CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761094Orig1s000

NON-CLINICAL REVIEW(S)
Tertiary Pharmacology/Toxicology Review

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA: 761094
Agency receipt date: December 22, 2017
Drug: OXERVATE (cenegermin)
Sponsor: Dompe Farmaceutica S.a.P.

Indication: Treatment of [redacted] neurotrophic keratitis

Reviewing Division: Division of Transplant and Ophthalmology Products

The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of OXERVATE for the indication listed above.

OXERVATE (cenegermin) is a recombinant human nerve growth factor (rhNGF) formulated as a topical eye drop. NGF is a member of the neurotrophin family that controls the differentiation, regeneration, and survival of sympathetic and sensory neurons of vertebrates. It is intended to be administered chronically to the affected eye(s) 6 times daily.

The nonclinical program consisted primarily of primary and safety pharmacology studies, repeat-dose ocular and subcutaneous toxicity studies in rats (up to 26 weeks) and rabbits (up to 4-months), and a complete battery of reproductive toxicity studies. Drug administration was not associated with ocular toxicity at exposure margins exceeding 3 times in rats and 23 times in rabbits. The only systemic toxicities identified were immunogenic responses, as well as ovarian effects, including persistent estrus, ovarian follicular cysts, atrophy/reduction of corpora lutea, and changes in ovarian weight, that occurred at doses > 100 times the maximum recommended ocular dose.

Genetic toxicity and carcinogenicity studies with cenegermin were not conducted given the negligible clinical systemic exposure to the drug and lack of concerning finding in the general toxicology studies.

Cenegermin was not associated with effects on fertility or postnatal development following subcutaneous administration. However, embryofetal development studies identified a slight increase in embryofetal resorption in rats and rabbits, cardiovascular anomalies in rabbits, and hydrocephaly and ureter anomalies in rats; these effects were observed at doses greater than 250 times the human dose.

Conclusion:
I agree with the Division pharmacology/toxicology conclusion that cenegermin can be approved from the nonclinical perspective. I have reviewed the proposed text for the nonclinical sections of the product label and agree with the Division recommendations.
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.

/s/

TIMOTHY J MCGOVERN
07/13/2018
DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: BLA 761094
Supporting document/s: SDN001
Applicant’s letter date: 5-31-2017
CDER stamp date: 5-31-2017
Product: Recombinant human nerve growth factor
Indication: Treatment of neurotrophic keratitis
Applicant: Dompe Farmaceutici S.p.A. (Milano, Italy)
Review Division: Division of Transplant and Ophthalmology Products
Reviewer: Aaron Ruhland, PhD
Supervisor/Team Leader: Lori Kutch, PhD, DABT
Division Director: Renata Albrecht, MD
Project Manager: Derek Alberding

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

9.2 1.1 Introduction

The Applicant has submitted a BLA application for Oxervate® [cenegermin; recombinant human nerve growth factor (rhNGF)] for the treatment of neurotrophic keratitis. The formulation is a topical eye drop to be administered chronically to the affected eye(s) 6 times daily.

Nerve Growth Factor is member of the neurotrophin family and controls the differentiation, regeneration and the survival of sympathetic and sensory neurons of vertebrates. Receptors for NGF include TrkA and the low-affinity nerve growth factor receptor p75. Nerve growth factor receptors have been found on the normal and abnormal cornea and conjunctiva. In vitro, NGF has been shown to induce proliferation and differentiation of rabbit corneal epithelial cells. In humans and in experimental animal models, both corneal and conjunctival layers (epithelial, stromal and endothelial cells) show the ability to produce and release NGF, and express TrkA and p75. Several publications have shown the role of NGF in corneal homeostasis, corneal wound healing and tear production.

9.3 1.2 Brief Discussion of Nonclinical Findings

- The ocular toxicity of recombinant human nerve growth factor (rhNGF) has been evaluated in rats and rabbits. Recombinant human nerve growth factor (also known as cenegermin) was not associated with adverse ocular toxicity following daily topical administration in rats and rabbits for periods up to 26-weeks and 125-days, respectively; at a rat dose corresponding to 3.8-fold the MRHOD and rabbit dose corresponding to 23-fold the MHROD.

- The systemic toxicity of rhNGF was evaluated in rats and rabbits after subcutaneous or ocular administration. Immunogenic response to rhNGF (a heterologous protein in animals) was reported in multiple studies following administration of rhNGF. In three of the pivotal toxicology studies, administration of rhNGF in females was associated with ovarian findings (Rabbit: 90-day subcutaneous administration and 2-month ocular administration; Rat: 26-week ocular administration). Findings included persistent estrus, ovarian follicular cysts, atrophy/reduction of corpora lutea, and changes in ovarian weight at doses ≥119-fold the MRHOD. The Applicant proposes that the observed ovarian findings in animals are consistent with mechanism of action. While plausible, no empirical data was provided to directly support that supposition.

- In reproductive toxicity studies, rhNGF did not produce effects on fertility or postnatal development in offspring. Following daily subcutaneous administration of rhNGF during the period of organogenesis, a slight increase in postimplantation loss (embryofetal resorption) was observed in rats and rabbits at all doses (≥42 µg/kg/day or 267-fold MRHOD); cardiovascular anomalies were observed in rabbits at 83 µg/kg/day (534-fold MRHOD) and hydrocephaly and ureter anomalies were observed in rats at 267 µg/kg/day (1709-fold MRHOD).
Fetal malformations were not observed at doses ≤42 µg/kg/day (267-fold MRHOD). In parental rats and rabbits, an immunogenic response to cenegermin was observed in all reproductive toxicity studies. Given that cenegermin is a heterologous protein in animals, this response may not be relevant to humans.

9.4 1.3 Recommendations

1.3.1 Approvability: Approvable from a Pharmacology/Toxicology perspective.

1.3.3 Labeling

1.3.3.1 Applicant’s Proposed Labeling (sections relevant to Pharmacology/Toxicology only)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary
There are no data from the use of OXERVATE in pregnant women to inform any drug associated risks.
8.2 Lactation
Risk Summary
There are no data on the presence of OXERVATE in human milk, the effects on breastfed infant, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered, along with the mother's clinical need for OXERVATE, and any potential adverse effects on the breastfed infant from OXERVATE.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
Nerve growth factor is an endogenous protein involved in the differentiation and maintenance of neurons, which acts through specific high-affinity (i.e., TrkA) and low-affinity (i.e. p75NTR) nerve growth factor receptors.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
Carcinogenesis and Mutagenesis
Animal studies have not been conducted to determine the carcinogenic and mutagenic potential of cenergermin.

Impairment of fertility
1.3.3.2 FDA Proposed Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary
There are no data from the use of OXERVATE in pregnant women to inform any drug associated risks.

Administration of cenegermin to pregnant rats or rabbits during the period of organogenesis did not produce adverse fetal effects at clinically relevant doses. In a pre- and postnatal development study, administration of cenegermin to pregnant rats throughout gestation and lactation did not produce adverse effects in offspring at clinically relevant doses.
Animal Data
In embryofetal development studies, daily subcutaneous administration of cenergermin to pregnant rats and rabbits throughout the period of organogenesis produced a slight increase in postimplantation loss at doses greater than or equal to 42 μg/kg/day (267 times the MRHOD). A no observed adverse effect level (NOAEL) was not established for postimplantation loss in either species. In rats, hydrocephaly and ureter anomalies were observed in fetuses at 267 μg/kg/day (1709 times the MRHOD). In rabbits, cardiovascular malformations, including ventricular and atrial septal defects, enlarged heart and aortic arch dilation were observed in fetuses at 83 μg/kg/day (534 times the MRHOD). No fetal malformations were observed in rats and rabbits at doses of 133 μg/kg/day and 42 μg/kg/day, respectively.

In a pre- and postnatal development study, daily subcutaneous administration of cenergermin to pregnant rats during the period of organogenesis and lactation did not affect parturition and was not associated with adverse toxicity in offspring at doses up to 267 μg/kg/day.

In parental rats and rabbits, an immunogenic response to cenergermin was observed. Given that cenergermin is a heterologous protein in animals, this response may not be relevant to humans.

8.2 Lactation
Risk Summary
There are no data on the presence of OXERVATE in human milk, the effects on breastfed infant, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered, along with the mother's clinical need for OXERVATE, and any potential adverse effects on the breastfed infant from OXERVATE.

12 CLINICAL PHARMACOLOGY
12.1 Mechanism of Action
Nerve growth factor is an endogenous protein involved in the differentiation and maintenance of neurons, which acts through specific high-affinity (i.e., TrkA) and low-affinity (i.e. p75NTR) nerve growth factor receptors.
13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis
Animal studies have not been conducted to determine the carcinogenic and mutagenic potential of cenegermin.

Impairment of fertility
Daily subcutaneous administration of cenegermin to male and female rats for at least 14 days prior mating, and at least 18 days post coitum had no effect on fertility parameters in male or female rats at doses up to 267 µg/kg/day (1709 times the MRHOD).

In general toxicity studies, subcutaneous and ocular administration of cenegermin in females was associated with ovarian findings including persistent estrus, ovarian follicular cysts, atrophy/reduction of corpora lutea, and changes in ovarian weight at doses greater than or equal to 19 µg/kg/day (119 times the MRHOD).

2 Drug Information

2.1 Drug

CAS Number: 86923-98-0

Proposed Trade Name: Oxervate

Code Names: cenegermin, cenegermin ophthalmic solution; recombinant human nerve growth factor (rhNGF)

Molecular Formula/Molecular Weight: \( \text{C}_{583}\text{H}_{908}\text{N}_{166}\text{O}_{173}\text{S}_{8} \sim 13.266 \text{kDa} \)

Structure or Biochemical Description: Recombinant protein expressed in E. coli

Pharmacologic Class: Recombinant growth factor

9.5 2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 115,892
9.6 2.3 Drug Formulation

The drug substance is a recombinant protein produced in *E. coli* as pro-NGF and converted to the human conformation through an enzymatic process.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Function</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhNGF</td>
<td>Drug substance</td>
<td>0.002%</td>
</tr>
<tr>
<td>Trehalose dihydrate</td>
<td></td>
<td>4.7%</td>
</tr>
<tr>
<td>Mannitol</td>
<td></td>
<td>1.22%</td>
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<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt; anhydrous</td>
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<tr>
<td>NaH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt; dihydrate</td>
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<tr>
<td>Hydroxypropylemethyl cellulose</td>
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<tr>
<td>PEG 6000</td>
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<td>0.1%</td>
</tr>
<tr>
<td>L-methionine</td>
<td></td>
<td>0.001%</td>
</tr>
</tbody>
</table>

- API: 0.002%

9.7 2.4 Comments on Novel Excipients

- Trehalose
- Methionine

A 2-month ocular toxicology study in rabbits showed no toxicity associated with the vehicle formulation containing methionine and trehalose (4.7%). A 26-week ocular toxicology study in rats used a formulation that lacked L-methionine but contained trehalose (4.7%); no toxicity was associated with the vehicle.

9.8 2.5 Comments on Impurities/Degradants of Concern

**Study title:** Safety assessment of two impurities occurring during the manufacturing of rhNGF. The assessment was done according to ICH Q5E and Q6B.

- Degradation during manufacturing results in the formation of
- Applicant states that the impurities are
- Applicant states that this class of impurities are common in manufacture of recombinant proteins and do not interfere with the biological activity or with the safety of the drug product (references provided).
- The Applicant proposes a specification of % for each impurity.
- In the pivotal chronic nonclinical studies, Batch no. RS 0313 (containing both impurities at an amount of %) was administered by subcutaneous route in
rabbits and rats up to a period of 90 days and 26 weeks, respectively, without inducing relevant adverse effects.

- **Nonclinical support**
  - **Systemic toxicity**
    - **Rabbit**
      - Study no.: M1305BPL (D68326): rhNGF: 90-day subcutaneous toxicity study in the New Zealand White rabbit with a 4-week recovery period.
      - NOAEL (reviewer’s conclusion) is 200 µg/animal in male rabbits (0.111 mg/kg) and 100 µg/animal in female rabbits (0.0555 mg/kg).
      - The NOAEL doses in rabbits correspond to an 11.5 and 23-fold margin over 100% absorption of the proposed human ocular dose in females and males, respectively.
    - **Rat**
      - Study D68304 (M1306BPL): Combined 8/26-week subcutaneous toxicity study in the Wistar rat with a 2/4-week recovery period.
      - The NOAEL in rats (0.667 mg/kg) corresponds to a 690-fold margin over 100% absorption of the proposed bilateral human ocular dose.
  - **Ocular toxicity**
    - **Rat**
      - Study 0472-2011 (A1213BPL/E): 26-week eye drop administration toxicity study in the Wistar rat followed by a 4-week recovery period.
      - Ocular NOAEL is 18 µg/eye/day, which corresponds to a 3.85-fold margin over the proposed clinical dose (4.68 µg/eye/day).
      - High dose (Batch RS1212): contained
      - Ocular NOAEL therefore supports
      - **Applicant proposes**
        - the total amount administered in patients will be still considerably below NOAEL in animals.

**Container Closure System**

Upon reviewing the leachables and extractables data for the container closure system, the CMC reviewer noted that the Applicant found \( (b)(4) \) in both the vials and stoppers for their drug product container closure system in an extractables study. The following data were provided:

- \( (b)(4) \) in vials \( (b)(4) \) ng/vial
- Stoppers \(\text{ng/stopper}\)

In the experiment conducted by the Applicant, the extractables study used PBS pH 7 buffer with 10% ethanol as a solvent for extraction. The CMC reviewer noted that compared to what is usually used as extraction solvents, this solvent is not considered to be very extreme/harsh.

Using Product Quality Research Institute recommendations for ophthalmic products (leachables above 1ppm should be reported), the CMC reviewer calculated a safety concern threshold (SCT) of \(\text{ng/day}\) for this product. Also, it seems that finding \(\text{ng/g}\) in glass is common as there is a USP limit for \(\text{ng/g}\) and this USP limit is included in the Applicant’s vial specifications.

The CMC reviewer stated that typically, this information is adequate to ensure low safety risk from an extractable. However, since it is \(\text{ng/g}\), the reviewer was not sure if different evaluation criteria should apply to determine the level of safety risk. The reviewer asked the Pharm/Tox discipline whether the levels of \(\text{ng/g}\) found in the DP container closure system are a safety concern.

The CMC reviewer notes that there is no risk of \(\text{ng/ml}\) leaching from the stopper contributing to the drug product. If \(\text{ng are present in each vial of the DP and equally distributes to the volume of DP in the vial (1mL) the concentration of \(\text{ng/mL}\) would be \(\text{ng/mL}\). For a \(\text{mL}\) drop size, this corresponds to a concentration of \(\text{ng/drop}\). Therefore, a single unilateral daily dose (6 drops) would expose the patient to \(\text{ng/day}\) or \(\text{ng/day}\) for bilateral administration. This amount is less than the SCT of \(\text{ng/day}\).

Per ICH Q3D: Elemental Impurities, \(\text{ng}\) is considered \(\text{ng/day}\). The Permissible Daily Intake of \(\text{ng}\) for oral administration is \(\mu\text{g/day}\). The amount of \(\text{ng}\) present in the daily dose, \(\text{ng}\), is much less than this amount (192-times lower).

The current drinking water standard, or Maximum Contaminant Level (MCL), from the U.S. Environmental Protection Agency (EPA) is \(\mu\text{g/L}\) or parts per million (ppm). This is equal to \(\mu\text{g/L}\) (micrograms per liter) or \(\text{ppb}\). Data from the U.S. National Health Nutrition Examination Survey for 2009 to 2012, found that adult men consume 117 ounces of water daily (\(\text{mL}\)), on average and women consume approximately 93 ounces (\(\text{mL}\)). Therefore, at the MCL, the average adult female (lower estimate) would consume approximately \(\text{ng}\) per day. The amount of \(\text{ng}\) a typical female consumes per day far exceeds the daily amount of \(\text{ng}\) that a patient would be exposed to daily if all \(\text{ng}\) leached from the glass vial to the drug product volume and was systemically absorbed (approximately 338-times the exposure).

There are no ocular exposure limits established for \(\text{ng}\). According to Principles and Practice of Ophthalmology: Clinical Practice Volume 3 (authors: D. Albert and F.
Jakobiec, W.B. Saunders Company, 1994), chronic exposure to inorganic results in a generalized bronze discoloration of the skin caused by increased deposition of melanin in keratinocytes. Involvement of the gastrointestinal tract, bone marrow, liver, and central nervous system causes a variety of symptoms. At the time of the 4 Month Safety Update, rhNGF eye drops using the container closure system had been marketed in Germany, UK and Italy. A total of 55 treatment supplies (8-week treatment cycles) had been distributed thus far. No adverse drug reaction (neither serious nor non serious) had been reported in the clinical trials conducted for approval or by prescribing physicians or patients in the post marketing setting as of 21 March 2018 which would include adverse ocular events associated with in the container such as skin discoloration or systemic toxicity.

9.9 2.6 Proposed Clinical Population and Dosing Regimen

Instill one drop of OXERVATE 6 times a day at 2 hourly intervals, Treatment should be continued for eight weeks.
- Maximum total daily dose = 0.02 mg/mL mL/drop 6 drops = μg/eye/day or μg/day total dose for bilateral administration (or μg/kg/day).

3 Studies Submitted

9.10 3.1 Studies Reviewed
Primary Pharmacology
- Report M1405: Study of the effects of topical recombinant human nerve growth factor (NGF) on Retinal ganglion cells (RGC) apoptosis and loss in a rat model of partial optic nerve injury
- Report M1307: Identification of an effective dose-regimen of rhNGF in the acute treatment of RCS in rats
- Report M1212: Efficacy of hr-NGF in a rat model of retinitis pigmentosa
- Report M1201: Evaluation of the effect of murine NGF in a rabbit model after photorefractive keratectomy
- Report M1109: Efficacy of rhNGF in a rat model of superior cervical ganglia hypertrophy
- Report A1578/E: Evaluation of the dosage efficacy of recombinant human Nerve Growth Factor (rhNGF) eye drops on rat conjunctiva in vivo
- Report A1569: Setting of a functional and quantitative test in PC12 cells, for the characterization of human and mouse recombinant NGF
- Report A1549e: Investigation of Nerve Growth Factor (NGF) effects on rabbit ovular cell cultures
- Report A1330E: Effect of human recombinant NGF and murine NGF on PC12 cell line
• Report A1235E: Effects of recombinant human NGF and murine NGF on a human neuroblastoma cell line SH-SY5Y
• Report A1226e: TrkA-NGF assay development study
• Report A1131: Rabbit SIRC cells proliferation assay induced by rhNGF and mNGF
• Report A1130: Human TF-1 cells proliferation assay induced [sic] by rhNGF and mNGF

Safety Pharmacology
• Study d39098 (A1129BPL/E): rhNGF-4 week eye drop administration toxicity study in the Wistar rat with a 2-week recovery period

Pharmacokinetics
• Study D69586: rhNGF-Toxicokinetic study in Wistar rat using eye drop or subcutaneous administration
• Study A1215: Enzyme-linked immunosorbent assay determination of rhNGF in rat serum sample of Study N. 0007-2012 carried out by Accelera
• Study 007-2012: Dosing and sampling to enable investigation of the absorption of rhNGF following ocular administration of two different formulation to the rat
• Study 8263061: [3H]-rhNGF: Disposition of radioactivity in the rat following ocular administration

Toxicology
• Single dose
  o Study D39054: rhNGF-intravenous (bolus)-dose escalation toxicity study in the New Zealand White rabbit
  o Study D39043: rhNGF-eyedrop administration MTD (dose escalation) toxicity study in the New Zealand White rabbit
  o Study D39076: rhNGF-intravenous (bolus)-dose escalation toxicity study in the Wistar rat
  o Study D39087: rhNGF-eye drop administration MTD (dose escalation) toxicity study in the Wistar rat
• Repeat dose
  o Rat
    ▪ Topical ocular administration
      • Study A1129BPL/E (D39098): rhNGD: 4-week eye drop administration toxicity study in Wistar rat with a 2-week recovery
      • Study A1213BPL/E (0472-2011): rhNGF: 26-week eye drop administration toxicity study in the Wistar rat followed by a 4-week recovery period
      • Study A1615BPL/E (513094): 31-day ocular and subcutaneous toxicity study with rhNGF followed by a 2-week recovery period in juvenile Wistar rats from PND 30 onwards
    ▪ Subcutaneous administration
• Study M1306BPL (D68304): Combined 8/26-week subcutaneous toxicity study in the Wistar rat with a 2/4-week recovery period
  o Rabbit
    ▪ Topical ocular administration
      • Study A1138BPL/E (D39065): rhNGF: 4-week eye drop administration toxicity study in the New Zealand White rabbit with a 2-week recovery period
      • Study A1412BPL/E (D23I32313): 2-month ocular tolerance study by daily instillations of rhNGF formulated with methionine in albino rabbits followed by a 2-week recovery period
      • Study A1302BPL/E (D23I27212): 2-month ocular tolerance/toxicity study by daily instillations of rhNGF eye drops in albino rabbits with a 2-week recovery period
      • Study A1314BPL/E (D23I07113): Evaluation of the ocular tolerance of a new formulation with methionine in albino rabbits following daily instillations for 14 days
      • Study A1315BPL/E (D23I07013): rhNGF: preliminary evaluation of the ocular tolerance of a new formulation with methionine in albino rabbits following multiple daily instillations for 5 days.
      • Study A1614BPL/E (513095): 125-day ocular and subcutaneous toxicity study with rhNGF followed by a 2-week recovery period in juvenile New Zealand White rabbits from PND 50 onwards
    ▪ Subcutaneous administration
      • Study M1305BPL (D68326): rhNGF: 90-day subcutaneous toxicity study in the New Zealand White rabbit with a 4-week recovery period

Reproductive and Developmental Toxicology
• Fertility and early embryonic development
  o Study A1462BPL/E (505338): Combined study of the effects of recombinant human nerve growth factor on fertility and embryo-fetal development in rats by subcutaneous administration

• Embryo-fetal development
  o Study A1463BPL/E (505336): Study of the effects of recombinant human nerve growth factor on embryo-fetal development in rabbits by subcutaneous administration

• Prenatal and postnatal development
  o Study A1442BPL/E (505340): Study of the effects of recombinant human nerve growth factor on pre- and postnatal development, including maternal function in rats by subcutaneous administration
9.11 3.2 Studies Not Reviewed

Pharmacology
- Report A1557: Comparison of two batches of TF-1 cell line the “Potency: TF-1 Proliferation bioassay” induced by rhNGF

Pharmacokinetics
- Study SWBQ16138: Validation of a method for the determination of anti-rhNGF in rabbit serum by an immunoassay
- Study SBQ6133: Validation of a method for the determination of anti-rhNGF in rat serum by an immunoassay
- Study D39032: rhNGF-Validation of an analytical method for the determination of binding antibodies in rabbit serum
- Study D39021: rhNGF-Validation of analytical method for determination of binding antibodies in rat serum
- Study D39008: rhNGF-Validation of an analytical method for the determination of rhNGF in rat and rabbit serum
- Study 505340: The validation of the determination of rhNGF in formulation us LC-DAD
- Study 1316BPL: Partial validation of an HPLC-UV test method for the quantitative determination of rhNGF in a new formulation with methionine at concentration of 1.2 mg/mL
- Study 1306BPL: Partial validation of an HPLC-UV test method for the quantitative determination of rhNGF in an aqueous formulation at concentration of 0.60 and 1.20 mg/mL
- Study 1205BPL: Validation report of the HPLC-UV test method for the quantitative determination of rhNGF in aqueous formulation (0.6 mg/mL)
- Study A1137: Validation report on the HPLC-UV test method for the quantitative determination of rhNGF in saline formulation (150 micrograms/mL)
- Study 508467: Development and validation of an analytical method for assessment of stability of recombinant human nerve growth factor in vehicle

Reproductive and Developmental Toxicology
- Study A1441/E (505334): Preliminary rabbit study of recombinant human nerve growth factor by subcutaneous administration to check systemic exposure

4 Pharmacology

9.12 4.1 Primary Pharmacology

Nerve growth Factor (NGF), is a neurotrophin involved in neuronal differentiation, neuronal protection and axonal regeneration. NGF is thought to act through two main classes of transmembrane receptors: the ~75 LNGFR (low-affinity nerve growth factor
receptor) or neurotrophin receptor (p75 TR) and the high affinity neurotrophin receptor TrkA. Both receptors are distributed in neuronal tissues and other normal and pathologic tissues including epithelial, endothelial or lymphoid tissues.

In vivo, the factor exerts physiological functions in the nervous system, by regulating growth and survival of sympathetic and sensory neurons. NGF provides trophic support after neuronal injuries and reverses pathologic changes induced by peripheral nerve injury. Additionally, NGF shows activity on a different cell types including epithelial cells, fibroblast and hematopoietic cells stimulating growth, migration and morphological changes.

The Applicant provides a number of in vivo and in vitro studies using rat and rabbit model systems, establishing support for pharmacologic activity in these species.

**Report A1226e: TrkA-NGF assay development study**

This study determined binding affinity of rhNGF on the TrkA receptor using the HTRF®/Tag-lite® assay. Briefly, the assay employed a construct of the TrkA receptor fused to a SNAP tag and expressed at the cell surface in HEK 293 cells. The SNAP tag was labelled with a fluorescent donor dye. rhNGF was labelled with a red acceptor dye. The binding of rhNGF on TrkA allowed receptor:ligand proximity creating fluorescence resonance energy transfer (FRET) when exciting the donor. The binding was measured by detecting the specific fluorescence emitted by the acceptor dye. A competition assay was developed to determine the binding affinity of rhNGF. Maximum binding was shown following overnight incubation resulting in IC\textsubscript{50} and K\textsubscript{i} values of 7.54 nM and 3.73 nM, respectively.
Report A1130: Human TF-1 cells proliferation assay induced by rhNGF and mNGF

This study compared the in vitro biological activity of rhNGF and murine NGF (mNGF) in human TF-1 cells. TF-1 cell proliferation was measured in a chromogenic assay following incubation with rhNGF or mNGF (0.3 pM to 6.76 nM). Mean EC$_{50}$ calculated for rhNGF was $19.2 \pm 1.6$ pM, whereas EC$_{50}$ calculated for mNGF was $237.4 \pm 59.1$ pM, indicating a reduced effect of mNGF in inducing proliferation of TF-1 cells.
Report A1131: Rabbit SIRC cells proliferation assay induced by rhNGF and mNGF

This study evaluated the *in vitro* biological activity of rhNGF and mNGF in rabbit SIRC cells (corneal cell line). SIRC cell proliferation was measured in a chromogenic assay following incubation with rhNGF or mNGF (0.3pM to 6.76nM, or 0.7pM to 13.52nM). Recombinant human NGF showed no proliferative effect on the rabbit cells whereas mNGF induced cells proliferation at the concentrations above 200 pM.

Report A1235E: Effects of recombinant human NGF and murine NGF on a human neuroblastoma cell line SH-SY5Y

This study evaluated the ability of rhNGF (25 ng/mL – 100 ng/mL) to induce neuronal differentiation of a human neuroblastoma cell line, SH-SY5Y. The cell line expresses both neurotrophin receptor p75 and TrkA. Differentiation was determined by fluorescent
staining for neuronal cell specific β-tubulin 3 and axonal GAP-43. β-tubulin 3 is an early neuronal differentiation marker, expression is associated with neurons which are exiting mitotic phases and entering the differentiation pathway. GAP-43 is an axonal marker that identifies growth cones at the beginning of differentiation and axons following differentiation. Microscopic examination of neurite formation (expressed as neurite number vs. the total cell number) and neurite length were also reported.
*Results reported following 7 days of incubation. N2 is a conditioned medium with specific activity in inducing neuronal differentiation. Unstimulated control included in graph.
Report A1330E: Effect of human recombinant NGF and murine NGF on PC12 cell line

This study characterized neuronal differentiation of rat PC 12 cells in response to rhNGF (25 – 100 ng/mL). Differentiation was determined by contrast microscopic examination of neurite formation morphometry expressed as neurite number vs. the total cell number. The neurite length was determined by comparing the neurite length with the mean diameter of cell soma and reported as neurite length/soma. Immunofluorescent staining of GAP 43, tubulin-3 and Neurofilament-200. Western blotting was used to semi-quantitatively determine NFkB, GAP43, Neu200 and tubulin-3 protein levels (relative band densities).

rhNGF induced dose-dependent neurite growth following 7-days of incubation. Neuronal differentiation marker localization and expression increased in response to differentiation (tubulin-3, GAP-43 and NF-200). Protein expression analysis showed a significant increase of tubulin-3 at the concentration range 25 – 100 ng/mL, while NF200 increased only in response to rhNGF at concentrations greater than 50 ng/mL. However, more than the quantitative data regarding the different markers, the localization and morphology of the treated cells appeared modified by the treatments.
Effect of rhNGF on neurite morphometry of rat PC12 cells
Effect of rhNGF on neurite differentiation markers in rat PC12 cells

Report A1569: Setting of a functional and quantitative test in PC12 cells, for the characterization of human and mouse recombinant NGF

This report details the development of a PC-12 cell line expressing a luciferase reporter activated in response to NGF (c-fos-luc). The assay was used to evaluate the activity of rhNGF, at different concentrations (range 0.3-3 ng/ml). Results showed that rhNGF strongly induced the expression of luciferase in a dose-dependent manner with an EC$_{50}$ at 1.4 ng/ml.

Report A1549e: Investigation of Nerve Growth Factor (NGF) effects on rabbit ovular cell cultures

Rabbit limbal-corneal tissue cultures from three different rabbits (LE-119, LE-120, and LE-121) were treated with NGF from human or mouse. Primary rabbit corneal epithelial
cultures were cultivated on irradiated 3T3-J2 cells and treated with different concentrations of rhNGF (100 pM, 6.8 nM, and 1.02 µM) given continuously or reapplied every three days (pulse-chase administration). Analysis included marker expression, dose/response (clonogenicity versus differentiation), long-term effects, and single cell clonal expansion.

NGF treatment by pulse/chase administration slightly increased the clonogenicity at low doses, while continuous NGF administration did not increase cell clonogenicity, but showed an increase in differentiation; these data are consistent with a small increase of expression of stem cell markers BMI-1 (marker of proliferation potential) and p63 alpha (marker for self-renewal) observed at low NGF concentration. NGF administration also significantly stimulated TrkA expression. Continuous long term exposure slightly increased aborted colonies (colonies no longer proliferating) suggesting an exhausted proliferation after long term repeated NGF administration. Stable expression of 14-3-3 sigma (an early differentiation marker) and intracellular pro-NGF was observed.

**Report A1578/E: Evaluation of the dosage efficacy of recombinant human Nerve Growth Factor (rhNGF) eye drops on rat conjunctiva in vivo**

Conjunctival goblet cells (CGC) express NGF receptors, and enhance mucin secretion when stimulated with NGF. A study was designed to compare the efficacy of two formulations of rhNGF at different concentrations (10 and 20 µg/ml) administered according to two different schedules (6 vs 3 times over 12 hours) in male Sprague-Dawley rats. Rats were sacrificed two hours after the last treatment (total experimental time is 14 hours). Impression cytology specimens were analyzed for the distribution of CGCs and the levels of goblet cell-specific mucin MUCSAC by periodic acid shift analysis and immunofluorescence. Fresh conjunctivas were processed for total protein extraction and measurement of MUCSAC concentration ELISA.

rhNGF increased density of CGCs with the greatest effect shown in rats treated 6-times with 20 µg/ml. rhNGF also increased the concentration of MUCSAC in conjunctiva protein extracts. Results were obtained through comparison with an untreated control eye (CoEye).
Report M1109: Efficacy of rhNGF in a rat model of superior cervical ganglia hypertrophy

The efficacy of rhNGF in expansion of the superior cervical ganglia (SCG) during postnatal development was determined in male neonatal Sprague-Dawley rats (n=18; subcutaneous rhNGF 1, 10 and 20 μg/day for 4 days). At 24 hours following the end of treatment, SCG weight, morphology and histology were determined. rhNGF induced dose-dependent SGC hypertrophy characterized by SGC weight increases directly correlated with the extent of neuronal tissue.

Report M1201: Evaluation of the effect of murine NGF in a rabbit model after photorefractive keratectomy

This study investigated the effect of a murine- nerve growth factor (NGF) on corneal wound healing, epithelial proliferation and nerve regeneration in a rabbit model after photorefractive keratectomy (PRK). Tear breakup time, Schirmer's test and in vivo corneal structural evaluation using corneal confocal microscopy were performed preoperatively, 15 days and 1 month postoperatively under general anesthesia. One eye received mNGF while the other served as control. There were no statistically significant differences in any of the tests one month postoperatively though the authors conclude this may be due to small sample size. Confocal microscopy resulted in poor quality images but may have qualitatively suggested that mNGF treated eyes presented better nerve regeneration when compared to control.

Report M1212: Efficacy of hr-NGF in a rat model of retinitis pigmentosa
This study investigated the effect of topical and intravitreal rhNGF administration on photoreceptor degeneration in the Royal College of Surgeons (RCS) rat: a well-characterized rat model of autosomal recessive retinitis pigmentosa (RP).

Rats were treated with a single dose (5µg / 5µL intravitreal injection) or multiple doses (20 µg / 10µL) three times daily for 20 days. Parameters measured include body weight, intraocular pressure, retinal cell viability, retinal NGF concentration (ELISA), retinal NGF, TrkA and phospho-TrkA (Western blot analysis), retinal FACS analysis (anti-rhodopsin), retinal confocal microscopy (anti-rhodopsin), and Caspase-3/Caspase-9 immunohistochemistry.

Results indicated that both topical and intravitreal administration of rhNGF protects RCS rats from photoreceptor degeneration. This hypothesis is supported by the observations that NGF:

- Significantly reduced the total number of dead retinal cells
- Increased in expression of TrkA and increased phosphor-TrkA (activated form)
- Elevated expression of rhodopsin
- Decreased Caspase 3 and 9 compared to untreated control animals, thus supporting the evidence for the anti-apoptotic effect in the retina of recombinant human NGF.

Administration of NGF topically or by intravitreal injection had no adverse effect on body weight or intraocular pressure.
Number of dead retinal cells per $8 \times 10^5$ total cells

Retinal NGF levels

Total TrkA expression

Phospho-TrkA Expression

Rhodopsin expression (FACS)

Caspase 3
Report M1307: Identification of an effective dose-regimen of rhNGF in the acute treatment of RCS in rats

This study investigated the activation of the NGF high affinity receptor (pTrkA) in the retina of the RCS rat (model of retinal degeneration) after treatment with topical ocular rhNGF. rhNGF was administered as a 10 μl eye drops (20 – 180 μg/ml single dose, or 60 – 180 μg/ml BID or TID for 3 days). Endpoints included:

- retinal accumulation of NGF
- presence of activated form of high-affinity NGF receptor (pTrkA)
- expression of Caspase 3, Bcl-2 and Bax
- number of pyknotic cells.

Only the high dose, 180 μg/ml (TID for 3 days), activated pTrkA and influenced apoptosis. Using this regimen, the expression of pTrkA in the retina increased; expression of Bcl-2, a molecule associated with cell survival, increased; expression of Bax and caspase 3, molecules associated with apoptosis, were down-regulated and the number of pyknotic cells decreased. The results suggest that ocular administration of rhNGF protected photoreceptor loss by reducing apoptotic cell death in the photoreceptor layer.

Report M1405: Study of the effects of topical recombinant human nerve growth factor (NGF) on Retinal ganglion cells (RGC) apoptosis and loss in a rat model of partial optic nerve injury

This study investigated the effect of topical recombinant human nerve growth factor (NGF) on retinal ganglion cell (RGC) apoptosis in vivo and RGC loss histologically in a rat model of partial optic nerve transection (pONT). A further assessment of whether any resultant effects of NGF were associated with primary or secondary degeneration was performed. The optic nerve of Dark Agouti (DA) rats was superiorly and partially transected in one eye [partial optic nerve transection (pONT)]. On the day of pONT animals were treated (6 animals/treatment) with:

1) No treatment: pONT only
2) Saline solution eye drops (0.01mL)
3) NGF eye drops: 180µg/ml of eye drops of NGF (0.01mL)
4) NGF eye drops: 60µg/ml of eye drops of NGF (0.01mL)

Treatments were administered to both eyes BID for 21 days. Retinal ganglion cells undergoing apoptosis in vivo were detected with an annexin V based assay. To evaluate effectiveness of treatment with rhNGF eye drops, the number of RGC was counted after 21 days of pONT. To assess RGC survival, whole retinal whole-mounts were stained with anti-mouse Brn3 (marker of retinal ganglion cells) and confocal images were analyzed.

Treatment with topical rhNGF significantly reduced annexin-v labelled cells at both concentrations in pONT operated eyes.

![Figure 1](image)

In the pONT eyes, treatment with rhNGF significantly reduced RGC loss in both treatment groups compared with no treatment group.

![Figure 2](image)

While no significant differences were seen in the superior sector of retinal whole mounts, analysis of inferior counts showed increased RGC survival in (p<0.01) at both rhNGF concentrations in pONT operated eyes.
9.13 4.3 Safety Pharmacology

**Study 39098-A1129BPL/E; rhNGF: 4-week eye drop administration toxicity study in the Wistar rat with a 2-week recovery period**

A modified Irwin test was performed in Study 39098-A1129BPL/E (rhNGF: 4-week eye drop administration toxicity study in the Wistar rat with a 2-week recovery period). This study tested doses of 0.6, 0.8 and 1.2 mg/mL as a bilateral 0.005 mL drop TID (0.12, 0.17, and 0.25 mg/kg/day) in Wistar rats (10/sex/group). A functional observational battery including appearance, behavior, reflexes, locomotor activity and grip strength was performed during Week 4. No effects of the treatment with test article were reported under study conditions.

5 Pharmacokinetics/ADME/Toxicokinetics

9.14 5.1 PK/ADME

**Study D69586: rhNGF-Toxicokinetic study in Wistar rat using eye drop or subcutaneous administration**

This study compared the toxicokinetic profile of rhNGF when administered subcutaneously or via eye drops using two different blood sampling routes (sublingual and tail vein) in Wistar rats. rhNGF was administered as a topical eye drop (0.8 mg/mL; 0.005 mL drop TID) or subcutaneous injection (1 mg/kg TID) for 3 days. Blood samples...
were collected from separate animals either via the sublingual vein or via the tail vein at 0, 2, 4, 8, and 24 hours after the first daily administration on days 1 and 3. Some toxicological endpoints were also reported.

Mortality
- All animals survived the treatment period

Clinical signs
- Animals receiving topical ocular rhNGF appears normal. Animals receiving rhNGF via subcutaneous injection presented with ruffled fur, decreased activity, reddened ears or localized swelling in both cheeks.

Toxicokinetics
- Differences in rhNGF levels were observed depending on the location of the blood draw. This was particularly evident after the eye drop administration and to a lesser extent after the subcutaneous route.
- After topical ocular administration, rhNGF levels reported from tail vein blood were much lower than those from the sublingual vein (approximately 40 – 400-fold lower).
- The Applicant explains this difference as the passage of the test article from the eyes through the nasolacrimal and nasopharyngeal ducts into the oral cavity and then locally reabsorbed leading to a very high local concentration in the blood and depicted by the sampling from the sublingual vein.
- On the other hand, after subcutaneous injection, rhNGF levels from tail vein blood were higher than those from the sublingual vein.
- The Applicant explains this difference as being variable depending on the site of bleeding and the differences in residence time in the different body areas.

<table>
<thead>
<tr>
<th>Period</th>
<th>Parameter</th>
<th>Units</th>
<th>Sublingual (Eye drop)</th>
<th>Tail vein (Eye drop)</th>
<th>Sublingual (Subcutaneous)</th>
<th>Tail vein (Subcutaneous)</th>
</tr>
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<tr>
<td>Day 1</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/mL</td>
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<td>0.10</td>
<td>69.5</td>
<td>174</td>
</tr>
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<td></td>
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<td>2</td>
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<tr>
<td></td>
<td>AUC&lt;sub&gt;t&lt;/sub&gt;</td>
<td>ng·h/mL</td>
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<td>1597</td>
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<td></td>
<td>F&lt;sub&gt;rel&lt;/sub&gt;</td>
<td>%</td>
<td>-</td>
<td>-</td>
<td>92.8</td>
<td>*</td>
</tr>
<tr>
<td>Day 3</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/mL</td>
<td>4.2</td>
<td>0.07</td>
<td>73.5</td>
<td>173</td>
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<td>0.37</td>
<td>277</td>
<td>555</td>
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<td></td>
<td>F&lt;sub&gt;rel&lt;/sub&gt;</td>
<td>%</td>
<td>-</td>
<td>-</td>
<td>36.5</td>
<td>*</td>
</tr>
</tbody>
</table>

# Calculated taking into account the following doses: 0.024 mg (eye drop) and 1 mg/kg (mean bodyweight provided by the sponsor)

*: Value higher than 120%
Study Accelera 0007- 2012 (M1202) - A1215 (Non-GLP)

This study determined the pharmacokinetics of rhNGF after topical administration to male Wistar rats with intact or abraded corneas. Rat were treated with a 5 µL drop of 0.8 mg/mL rhNGF twice (3 hours between doses; 8µg/2 administrations). Blood was collected from the tail vein 2 hours and 4 hours after the first dose. In rats with intact corneas, serum concentrations of rhNGF were not detected at both 2 and 4 hours after the first treatment. In rats with abraded corneas, serum levels were 0.179 – 0.266 ng/mL at 2 hours after the first administration and 0.188 – 0.676 ng/mL at 4 hours (after 2 administrations).

Study 8263061 (A1120BPL/E)

Five male rats each received a single 5 µL administration of [³H]-rh-NGF (20 µg/mL) to each eye (conjunctival sac) six times in one day (one every 2 hours; total 0.6 µg/day). Autoradiographs were obtained and tissues were sampled at 2, 4, 8, 12 and 24 hours post-dose. The only tissues that contained visible levels of radioactivity at each sampling time were the kidney (cortex and medulla), the urinary bladder (wall and contents), nasal mucosa and regions of the gastrointestinal tract. Although radioactivity was visible in the kidney, it was only present at quantifiable levels in the kidney medulla at 4 hours. The presence of radioactivity in the nasal cavity and levels associated with the esophageal wall suggest that some of the ocular administered dose was ingested either from the animal preening or by transfer via the nasolacrimal duct into the nasal cavity, buccal cavity and esophagus. The Applicant states that there was no specific evidence of distribution of radioactivity into the eye but did not list the tissues of the eye which were assayed. Radioactivity was also present at detectable levels in serum, suggesting there was some absorption and systemic distribution of [³H]-rh-NGF following ocular administration to the rat.

6 General Toxicology

9.15 6.1 Single-Dose Toxicity

Study D39054 (A1120BPL/E): rhNGF-intravenous (bolus)-dose escalation toxicity study in the New Zealand White rabbit

- GLP, QA statement signed (b) (4); start date 9-29-2011)
- Intravenous rhNGF:
  - One male and one female New Zealand White rabbit treated at ascending doses of 0.3, 0.6, 1.2, and 2.4 mg/kg with 3- day wash-out periods between escalating dose administrations.
- Subcutaneous rhNGF:
Four days after the last intravenous both animals were administered rhNGF by subcutaneous injection with 2.4 mg/kg.

- Parameters monitored:
  - Ophthalmoscopic examination (after each administration)
    - No adverse ophthalmic findings were attributed to the test article
  - Mortality (twice daily)
    - Neither animal died or sacrificed prior to schedule
  - Clinical signs (twice daily)
    - No adverse clinical findings were attributed to the test article
  - Food consumption and body weights (once daily)
    - Minimal decrease in the male during the 24 hours after each administration; no effect in female
  - Necropsy / Tissue preservation: (Four days after the subcutaneous treatment; Brain, eyes, Harderian gland, heart, injection site, kidneys, livers, lungs, ovaries, spleen, testes, and gross lesions retained for potential future analysis).
    - Dark red discoloration of the lungs and the medulla region of the kidneys were recorded in the male animal. No macroscopic findings were recorded in the female
  - Toxicokinetics: Blood samples were collected from the ear vein from all animals before (0h) administration and 0.167 h (10min), 0.5, 1, 2, 3, 4, 6, 8, and 24 hours after the first four administrations.
## rhNGF: Effect of Dose

| Dose (mg/kg) | Ratio | AUC_{0-4} (ng.h/mL) | Ratio | AUC_{0-4 norm} | Ratio |
|-------------|-------|---------------------|-------|----------------|--|------|
| Male        |       |                     |       |                |     |
| 0.3         | ---   | 2130                | ---   | 7100           | --- |
| 0.6         | 2.0   | 5540                | 2.6   | 9230           | 1.3 |
| 1.2         | 2.0   | 15000               | 2.7   | 12500          | 1.4 |
| 2.4         | 2.0   | 29600               | 2.0   | 12300          | 0.98|
| max/min     | 8.0   |                     | 1.4   |                | 1.7 |
| Female      |       |                     |       |                |     |
| 0.3         | ---   | 1590                | ---   | 5300           | --- |
| 0.6         | 2.0   | 5330                | 3.4   | 8880           | 1.7 |
| 1.2         | 2.0   | 3900                | 0.73  | 3250           | 0.37|
| 2.4         | 2.0   | 7780                | 2.0   | 3240           | 1.0 |
| max/min     | 8.0   |                     | 4.9   |                | 0.61|

## Study D39043 (A1118/E): rhNGF-eyedrop administration MTD (dose escalation) toxicity study in the New Zealand White rabbit

- Non-GLP [date 9-29-2011](b) (4)
- Topical ocular rhNGF:
  - Bilateral administration (0.03 mL/drop) to one male and one female New Zealand White rabbit at ascending doses of 0.2, 0.4, 0.6, 0.8 and 1.2
mg/mL (3 times a day, 3 hours apart) with 3-day wash-out periods between all administrations.

- Parameters monitored:
  - Ophthalmoscopic examination (after each administration)
    - No adverse ophthalmic findings were attributed to the test article
  - Mortality (twice daily)
    - Neither animal died or sacrificed prior to schedule
  - Clinical signs were recorded twice daily.
    - No adverse clinical findings were attributed to the test article
  - Food consumption and body weights were recorded once daily during the treatment period.
    - No effect attributed to the test article
  - Necropsy / Tissue preservation: (Four days after the subcutaneous; Brain, eyes, Harderian gland, heart, injection site, kidneys, livers, lungs, ovaries, spleen, testes, and gross lesions retained for future analysis.
    - No macroscopic findings attributed to the test article

- Toxicokinetics / Immunogenicity: Blood samples were collected from the ear vein from all animals before (0h) administration and after the first dose at 2 h, 6h (i.e. 3h after 2nd second administration), 9h (i.e. 3h after 3rd administration), 12h (i.e. 6h after 3rd administration), and 15 hours (i.e. 9h after 3rd administration) after each dose level. LLOQ of assay is 8 pg/mL.

<table>
<thead>
<tr>
<th>Time (h)</th>
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<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>2</th>
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</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>0.105</td>
<td>1.33</td>
<td>0.515</td>
<td>0.478</td>
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</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)/dose (ng/mL)</td>
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<td>3.33</td>
<td>0.838</td>
<td>0.598</td>
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<tr>
<td>ratio dose / low dose</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>6.0</td>
<td>---</td>
</tr>
<tr>
<td>ratio C&lt;sub&gt;max&lt;/sub&gt; / C&lt;sub&gt;max&lt;/sub&gt; (low dose)</td>
<td>13</td>
<td>4.9</td>
<td>4.6</td>
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<td>---</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>15</td>
<td>9.0</td>
<td>2.0</td>
<td>9.0</td>
<td>---</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0&lt;/sub&gt; (ng.h/mL)</td>
<td>0.138</td>
<td>6.05</td>
<td>2.22</td>
<td>0.717</td>
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<tr>
<td>AUC&lt;sub&gt;0-15h&lt;/sub&gt; (ng.h/mL)</td>
<td>0.790</td>
<td>15.1</td>
<td>3.70</td>
<td>0.896</td>
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</table>
Study D39076 (A1121BPL/E): rhNGF-intravenous (bolus)-dose escalation toxicity study in the Wistar rat

- GLP, QA statement signed, start date 9-27-2011
- Intravenous rhNGF:
  - Administration to two male and two female Wistar rats at ascending doses of 0.3, 0.6, 1.2, and 2.4 mg/kg with a 3-day wash-out periods between all administrations.
- Subcutaneous rhNGF:
  - Four days after the last intravenous administration all four animals were administered once by subcutaneous injection with 2.4 mg/kg.
- Parameters monitored:
  - Mortality (twice daily).
    - Neither animal died or sacrificed prior to schedule
  - Clinical signs were recorded twice daily.
    - No adverse clinical findings were attributed to the test article
  - Food consumption and body weights were recorded once daily during the treatment period.
    - Slight to moderate decrease of the food consumption in animals of both sexes during the 24 hours after each intravenous administration.
    - No effects on food consumption were reported after subcutaneous administration.
    - A minimal decrease of the body weight was recorded in animals of both sexes on the day after each administration.
  - Necropsy / Tissue preservation: (Four days after the subcutaneous; Brain, eyes, Harderian gland, heart, injection site, kidneys, livers, lungs, ovaries, spleen, testes, and gross lesions retained for future analysis).
    - No macroscopic findings attributed to the test article
Toxicokinetics: Blood samples were collected from the ear vein from all animals before (0h) administration and after the first dose at 2 h, 6h (i.e. 3h after 2nd second administration), 9 h (i.e. 3h after 3rd administration), 12 h (i.e. 6 h after 3rd administration), and 15 hours (i.e. 9 h after 3rd administration) after each dose level. LLOQ of assay is 8 pg/mL

<table>
<thead>
<tr>
<th>rhNGF Dose (mg/kg)</th>
<th>0.3</th>
<th>1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>1310</td>
<td>4210</td>
</tr>
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<td>$C_{max}_norm$</td>
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<tr>
<td>$t_{max}$</td>
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<td>AUC$_{0-inf}$</td>
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<tr>
<td>$t_{1/2,z}$</td>
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<td>3.8*</td>
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<tr>
<td><strong>Female</strong></td>
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<td>$C_{max}$</td>
<td>928</td>
<td>3950</td>
</tr>
<tr>
<td>$C_{max}_norm$</td>
<td>3090</td>
<td>3290</td>
</tr>
<tr>
<td>$t_{max}$</td>
<td>0.17</td>
<td>0.50</td>
</tr>
<tr>
<td>AUC$_{0-t}$</td>
<td>666</td>
<td>3800</td>
</tr>
<tr>
<td>AUC$_{0-t}_norm$</td>
<td>2220</td>
<td>3170</td>
</tr>
<tr>
<td>AUC$_{0-inf}$</td>
<td>666</td>
<td>3810</td>
</tr>
<tr>
<td>$t_{1/2,z}$</td>
<td>3.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

1 (ng/mL)/(mg/kg)
2 (ng.h/mL)/(mg/kg)
* unreliable, r$^2 < 0.900$

Study D39087 (A1122/E): rhNGF-eye drop administration MTD (dose escalation) toxicity study in the Wistar rat

- Non-GLP start date 9-29-2011
- Topical ocular rhNGF:
  - Bilateral administration (0.005 mL/drop) to two male and two female Wistar rats at ascending doses of 0.2, 0.4, 0.6, 0.8, and 1.2 mg/mL (3 times a day, 3 hours apart), with a 3- day wash-out periods between all administrations.
- Parameters monitored:
  - Mortality (twice daily).
    - Neither animal died or sacrificed prior to schedule
Clinical signs were recorded twice daily.
- No adverse clinical findings were attributed to the test article
Food consumption and body weights were recorded once daily during the treatment period.
- No changes in food consumption or body weight attributed to the test article
Ophthalmoscopy (Draize scoring)
- No changes in ophthalmologic findings attributed to the test article
Necropsy / Tissue preservation: (Four days after the subcutaneous; Brain, eyes, Harderian gland, heart, injection site, kidneys, livers, lungs, ovaries, spleen, testes, and gross lesions retained for future analysis).
- No macroscopic changes attributed to the test article.

Toxicokinetics / Immunogenicity: Blood samples were collected from the ear vein from all animals before (0h) administration and 0, 3, 6, 9, and 12 hours after the first, third, and fourth administration for serum level determination. LLOQ of assay is 8 pg/mL

<table>
<thead>
<tr>
<th>rhNGF Dose (mg/mL)</th>
<th>0.2</th>
<th>0.6</th>
<th>0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>0.250</td>
<td>66.5</td>
<td>3.10</td>
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<tr>
<td>C&lt;sub&gt;max norm&lt;/sub&gt;</td>
<td>1.25</td>
<td>111</td>
<td>3.88</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>9.0</td>
<td>6.0</td>
<td>3.0</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt; (ng h/mL)</td>
<td>0.741</td>
<td>133</td>
<td>21.2</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t norm&lt;/sub&gt;</td>
<td>3.71</td>
<td>222</td>
<td>26.5</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t inf&lt;/sub&gt; (ng h/mL)</td>
<td>n.c.</td>
<td>0.79*</td>
<td>25.5</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>n.c.</td>
<td>7.9</td>
<td>3.9</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>0.500</td>
<td>9.50</td>
<td>3.30</td>
</tr>
<tr>
<td>C&lt;sub&gt;max norm&lt;/sub&gt;</td>
<td>2.50</td>
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<tr>
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<td>6.0</td>
<td>9.0</td>
</tr>
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<td>1.18</td>
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<td>13.4</td>
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<tr>
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<tr>
<td>AUC&lt;sub&gt;0-t inf&lt;/sub&gt; (ng h/mL)</td>
<td>n.c.</td>
<td>28.3*</td>
<td>n.c.</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>n.c.</td>
<td>1.4*</td>
<td>n.c.</td>
</tr>
</tbody>
</table>

1 (ng/mL)/(mg/mL)
2 (ng h/mL)/(mg/mL)
n.c. not calculated
* unreliable, r² < 0.900

- No antibodies to rhNGF were found.

Dose Solution Analysis
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Date of sample</th>
<th>Nominal Concentration (µg/mL)</th>
<th>Measured Concentration (µg/mL)</th>
<th>Theoretical Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>D39087 06/10/2011 vor</td>
<td>06-Oct-2011, before admin.</td>
<td>200</td>
<td>203.48</td>
<td>101.7%</td>
</tr>
<tr>
<td>D39087 06/10/2011 nach</td>
<td>06-Oct-2011, after admin.</td>
<td>200</td>
<td>214.10</td>
<td>107.1%</td>
</tr>
<tr>
<td>D39087 10/10/2011 vor</td>
<td>10-Oct-2011, before admin.</td>
<td>400</td>
<td>100.35</td>
<td>25.1%</td>
</tr>
<tr>
<td>D39087 10/10/2011 nach</td>
<td>10-Oct-2011, after admin.</td>
<td>400</td>
<td>84.30</td>
<td>21.1%</td>
</tr>
<tr>
<td>D39087 14/10/2011 vor</td>
<td>14-Oct-2011, before admin.</td>
<td>600</td>
<td>116.93</td>
<td>19.4%</td>
</tr>
<tr>
<td>D39087 14/10/2011 nach</td>
<td>14-Oct-2011, after admin.</td>
<td>600</td>
<td>216.48</td>
<td>36.1%</td>
</tr>
<tr>
<td>D39087 18/10/2011 vor</td>
<td>18-Oct-2011, before admin.</td>
<td>800</td>
<td>281.25</td>
<td>35.2%</td>
</tr>
<tr>
<td>D39087 18/10/2011 nach</td>
<td>18-Oct-2011, after admin.</td>
<td>800</td>
<td>703.06</td>
<td>87.9%</td>
</tr>
<tr>
<td>D39087 22/10/2011 vor</td>
<td>22-Oct-2011, before admin.</td>
<td>1200</td>
<td>568.68</td>
<td>47.4%</td>
</tr>
<tr>
<td>D39087 22/10/2011 nach</td>
<td>22-Oct-2011, after admin.</td>
<td>1200</td>
<td>587.15</td>
<td>48.9%</td>
</tr>
</tbody>
</table>

- Formulation analysis results indicate a significant loss of actual concentration (compared to nominal) over the course of dose escalation.

### 9.16 6.2 Repeat-Dose Toxicity

**Systemic Toxicity:**

**Study title: rhNGF: Combined 8/26-week subcutaneous toxicity study in the Wistar rat with a 2/4-week recovery period**

<table>
<thead>
<tr>
<th>Study no.</th>
<th>D68304 (M1306BPL)</th>
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</thead>
<tbody>
<tr>
<td>Study report location:</td>
<td>SDN001 (5-31-2017); Section 4.2.3.2</td>
</tr>
<tr>
<td>Conducting laboratory and location:</td>
<td>[Redacted]</td>
</tr>
<tr>
<td>Date of study initiation:</td>
<td>6-6-2013</td>
</tr>
<tr>
<td>GLP compliance:</td>
<td>Yes</td>
</tr>
<tr>
<td>QA statement:</td>
<td>Yes; signed</td>
</tr>
<tr>
<td>Drug, lot #, and % purity:</td>
<td>rhNGF; RS0313; 90.2%; rhNGF; RS1013; 87.3%</td>
</tr>
</tbody>
</table>

Reference ID: 4274484
Key Study Findings

- A NOAEL is established as the high dose, 100 μg/animal/day (667 μg/kg/day for 150 g rat). All changes reported were either not adverse or secondary to the pharmacological activity of the test item, and all reversible following 2 or 4 weeks recovery periods.
- The rat NOAEL corresponds to a margin of 4275-fold over a presumed 100% absorption of the proposed bilateral human ocular dose (0.00936 mg/day or 0.156 μg/60kg adult/day)

Methods

- **Doses:** 0, 50 or 100 μg/animal/day
- **Frequency of dosing:** Once daily for 26 weeks
- **Route of administration:** Subcutaneous injection
- **Dose volume:** 2 mL (2 different injection sites on same flank, therefore 1mL/site)
- **Formulation/Vehicle:** Saline
- **Species/Strain:** Rat; Wistar
- **Number/Sex/Group:** Control: 54/sex
  - Low-dose: 40/sex
  - High-dose: 57/sex
- **Age:** 6 weeks
- **Weight:** Males: 133.9 – 174.1 g (mean 154.5 g)
  - Females: 112 – 147.2 (mean 125.2)
- **Deviation from study protocol:** None which affected conduct or interpretability

Observations and Results

Mortality (twice daily)

- **Low-dose**
  - Male no. 92: sacrificed on treatment day 136 due to moribund condition. The animal showed the following clinical signs: convulsions, sedation, prostration and bradypnea. At necropsy, no macroscopic findings were recorded. Congestion, grade 2, was found during histopathological examination

Clinical Signs (at acclimation, twice daily on Days 1 – 3, once daily thereafter through recovery period)

- **Localized swellings of cheeks, legs, paws, nose, and/or tail as well as erythema on the ears and tail**
  - Recorded in almost all test item treated animals at both dose levels.
  - Attributed to scratching
- **Scabs, sores, wounds, hair loss, and/or injured legs**
  - Low-dose: 16 out of 40 males (40%) and 8 out of 40 females (20%)
  - High dose 40 out of 57 males (70%) and 28 out of 57 females (49%)
Except for hair loss in a few animals, all clinical signs recorded were completely reversible following the respective recovery periods either of 2 or 4-week.

Attributed to scratching

**Body Weights (weekly)**

- **Low-dose:**
  - Decreased in animals of both sexes (-9% in males and -3% in females at the end of the treatment period)

- **High-dose:**
  - Decreased in animals of both sexes (-13% in males and -5% in females at the end of the treatment period).
  - Trend of recovery, but body weights of males previously treated with 100 μg/animal/day were still slightly lower at the end of the recovery period

**Feed Consumption (weekly)**

- Decreased absolute feed consumption was reported in animals of both sexes treated with 50 and 100 μg/animal/day during the entire treatment period. Food consumption in high-dose recovery animals previously treated was similar to or slightly higher than in the controls during the recovery periods.

**Ophthalmoscopy (direct ophthalmoscopy; Week 8 and 26)**

- No ophthalmoscopic findings related to the treatment with rhNGF were observed.

**Hematology [at 8 and 26 weeks and after recovery (Week30)]**

- White blood cell count (lymphocytes)
  - Possibly related with the sore, scab formation, wounds and injured legs
  - High-dose
    - 8 weeks
- Males only
  - Increase in lymphocytes (+27%)
  - Changes were reversible after the 2-week recovery period.

  26 weeks
  - Females only
    - Increase of lymphocytes (+30%),
    - Change was reversible after the 4-week recovery period.

Clinical Chemistry [at 8 and 26 weeks and after recovery (Week30)]
- Phosphorus (not considered by Sponsor as toxicologically relevant) - Absence of any microscopic correlate
  - Changes were recoverable
  - High-dose
    - 8-weeks
      - Both sexes
      - Increase (males: +14%; females: +17%)
    - 26-weeks
      - Both sexes
      - Increase (males: +15%; females: +14%)
  - Low-dose
    - 8-weeks
      - Males only
      - Increase +10%
    - 26-weeks
      - Males only
      - Increase +7%

Urinalysis [at 8 and 26 weeks and after recovery (Week30)]
- No changes in urinalysis parameters attributed to the test article.

Gross Pathology
- Sores in the nose region
  - High dose
    - 8-weeks
      - one female and two males
- Sore in the region of the auricle
  - High-dose
    - 8-weeks
      - one female and two males
- Dark red discoloration of injection area
  - High-dose
    - 26-weeks
      - Females only
• Higher incidence than control

Organ Weights

• Adrenal gland
  o High-dose
    ▪ 26-weeks
    • Both sexes
    • Increased absolute weights
      o Males: +28%
      o Females: +17%
    • Increased relative adrenal weights
      o Males: +38%
      o Females: +17%
    • No histologic correlated
    • Considered stress related
  o Low-dose
    ▪ 26-weeks
    • Males only
    • Increased absolute weight (+13%)
    • Increased relative adrenal weights (+15%)
    • No histologic correlate
    • Considered stress related

• Thymus weights
  o High-dose
    ▪ 26-weeks
    • Females only
    • Decrease in absolute weight (-15%)
    • Decrease relative to brain weight (-16%)
    • No histological correlate
    • Considered to be stress-related.

• Other organ weights (organ/body weight ratios of brain, heart, lungs, liver, spleen, kidney and/or testes)
  o High and low-dose
  o Considered related to by lower body weights in animals.

Histopathology

Adequate Battery: Yes; standard

Peer Review: yes

Histological Findings

• Mixed cell infiltration at the skin injection site
  o High-dose
    ▪ 8-weeks
Male only
- Minimal increase of incidence and severity
  - End of recovery (Interim)
  - Females only
  - Higher incidence

Special Evaluation
- Immunogenicity (binding assay; Weeks 1, 5, 9, 13 and 26: Hours 0, 2, 4, 6, 8, and 24 after dose application)
  - A total of 1/6 animals in low-dose group and 3/6 animals in high-dose group developed binding antibodies. In control animals, no binding antibodies were detected.
  - Immunogenic potential of rhNGF in the rat when administrated daily subcutaneously

Toxicokinetics [Weeks 1, 5, 9, 13 and 26: Hours 0, 2, 4, 6, 8, and 24 after dose administration; blood collected from sublingual vein (reviewer note: it is unclear why blood was collected from the sublingual vein since the Applicant provided empirical PK data showing that tail vein collection provides a more appropriate sampling site for systemic route administration)]
### Dosing Solution Analysis

- All dose solutions were within the variation limit of 10% from the time-zero value, except for one analysis of dosing solution (low-dose) which showed a decrease in potency of 12.9% near the end of the dosing period.

### Study title: rhNGF: 90-day subcutaneous toxicity study in the New Zealand White rabbit with a 4-week recovery period

- **Study no.:** D68326 (M1305BPL)
- **Study report location:** SDN001 (5-31-2017); Section 4.2.3.2
- **Conducting laboratory and location:** (b) (4)
- **Date of study initiation:** 6-11-2013
- **GLP compliance:** Yes
- **QA statement:** Yes; signed
- **Drug, lot #, and % purity:** rhNGF; RS0313; 90.2%
  rhNGF; RS1013; 87.3%
Key Study Findings

- Hilar cell proliferation, ovarian cyst, bilateral corpora lutea
  - Higher severity and incidence in high-dose females compared to control females
  - Applicant considers the findings incidental though cannot exclude that the cause might have been the effect of the pharmacological activity of rhNGF.
    - NGF in humans stimulates the secretion of estradiol and expression of FSH Receptor. In rats, NGF stimulates the FSHR and the initiation of follicular growth. Hilus cells (which produce 20α-dihydroprogesterone) are more abundant in the estrus phase of the rabbit and are similar to the interstitial Leydig cell of the testes. Hemorrhage into an ovarian follicle occurs in all species during ovulation; also rarely in anovulatory follicles. Therefore, the Applicant concludes that the changes observed in the ovaries of female rabbits at the dose of 200 µg/animal should not be considered as an adverse toxicological effect of rhNGF but likely the result of its pharmacological action that might have stimulated the follicle maturation thus ovulation, and the proliferation of interstitial cells. Rabbits usually do not present spontaneous ovulation but only after mating or artificial induction (e.g. LH administration).

- Sponsor establishes NOAEL at 200 µg/animal/day

- Reviewer established NOAEL:
  - Males: 200 µg/animal/day (111 µg/kg/day for 1.8 kg rabbit)
  - Females: 100 µg/animal/day (55.6 µg/kg/day for 1.8 kg rabbit)

- NOAELs correspond to a margin of 712-fold and 356-fold for males and females, respectively, over the proposed clinical dose (9.36 µg/day or 0.156 µg/60kg adult/day).

Methods

- Doses: 0, 100 or 200 µg/animal/day (or 56 µg/kg/day and 111 µg/kg/day)
- Frequency of dosing: Once daily for 26 weeks
- Route of administration: Subcutaneous injection
- Dose volume: 5mL (2 alternating injection sites)
- Formulation/Vehicle: Saline
- Species/Strain: Rabbit; New Zealand White
- Number/Sex/Group:
  - Control: 7/sex
  - Low-dose: 4/sex
  - High-dose: 7/sex
- Age: 10 weeks
- Weight:
  - Males: 1.611 – 2.276 kg
  - Females: 1.746 – 2.230 kg
- Deviation from study protocol: None which affected conduct or interpretability
Observations and Results

Mortality (twice daily)
- All animals survived until scheduled sacrifice

Clinical Signs (at acclimation, twice daily on Days 1 – 3, once daily thereafter through recovery period)
- Thickened flank, shoulder or dorsum (recoverable)
  - High-dose
    - Males (2)
    - Females (1)
  - Low-dose
    - Female only (1)

Body Weights (weekly)
- Body weight gain (reviewer calculated for end of treatment period only)
  - High-dose
    - Males
      - Decrease (-8%)
      - Recoverable
    - Females
      - Decrease (-20%)
      - Recoverable
  - Low-dose
    - Females only
      - Decrease (-28%)

Feed Consumption (weekly)
- No changes in feed consumption were attributed to the test article

Ophthalmoscopy (direct ophthalmoscopy; acclimation, Week 13 and at recovery)
- No changes in ophthalmoscopic observations were attributed to the test article

Hematology [13 weeks and after recovery (Week 17)]
- No changes in hematological parameters were attributed to the test article

Clinical Chemistry [13 weeks and after recovery (Week 17)]
- No changes in clinical chemistry parameters were attributed to the test article

Urinalysis [13 weeks and after recovery (Week 17)]
• No changes in urine parameters were attributed to the test article

**Gross Pathology**

• Mass in the subcutis
  - High-dose male (1)
  - Correlated with a granuloma

• Ovarian cysts
  - High-dose female (1)
    - Black brown in color

• Dark red discoloration in the skin
  - High-dose
    - Females (2)
      - Correlated either with inflammation or hemorrhage.
  - Low-dose
    - Male (1)
  - Dark red discoloration of ventral muscle
    - Low-dose
    - Male (1)
  - Dark/light red discoloration of treated skin
    - Correlated with inflammation
      • Control (1)
        - Female (1)
      • High-dose
        - Female (1)
  - Yellowish mass in the left flank
    - High-dose recovery
      • Female (1).
      • Considered likely related to the treatment procedure.

**Organ Weights**

• Ovary weight
  - High-dose
    - Increased absolute ovary weights (+60%) as well as ovary weights relative to body (+50%) and brain (+64%) weight
    - Ovarian mean weights of females of recovery group were not significantly different from the weights of recovery controls.

**Histopathology**

Adequate Battery: Yes; standard

Peer Review: yes

Histological Findings:

**End of treatment period (Group 1: control; Group 2: Low-dose; Group 3: High-dose)**
• Hilus cell proliferation
  o High-dose
    ▪ All females (4); mean severity = 3.0
  o Low-dose
    ▪ Female (1); severity = 1.0
  o Control
    ▪ Female (1); severity = 2.0
  o At the end of the 4-week recovery period, hilus cell proliferation was seen in two females of control and high-dose with a slight higher severity in the treated group.
• Hemorrhagic ovarian cyst
  o High-dose
    ▪ Female [2: unilateral (1) and bilateral (1)]
  o Not noted in recovery groups
• Bilateral corpora lutea in one female;
  o High-dose
    ▪ Female (1)
  o Not reported in recovery groups

<table>
<thead>
<tr>
<th>Females (K0)</th>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose Group µg/animal</td>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Animals Examined</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Hilus Cells Prolif.</td>
<td>1/2.0</td>
<td>1/1.0</td>
<td>4/3.0</td>
<td></td>
</tr>
<tr>
<td>Cyst. Hemorrhagic</td>
<td>-</td>
<td>-</td>
<td>2/2.0</td>
<td></td>
</tr>
<tr>
<td>Corpora Lutea</td>
<td>-</td>
<td>-</td>
<td>1/3.0</td>
<td></td>
</tr>
</tbody>
</table>

• End of recovery (Group 1: control; Group 2: Low-dose; Group 3: High-dose)

<table>
<thead>
<tr>
<th>Females (R1)</th>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose Group µg/animal</td>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Animals Examined</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hilus Cells Prolif.</td>
<td>2/3.0</td>
<td>-</td>
<td>2/3.5</td>
<td></td>
</tr>
<tr>
<td>Cyst. Hemorrhagic</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Corpora Lutea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

• Inflammation at injection site
  o High-dose
Males
- Minimal
- A granuloma in the injection site of one male correlated with the mass reported macroscopically in this animal, was considered incidental and probably caused by the syringe (accidental inoculation of bacteria).

Special Evaluation
- Immunogenicity (binding assay; at acclimation and Weeks 4, 13, and end of recovery: Hours 0, 2, 4, 6, 8, and 24 after dose application)
  - A total of 4/8 animals in low-dose group and 12/14 animals in high-dose group developed binding antibodies. In control animals, no binding antibodies were detected.
  - Immunogenic potential of rhNGF in the rabbit when administrated daily subcutaneously.

Toxicokinetics (Days 1/2, 3, 86, 87/88: Hours 0, 0.5, 2, 4, 8, and 24 after dose administration; blood collected from ear vein)

<table>
<thead>
<tr>
<th>Period</th>
<th>Parameter</th>
<th>Units</th>
<th>100 µg/animal/day Group 2</th>
<th>200 µg/animal/day Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Day 1/2</td>
<td>( t_{\text{max}} )</td>
<td>h</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>( C_{\text{max}} )</td>
<td>ng/mL</td>
<td>2.34</td>
<td>2.97</td>
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<tr>
<td></td>
<td>( \text{AUG} )</td>
<td>ng h/mL</td>
<td>17.49</td>
<td>18.60</td>
</tr>
<tr>
<td>Day 87/88</td>
<td>( t_{\text{max}} )</td>
<td>h</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>( C_{\text{max}} )</td>
<td>ng/mL</td>
<td>4.18</td>
<td>10.26</td>
</tr>
<tr>
<td></td>
<td>( \text{AUG} )</td>
<td>ng h/mL</td>
<td>23.65</td>
<td>47.64</td>
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<tr>
<td></td>
<td>( R_{\text{ex}} )</td>
<td>-</td>
<td>1.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Dosing Solution Analysis
- The rhNGF concentrations in the dose formulations ranged from 90.1% to 104.4% with reference to the nominal and were within the accepted range of ±10%, with exception of a sample of low-dose formulation after treatment on day 1 (40.6%) and samples of low-dose formulation prepared in week 13 which ranged from 81.0% to 88.3%.

Ocular Toxicity
Study title: rhNGF: 26-week eye drop administration toxicity study in the Wistar rat followed by a 4-week recovery period

Study no.: 0472-2011 (A1213BPL/E)
Study report location: SDN001 (5-31-2017); Section 4.2.3.2
Conducting laboratory and location: [Redacted]
Date of study initiation: 4/5/2012 (males); 4/13/2012 (females)
GLP compliance: Yes
QA statement: Yes; signed
Drug, lot #, and % purity: RS0812: 108 – 127%

Key Study Findings

- In Wistar rats, no adverse ocular findings were associated with the bilateral administration of the high-dose, 1.2 mg/mL TID. The NOAEL for ocular effects, 1.2% TID (18 µg/eye/day), exceeds the clinical dose (4.68 µg/eye/day) by 3.85-fold.
- In males, no adverse systemic findings were associated with the test article. The high dose, 36 µg/day (240 µg/kg/day), corresponds as a margin of 1538-fold over the proposed human dose of 9.36 µg/day (0.000156 mg/kg).
- In females, the high dose was associated with an increase in ovarian cysts and ovarian atrophy. The mid-dose, 24µg/day (160 µg/kg/day for a 0.15 kg rat), represents a 1026-fold margin over the proposed human dose (0.156 µg/kg/day).
Methods

**Doses:** 0.6, 0.8, or 1.2 mg/mL
Total daily dose: 9, 12, 18 µg/eye/day, respectively, or 18, 24, 36 µg/day total daily dose, respectively.

**Frequency of dosing:** Three times daily; bilateral; 26-weeks

**Route of administration:** Topical ocular drop

**Dose volume:** 0.005 mL/drop

**Formulation/Vehicle:** not the final clinical formulation (lacking L-methionine)

**Species/Strain:** Rat / Wistar

**Number/Sex/Group:** 20/sex/group

**Age:** 6 weeks

**Weight:** Males: 174 – 237 g
Females: 178 – 230 g

**Satellite groups:** Toxicokinetics: Control: 6/sex; Treatment groups: 9/sex

**Unique study design:** As per Amendment 1, systemic exposure evaluation of Day 1 was not performed due to technical difficulties related to the use of an Accelera non-standard blood sampling site for repeated collection of a relatively large amount of blood in small sized animals (about 200 g). For this reason, six additional Rcc-Han Wistar rats/sex/dose, with a body weight of about 350 g, received two treatments 3h-apart with rhNGF at the concentrations of 0.8 (Group 7) and 1.2 mg/mL (Group 8), 5 µL/drop, both eyes; they were used for blood sampling to evaluate Day 1 systemic exposure and were discarded after the last sampling.

**Deviation from study protocol:** None which affected conduct or interpretability of the study

Observations and Results

**Mortality (Once daily during pretest period; twice daily on treatment period)**

- No treatment-related death was observed during the study.
  - Control male No. 4434 was sacrificed moribund on Day 43, due to accidental lesions occurred during restraining for treatment.
  - Animals Nos. 4401(vehicle control, male) and 4482 (High-dose, male) died on Day 57 following blood sampling.
  - Satellite animals Nos. 4638 and 4639 (males, low-dose, TK group) were found dead on Day 57, following anesthesia and blood sampling for systemic exposure evaluation.
Female No. 4689 (High-dose, TK group) was sacrificed on humane reason due to the presence of an ulcerated mass in the inguinal region. At necropsy the animal showed good general condition, and the presence of a whitish ulcerated mass, 30x15 mm, was noted in the skin and subcutis of the inguinal region which correlated with a spontaneous basal cell carcinoma.

Clinical Signs (Once daily during pretest period. At least twice daily on treatment period)
- No changes in clinical signs attributed to the test article

Body Weights (Once during the pretest period, then weekly)
- No changes in body weights attributed to the test article

Feed Consumption (Once weekly during treatment and recovery period)
- No changes in feed consumption attributed to the test article

Ophthalmoscopy (Once pre-test, then on weeks 8 (interim sacrifice), 13, 26 (end of treatment) and 30 (end of recovery); Graded according to Draize scoring system; examined: conjunctiva, cornea, sclera, anterior chamber, iris, lens, vitreous body and fundus using a binocular ophthalmoscope, an indirect ophthalmoscope and a slit lamp when necessary)
- All ophthalmic findings were considered related to chance since present occasionally, often unilateral, in animals from all groups including controls, without time relationship, slight in severity and the different incidence in the groups was minor.
  - Reviewer finds some findings particularly conjunctival redness and ocular discharge to be possibly related to treatment since they occurred with highest frequency in the high dose group. They are not considered adverse.
Hematology (Weeks 8, 13, 26 and 30)
• No changes in hematological parameters attributed to the test article

Clinical Chemistry (Weeks 8, 13, 26 and 30)
• No changes in clinical chemistry parameters attributed to the test article

Urinalysis (Weeks 8, 13, 26 and 30)
• No changes in urinalysis parameters attributed to the test article

Gross Pathology (sacrifice days: 57 (interim), 183 (end of treatment) and 211 (end of recovery))
No changes in macroscopic pathology were specifically attributed to the test article. The following represent scattered, abnormal findings:

• Interim sacrifice (Day 57)
  o Control:
    ▪ Firm consistency of the bone marrow was noted in one control female (No. 4520).
  o High dose (1.2 mg/mL TID)
    ▪ Unilateral enlargement of the left kidney with a cystic appearance was observed in a male (No. 4484).
    ▪ Small seminal vesicle was noted unilaterally (No. 4481).
• End of treatment sacrifice (Day 182 – 186)
  o Control
    ▪ Males
      • An enlarged para-aortic lymph node with yellowish discoloration; n=1
      • Absence of the left eyeball; n=1
    ▪ Females:
      • A soft rounded soft pink nodule, 2 mm in diameter, was noted in the serosal surface of one uterine horn
      • Firm consistency of the bone marrow; n=2
      • Dark irregular mass in the peritoneal cavity, with one portion showing presence of soft greenish material; n=1
  o Low-dose
    ▪ Male:
      • Dilated pelvis (unilaterally, affecting the right or left kidney); n=1
    ▪ Female
      • Dilated pelvis (unilaterally, affecting the right or left kidney); n=1
      • Firm consistency of the bone marrow; n=1
  o Mid-dose
    ▪ Males:
      • Dilated pelvis (unilaterally, affecting the right or left kidney); n=1
      • Enlarged spleen; n=1
  o High dose
    ▪ Males
      • Small thymus, small testes and epididymides with adhesions between them and with adjacent tissues, multifocal alopecic areas on the muzzle and in the peri-orbital region, and bilateral thickening of the ears; n=1
      • Small and flaccid left testis; n=1
      • Dilated pelvis (unilaterally, affecting the right or left kidney); n=1
  • Recovery Phase (Day 211)
    o Control
      ▪ Small size of the ovaries was noted unilaterally in the control female No. 4548
    o High-dose
      ▪ Unilateral enlargement of the left kidney, oozing brownish liquid to granular material on cut surface, enlarged spleen and small size of right seminal vesicle.
      ▪ Enlarged spleen (n=1).

**Organ Weights**

• Ovaries
End of treatment phase:
- A minimal to slight decrease in the absolute and relative average weight of the ovaries was noted in treated females compared with concurrent controls albeit without statistical significance.

End of Recovery phase:
- A decrease in the average absolute and relative weight of the ovaries (-28% and -24% respectively) was observed in females from the 1.2 mg/mL dose group compared with concurrent controls, with statistical significance ($P \leq 0.05$) for the absolute value only.
- A statistically significant increase in the average absolute and relative weight of the spleen (+24% with $P \leq 0.05$ and +26% with $P \leq 0.01$ respectively) was observed in high dose treated males compared with their respective controls. However, this variation in weight of the spleen was judged within the range of biological variation.

Histopathology
Adequate Battery: Yes; full panel: high-dose and control only; select tissues in females (uterus including oviduct and cervix, ovaries, vagina, lesions): all dose groups and control

Peer Review: Yes

Histological Findings:
- Interim Sacrifice
  - No changes in histopathology were attributed to the test article
- End of treatment
  - The following findings occurred with higher frequency than control. The Sponsor does not attribute the changes to the test article and attributes changes to senescence and within the range of the expected biological variation for animals of this age.
  - Reviewer maintains that given dose related findings and similar findings in recovery animals (see below) that the changes are likely related to the test article.
  - Ovarian follicular cyst / Ovarian Atrophy (absence of corpora lutea)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Dose (mg/mL)</td>
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<tr>
<td>No. examined</td>
<td>20</td>
</tr>
<tr>
<td>OVARIES</td>
<td></td>
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<tr>
<td>Follicular Cysts</td>
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</tr>
<tr>
<td>- Minimal</td>
<td>4</td>
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<tr>
<td>- Slight</td>
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<td>Total incidence of finding observed</td>
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<tr>
<td>Atrophy</td>
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</tr>
<tr>
<td>- Minimal</td>
<td>1</td>
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<tr>
<td>- Slight</td>
<td>2</td>
</tr>
<tr>
<td>Total incidence of finding observed</td>
<td>3</td>
</tr>
</tbody>
</table>

- Persistent estrus
End of Recovery

- The Sponsor states that “The physiological status of the ovaries of control recovery females was unusually active for rats of this age. This ovarian status was the cause of the differences seen between control and treated animals both in the weight of the organ and in the morphological appearance; therefore, these changes were considered within the biological variation for female rats of this age, and thus unrelated to the treatment with the test item.”
- Reviewer’s opinion is that changes are likely related to the test article.
- Follicular cyst / Ovarian atrophy (absence of corpora lutea)

<table>
<thead>
<tr>
<th>Group</th>
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<th>4</th>
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</thead>
<tbody>
<tr>
<td>Dose (mg/mL)</td>
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<td>1.2</td>
</tr>
<tr>
<td>No. examined</td>
<td>10</td>
<td>10</td>
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</table>

<table>
<thead>
<tr>
<th>STAGE OF ESTRUS</th>
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<tbody>
<tr>
<td>Follicular Cysts</td>
</tr>
<tr>
<td>Total incidence of finding observed</td>
</tr>
<tr>
<td>Ovarian atrophy</td>
</tr>
<tr>
<td>Total incidence of finding observed</td>
</tr>
</tbody>
</table>

Persistent estrus

- Follicular cysts findings were correlated with observed persistent estrus.

Immunogenicity (acclimatization, on week 8 and on week 26; binding and neutralizing assay)

Binding antibodies against rhNGF were found in one animal of the mid-dose group (week 8 only) and in 2 animals of high-dose group (Week 8 and Week 26). Given a low responder rate and scattered results, no clear conclusion can be made regarding the
immunogenic potential of rhNGF, when administrated daily by eye drops over a period of 26-weeks in Wistar rat.

**Toxicokinetics** [Days 1, 15, 56, 91, 182 (males) or 181 (females); Day 1: 0.5, 2, 4 h after 1st dosing; Day 15: 0.5, 2, 4, 8h after 1st dosing; Day 56 and subsequent days: 0.5, 2, 4, 8 and 24 h after 1st dosing]

### Table 1. Exposure parameters of rhNGF after single dose in rats

<table>
<thead>
<tr>
<th>Week</th>
<th>Day</th>
<th>Treatment (µg TID)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (Hours)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (pg/mL)</th>
<th>SD of C&lt;sub&gt;max&lt;/sub&gt; (pg/mL)</th>
<th>AUC0-4 (pg·Hours/mL)</th>
<th>SE of composite AUC0-4 (pg·Hours/mL)</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>8</td>
<td>4.00</td>
<td>9074.46</td>
<td>8720</td>
<td>12700</td>
<td>6180*</td>
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<tr>
<td>1</td>
<td>1</td>
<td>12</td>
<td>0.50</td>
<td>42400.9</td>
<td>42200</td>
<td>54800</td>
<td>23100*</td>
</tr>
</tbody>
</table>

* SEM of mean AUC

Taking into account the inter-animal variability of the estimated parameters (particularly high variability in male exposure), no significant gender differences were reported after a single dose. The difference in means in the high dose group was at 4.2-fold for C<sub>max</sub> and 3.4-fold for AUC<sub>0-4</sub>.

### Table 2. Overall mean of week 8, 13 and 26 C<sub>max</sub> and AUC0-24 of rhNGF in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 8, 13 and 26 overall C&lt;sub&gt;max&lt;/sub&gt; (pg/mL)</th>
<th>Week 8, 13 and 26 overall AUC0-24 (pg·Hours/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>low-dose</td>
<td>6446</td>
<td>7907</td>
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<tr>
<td>mid-dose</td>
<td>9868</td>
<td>9017</td>
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<tr>
<td>high-dose</td>
<td>22057</td>
<td>16893</td>
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</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 8, 13 and 26 overall C&lt;sub&gt;max&lt;/sub&gt; (pg/mL)</th>
<th>Week 8, 13 and 26 overall AUC0-24 (pg·Hours/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>low-dose</td>
<td>6054</td>
<td>3583</td>
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<tr>
<td>mid-dose</td>
<td>15975</td>
<td>7256</td>
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<tr>
<td>high-dose</td>
<td>5504</td>
<td>2616</td>
</tr>
</tbody>
</table>

No significant gender differences for rhNGF C<sub>max</sub> and AUC0-24 were found though high variability may have contributed. At high-dose, rhNGF C<sub>max</sub> and AUC0-24 were 4.0 and 2.1-fold higher in males than in females, respectively.
Dosing Solution Analysis

- rhNGF concentration in all the tested formulation samples was within the acceptance criteria outlined in the Study Plan (± 20% of the expected nominal content), except for sample N. 2A (Day 1; nominal concentration 0.8 mg/mL) where obtained value was 27% higher than the nominal content and for sample N. 3 (Day 182) where obtained value was 79% of the nominal content.

Study title: 2-month ocular tolerance/toxicity study by daily instillations of rhNGF eye drops in albino rabbits with a 2-week recovery period

<table>
<thead>
<tr>
<th>Study no.</th>
<th>D23127212 / A1302BPL/E</th>
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<tbody>
<tr>
<td>Study report location</td>
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<td>Date of study initiation</td>
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<td>GLP compliance</td>
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<tr>
<td>QA statement</td>
<td>Yes</td>
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<tr>
<td>Drug, lot #, and % purity</td>
<td>rhNGF; RS1212: 95.3%; RS0503: 95.6%</td>
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</table>

Key Study Findings

Ocular:
- No adverse ocular effects were associated with rhNGF.
- The ocular NOAEL is 108 µg/eye/day which represents a 23-fold margin over the proposed clinical dose (4.68 µg/eye/day)

Systemic:
- rhNGF elicited an antibody response evidenced by the presence of anti-rhNGF in the plasma. Although an assessment of the neutralizing capacity of the ADA response cannot be made, it is not suggested based on limited data.
- In one female from the low-dose group, multiple unilateral ovarian cysts were reported macroscopically at the end of the treatment period
- In one female of the high-dose group, single bilateral ovarian cysts were found at the end of recovery.
- A NOAEL was not established for systemic effects, due to presence of ovarian cysts at the low dose. The low-dose (~18.6 µg/kg/day for a 2.9 kg rabbit) represents an 119-fold margin over the maximum proposed clinical dose (9.36 µg/day or 0.156 µg/kg/day).
Methods

Doses: 0.6 and 1.2 mg/mL (~54 or 108 µg/eye/day; ~18.6 or 37.2 µg/kg/day, based on the upper bound rabbit weight of 2.9 kg)

Frequency of dosing: Three times daily; right eye only*

Route of administration: Topical ocular

Dose volume: 30 µL

Formulation/Vehicle: Not the final clinical formulation (without L-methionine)

Species/Strain: Rabbit / New Zealand White

Number/Sex/Group:
- Main study: 3/sex/group
- Recovery: 2/sex/group

Age: 2 – 3 months

Weight: 2.4 – 2.9 kg

Observations and Results

Mortality (daily)
- All animals survived to scheduled sacrifice

Clinical Signs (daily)
- No changes attributed to test article

Body Weights (weekly)
- No changes in body weight were attributed to the test article

Feed Consumption (weekly)
- No changes in feed consumption were attributed to test article

Ophthalmoscopy [both eyes; baseline, then on Day 1, Day 29, Day 57 at 5 min after the last administration of the day, and Week 11 (recovery); graded per Draize scale]
- No test article associated changes

Ocular Examination (Slit-Lamp; both eyes; once a week from Week 1 to Week 9, in the morning before the first administration of the day; graded per McDonald-Shadduck’s scale)
- No test article associated changes

IOP [Day 1: 0.5 and 1 hour post-dose 1, Day 72 (recovery)]
• No effect on intraocular pressure was attributed to the test article

**ERG (Scotopic: Day 31, Day 53 – 56, Day 71 (recovery))**

• No changes in electroretinography were attributed to test article

*Reviewer’s note:* it is not clear why photopic ERG was not conducted.

**Hematology** (Day 30, Day 59 and at the end of the recovery period)

• No changes in hematological parameters were attributed to test article

**Clinical Chemistry** (Day 30, Day 59 and at the end of the recovery period)

• No changes in clinical chemistry were attributed to test article

**Immunogenicity**

• Week 4:
  o Low-dose: 3/6 positive for binding antibody.
  o High-dose: 9/10 positive for binding antibody.

• Week 8:
  o Low-dose group: 6/6 positive for binding antibody.
  o High-dose: 9/10 positive for binding antibody.

*Reviewer’s note:* Based on TK results, an assessment of the presence of neutralizing antibodies cannot be made since the ADA were not further characterized but is not suggested by the data. On Day 1, 1/6 and 0/6 animals had detectable rhNGF in the low-dose group and high-dose groups, respectively. After 4 weeks of administration (Day 30), 0/6 and 2/9 animals had detectable rhNGF in the low- and high-dose groups, respectively. At that time-point, the two animals with detectable rhNGF in the high dose group also were positive for ADA. Samples for TK analysis were only obtained at 1 hour post-dose on Day 30 and Day 59.

**Urinalysis (Day 59 and Day 73 (recovery animals))**

• No changes in clinical chemistry were attributed to test article

**Gross Pathology**

• End of treatment phase:
  o Ovaries
    ▪ Low-dose (0.6 mg/mL):
      • Female: (#26): Described as an irregular form of the left ovary with a dark mark and many cysts.* (see Histopathology below)

• End of recovery:
  o Ovaries
    ▪ High-dose (1.2 mg/mL):
• Female (#15): cysts (1 mm of diameter) on both ovaries.

**Organ Weights**
- No change in organ weights were attributed to test article

**Histopathology**
Adequate Battery: Yes
Peer Review: No

**Histological Findings**
- Ovaries
  - Low-dose (0.6 mg/mL):
    - Female (#26): In the summary, the pathologist notes that there was no microscopic correlate upon examination of the left ovary (the right ovary was not evaluated).
  - High-dose (1.2 mg/mL):
    - Female (#15): Ovaries noted as no pathologic findings noted

**Special Evaluation**

**Toxicokinetics** [Day 1 (1 h, 2 h, 4 h, 8 h and 24 h after the first administration), Day 30/31 and Day 59 (1 h after the first administration)].
- Low-dose
  - Day 1: rhNGF was only detectable only at 24 hours in 1/10 animals (0.08 ng/mL). All other samples tested BLQ (< 8 pg/mL)
  - Day 30: All levels BLQ
  - Day 59: All levels BLQ
- High-dose
  - Day 1: rhNGF was detectable only at 24 hours in 3/9 animals with $C_{\text{max}}$ values of 0.106, 0.243 and 0.089 ng/mL. All other samples tested BLQ.
  - Day 30: rhNGF was detectable at 1 hour after administration in 2/9 samples (0.041 and 0.026 ng/mL). Both animals were ADA positive.
  - Day 59: All levels BLQ.
Dosing Solution Analysis

- Dose formulations met acceptance criteria of Study Plan (± 20%)

Study title: rhNGF: 2-month ocular tolerance study by daily instillations of rhNGF formulated with methionine in albino rabbits followed by a 2-week recovery period

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<tr>
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<td>SDN001 (5-31-2017); Section 4.2.3.2</td>
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<td>GLP compliance:</td>
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<td>QA statement:</td>
<td>Yes; signed</td>
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<tr>
<td>Drug, lot #, and % purity:</td>
<td>rhNGF: RS0114, 87.8%</td>
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</table>

Key Study Findings

- No adverse ocular findings were associated with the test article
- The NOAEL 1.2 mg/mL (108 µg/eye/day rhNGF containing 0.1% L-methionine) corresponds to a margin of 23-fold over the proposed clinical dose (4.68 µg/eye/day)
- The findings qualify L-methionine for topical ocular administration up to a concentration of 0.1%

Methods

- Doses: rhNGF: 1.2 mg/mL (0.25 mg/mL L-methionine)
  rhNGF: 1.2 mg/mL (1.0 mg/mL L-methionine)
- Frequency of dosing: TID; unilateral (OD) for 2 months.
- Route of administration: Topical ocular drop
- Dose volume: 0.03mL/drop
- Formulation/Vehicle: Clinical vehicle containing L-methionine
- Species/Strain: Rabbit / New Zealand White
- Number/Sex/Group: Main study: 3 females/group
  Recovery: 2 females/group
- Age: 2 – 3 months
- Weight: 2.0 – 2.5 kg
- Deviation from study protocol: None which affected conduct or interpretability of study

Observations and Results

Mortality (daily)

- No animals died or were sacrificed prior to schedule

Clinical Signs (daily)
No adverse clinical signs were attributed to the test article

**Body Weights (weekly)**

- No changes in body weight were attributed to the test article

**Ophthalmoscopy (Baseline; daily (ophthalmoscope examination; Draize scoring); baseline, weekly (slit-lamp examination; McDonald-Shadduck scoring)**

**Ophthalmoscope findings**

- Vehicle (containing 1 mg/mL L-methionine)
  - Day 22
    - Conjunctival redness OD (score 1); n=1
- rhNGF (containing 0.25 mg/mL L-methionine)
  - No adverse ophthalmic findings reported
- rhNGF (containing 1.0 mg/mL L-methionine)
  - Conjunctival redness OD (score 1); n=1

**Slit-Lamp findings**

- Vehicle (containing 1 mg/mL L-methionine)
  - Week 10 (recovery)
    - Iris hyperemia OD (score 1; OD); n=1
- rhNGF (containing 0.25 mg/mL L-methionine)
  - Fibrin filament in the anterior chamber of two animals throughout study. No toxicological significance was attributed to the finding.
    - In one animal, presence of filament was seen in both eyes (i.e. untreated eye also)
    - In other animal, fibrin filament found in treated eye only
- rhNGF (containing 1.0 mg/mL L-methionine)
  - Week 1
    - Iris hyperemia (score 1; OD); n=1
  - Week 6
    - Iris hyperemia (score 1; untreated OS); n=1

**Gross Pathology**

- No adverse findings were found upon macroscopic examination

**Histopathology**

Adequate Battery: Brain and ocular tissues only

Peer Review: Yes
Histological Findings: No adverse microscopic findings attributed to the test article

Immunogenicity (Day 1 and Day 56)
- All animals treated for 56 days with rhNGF formulated with L-methionine were analyzed as positive for binding antibodies against rhNGF and were confirmed as positive in a immunodepletion assay.

Toxicokinetics (Blood collected from ear vein; Day 1: 1 hr after first dose, hour 4 (immediately after second daily dose) and hour 24 (before first daily dose on Day 2); Day 56: immediately before first daily dose, 1 hr after first daily dose, hour 4 (immediately after second daily dose) and hour 24. LLOQ = 8 pg/mL

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<th>Time [h]</th>
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<td>rhNGF Individual Conc. [ng/mL]</td>
<td>rhNGF Mean Conc. [ng/mL]</td>
<td>rhNGF Individual Conc. [ng/mL]</td>
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* reanalysis was performed out of stability period, mean based on repeated value
BLQ values were set to zero for calculations of mean
Dosing Solution Analysis

- rhNGF concentration in all the tested formulation samples was ± 20% of the expected nominal content.

9.17
Study title: 125-day ocular and subcutaneous toxicity study with rhNGF followed by a 2-week recovery period in juvenile New Zealand White rabbits from PND50 onwards

- Study no.: 513095 (A1614BPL/E)
- Study report location: SDN001 (5-31-2017); Section 4.2.3.2
- Conducting laboratory and location: [Redacted]
- Date of study initiation: April 7, 2016
- GLP compliance: Yes
- QA statement: Yes; signed
- Drug, lot #, and % purity: Topical ocular rhNGF: Batch R0716; 87.1% (reverse phase HPLC)
  Subcutaneous rhNGF: Batch 0415; 88.9% (reverse phase HPLC)

Key Study Findings

- Topical ocular administration of rhNGF (lacking L-methionine) was associated with increased minimal conjunctival irritation.
- No other ocular or systemic toxicity was associated with the test article.
- The ocular NOAEL of the study is 108 µg/eye/day. This corresponds to a margin of 23-fold over the intended clinical dose.
- The systemic NOAEL is 28 µg/kg/day (subcutaneous dose of 50 µg/animal/day). This dose corresponds to a margin of 180-fold over the intended clinical dose, presuming 100% systemic absorption.

Methods

- Doses: Topical ocular: 0, 0.6, 0.8 and 1.2 mg/mL (0, 54, 72 and 108 µg/eye/day)
  Subcutaneous: 50 µg/animal/day (or 28 µg/kg/day)
- Frequency of dosing: Topical ocular: bilateral; TID for 125 days
  Subcutaneous: QD for 125 days
- Route of administration: Topical ocular drop or subcutaneous injection
- Dose volume: Topical ocular: 0.03 mL/drop
  Subcutaneous: 3mL
- Formulation/Vehicle: Not the final clinical formulation (lacking L-methionine)
- Species/Strain: Rabbit / New Zealand White
- Number/Sex/Group: Main study: 4/sex/group
  Recovery: 3/sex/group
- Age: PND 50
- Weight: Males: 1.5 kg
  Females: 1.7 kg
- Deviation from study protocol: None which affected integrity of study conduct or interpretability of results
Observations and Results

Mortality (twice daily)

Subcutaneous:
- Two animals were killed in extremis due to severe complications at the injection sites (extensively thickened area of the flank region), causing a deteriorated physical condition of these animals:
  - Recovery group female No. 58 on Day 96.
  - Main study group male No. 25 on Day 32
    - Dark red or reddish foci and/or discoloration (green or red) of organs of the gastro-intestinal (GI)-tract and of the local lymph nodes were observed additionally. Infection of the subcutis of the injection sites (inflammation of mixed inflammatory cells and/or abscess formation) was the microscopic correlate to the injection sites findings in both animals. In addition, marked ulceration of the stomach correlated with the many dark red foci that were recorded at necropsy.
    - Both animals showed decreased food intake with correlating (slight) body weight loss prior to sacrifice.
    - Macroscopic findings in these animals were mostly related to these injection site complications and included nodules and/or thickening and/or reddish discoloration of the injection sites and the underlying tissues.
    - Lymphoid hyperplasia of the draining lymph nodes, germinal center formation and/or lymphoid hyperplasia and/or increased erythropoiesis in the spleen and increased cellularity (all cell lines) of the bone marrow (sternum).
    - The moribundity of these two animals was considered to be related to complications at the injection sites (infection with inflammation and/or abscesses) elicited by the repetitive daily subcutaneous injections, rather than being directly related to the test item.

Clinical Signs (once daily)

Topical ocular:
- General erythema in the eyes and/or eyelids was prevalent and watery discharge from the eye was occasionally seen across the ocular dosed animals from all groups, including controls. During the recovery period, ocular changes decreased and had mostly resolved towards the end of the 2-week recovery period.

Subcutaneous:
- Blue discoloration was occasionally seen at the injection site(s)
Most prevalent towards the end of the treatment period. The blue discoloration at the injection site(s) was no longer observed by the end of the recovery period.

**Body Weights (twice weekly up to end of Week 4, then weekly)**
- Slightly lower body weights and body weight gain (although not reaching statistical significance on most occasions) were noted for the subcutaneously dosed animals throughout the treatment period. During the recovery period, body weight gain was still slightly lower, albeit not statistically significantly.

**Feed Consumption**
- No changes in feed consumption attributed to the test article.

**Ophthalmoscopy (pre-treatment, end of treatment (18 weeks); slit lamp examination, Draize scoring)**
- Conjunctival Irritation
  - Increased in all groups treated with topical ocular rhNGF compared to control

**Hematology**
- Increased fibrinogen
  - Subcutaneous administration
    - +22% (males only)
    - Finding not reported in recovery animals
  - Topical ocular administration
    - High dose
      - Males: +17%
      - Females +16%:
    - Finding not reported in recovery animals

**Clinical Chemistry**
- No changes in clinical chemistry parameters attributed to the test article.

**Gross Pathology**
- Subcutaneous administration
  - Dark red/reddish foci
    - Recorded in the left injection site in 3/4 males and 1/4 females and in the right injection site in 1/4 males and 2/4 females.

**Organ Weights**
- Decreased adrenal weights
Subcutaneous administration
- Males: - 38%
- Females: - 10%

Topical ocular administration
- Low dose
  - Males: - 34%
  - Females: - 14%
- Mid-dose
  - Males: - 26%
  - Females: +9%
- High dose
  - Males: -38%
  - Females -10%

Histopathology
Adequate Battery: Yes; full battery of systemic tissues and ocular tissues
Peer Review: Yes

Histological Findings:

- **Lenticular changes**
  - Minimal bilateral swelling/vacuolation of lens fibers of the lens bow area was recorded at the end of the treatment period in two high-dose topical ocular treated males.
  - After recovery period, unilateral slight swelling/vacuolation of lens fibers was recorded in 1/3 high-dose males.

![Histology Table]

- **Injection site reaction**
  - There were minor local microscopic findings in the injection sites of the subcutaneous treated animals, consisting of a minimal or slight degree of inflammation of mixed cells in 2/4 males and 2/4 females (left injection site) and 2/4 males and 3/4 females (right injection site). Hemorrhage was recorded in 2/4 males and 1/4 females (left injection site) and in 1/4 females (right injection site).
  - Finding not reported in recovery animals.
Immunogenicity (pre-treatment, Week 19)

- Only 2 of the samples from topical ocular treated animals (mid-dose female and high-dose male No. 18) were concluded to be confirmed positive for anti-rhNGF antibodies.
- Data show low immunogenic potential of rhNGF following topical ocular administration in juvenile rabbits

Toxicokinetics (Day 1, Weeks 9 and 18; Topical ocular groups: 1 h after each daily application. Subcutaneous injection group: Predose, 0.5 h, 2 h, 4 h, 8 h, and 24 h; blood collected from ear artery)

- Subcutaneous administration

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| Week 9     | n=6     | n=7          |
| Cmax       | h       | 24           | 24           |
| t1/2       | h       | 2.4±       | 2.4±         |
| Cmax,D     | pg/mL   | 14500*      | 6500         |
| AUCmax     | hpg/mL  | 290          | 130          |
| AUCmax,D   | hpg/mL  | 76200        | 42600        |
| t1/2       | h       | 1520         | 852          |
|            | 4.23     | 3.37         |

| Week 18    | n=6     | n=6          |
| Cmax       | h       | 24           | 24           |
| t1/2       | h       | 2.4±        | 2.4±         |
| Cmax,D     | pg/mL   | 7010         | 6480         |
| AUCmax     | hpg/mL  | 140          | 130          |
| AUCmax,D   | hpg/mL  | 41100        | 39000        |
| t1/2       | h       | 821          | 780          |
|            | 4.40     | 2.72         |

Dosing Solution Analysis

- Topical ocular administration
  - The Applicant reports that the measured rhNGF plasma concentrations after ocular administration were extremely variable. At one hour after ocular administration, the average plasma concentrations ranged between 0.00 to 4950 pg/mL (Day 1 and Weeks 9 and 18, data combined). Generally, the data show that the rhNGF treated animals were exposed to rhNGF confirming ocular administration and systemic absorption.
The achieved content of rhNGF in the formulation samples met the acceptance criteria outlined in the Study Plan (± 20% of the nominal content).

9 Reproductive and Developmental Toxicology

9.18 9.1 Fertility and Early Embryonic Development

Study title: Combined study of the effects of recombinant human nerve growth factor on fertility and embryo-fetal development in rats by subcutaneous administration

Study no.: A1462BPL/E (505338)
Study report location: EDR: BLA 761094/eCTD seq.0001 / 4.2.3.5.1
Conducting laboratory and location: [redacted]
Date of study initiation: 7-31-2014 (allocation animals)
8-4-2014 (start treatment)
GLP compliance: Yes; signed
QA statement: Yes; signed
Drug, lot #, and % purity: rhNGF: 205542/C, Batch 0413, 91.7%
205542/D, Batch 0813, 88.3%
205542/E, Batch 0414, 90.7%

Key Study Findings

- Early resorptions were statistically increased at 20 µg/animal (lowest dose).
- Hydrocephaly and ureter anomalies were present at 40 µg/animal (high dose).
- No effect on fertility was observed up to the highest dose level tested (40 µg/animal).
- Immunogenicity evaluation:
  - 1 out of 53 animals in each group at predose tested positive
  - 9 out of 53 animals (17%; group 2) and 17 out of 53 animals (32.1%; group 3) at necropsy were positive for binding antibodies against rhNGF indicating immunogenic potential of rhNGF, when administered once daily for 7 days per week to rats.
- The LOAEL dose, 20 µg/day (133 µg/kg/day), represents a 852-fold margin over presumed 100% absorption of the proposed ocular dose (9.36 µg/day or 0.156 µg/kg/day)
Methods

Doses: 20 or 40µg
Frequency of dosing:
Males: Daily from 2-weeks prior to mating throughout mating until one day prior to euthanasia (total: 42 – 43 days)
Females: Daily from 2 weeks prior to mating throughout mating and continuing until Day 17 post-coitum (total: 33 – 46 days)
Dose volume: 2 mL/animal (2 different injection sites)
Route of administration: Subcutaneous injection
Formulation/Vehicle: 0.9% NaCl
Species/Strain: Rat / Crl:WI (Han)
Number/Sex/Group: 24/sex/group
Age: ~11 weeks
Body weight
Males: ~320g
Females: ~210g
Satellite groups
Control: 3 females
Treated: 5 females/group
Study design: Combined Fertility/embryo-fetal development
(see separate section below for embryo-fetal development results)

Deviation from study protocol:

Observations and Results

Mortality (twice daily)
- No animals died or were sacrificed prior to schedule

Clinical Signs (once daily)
- Swelling and general erythema of the head, ears, tail and/or legs
  - Observed 2 to 4 hours after treatment in a dose related manner.
  - At 20 µg, slight general erythema of the tail was noted for one male (1 day), slight to moderate general erythema of the ear(s) was noted for 18 males and 11 females (1 to 6 days), slight to moderate swelling of the head was noted for 5 males and 4 females (1 to 5 days), and slight swelling of the hindlimb was noted for one male (1 day).
  - At 40 µg, slight to moderate general erythema of the tail was noted for 4 males and 13 females (1 to 24 days), slight to moderate general erythema of the ear(s) was noted for 23 males and 22 females (1 to 32 days), slight general erythema of the legs was noted for one female (2 days), slight to severe swelling of the head was noted for 20 males and 20 females (1 to 13 days), and slight to severe swelling of the leg(s) was noted for 14 males and 19 females (1 to 10 days). In addition, one male showed slight focal erythema of the snout (1 day), one male showed abnormal posture of the hindlimb (18 days), and three males and one female showed slight to moderate abnormal gait (1 to 9 days).
Control: General erythema of the ears (slight) was also noted for two females for 1 to 3 days.

- Other findings were considered incidental (occurred within background range of occurrence) and included alopecia, scabs, chromodacryorrhea, scales, diarrhea, piloerection, hunched posture, and rales.

Body Weight (Males/females weighed on the first day of exposure and biweekly thereafter. Mated females were weighed on Days 0, 3, 6, 9, 12, 15, 17, 18, 19 and 20 post-coitum)

- Males
  - High-dose (40 μg)
    - Statistically significant decrease was noted for mean body weights and body weight gain during the complete study period. An initial body weight loss (followed by body weight gain) was noted for seven males. One male (no. 52) showed a continuous low body weight and gained only 5% during the complete treatment period.
  - Low-dose (20 μg)
    - Statistically significantly reduction in mean body weight gain was reported during the full treatment period. Mean body weights were statistically significantly decreased on Days 4-11 of the mating period and on Day 4 of post mating.

- Females
  - High-dose
    - An initial body weight loss was noted for almost all animals; this recovered within the first two weeks. A statistically significant difference was noted for body weight gain on Days 4 of premating until Day 4 of mating.
  - Low-dose
    - An initial body weight loss was noted for almost all animals; which mostly recovered within the first week of treatment. A statistically significant difference was noted for body weight gain on Days 4 of premating until Day 1 of mating.

- All changes were not regarded toxicologically relevant as amplitude of decrease was considered small and no clear dose response was found.

Feed Consumption (Biweekly, except for males and females which were housed together for mating and for females without evidence of mating. Food consumption of mated females was measured on Days 0, 3, 6, 9, 12, 15, 17 and 20 post coitum)

- High-dose: Food consumption was slightly reduced.
  - Males
    - For males, this was statistically significant for absolute food consumption on Days 1 to 15 of the pre-mating period and on Days
1 to 11 of the post-mating period, and for relative food consumption on Days 1 to 4 and 8 to 15 of pre-mating.

- **Females**
  - Statistical significance was reached during pre-mating on Days 1 to 4 and 11 to 15.

**Toxicokinetics (blood collected from jugular vein; Day 16 post-coitum at predose and 2, 4, 6, 8, and 24 hours post daily-dose)**

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# : dose-normalized to 1 mg/kg b.w.

**Immunogenicity (baseline and necropsy)**

Positive response (OD > the pre-determined cut point):

- **Baseline (pre-screening)**
  - **Control**
    - 3 out of 24 (12.5%) males, and 1 out of 27 (3.7%) females of group 1
  - **Low-dose**
    - 1 out of 24 (4.2%) males, and 1 out of 29 (3.4%) females of group 2
  - **High-dose**
    - 1 out of 24 (4.2%) males, and 1 out of 29 (3.4%) females of group 3

- **Necropsy**
  - **Control**
    - 2 out of 24 (8.3%) males, and 3 out of 27 (11.1%) females of group 1
  - **Low-dose**
    - 4 out of 24 (16.7%) males, and 8 out of 29 (27.6%) females of group 2
  - **High-dose**
    - 7 out of 24 (29.2%) males, and 10 out of 29 (34.5%) females of group 3

- The putative positive samples were analyzed in titration binding assays.
  - **Baseline samples**
Control

- No animals confirmed positive.

Low-dose

- One male and one female confirmed positive

High-dose

- One male confirmed positive

Necropsy

- None confirmed to be positive

Low-dose

- 2 out of 4 (50%) males and 8 out of 8 (100%) females confirmed to be

High-dose

- 7 out of 7 (100%) males and 10 out of 10 (100%) females confirmed positive for binding antibodies against rhNGF.

The confirmed positive samples were further analyzed with an excess of rhNGF (an immunodepletion experiment) to confirm the specificity of the signal.

Baseline:

- Low-dose:
  - The single positive bindings pre-dose sample confirmed positive for immunodepletion

- High dose:
  - The single positive bindings pre-dose sample confirmed positive for immunodepletion

Necropsy,

- Low-dose: 9 out of 10 (90.0%) animals which tested positive in the titration assay were confirmed positive for immunodepletion

- High-dose:
  - 17 out of 17 (100%) animals which tested positive in the titration assay were confirmed to be positive for immunodepletion.

Overall, 1 out of 53 animals from the high- and low-dose groups at predose and 9 out of 53 animals (17%; low-dose) and 17 out of 53 animals (32.1%; high-dose) at necropsy were positive for binding antibodies against rhNGF.

Dosing Solution Analysis

Formulations remained within acceptable criteria (90-110%) except one pre-dose sample that showed a mean accuracy of 88.9%. Since the post-dose samples were within the accepted range, this slightly low value was not considered significant.

Necropsy
Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Males:
- No toxicologically relevant changes were noted for sperm motility up to 40 μg.
- There were no test item-related microscopic observations in the testes.
- Spermatogenic staging profiles were normal for all treated males examined.

Females:
- Early resorptions were statistically increased in treated females. In both low and high-dose animals, an early resorption rate of 8.4% was reported compared to 2.4% in controls.
- No other toxicologically relevant effects on reproductive parameters were noted up to 40 μg. Mating, fertility and conception indices, precoital time, numbers of corpora lutea and implantation sites, and pre-implantation loss were unaffected by treatment.
Offspring (Malformations, Variations, etc.)

- No malformations or variations were attributed to the test article at 20 µg/animal.
At 40 μg/animal, soft tissue malformations present in high-dose offspring that were not found at low-dose or controls included convoluted ureter (3 fetuses/2 litters) and dilated ureter (1 fetus/litter); and hydrocephaly (1 fetus/1 litter). The incidence of these anomalies exceeded that of provided historical control, and were considered treatment-related.

Study title: Study of the effects of recombinant human nerve growth factor on embryo-fetal development in rabbits by subcutaneous administration

Study no.: 505336 (A1463BPL/E)
Study report location: EDR: BLA 761094/eCTD seq.0001 / 4.2.3.5.2
Conducting laboratory and location: [redacted]
Date of study initiation: September 1, 2014
GLP compliance: Yes
QA statement: Yes; signed
Drug, lot #, and % purity: rhNGF, Batch 0414

Key Study Findings

- No maternal toxicity was observed in the 75 and 150 μg/animal/day groups
- An increase in fetal cardiovascular effects was observed at 150 μg/animal/day (83 μg/kg/day for a 1.8 kg rabbit) and increased postimplantation loss at 75 μg/animal/day.
- The LOAEL for rhNGF was 75 μg/animal/day (42 μg/kg/day for a 1.8 kg rabbit). This dose corresponds to a margin of 267-fold over presumed 100% absorption of the proposed clinical dose (9.36 μg/day, or 0.156 μg/kg/day for a human weighing 60kg).

Methods

- Doses: 75 or 150 μg/animal day
- Frequency of dosing: Once daily on Gestational Days (GD) 7 – 20
- Dose volume: 5 mL
- Route of administration: Subcutaneous injection
- Formulation/Vehicle: Saline
- Species/Strain: Rabbit / New Zealand White
- Age: 17 – 19 weeks at delivery
- Weight: ~3.7 kg
- Number/Sex/Group: 22/females/group
- Deviation from study protocol: None which affected conduct or interpretability

Observations and Results

Mortality (twice daily)
• No unscheduled mortalities occurred during the study period

**Clinical Signs (once daily)**

• No adverse clinical signs attributed to the test article

**Body Weight (Gestational Days 0, 4, 7, 10, 13, 16, 20, 21, 23, 26, 29)**

• No changes in body weight were attributed to the test article

**Feed Consumption (Gestational Days 0-4, 4-7, 7-10, 10-13, 13-16, 16-20, 20-23, 23-26 and 26-29)**

• No changes in feed consumption were attributed to the test article

**Toxicokinetics (Ear artery; Day 7 (before dosing period) and Day GD20; pre-dose, 0.5, 2, 4, 8, and 24 hours post-dose)**

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<td>t&lt;sub&gt;max&lt;/sub&gt;</td>
<td>(h)</td>
<td>2</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>(ng/mL)</td>
<td>6.72</td>
</tr>
<tr>
<td>#C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>(kg·ng/mL/μg)</td>
<td>0.374</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-8&lt;/sub&gt;</td>
<td>(h·ng/mL)</td>
<td>32.6</td>
</tr>
<tr>
<td>#AUC&lt;sub&gt;0-8&lt;/sub&gt;</td>
<td>(h·kg·ng/mL/μg)</td>
<td>1.82</td>
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<tr>
<td>AUC&lt;sub&gt;last&lt;/sub&gt;</td>
<td>(h·ng/mL)</td>
<td>57.7</td>
</tr>
<tr>
<td>#AUC&lt;sub&gt;last&lt;/sub&gt;</td>
<td>(h·kg·ng/mL/μg)</td>
<td>3.21</td>
</tr>
<tr>
<td>t&lt;sub&gt;50&lt;/sub&gt;</td>
<td>(h)</td>
<td>3.53</td>
</tr>
</tbody>
</table>

<sup>$</sup> range

<sup>#</sup> dose-normalized to 1 μg/kg b.w.

<sup>n/c</sup> could not be calculated
Immunogenicity (Ear artery; Day 7 (before dosing period) and Day GD20)

In total, 2 out of 22 animals of Control group (9%) were positive for binding antibodies against rhNGF on day 7 post-coitum (pretest) and on day 20 post-coitum. In LD animals, only 1 out of 22 animals (4.5%) was positive on day 7 post-coitum (pretest) for binding antibodies against rhNGF, whereas on day 20 post-coitum, 18 out of 22 (81.8%) were positive for binding antibodies against rhNGF. In HD animals, 6 out of 22 animals (27.3%) on day 7 post-coitum (pretest) and 17 of 22 (77.3%) on day 20 were positive for binding antibodies against rhNGF.

Dosing Solution Analysis

- Low-dose formulation samples collected at the end of the dosing period showed mean accuracies between 64.3 and 77.0%, and the samples collected at the high-dose showed mean accuracies between 83.8 and 84.3%. All samples were showing accuracies below the lower acceptance range (90%). The lower accuracy obtained for the formulations was attributed to the thawing process of rhNGF (as the formulation samples were supplied on dry ice to the test site). Moreover, additional formulation analyses on fresh prepared formulations at the test facility confirmed that the formulations are stable for at least 4 days when kept at room temperature. In practice, all formulations were prepared fresh daily, kept at room temperature and used within 5 hours. Furthermore, all data show formulations were weighed and prepared correctly, and thus there was no influence on the study integrity.

Necropsy

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- Litter size and sex ratio were not affected by treatment
- Compared to control animals, a dose-dependent increase in early/late resorptions (post-implantation loss) was observed at ≥75 µg/day.
## Dose Group 1

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td><strong>Females on Study</strong></td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td><strong>Females that aborted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Or delivered</td>
<td>1</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Females that died</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Females that aborted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonrhagid</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Gravid</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Females that were euthanized</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gravid</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Females Examined at Scheduled Necropsy</strong></td>
<td></td>
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<tr>
<td>21</td>
<td>95.5</td>
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<td>100.0</td>
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<tr>
<td>Nonrhagid</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
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<tr>
<td>Gravid</td>
<td>21</td>
<td>100.0</td>
<td>21</td>
</tr>
<tr>
<td>With Resorptions Only</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
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<tr>
<td>With Viable Fetuses</td>
<td>21</td>
<td>100.0</td>
<td>21</td>
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<tr>
<td><strong>Total Females Gravid</strong></td>
<td>22</td>
<td>100.0</td>
<td>21</td>
</tr>
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</table>

### 1-0 µg/Animal/Day  2-75 µg/Animal/Day  3-150 µg/Animal/Day

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Viable</th>
<th>Dead</th>
<th>Resorptions</th>
<th>Implantation Sites</th>
<th>Lutea</th>
<th>Loss Sites</th>
<th>Corpora Lutea</th>
<th>Weights</th>
<th>Gravid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>TOTAL</td>
<td></td>
<td>109</td>
<td>191</td>
<td>0</td>
<td>1</td>
<td>0.6</td>
<td>192</td>
<td>204</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>Mean</td>
<td>5.2</td>
<td>3.9</td>
<td>9.1</td>
<td>0.0</td>
<td>0.0</td>
<td>9.1</td>
<td>9.7</td>
<td>0.6</td>
<td>40.1</td>
<td></td>
</tr>
<tr>
<td>St.D.</td>
<td>1.81</td>
<td>1.55</td>
<td>1.95</td>
<td>0.0</td>
<td>0.22</td>
<td>0.0</td>
<td>1.90</td>
<td>1.90</td>
<td>0.60</td>
<td>5.94</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>89</td>
<td>200</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>207</td>
<td>214</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Mean</td>
<td>5.3</td>
<td>4.2</td>
<td>9.5</td>
<td>0.0</td>
<td>0.3</td>
<td>9.9</td>
<td>10.2</td>
<td>0.3</td>
<td>39.1</td>
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<tr>
<td>St.D.</td>
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<td>2.00</td>
<td>1.17</td>
<td>0.0</td>
<td>0.58</td>
<td>0.58</td>
<td>1.11</td>
<td>1.33</td>
<td>0.58</td>
<td>3.77</td>
</tr>
<tr>
<td>3</td>
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<td>99</td>
<td>214</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>223</td>
<td>233</td>
<td>10</td>
<td>22</td>
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<tr>
<td>Mean</td>
<td>5.2</td>
<td>4.5</td>
<td>9.7</td>
<td>0.0</td>
<td>0.3</td>
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<td>10.1</td>
<td>10.6</td>
<td>0.5</td>
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<tr>
<td>St.D.</td>
<td>1.77</td>
<td>1.07</td>
<td>1.91</td>
<td>0.00</td>
<td>0.57</td>
<td>0.29</td>
<td>0.59</td>
<td>1.73</td>
<td>1.92</td>
<td>3.67</td>
</tr>
</tbody>
</table>

None significantly different from control group

NA = Not Applicable

Mean Number of Viable Fetuses, Mean Number of Implantation Sites, Mean Number of Corpora Lutea, Fetal Weights Compared Using Dunnett's Test
Offspring (Malformations, Variations, etc.)

- No adverse effects on fetal weight or skeletal formation were attributed to the test article.
- Cardiovascular effects, including ventricular and atrial septal defects, enlarged heart and aortic arch dilation were reported at 150 µg/day (in two fetuses from 2 litters [A045 and A066]). The incidence of these effects exceeded that of the provided historical control data, and were considered treatment-related.
<table>
<thead>
<tr>
<th>GROUP:</th>
<th>0 μg/ANIMAL/DAY</th>
<th>75 μg/ANIMAL/DAY</th>
<th>150 μg/ANIMAL/DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEMALE FETAL WEIGHTS (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEAN</td>
<td>35.7</td>
<td>37.9</td>
<td>36.0</td>
</tr>
<tr>
<td>S.D.</td>
<td>5.44</td>
<td>5.06</td>
<td>4.30</td>
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<tr>
<td>N</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>COMBINED FETAL WEIGHTS (g)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MEAN</td>
<td>40.1</td>
<td>35.1</td>
<td>36.8</td>
</tr>
<tr>
<td>S.D.</td>
<td>5.94</td>
<td>3.77</td>
<td>3.96</td>
</tr>
<tr>
<td>N</td>
<td>21</td>
<td>21</td>
<td>21</td>
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</tbody>
</table>

PROPORTIONAL (%) DATA COMPARED USING THE MANN-WHITNEY TEST
FETAL WEIGHTS COMPARED USING DUNNETT’S TEST
None significantly different from control group

<table>
<thead>
<tr>
<th>Dose Group:</th>
<th>Fetuses</th>
<th>Litters</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

NUMBER EXAMINED EXTERNALLY

<table>
<thead>
<tr>
<th>Condition</th>
<th>0 μg/ANIMAL/DAY</th>
<th>75 μg/ANIMAL/DAY</th>
<th>150 μg/ANIMAL/DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exencephaly</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eye(s): Open</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cleft Palate</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Euthyphragm</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ventricular Septum Defect</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Heart: Atrium Enlarged</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lung: Absent and/or Small</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aortic Arch: Dilated</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Heart: Enlarged</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lung: Small Lung Lobe(s)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Atrial- Septum Defect</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
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<td>0</td>
<td>0</td>
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</tbody>
</table>

NUMBER EXAMINED SKELETALLY

<table>
<thead>
<tr>
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<th>75 μg/ANIMAL/DAY</th>
<th>150 μg/ANIMAL/DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bent Long Bone(s)</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sternebra(e) Malloriated (Severe)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sternebra(e) Fused</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Skull Anomaly</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sterno-occipital</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Caudal Vertebra Anomaly</td>
<td>1</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

TOTAL NUMBER WITH MALFORMATIONS

<table>
<thead>
<tr>
<th>Type</th>
<th>0 μg/ANIMAL/DAY</th>
<th>75 μg/ANIMAL/DAY</th>
<th>150 μg/ANIMAL/DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>External</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soft Tissue</td>
<td>4</td>
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<td>4</td>
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<tr>
<td>Skeletal</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Combined</td>
<td>7</td>
<td>6</td>
<td>8</td>
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### 9.19 Prenatal and Postnatal Development

**Study title:** Study of the effects of recombinant human nerve growth factor on pre- and postnatal development, including maternal function in rats by subcutaneous administration

<table>
<thead>
<tr>
<th>DOSE GROUP:</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NUMBER OF LITTERS EXAMINED EXTERNALLY</strong></td>
<td>21</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>EXENCEPHALY</td>
<td>MEAN</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2.18</td>
<td>0.00</td>
</tr>
<tr>
<td>EYE (S) - OPEN</td>
<td>MEAN</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2.18</td>
<td>0.00</td>
</tr>
<tr>
<td>CLEFT PALATE</td>
<td>MEAN</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2.18</td>
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</tr>
<tr>
<td>BRAFACRATURY</td>
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<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2.18</td>
<td>0.00</td>
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</table>

1-0 μg/ANIMAL/DAY  2-75 μg/ANIMAL/DAY  3-150 μg/ANIMAL/DAY

None significantly different from control group

<table>
<thead>
<tr>
<th>DOSE GROUP:</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td><strong>NUMBER OF LITTERS EXAMINED VISCERALLY</strong></td>
<td>21</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>LUNG- ABSENT LUNG LOBE (S)</td>
<td>MEAN</td>
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</tr>
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<td></td>
<td>S.D.</td>
<td>3.41</td>
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<tr>
<td>VENTRICULAR SEPTUM DEFECT</td>
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<tr>
<td></td>
<td>S.D.</td>
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<td>0.00</td>
</tr>
<tr>
<td>HEART- ATRIUM ENLARGED</td>
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<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
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<td>0.00</td>
</tr>
<tr>
<td>EYE (S) - ABSENT AND/OR SMALL</td>
<td>MEAN</td>
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<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
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</tr>
<tr>
<td>LUNG- ABNORMAL LOCATION</td>
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<tr>
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<td>S.D.</td>
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<td>AORTIC ARCH- DILATED</td>
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<td>0.00</td>
</tr>
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<td>HEART- ENLARGED</td>
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<td>0.0</td>
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<td>LONG- SMALL LUNG LOBE (S)</td>
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<td>0.00</td>
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<tr>
<td>ATRIAL- SEPTUM DEFECT</td>
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</tr>
<tr>
<td></td>
<td>S.D.</td>
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<td>0.00</td>
</tr>
<tr>
<td>TETRALOGY OF FALLOT</td>
<td>MEAN</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2.18</td>
<td>0.00</td>
</tr>
</tbody>
</table>

1-0 μg/ANIMAL/DAY  2-75 μg/ANIMAL/DAY  3-150 μg/ANIMAL/DAY

None significantly different from control group

---

**Reference ID:** 4274484
Key Study Findings

- No toxicological significant findings in pre- and postnatal development on F₀ dams and F₁ pups.
- Immunogenicity was observed in F₀ and F₁ pups in both treatment groups.
- NOAEL is high dose: 40 µg/day (267 µg/kg/day)
- The NOAEL (267 µg/kg/day), represents a 1711-fold margin over presumed 100% absorption of the proposed ocular dose (9.36 µg/day or 0.156 µg/kg/day)

Methods

<table>
<thead>
<tr>
<th>Doses:</th>
<th>20 or 40µg (133 or 267 µg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of dosing:</td>
<td>Daily from post-coitum Day 6 through lactation</td>
</tr>
<tr>
<td></td>
<td>Day 21 (if females delivered) or through Day 25</td>
</tr>
<tr>
<td></td>
<td>– 27 (if females failed to deliver)</td>
</tr>
<tr>
<td>Dose volume:</td>
<td>2 mL (2 sites/animal)</td>
</tr>
<tr>
<td>Route of administration:</td>
<td>Subcutaneous injection</td>
</tr>
<tr>
<td>Formulation/Vehicle:</td>
<td>0.9% NaCl</td>
</tr>
<tr>
<td>Species/Strain:</td>
<td>Rat / Crl:WI (Han)</td>
</tr>
<tr>
<td>Number/Sex/Group:</td>
<td>24 females/group</td>
</tr>
<tr>
<td>Satellite groups:</td>
<td>10 F₁ culled on PND 4 – 6 for blood sampling</td>
</tr>
<tr>
<td>Deviation from study protocol:</td>
<td>Did not affect interpretability or integrity of study</td>
</tr>
</tbody>
</table>

Observations and Results
**F₀ Dams**

**Survival:** All females survived to scheduled sacrifice

**Clinical signs:**
- Swelling, scales, and/or general erythema of the head, ears, tail and/or legs were observed 2 to 4 hours after treatment in a dose related matter
  - 20 μg/day: slight swelling of the ears was noted for one female, slight to moderate general erythema of the ear(s) was noted for 23 females, slight general erythema of the tail was noted for 7 females, scales at the ears (slight) was noted for one female, and ten females showed scales at the tail (slight to moderate).
  - 40 μg/day: slight to severe swelling of several body parts (head, ears, snout and/or legs) was noted for 21 females, slight general erythema of the head was seen for one female, slight to severe general erythema of the ears was noted for 23 females, slight general erythema of the tail was noted for 14 females, slight general erythema of the legs was observed for 15 females, scales at the ears (slight) was noted for one female, scales at the tail (slight) was noted for 19 females, and scabs at the ears (slight) was noted for one female.

**Body weight:**
- 40 μg/day: body weight gain was slightly reduced on Days 9 to 20 post-coitum.

**Feed consumption:**
- 40 μg/animal, food consumption before or after allowance for body weight was slightly reduced on Days 6 to 15 post-coitum.

**Uterine content:** number of corpora lutea, implantation sites and number of pregnant females were similar between control and treated animals. No signs of abortion or premature delivery were seen.

**Necropsy observation:** no treatment-related macroscopic findings

**Toxicokinetics (jugular vein):**
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>rhNGF mean concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F₀ Generation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PND 21</td>
<td>BLQ</td>
</tr>
<tr>
<td>20 µg/day</td>
<td>PND 21 - 23</td>
<td>25.7</td>
</tr>
<tr>
<td>40 µg/day</td>
<td>PND 21 – 23</td>
<td>86.9</td>
</tr>
<tr>
<td><strong>F₁ Generation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PND 21</td>
<td>BLQ</td>
</tr>
<tr>
<td>20 µg/day</td>
<td>PND 21 - 23</td>
<td>5.7</td>
</tr>
<tr>
<td>40 µg/day</td>
<td>PND 21 – 23</td>
<td>BLQ</td>
</tr>
</tbody>
</table>

Immunogenicity (total positive samples after immunodepletion to confirm specificity):

<table>
<thead>
<tr>
<th>Generation</th>
<th>Day</th>
<th>Control</th>
<th>20 µg/day</th>
<th>40 µg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₀ females</td>
<td>Day 6 post coitum</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PND 21 – 23</td>
<td>0</td>
<td>10/22</td>
<td>15/23</td>
</tr>
<tr>
<td>F₁ pups</td>
<td>PND 21 – 23</td>
<td>0</td>
<td>12/22</td>
<td>18/23</td>
</tr>
</tbody>
</table>

Dosing Solution Analysis:
Formulation samples showed mean accuracies for Group 2 between 51.6% and 69.7% and for Group 3 between 66.0% and 91.3%. Most samples had accuracies below the lower acceptance range (90%). The lower accuracy levels can be attributed to the thawing process of rhNGF (as the formulation samples were supplied on dry ice to the test site).
F<sub>1</sub> Generation [Sacrificed PND 4 – 6 (culled) or PND 21 – 23]

**Survival:** Pup loss was within normal range through PND 4 and no dose related trend in pup loss was observed. No premature deaths occurred thereafter.

**Clinical signs:** No treatment-related clinical signs were noted

**Body weight:** No toxicologically relevant effect was noted for pup body weights

**Feed consumption:** No toxicologically relevant effect was noted for pup feed consumption

**Physical development:** Males (Balanopreputial separation): comparable to control

Females (Vaginal opening): comparable to control

**Neurological assessment:** Acoustic startle response (PND 60): unaffected by treatment

Locomotor activity (PND 61): unaffected by treatment

Learning and memory test (PND 62 – 68; Biel maze): unaffected by treatment

**Reproduction:** no toxicologically relevant effects on precoital time, indices for mating, fertility and conception, and numbers of corpora lutea, normal implantation sites or resorptions

F<sub>2</sub> Generation:
- dated not collected
11 Integrated Summary and Safety Evaluation

Ocular and systemic safety margins calculated from the NOAELs of the nonclinical studies

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Species</th>
<th>NOAEL or LOAEL (µg/kg)</th>
<th>Safety Margin (based on direct dose comparison of animal to human*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic toxicity</td>
<td>Rat (26-weeks; subcutaneous)</td>
<td>Both sexes: NOAEL is 667 µg/kg</td>
<td>4275-fold</td>
</tr>
<tr>
<td></td>
<td>[333 or 667 µg/kg/day]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rabbit (90-days; subcutaneous)</td>
<td>Male: NOAEL is 111 µg/kg</td>
<td>711-fold</td>
</tr>
<tr>
<td></td>
<td>[56 µg/kg/day and 111 µg/kg/day]</td>
<td>Female (ovarian findings): NOAEL is 56 µg/kg/day, based on ovarian findings at 111 µg/kg/day.</td>
<td>356-fold</td>
</tr>
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<tr>
<td></td>
<td>Rat (26-weeks; ocular)</td>
<td>Male: NOAEL is 240 µg/kg/day</td>
<td>1538-fold</td>
</tr>
<tr>
<td></td>
<td>[120, 160 and 240 µg/kg/day]</td>
<td>Female: NOAEL is 160 µg/kg/day, based on ovarian findings at 240 µg/kg/day.</td>
<td>1026-fold</td>
</tr>
<tr>
<td></td>
<td>Rabbit (2-month ocular)</td>
<td>Male: NOAEL is 37 µg/kg/day</td>
<td>237-fold</td>
</tr>
<tr>
<td></td>
<td>[19 or 37 µg/kg/day]</td>
<td>Female: LOAEL is 19 µg/kg/day, based on ovarian findings.</td>
<td>119-fold</td>
</tr>
<tr>
<td>Toxicity Type</td>
<td>Species</td>
<td>Dose Range</td>
<td>NOAEL</td>
</tr>
<tr>
<td>-------------------------------------</td>
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</tr>
<tr>
<td><strong>Ocular toxicity</strong></td>
<td>Rat (26-week ocular)</td>
<td>[9, 12 and 18 µg/eye/day]</td>
<td>18µg/eye/day</td>
</tr>
<tr>
<td></td>
<td>Rabbit (125-day ocular)</td>
<td>[54, 72 and 108 µg/eye/day]</td>
<td>108 µg/eye/day</td>
</tr>
<tr>
<td><strong>Developmental and Reproductive Toxicity</strong></td>
<td>Rat</td>
<td>[20 or 40µg/animal/day]</td>
<td></td>
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<tr>
<td></td>
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</tr>
<tr>
<td>Rabbit</td>
<td>Embryo-fetal: NOAEL is 42 µg/kg/day for fetal malformations, based on increased incidence of cardiac anomalies at 83 µg/kg/day (534-fold human dose)</td>
<td></td>
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<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td></td>
<td>LOAEL is 42 µg/kg/day for postimplantation loss</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

267-fold

*Proposed human dose: 4.68 µg/eye/day, or 9.36 µg/day (0.156 µg/kg/day) total daily dose following bilateral administration*
This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

AARON M RUHLAND
06/07/2018

LORI E KOTCH
06/07/2018
I concur with the Pharmacology/Toxicology review conclusions and recommendations.