (Brilliant Blue G Ophthalmic Solution) 0.025% is supplied in 2.25 mL syringes filled to a volume of 0.5 mL (0.125 mg). TissueBlue 0.025% is carefully injected into the BSS-filled vitreous cavity using a blunt cannula attached to the syringe, without allowing the cannula to contact or damage the retina or allowing TissueBlue to get under the retina. Sufficient staining is expected within a few seconds. Following the surgical procedure, all excess dye should be removed from the vitreous cavity.

### 2.7 Regulatory Background

- Pre-IND (117753) submission submitted on 3-18-2013; sponsor meeting held on 4-16-2013
  - The Division agreed the nonclinical data provided (a combination of literature and sponsor's owned studies) was adequate to support the intended clinical use.

- Pre-NDA submission received on 10-6-2016; sponsor meeting was cancelled as preliminary comments sufficiently addressed the sponsor's questions
  - The Division agreed with the proposed CTD structure.
  - The Division conveyed the standards nonclinical recommendations for a 505(b)(2) NDA.

### **3 Studies Submitted**

#### **3.1 Studies Reviewed**

- A 2-Week Intravitreal Injection and Subretinal Injection Study of an ILM Staining Dye in the Dutch Belted Rabbit (Study # 570151)
- Bacterial Mutagenicity Test- Ames Assay (Study # 303161 [53899])
- In Vitro Mouse Lymphoma Assay (Study # 303211 [53900])
- In Vivo Mouse Micronucleus Test (Study # 30324G)
- ISO Ocular Irritation Study (Study # TI253\_800)
- In Vivo Skin Irritation Test in Albino Rabbits (Study # S-2018-00490)

#### **3.2 Studies Not Reviewed**

- Buehler Dermal Sensitization Test (Study # 900890L)
- Delayed Hypersensitivity Test in Guinea Pigs (Study # S-2018-00489 AM)
- ISO Agarose Overlay Using L-929 Mouse Fibroblast Cells (Study # 140150K)
- Cytotoxicity by Direct Contact Test (Study # S-2018-00488 AM)

#### **3.3 Previous Reviews Referenced**

None

### **4 Pharmacology**

#### **4.1 Primary Pharmacology**

The use of BBG in protein assays was described more than thirty years ago and is considered a standard tool for biologists and chemists<sup>1</sup>. The binding of the dye to protein causes a shift in the spectrophotometric absorption maximum of the dye from 465 to 595 nm, enabling visualization of the protein.

BBG has been shown to selectively stain the ILM but not the epiretinal membrane nor the retina, making it an easier to visualize for removal. The staining mechanism of BBG at the ILM still remains unknown.

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<sup>1</sup> Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem, 72, 248-54.

The Applicant summarized the results from published studies demonstrating the BBG has ILM staining ability and lower toxicity compared to other alternatives:

- In the primate eyes, the ILM was clearly visualized after the intravitreal injection of BBG (0.5 mg/mL) and was easily peeled away from the retina. No adverse findings were observed in the retina during the 6-month follow-up period<sup>2</sup>.
- Among 13 vital dyes, BBG showed the best ILM staining in enucleated porcine eyes and human donor eyes<sup>3</sup>.
  - Note: The Applicant provided the wrong citation for this publication.

## 4.2 Secondary Pharmacology

Published studies support that BBG exerts therapeutic effects in clinically relevant models of neurodegenerative diseases<sup>4</sup>. A substantial neuroprotective effect of BBG was observed in mouse primary retinal cells at a dose of 10  $\mu\text{mol/L}$  (8.5  $\mu\text{g/mL}$ ), which is considerably lower than the concentration recommended for use during chromovitrectomy (293  $\mu\text{mol/L}$  [250  $\mu\text{g/mL}$ ]). In the same study, intraocular administration of 500  $\mu\text{mol/L}$  (427  $\mu\text{g/mL}$ ) BBG inhibited photoreceptor apoptosis caused by intraocular injection of 20 mmol/L BzATP (P2RX7 agonist) into the eyes of C57BL/6 mice. BBG has been characterized as a P2RX7 antagonist ( $\text{IC}_{50}$ : 10 to 200 nM [8.5 ng/mL to 171 ng/mL])<sup>5</sup>. An  $\text{IC}_{50}$  value of 265 nM (226 ng/mL) have been reported in humans.<sup>6</sup> P2X7 receptors are ionotropic ATP-gated receptors found in cells of neuronal and hemopoietic lineage and mediate influx of  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  and the release of pro-inflammatory cytokines.

## 4.3 Safety Pharmacology

No studies have been conducted.

# 5 Pharmacokinetics/ADME/Toxicokinetics

## 5.1 PK/ADME

The plasma concentration of BBG was monitored after a single intravitreal (IVT) (25  $\mu\text{g}$  and 50  $\mu\text{g}$ ) and subretinal (12.5  $\mu\text{g}$  and 25  $\mu\text{g}$ ) injection in rabbits (See Study # 570151 below). Plasma concentrations of BBG measured from 24 hours postdose through Day 14 were below the limit of quantitation (< 10 ng/mL) for both IVT and sub-

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<sup>2</sup> Enaida, H., et al., 2006, Preclinical investigation of internal limiting membrane staining and peeling using intravitreal Brilliant Blue G, *Retina*, 26(6): 623-630.

<sup>3</sup> Rodrigues, E.B., et al., 2010, Ability of New Vital Dyes to Stain Intraocular Membranes and Tissues in Ocular Surgery, *Am J Ophthalmol*, 149(2): 265-277.

<sup>4</sup> Notomi S, et al., 2011, Critical Involvement of Extracellular ATP Acting on P2RX7 Purinergic Receptors in Photoreceptor Cell Death. *Am J Pathol*. 179(6): 2798.

<sup>5</sup> Jiang, L., et al., 2000, Brilliant Blue G Selectively Blocks ATP-Gated Rat P2X7 Receptors, *Mol Pharmacol*, 58(1): 82 – 88.

<sup>6</sup> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5090090/>



retinal-dosed animals. These data support the view that systemic uptake of BBG during a normal BBG-assisted vitrectomy is expected to be negligible.

Ocular tissue distribution was also assessed in Study # 570151. The main results were the following:


### **BBG concentrations in ocular tissues (Day 14)**

- Intravitreal injections:
  - BBG was observed with the rank order retina > choroid ≈ optic nerve > vitreous.
  - Concentrations of ≤ 3440 ng/mL and ≤ 10100 ng/mL (retina), and ≤113 ng/mL and ≤555 ng/mL (choroid) were observed at the low and high dose, respectively.
  - BBG levels were near or below the limit of quantitation (10 to 101 ng/mL depending on the tissue) in the aqueous, iris, and lens.
- Subretinal injections:
  - BBG was observed with the rank order retina > choroid > vitreous.
  - Concentrations of ≤ 4620 ng/mL and ≤ 7810 ng/mL (retina) and ≤150 ng/mL and ≤620 ng/mL (choroid) were observed in the low and high dose, respectively.
  - BBG levels were below the limit of quantitation in the aqueous, iris, lens, and optic nerve.

## **6 General Toxicology**

### **6.1 Single-Dose Toxicity**

#### **Study title: A 2 Week Intravitreal Injection and Subretinal Injection Study of an ILM Staining Dye in the Dutch Belted Rabbit**

Study no.:	570151
Study report location:	EDR Module 4.2.3.1
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	November 20, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A0001 (Brilliant Blue G) 0.25 mg/mL, labelled as DYME BBG250, lot # 176-185-003, 107% pure

A0001 (Brilliant Blue G) 0.5 mg/mL,  
labelled as DYME BBG250, lot # 175-  
185-002, assumed 108% pure

**Note:** DYME is not the intended clinical formulation. The composition is different and does not contain PEG3350.

## Key Study Findings

### Methods

Doses: See Study Design Table below.  
 Frequency of dosing: Single bilateral administration on Day 1  
 Route of administration: Intravitreal or subretinal injection  
 Dose volume: See Study Design Table below.  
 Formulation/Vehicle: The test article was used as supplied.  
 Species/Strain: Dutch-Belted rabbit  
 Number/Sex/Group: 4 males  
 Age: 6 months old  
 Weight: 1.7 to 2.1 kg  
 Satellite groups: None  
 Unique study design: The intravitreal and subretinal route were selected to maximize exposure to the dye. The intravitreal route was selected as this route provided broad retinal exposure and the subretinal route, local exposure to the retina and retinal pigment epithelium.  
 Deviation from study protocol: None with an impact on study validity

### Study Design

Group No. Identification	Dose Per Eye ug/eye		Dose Volume uL/eye		Number of Animals (♂)	
	Intravitreal	Subretinal	Intravitreal	Subretinal	Intravitreal	Subretinal
1-Saline Control	0	0	100	50	4	4
2-Low Dose	25	12.5	100	50	4	4
3-High Dose	50	25	100	50	4	4

## Observations and Results

### Mortality (2X/day)

None

**Clinical Signs (2X/day; detailed examinations, weekly)**

Nictitating membrane protruding from left and/or right eye was noted on Day 14 in one low-dose rabbit treated IVT and on Days 7, 14 and 15 in one high-dose rabbit treated subretinally. Based on the low incidence, sporadic nature, and lack of a clear dose response, the finding could be related to experimental procedures.

**Body Weights (Weekly)**

No test article-related effects in body weight and body weight gains.

**Feed Consumption (Daily; qualitatively)**

Per Summary information (no data provided), there were no test article-related effects.

**Ophthalmoscopy (Pretreatment, immediately following dosing [indirect ophthalmoscopy only], Days 2, 7, and 13; slit lamp and indirect ophthalmoscopy)**

Intravitreal Injection: All animals were noted to have a bluish vitreous on Day 1, decreasing in intensity with time, and still apparent in the vitreous in two high-dose animals on Day 13. For all animals, a bluish haze was observed at the bottom of the physiological cup of the optic nerve on Day 13.

Subretinal Injection: Anterior segment inflammation (flare, fibrin and hyphema, conjunctival swelling and/or hyperemia) as well as posterior segment findings ('hazy view' of vitreous and/or vitreous opacity and/or presence of vitreal hemorrhage) were noted in all groups and considered by the Applicant likely associated with the surgical procedure. However, there was a higher incidence of anterior chamber inflammation in test-article treated groups, suggesting a contribution by the test article. Anterior chamber inflammation improved with time or had resolved in some animals by Day 7.

In some eyes, vitreal hemorrhage was only noted on Day 2 and was not present at time of surgery (post-surgical hemorrhage). Observation of vitreal hemorrhage improved with time in all groups.

For test article-treated animals, the area where the subretinal space was injected appeared as a focal bluish area which remained present after retinal reattachment. All retinas that could be visualized were reattached (resolved surgical bleb) by Day 7. The retina overlying the injection site appeared to be altered by the test-article and/or iatrogenic (surgical retinal detachment).

On Day 13, the fundus of 1 eye in the saline control, 3 eyes in the low-dose and 4 eyes in the high-dose group could not be examined due to the presence of opacities in the vitreous. The higher incidence in the test article-treated groups suggests a contribution by the test article to the vitreal opacity.



**Tonometry (Pre-treatment and on Days 2, 7 and 13)**

Fluctuations in IOP (increased or decreased) were noted in some animals following intravitreal or subretinal injection but the changes were considered likely related to the surgical procedure. No obvious trend was noted in any groups regarding changes in IOP.

**Electroretinography (Predose and Day 14; scotopic and photopic conditions)**

Compared to the concurrent control, reductions in scotopic a-wave and/or b-wave amplitude (single-flash 0dB b-wave, scotopic single flash -10dB b-wave, scotopic single flash 0dB - a-wave, scotopic single flash 0dB - b-wave, photopic 1Hz flicker - a-wave, photopic 1Hz flicker - b-wave, and/or photopic 29Hz Flicker - b-Wave) were observed at both IVT doses of the test article (with no dose relationship) and at the high subretinal dose.

If the baseline value is considered, as well as the current control range, it appears that 1 low dose animal at each administration route (# 203 and # 215) and 2 high-dose animals at the IVT (# 301 and # 304) route and 1 high-dose animal at the subretinal route (# 305) were primarily affected.

It was stated in the Study Report that the blue color in the vitreous likely played a role in the ERG changes that were seen at both IVT dose levels and the high subretinal dose, where reflux into the vitreous was more probable (see further details under Section 11 of this review). However, a direct effect of the test article cannot be ruled out.

**Gross Pathology (Day 15)**

No test article-related findings

**Histopathology (Left eye and optic nerve; right eye was used for assessment of ocular tissue distribution of the test article)**

Adequate Battery – Yes, based on the negligible BBG systemic exposure

Peer Review: No

Histological Findings: There were no test article-related microscopic findings in eyes dosed IVT. In eyes treated subretinally, minimal to marked retinal changes, detachment and degeneration were observed. These changes were present in both control and treated groups (Table 2). However, the severity was increased in test article-treated groups, compared to the saline control group (i.e., minimal to slight in controls, minimal to severe in test article-treated groups). The findings were considered likely due to the surgical procedure, but a test-article related effect cannot be excluded based on the increase in severity noted in the treated groups.

**Table 2: Incidence of Microscopic Findings - Single Subretinal Dose in Rabbits**

DOSE GROUP		1	2	3
NUMBER OF ANIMALS EXAMINED		4	4	4
EYE, LEFT	EXAMIN:	4	4	4
- Eosinophilic material:	vitreous body	4	4	4
- Degeneration:	retina	3	4	4
- Detachment:	retina	3	4	4
- Needle tract lesion		-	3	-

In one saline control animal (# 106), two low-dose animals (# 207 and # 208) and two high-dose animals (# 305 and # 306), the retinal changes observed were associated with focal/multifocal subretinal and/or retinal hemorrhage. Minimal to moderate retinal detachment was usually seen in combination with hypertrophy of pigmented epithelium. Eosinophilic material often in combination with hemorrhage and pigmented inflammatory cells was observed in the vitreous body. One low-dose animal (# 207) had similar eosinophilic material present in the anterior chamber.

**Special Evaluation - BBG concentrations in ocular tissues (Day 14)** – See Section 5.1 PK/ADME above.

#### **Toxicokinetics (24 and 48 hours postdose, Days 8 and 14)**

Plasma concentrations of BBG from 24 hours postdose through Day 14 were below the limit of quantitation (< 10 ng/mL) for both intravitreal and sub-retinal-dosed animals. In contrast, high levels of BBG were still present in ocular tissues on Day 14 (see Section 5.1 PK/ADME above).

#### **Dosing Solution Analysis**

The test article dose formulations were used as supplied by the Sponsor.

## 7 Genetic Toxicology

### 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

#### Study title: Bacterial Mutagenicity Test- Ames Assay

Study no.: 30316I (53899)  
 Study report location: EDR Module 4.2.3.3.1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: 1-31-2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: DYME (BBG250 in sterile ophthalmic solution), Lot # 304AQU06-2, % purity not specified

#### Key Study Findings

- DYME (BBG 250 in sterile ophthalmic solution) was negative for the induction of mutagenicity, under the conditions of the assay.
- Dosing solution analysis was not performed and only 3 concentrations were evaluated for mutagenicity in the definitive assay. Based on the existent marketing experience with the proposed clinical formulation and other BBG formulations, repetition of this assay is not considered needed for the intended indication and dosing regimen.

#### Methods

Strains: *Salmonella typhimurium* (TA97a, TA98, TA100, TA102 and TA1535 ± S9 activation system (S9 mix))

Concentrations in definitive study: 0.501, 1.582, and 5.000 mg/plate

Basis of concentration selection: Range-finding mutagenicity assay in strain TA100 – 0.016 to 5.000 mg/plate – There was no cytotoxicity at any dose level ± S9 mix (measured as reduction of background lawn or in the number of spontaneous mutations). There was no increase in revertant colonies.

Negative control: 0.9% sodium chloride for injection (saline)

Positive control:

CONTROL	STRAIN	METABOLIC ACTIVATION	CONCENTRATION
ICR-191 Acridine	TA97a	No	1.0 µg/plate
2-nitrofluorene	TA98	No	10.0 µg/plate
Sodium azide	TA100 and TA1535	No	1.5 µg/plate
Cumene	TA102	No	200.0 µg/plate
2-aminoanthracene	all strains (except TA1535)	Yes	10.0 µg/plate
2-aminoanthracene	TA1535	Yes	1.6 µg/plate



Formulation/Vehicle: 0.9% sodium chloride for injection (saline)  
Incubation & sampling time: Plate incorporation method – plates incubated for 48-72 hours at 37 ± 2°C after agar solidification


**Study Validity:** All negative controls were within or slightly below (TA102 and TA1535) normal ranges and all positive controls showed the expected increase in reversion rates, indicating a sensitive assay. Data/information on test article precipitation was not reported. No cytotoxicity was observed at concentrations up to the maximum recommended (5 mg/plate). However, the Applicant did not include dosing solution analysis data, and only 3 concentrations were evaluated in the definitive assay (we typically expect at least 5 concentrations to be tested). Thus, it is uncertain if adequate test article concentrations were evaluated. This reviewer believes there is no need to repeat the assay based on the marketing experience and long history of use of BBG, supporting a lack of genotoxic concern for the proposed indication and usage.

### Results:

- The test article did not induce a significant increase in revertant colonies.
- The background lawn appeared normal, the test article did not induce significant cytotoxicity.

## 7.2 *In Vitro* Assays in Mammalian Cells

### Study title: *In Vitro* Mouse Lymphoma Assay

Study no.: 30321I (53900)  
Study report location: EDR Module 4.2.3.3.1  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: 1-31-2007  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: DYME (BBG250 in sterile ophthalmic solution), Lot # 304AQU06-2, % purity not specified

### Key Study Findings

- DYME (BBG250 in sterile ophthalmic solution) was negative for the induction of mutagenicity, under the conditions of the assay.

### Methods

Cell line: L5178Y TK<sup>+</sup> mouse lymphoma cells  
Concentrations in definitive study: 0.158, 0.501, 1.582, and 5.000 mg/mL

Basis of concentration selection: Dose-range finding assay – ten dilutions ranging from 0.0002 to 5.0000 mg/mL – No noticeable change in relative suspension growth was observed, except for the high dose; precipitation was observed at all concentrations (the cultures turned an intense blue).

Negative control: Saline

Positive control: No S9 mix: Methylmethanesulfonate (MMS), 5 µg/mL or 15 µg/mL  
With S9 mix: Cyclophosphamide (CP), 3 µg/mL or 5 µg/mL

Formulation/Vehicle: Saline

Incubation & sampling time: Test article treatment:

- 4 hours ± S9 mix
- 28 hours without S9 mix

Mutant frequency:

- After completion of the 10 to 12-day incubation period, the colonies are counted using software for discrimination of colony size.

**Study Validity:** The positive and negative controls showed the expected results. Dosing analysis was not conducted. However, the higher 2 doses in the definitive assay showed decreased cell density (37% to 84%), indicating cytotoxicity. Therefore, the limitation is not considered to affect the validity of the assay.

## Results

- At 1.58 mg/mL and 5.0 mg/mL in the 4 hours + S9 incubation and 28-hours continuous incubation, there were lower levels of cell density on both Day 1 and Day 2, during the expression and recovery period. However, most cultures recovered and the average cloning efficiencies were within normal limits. As noted in the Study Report, these data suggest that toxicity did not substantially affect the ability of the test system to accurately detect mutagens for these dose groups.
- None of the test-article treatments resulted in a substantial increase in mutant frequencies compared to the concurrent negative control. Actual colony counts do not show relevant increases in absolute numbers of colonies present in any test article extract treated preparation.

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

#### Study title: *In Vivo* Mouse Micronucleus Test

Study no: 30324G  
 Study report location: EDR Module 4.2.3.3.2  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: 7-6-2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: Purified BBG 250, lot # and % purity not specified

#### Key Study Findings

DYME (BBG250 in sterile ophthalmic solution) was not clastogenic under the conditions of the assay.

#### Methods

Doses in definitive study: 100.1, 316.5, and 1000.0 mg/kg IP  
 Frequency of dosing: Single dose  
 Route of administration: Intraperitoneal (IP) injection  
 Dose volume: 20 mL/kg  
 Formulation/Vehicle: 0.9% sodium chloride for injection (saline)  
 Species/Strain: CD-1 mice  
 Number/Sex/Group: 10 (saline control and test article-treated groups)  
 5 (positive control group)

Five mice/sex in the negative control and test article-treated groups were sacrificed at 24 hours and 48 hours postdose; the positive control group mice were sacrificed at 24 hours postdose.

Satellite groups: None  
 Basis of dose selection: Initial dose range study – 10 to 1000 mg/kg IP – Per summary information (data not shown) - Immediately post dosing, high-dose mice were slightly lethargic and had raised fur. By 3 hours, all animals were active and appeared healthy. The mice dosed with the top two doses were starting show a slight blue hue.  
 Negative control: Saline  
 Positive control: Cyclophosphamide, 15 mg/kg and 75 mg/kg

#### Study Validity

The positive and negative controls showed the expected results. Dosing analysis was not conducted. However, clinical signs of lethargy, raised fur, and blue hue noted at the mid and/or high dose, indicate adequate systemic exposure to the test article. Therefore, the limitation is not considered to affect the validity of the assay.

## Results

- Immediately after dosing, high-dose mice were lethargic and had raised fur. Three hours post dose, all animals were active. Mid-dose females were also lethargic. The mice extremities developed a blue hue.
- None of the test article treated groups showed significant increases in micronucleated polychromatic erythrocytes (mPCE) compared to the concurrent negative control.
- The erythropoietic ratios (PCE:RBC) in test-article treated animals were not significantly different from those in the negative controls, indicating lack of bone marrow toxicity.

## 8 Carcinogenicity

No carcinogenicity data was submitted. Based on the single-dose use, carcinogenicity studies are not considered necessary.

## 9 Reproductive and Developmental Toxicology

Reproductive toxicology studies have not been conducted by the Applicant. Because systemic absorption is expected to be negligible, the studies are not considered necessary. The drug product is indicated for single use and remains in the eye for a very short period. In typically less than 1 min after administration, the excess dye is irrigated. It is estimated that only minute amounts of dye remain in the eye bound to the ILM. The ILM is then removed.

## 10 Special Toxicology Studies

**ISO Ocular Irritation Study (Study # TI253\_800; Module 4.2.3.6; GLP)** - DYME - Purified BBG250 (Lot # 176-185-003; 96.5% pure) was evaluated for its potential to cause primary ocular irritation per requirements of ISO 10993. New Zealand White rabbits (3 females) received a 0.1 mL dose of BBG solution instilled into the lower conjunctival sac of the right eye. The left eye served as the untreated control. Ocular reactions were evaluated at 1, 24, 48, and 72 hours postdose. No irritation was observed.

**In Vivo Skin Irritation Test in Albino Rabbits (Study # S-2018-00490; Module 4.2.3.6; GLP)** - This study was conducted per procedures described in ISO 10993-10. New Zealand White rabbits (3 males) were used.



Each rabbit was treated with 0.5 ml of the test sample (ILMBLUE US; batch # 19317) applied with a gauze (25 mm x 25 mm) directly to the skin on two sites of the dorsum and covered with non-occlusive dressing. A no irritant gauze (25 mm x 25 mm) humidified with sodium chloride injection, used as control, was applied to 2 other sites in the rabbit dorsum. Reactions were evaluated 1 hour following the removal of the patches and 24, 48 and 72 hours after treatment.

A slight erythema was observed in test article-treated animals 60 minutes postdose. The finding was still present in 2 test article-treated animals at 24 hours postdose. No signs of erythema were observed at the 48-hour and 72-hour evaluations. The test article was considered as negligible irritant.

**Published Literature:** The Applicant submitted an integrated summary of nonclinical literature being relied upon to support this marketing application. A tabular integrated summary (as provided by the Applicant) can be found in Table 3.

Among these published studies, 2 publications evaluated the proposed formulation and are further discussed below:

**Award D et al, 2011<sup>7</sup>** - The toxicity in ARPE-19 human retinal pigment epithelium cells of trypan blue (TB) at 0.15% and 0.25% concentration, BBG at 0.025% and 0.05%, their combination, and the effect of the addition of 4% polyethyleneglycol (PEG) was investigated. Cells were exposed for 5 and for 30 minutes to the different preparations. Cell viability was measured with the WST-1 assay measuring intracellular dehydrogenase activity.

### Key Results:

- At 5 minutes, no toxicity was observed for any of the preparations.
- At 30 minutes, solutions containing PEG with BBG (0.025%; [REDACTED] (b) (4), TB (0.15%), and mixtures of BBG (0.025%) with TB (0.15% and 0.25%) were the least toxic of the preparations as well as preparations of BBG at 0.025% in phosphate-buffered saline solution, while TB at 0.25% in phosphate buffered saline solution appeared the most toxic (Table 2 in the publication, copied below).

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<sup>7</sup> Awad D, et al., 2011, Comparative toxicology of trypan blue, brilliant blue G, and their combination together with polyethylene glycol on human pigment epithelial cells. Invest Ophthalmol Vis Sci. Jun 9; 52(7): 4085.

TABLE 2. Cell Viabilities after an Incubation Time of 30 Minutes and P Values of Two-Sample *t*-Tests of Solutions Tested

	TB0.25BBG0.025PEG	TB0.15BBG 0.025PEG	Brilliant Peel, BBG0.025 (Fluoron)	BBG0.025PEG	BBG0.025	TB0.15PEG	TB0.25PEG	BBG0.05	BBG0.05PEG	MembraneBlue TB0.15 (DORC)	TB0.15	TB0.25	MonoBlue TB0.25 (Arcadophta)
Cell Viability	1.137	1.133	1.084	1.083	1.039	1.034	0.953	0.945	0.942	0.900	0.894	0.804	0.792
95% Confidence Interval	0.082	0.083	0.031	0.050	0.096	0.140	0.033	0.019	0.076	0.079	0.056	0.013	0.083

- The addition of PEG reduced the toxicity of preparations containing TB either alone or in combination with BBG.

Januschowski K, et al., 2012<sup>8</sup> - The cytotoxicity of ILM-Blue<sup>®</sup> (BBG with 4% PEG; (b) (4)) and MembraneBlue Dual<sup>®</sup> (TB and 4% PEG), CE-approved products marketed in Europe, on monolayer cultures of bovine retinal ganglion cells (RGC5) was evaluated after incubation for 30, 60, 120, and 320 seconds. For functionality testing, bovine retinas were isolated and superfused with an oxygen-saturated nutrient solution, and an electroretinogram (ERG) was recorded. The two dye solutions were applied epiretinally for 30, 60 or 120 seconds. ERG recovery was monitored for up to 75 minutes.

### Key Results:

- No significant difference in viability of RGC5 cells following exposure to ILM-Blue<sup>®</sup> or MembraneBlue Dual<sup>®</sup> for any staining period ranging from 30 to 320 seconds was observed compared to the control group.
- After staining with ILM-Blue<sup>®</sup>, no statistically significant reduction of a- or b-wave amplitudes were recorded.
- For MembraneBlue Dual<sup>®</sup>, significant changes in a-wave and/or b-wave amplitudes were observed after 60 and 120 seconds of application. Except for the washout period after 30 seconds application for a-wave amplitude, no significant effects were observed at the end of the 75-minute washout phases.
- PEG 4% alone did not show any effect on the ERG recovery and cell viability at any of the incubation times tested.

<sup>8</sup> Januschowski K, et al. (2), 2012, Investigating the biocompatibility of two new heavy intraocular dyes for vitreoretinal surgery with an isolated perfused vertebrate retina organ culture model and a retinal ganglion cell line. Graefes Arch Clin Exp Ophthalmol. Apr; 250(4): 533.

- The authors concluded that both Brilliant Blue G 0.025% Solution and MembraneBlue Dual® appear to be safe for clinical use for staining periods (retina exposure times) of 120 seconds and possibly up to 320 seconds.

**Table 3: Literature That Support the Nonclinical Safety of Brilliant Blue G 0.025% Solution**

Publication Authors	Nonclinical Topic	Model	Route of Administration	Dose	BBG Source Used (if known)	Noteworthy Findings
<a href="#">Awad D, et al., 2011</a>	Other Toxicity Studies (In-Vitro)	ARPE-19 cells	Incubation	0.025% and 0.05% (0.25 and 0.5 mg/mL)	Technical grade (Supplier not specified)	The inclusion of PEG into the formulation for staining was suggested to be beneficial not only for the wanted effect of a denser and more viscous solution, but also because of further reduced toxicity of the dye.
<a href="#">Balaiya S, et al., 2011</a>	Other Toxicity Studies (In-Vitro)	Rat RGC-5 and human ARPE-19 cells	Incubation	0.025% and 0.05% (0.25 and 0.5 mg/mL)	Technical grade (Sigma-Aldrich <sup>1</sup> )	A favorable safety profile of IfCG, BBG, and BPB compared with ICG in vitro was reported.
<a href="#">Creuzot-Garcher C, et al, 2010</a>	Other Toxicity Studies (In-Vivo)	Rat	Intravitreal	0.025% (0.25 mg/mL)	Brilliant Peel EU Commercial Product (Fluoron)	BBG did not show any morphologic alterations.
<a href="#">Ejstrup R, et al., 2012</a>	Other Toxicity Studies (In-Vivo)	Pig	Subretinal	0.025% (0.25 mg/mL)	Technical grade, Acros Organics	BBG did not alter the mfERG response or retinal structure.
<a href="#">Enaida H and Ishibashi T., 2008</a>	Single-Dose Toxicity	Rat  Primate	Rat: Intravitreal  Primate: Subretinal	Rat: 0.001% to 1% (0.01 to 10 mg/mL)  Primate: 0.025% (0.25 mg/mL)	Technical grade (Sigma-Aldrich <sup>1</sup> )	No toxic effects were observed over a period of 2 months. Visualization was clear. Postoperative toxic effects of BBG such as a corneal edema, severe retinal edema, and endophthalmitis were not observed at day 14.
<a href="#">Hisatomi, T., et al., 2006</a>	Single-Dose Toxicity	Rat	Intraocular (anterior chamber)	0.001% to 1% (0.01 to 10 mg/mL)	Technical grade (Sigma-Aldrich <sup>1</sup> )	BBG remained in the anterior chamber and was followed by biomicroscopic examination for 2 months. The cornea showed no remarkable changes; no endothelial cell loss or corneal edema. The lamellar collagen layers, stromal cells, and epithelial cell layer were well preserved. No inflammatory cell infiltration was observed in any corneal layers. No degenerative changes in any tissue were observed with electron microscopy.
<a href="#">Iriyama A, et al., 2012</a>	Other Toxicity Studies (In-Vitro and In-Vivo)	Rat RGC cells	Incubation	0.25 mg/mL	Technical grade (Supplier not specified)	BBG was not toxic over a long exposure of 72 hours to RGCs both in vivo and in vitro

Cont. Table 3

Publication Authors	Nonclinical Topic	Model	Route of Administration	Dose	BBG Source Used (if known)	Noteworthy Findings
		Rat	Intravitreal	3 µL of 0.0025 mg/mL	specified)	at concentrations clinically used. Brilliant Blue G was less toxic than ICG at the same concentration and the same exposure time.
Januschowski K, et al. (1), 2012	Other Toxicity Studies (In-Vitro)	Bovine and human retinas	Epiretinal incubation	0.25 mg/mL (+13% D <sub>2</sub> O)	Brilliant Peel EU Commercial Product (Fluoron)	Reductions of the a- and b-wave amplitudes directly after exposure with BBG and D <sub>2</sub> O. Effects on the ERG were rapidly and completely reversible within the recovery time for all exposure times. ERG amplitudes at the end of the washout did not differ from before staining.
Januschowski K, et al. (2), 2012	Other Toxicity Studies (In-Vitro)	RGC-5 cells	Incubation	0.025% (0.25 mg/mL)	ILM-Blue (0.025% BBG) and MembraneBlue Dual (0.15% Trypan Blue + 0.025% BBG) Commercial Product (DORC International)	No significant difference in cell viability after 30, 60, 120 and 320 seconds was reported.
Jiang, L., et al., 2000	Primary Pharmacodynamics	HEK293 Cells	Incubation	Up to 10 µM <sup>(b)</sup>	Technical grade (Supplier not specified)	BBG selectively and noncompetitively inhibits P2X7 receptor (purinoreceptor) with rat and human IC <sub>50</sub> values of 10 and 200 nM, respectively.
Kawahara S et al., 2007	Other Toxicity Studies (In-Vitro)	Human Muller Cells	Incubation	0.025% (0.25 mg/mL)	Technical grade (Sigma-Aldrich <sup>1</sup> )	BBG did not cause apoptosis and was suggested as a safer adjuvant to ICG.
Kimble DE, et al., 2012	Other Toxicity Studies (In-Vivo)	Mouse	Intravenous	Up to 100 mg/kg	Technical grade (Acros Organics)	Both intravenous and oral BBG administrations were well-tolerated and differences in locomotor activity or body weights were not observed.
Lüke M et al., 2008	Other Toxicity Studies (In-Vitro)	Bovine retina	Epiretinal incubation	0.025% (0.25 mg/mL)	Brilliant Peel EU Commercial Product (Fluoron)	BBG epiretinally applied led to a current depression of the b-wave, which did not show a difference with regard to the diverse retinal exposure time. BBG was suggested to be an alternative vital staining dye with a good biocompatibility.
Mennel S et al., 2008	Other Toxicity Studies (In-Vitro)	ARPE-19 cells	Incubation	Up to 2.4 mg/mL	Technical grade (Supplier not	A moderate decrease in transepithelial resistance (TER) after 1.5 and 3 h was



Cont. Table 3

Publication Authors	Nonclinical Topic	Model	Route of Administration	Dose	BBG Source Used (if known)	Noteworthy Findings
					specified)	observed. After 24 h, there was no difference between the control group and the BBG group at both concentrations. No structural changes of the cells, cell compartments and intercellular structures were seen.
Morales, MC, et al., 2010	Other Toxicity Studies (In-Vitro)	ARPE-19 cells	Incubation	0.50 mg/mL and 5 mg/mL	Technical grade (Sigma-Aldrich <sup>1</sup> )	BBG did not lead to apoptosis or necrosis.
Notomi S, et al., 2011	Safety Pharmacology	Mouse primary retinal cells	Incubation and intra-ocular	Incubation cells: up to 0.854 µg/mL Intra-ocular (IO): 500 µM(b)	Technical grade (Supplier not specified)	Minimal toxicity of BBG with the primary culture was observed.
Peng, W., et al., 2009	Repeat Dose Toxicity	Rat	Intravenous	Up to 3 x 50 mg/kg <sup>(c)</sup> (repeat doses)	Technical grade (Sigma-Aldrich <sup>1</sup> )	BBG had no effects on behavior, weight, survival, or other physiological parameters, including body temperature, blood pH, blood gases, or blood pressure.
Remy, M., et al., 2008	Single Dose Toxicity	Rat	Intravitreal	0.25 mg/mL (final concentration in vitreous by injecting 2 µL 0.25% BBG)	Brilliant Peel EU Commercial Product (Fluoron)	In BBG injected eyes no statistically significant differences in Rat Ganglion Cell densities were observed compared with Balanced Salt Solution injected eyes. No histological changes were observed to retinal cells.
Ridderström, M., and M. Ohlsson, 2014	Repeat Dose Toxicity	Rat	Intravenous	50 mg/kg	Technical grade (Sigma-Aldrich <sup>1</sup> )	Repeat dosing of BBG in a rat model of optic nerve injury did not report adverse events associated with repeat intravenous administration.
Rodrigues EB, et al., 2009b	Other Toxicity Studies (In-Vivo)	Rabbit	Intravitreal	50 µL of 0.5 and 5 mg/mL	Technical grade (Merck)	BBG showed good staining characteristics of the ILM and was biocompatible.
Takayama K, et al., 2012	Other Toxicity Studies (In-Vitro)	ARPE-19 cells	Incubation	0.5 mg/mL	Technical grade (Supplier not specified)	BBG is stable to light irradiation and less likely to produce cytotoxic products by light exposure than ICG. BBG is also expected to be rapidly cleared by intraocular fluid circulation due to its solubility in an aqueous solution.
Ueno, A., et al., 2007	Single Dose Toxicity	Rat	Subretinal	0.25 mg/mL	Technical grade (Sigma-Aldrich <sup>1</sup> )	After both 2 weeks and two 2 months, BBG had no detectable toxic effects with no sign

Publication Authors	Nonclinical Topic	Model	Route of Administration	Dose	BBG Source Used (if known)	Noteworthy Findings
						of apoptotic cell death detected in the inner and outer nuclear layers and the retinal pigment epithelial layer.
Yuen D, Gonder J, Proulx A, Liu H, Hutnik C., 2009	Other Toxicity Studies (In-Vitro)	ARPE-19 cells	Incubation	Up to 10 mg/mL	Technical grade (Supplier not specified)	BBG demonstrated dose- and time-dependent toxicity, although the results were not statistically significant at surgically relevant concentrations.

(b) (4)

As noted in Table 3, in several publications BBG concentrations up to 0.025% (0.25 mg/mL) were found to be nontoxic. Particularly, in vivo studies showed no adverse findings at the proposed concentration. Some key studies are further described below:

- Following vitrectomy, BBG solution was injected into the vitreous cavity of rats at concentrations of 0.001% to 1%<sup>9</sup>. No toxic effects, such as necrosis, apoptosis, corneal edema, retinal edema, or endophthalmitis were observed over a period of 14 days or 2 months. The 1% solution of BBG induced vacuolization in the inner retinal cells both on Day 14 and 2 months, though apoptosis was not detected. The same changes were also found in the group injected with 0.1% BBG, the grade of vacuolization was less than at 1%. This effect was not seen at concentrations  $\leq$  0.01%. There was no significant reduction in the amplitude of the ERG waves at both doses evaluated (0.1% and 1%).
- The ability of BBG to stain the ILM was investigated in cynomolgus injected a 0.05% IVT injection of BBG<sup>10</sup>. Toxic effects such as a corneal edema, severe retinal edema, and endophthalmitis were not observed at Day 14. Fluorescein angiography also revealed no apparent retinal damage by BBG on Day 14. Further ophthalmoscopic examinations showed no further changes in the retina during the 6-month follow-up period.
- BBG (0.25%; 0.025% final intravitreal concentration) injected into rat eyes showed no decrease in retinal ganglion cell counts or effects on retinal morphology visualized by light microscopy 7 days later<sup>11</sup>.
- The biocompatibility of BBG (0.025%) was determined following subretinal injection in rats. After 2 weeks or 2 months, BBG had no detectable toxic effects with no signs of apoptotic cell death detected in the inner and outer nuclear layers and the retinal pigment epithelial layer<sup>12</sup>.
- After IVT injection of BBG at 0.05% or 0.5%, remarkable changes within the photoreceptors were noted at 24 hours after injection (vacuolization and cellular edema)<sup>13</sup>. These changes decreased in severity after a 7-day follow up. Reduction in b-wave amplitude in rod testing, OPs and/or 30-Hz flicker testing in vivo in rabbit eyes injected with 0.05% or 0.5% BBG IVT at 7-day postdose.

Adverse findings reported in in vitro publications included transient effects in the ERG (reductions in a- and b-wave amplitude) in bovine retina preparations at 0.25 mg/mL; moderate decrease in transepithelial resistance (TER) in ARPE-19 human retinal pigment epithelium cell line at 0.25 mg/mL or 2.4 mg/mL, which was reversible by 24-hours postdose; dose- and time-dependent toxicity in ARPE-19 cells and/or a murine retinal ganglion/Muller glial mixed primary cell culture, at  $\geq$ 0.125 mg/mL in ARPE-19 cells after 30 minute exposure (not toxic at  $\leq$ 0.25 mg/mL after a 3 minute exposure) and at  $\geq$ 2.5 mg/mL in Muller glial cells after 30 minute exposure; necrosis in ARPE-19 and rat retinal ganglion cells (RCG-5) at exposure time periods beyond 5 minutes at 0.25 mg/mL and 0.5 mg/mL; etc. Overall, the published in vitro data support that Brilliant Blue G

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<sup>9</sup> Enaida, H., et al., 2006, Preclinical investigation of internal limiting membrane staining and peeling using intravitreal Brilliant Blue G, *Retina*, 26(6): 623 – 630.

<sup>10</sup>Ibid

<sup>11</sup> Remy, M., et al., 2008, An in vivo evaluation of Brilliant Blue G in animals and humans, *Br J Ophthalmol*, 92(8): 1142 - 1147.

<sup>12</sup> Ueno, A., et al., 2007, Biocompatibility of Brilliant Blue G in a rat model of subretinal injection, *Retina*, 27(4): 499 - 504.

<sup>13</sup> Rodrigues EB, et al., 2009a, Preclinical investigation of the retinal biocompatibility of six novel vital dyes for chromovitrectomy. *Retina*. Apr; 29(4): 497- 510.

0.025% has an acceptable safety profile for the intended clinical concentration and short-term ocular exposure.

## 11 Integrated Summary and Safety Evaluation

The pivotal nonclinical information includes the following study reports: a single dose ocular toxicity study in rabbits after intravitreal (25 and 50 µg/eye) or subretinal (12.5 and 25 µg/eye) administration, including a 14-day follow-up period, with ocular tissue distribution evaluation, full battery of genotoxicity tests (bacterial mutagenicity test, in vivo mouse micronucleus test and in vitro mouse lymphoma assay), and ocular irritation and skin irritation studies in rabbits.

The main findings of these studies include:

- In eyes treated by subretinal administration, anterior segment inflammation, vitreous opacity and/or presence of vitreal hemorrhage in saline control and BBG-treated groups. However, the increased incidence and/or severity of effects in the BBG-treated groups suggests a contribution by the test article.
- Reductions in scotopic a-wave and/or b-wave amplitude at both test-article routes of administration and at both dose levels.
  - Effects on the ERG have been reported in nonclinical studies from the published literature.
  - In in vitro studies, these effects have been shown to be reversible after a washout period<sup>14</sup>. The authors believed the reduction of the ERG amplitudes can be explained by absorption of the applied light by the dye. The applied light is initially absorbed by the dye before reaching the retina.
- In eyes treated by subretinal administration, minimal to marked microscopic retinal changes (detachment and degeneration) were observed. These changes were present in both control and treated groups, but with increased severity in test article-treated groups.
- Plasma concentrations of BBG from 24 hours postdose through Day 14 were below the limit of quantitation (< 10 ng/mL) for both intravitreal and subretinal route of administration. In contrast, high levels of BBG were still present in ocular tissues (primarily in the retina, i.e., ≤10100 ng/mL after IVT injection and ≤7810 ng/mL after subretinal injection) on Day 14.
- Negative genetic toxicity results, under the conditions of the assays
- No ocular irritation in the ocular irritation study
- Slight skin irritation in the skin irritation test

Overall, except for the ERG changes, the findings were considered mainly associated with the surgical procedure, but a contribution by the test-article cannot be

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<sup>14</sup> Lüke M et al., 2008, Electrophysiological effects of Brilliant Blue G in the model of the isolated perfused vertebrate retina, *Graefes Arch Clin Exp Ophthalmol*, 246 (6): 817-822.

excluded based on the increased incidence and/or severity in test article-treated groups. At the intended clinical dose regimen, the total dose to be administered to the human eye is 0.125 mg. Therefore, there is no exposure margin at any of the doses used in the ocular toxicity study ( $\leq 50 \mu\text{g}/\text{eye}$ ). However, the drug product is indicated for single use and is administered for a very short period. In typically less than 1 min after administration, the excess dye is irrigated. It is estimated that only minute amounts of dye remain in the eye bound to the ILM. The ILM is then removed from the eye. Therefore, the amount of test article remaining in the eye is expected to be negligible. As such, the design of the ocular toxicity study maximized exposure to the dye and it is considered acceptable for risk assessment.

A limitation of the ocular toxicity study is that it was conducted with a purified BBG preparation (DYME) (b) (4). (b) (4) The composition of the DYME preparation is different than that of the proposed clinical product. However, there is marketing experience with the proposed clinical formulation to support its safe use in humans (see below).

BBG has a long history of clinical use. BBG has been commercially available since 2007 in several countries outside the USA for visualization of ILM during vitreo-retinal surgery. The company Fluoron GmbH received CE approval (European Conformance, i.e., product complies with the European Directives to be marketed in the European Union) for the product Brilliant Peel as a class IIA medical device by the notified body EUROCAT dated June 28, 2007 (CE-0535). DORC received CE approval for Brilliant Blue G 0.025% Solution (marketed as ILM-Blue<sup>®</sup>) as a class IIA medical device by the notified body DEKRA in August 2010 (CE-0344). DORC claims that since the market introduction, over (b) (4) units of CE-approved ILM-Blue<sup>®</sup> have been distributed in the European Union and no reports of adverse effects or complaints related to the use of the product have been received.

The Applicant provided a review of scientific publications of in vitro cytotoxicity studies in several animal and human eye cell lines (rat retinal ganglion cells, human retinal pigment epithelium cells, human Muller cells), and tissues (bovine and human retinas) and in vivo studies in several animal species (rat, monkeys, mouse and pigs) to support proof of concept and the safety of ocular administration of BBG. (b) (4) (b) (4) (BBG with 4% PEG) showed no toxicity in ARPE-19 human retinal pigment epithelium cells up to 30 minutes postdose and in RGC5 bovine retinal ganglion cells up to 320 seconds postdose. Overall, these studies support the safety of the proposed concentration (0.025% or 0.25 mg/mL) and short-term ocular exposure (see further details under Section 10 Special Toxicology Studies above).

Given the intended single-dose use (with subsequent removal of most of the dye), repeat-dose and carcinogenicity studies are not considered relevant. Reproductive and developmental toxicity are also not considered relevant given that systemic absorption is expected to be negligible.



The reviewer believes that the nonclinical study reports provided, the nonclinical literature cited, together with the existent marketing experience for Brilliant Blue G 0.025% Solution (over (b) (4) units distributed in Europe; marketed as ILM-Blue® outside of the United States), provide adequate safety support for the intended indication. Approval of the NDA is recommended.

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**This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.**  
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/s/  
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MARIA I RIVERA  
10/09/2019 04:29:19 PM

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10/09/2019 06:02:57 PM