

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**761092Orig1s000**

**NON-CLINICAL REVIEW(S)**

## **Tertiary Pharmacology/Toxicology Review**

**From:** Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**BLA:** 761092

**Agency receipt date:** October 24, 2017

**Drug:** Revcovi (elapegamase [SC-PEG rADA])

**Sponsor:** Leadiant Biosciences, Inc.

**Indication:** Treatment of adenosine deaminase severe combined immune deficiency (ADA-SCID)

**Reviewing Division:** Division of Transplant and Ophthalmology Products

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

Revcovi (elapegamase [SC-PEG rADA]) is a PEGylated recombinant adenosine deaminase. The only previously approved enzyme replacement therapy for the treatment of ADA-SCID is Adagen, approved in 1990, also owned by the applicant. Revcovi was developed [REDACTED] (b) (4) with the key differences being the replacement of bovine intestinal adenosine deaminase with a recombinant product and the succinimidyl succinate linker with [REDACTED] (b) (4) succinimidyl carbamate (SC) linker. Each vial of drug product contains 1.5 mL of a 1.6 mg/mL solution that is intended for intramuscular injection.

The nonclinical program for Revcovi consisted of a bridging program to Adagen given the structural and pharmacological comparability with Adagen, the use of [REDACTED] (b) (4) PEG SC linker in FDA approved products, and the extensive clinical experience with Adagen. Pharmacokinetic studies demonstrated a longer terminal half-life and higher systemic exposure with SC-PEG rADA compared to Adagen. However, the toxicity profiles in 4-week studies were comparable; the primary finding was a slight increase in APTT at doses that resulted in systemic exposures that were 0.5 to 1.9 times the anticipated clinical exposure.

### **Conclusion:**

I agree with the Division pharmacology/toxicology conclusion that Revcovi can be approved from the nonclinical perspective. I have reviewed the proposed text for the nonclinical sections of the product label and have discussed them with the Division review team.

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/s/  
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TIMOTHY J MCGOVERN  
08/20/2018

**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: 761092

Supporting document/s: SD # 1: Nonclinical Information  
SD # 5: New BLA (Labeling)

Applicant's letter date: SD # 1: 7-14-2017  
SD # 5: 10-24-2017

CDER stamp date: SD # 1: 7-14-2017  
SD # 5: 10-24-2017

Product: REVCOVI (Elapegademase [SC-PEG rADA])

Indication: Treatment of adenosine deaminase severe  
combined immune deficiency (ADA-SCID)

Applicant: Leadiant Biosciences, Inc. (Leadiant, formerly  
Sigma-Tau Pharmaceuticals, Inc.)

Review Division: Transplant and Ophthalmology Products

Reviewer: María I. Rivera, PhD

Supervisor/Team Leader: Lori E. Kotch, PhD, DABT

Division Director: Renata Aldrich

Project Manager: Jacquelyn Smith

*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

Leadiant Biosciences, Inc. (Leadiant) has developed a PEGylated recombinant adenosine deaminase (SC-PEG rADA) for the treatment of adenosine deaminase severe combined immune deficiency (ADA-SCID). This proposed indication is the same as that of Leadiant's Adagen® drug product (NDA 19-818; approved in 1990). The key differences between SC-PEG rADA and Adagen® are the replacement of bovine intestinal adenosine deaminase (ADA) with a recombinant product (manufactured in *E. coli*) and the succinimidyl succinate (SS) linker by (b) (4) succinimidyl carbamate (SC) linker.

Leadiant received Orphan Drug Designation for SC-PEG rADA for this indication on March 19, 2015 (Orphan Drug Application 14-4675). The application also received Priority review classification (correspondence filed 7-27-2018).

## 1.2 Brief Discussion of Nonclinical Findings

A pharmacokinetic (PK) and pharmacodynamic (PD) bridging toxicology strategy was used in the nonclinical evaluations given (1) the 25-year successful clinical history with Adagen®, (2) the structural and pharmacological comparability of Adagen® and SC-PEG rADA and (3) the safe use of the stable PEG SC linker in other FDA approved biologics.

The nonclinical PK studies showed a longer terminal half-life ( $t_{1/2}$ ) and systemic exposure (AUC), as measured by ADA enzymatic activity, for SC-PEG rADA compared to Adagen®. These PK differences did not result in increased toxicity in the 4-week general toxicology studies (see below). Moreover, SC-PEG rADA was slightly more efficacious than Adagen® in the ADA-deficient mouse model, i.e., ADA-deficient mice treated with SC rPEG-ADA showed a longer life span than those treated with Adagen®. These results likely reflect SC-PEG rADA more favorable PK.

Early nonclinical studies were not conducted with the to-be-marketed formulation. These early lots of SC-PEG rADA were well-tolerated in repeat-dose toxicity studies in rats and dogs when given every 3 to 4 days for 4 weeks (nine doses total). Drug-related findings in rats and dogs were limited to a slight increase in APTT that was reversible or partially reversible during a 4-week recovery period. The NOAEL in both rats and dogs was the highest dose, 300 U/kg 2x/week (equivalent to 1.92 mg/kg) SC-PEG rADA. These doses resulted in exposure margins of 0.54-fold (rat) and 1.86-fold (dogs) the mean human AUC normalized to the dose of SC-PEG rADA administered per patient.

The to-be-marketed formulation given every 3 or 4 days for 4 weeks (eight doses total) at a dose of 500 U/kg to rats did not result in drug related adverse effects. As noted in earlier studies, a slight prolongation of APTT was observed. This study also included an arm with SC-PEG rADA spiked with (b) (4) % (b) (4) impurity excess. A (b) (4) % (b) (4) (b) (4) excess of impurity was used to give an adequate exposure margin for establishing

impurity specification limits. The current specification limit for the impurity is not more than (NMT) (b) (4)%. The presence of (b) (4)% impurities in the test article had no effects on safety profile, TK, or immunogenicity, compared to unspiked SC-PEG rADA. The results from this study show that the safety profile of the to-be-marketed product is the same as the safety profile from earlier lots of SC-PEG rADA. The NOAEL for this study was 500 U/kg 2x/week (3.22 mg/kg 2x/week). Based on AUC, this dose resulted in an exposure margins of 0.71-fold the mean human AUC normalized to the dose of SC-PEG rADA administered per patient.

The applicant conducted a dose-ranging embryo-fetal development study. No adverse maternal or embryofetal findings were observed. The study was not sufficiently powered to establish a reliable NOAEL, based on the low number of animals evaluated per group. The highest dose tested in this study was 500 U/kg/day (nominal 1.48 mg/kg/day [2.96 mg/kg/week] per this reviewer’s calculations; 1.76 mg/kg/day [3.52 mg/kg/week] using actual protein concentration, (b) (4)

The conducted nonclinical studies established comparable pharmacological and toxicological profiles for SC-PEG rADA and Adagen®. As such, the 25-year clinical experience with Adagen® use provides additional support for the safety of chronic treatment with SC-PEG rADA. Overall, the nonclinical data presented support the approval of the marketing application of SC-PEG rADA for the treatment of SCID due to ADA deficiency. Given the structural and pharmacological comparability and similar toxicity profile of Adagen® and SC-PEG rADA, additional nonclinical reproductive studies are not considered necessary for approval. Pharmacovigilance data can be monitored for potential drug-related effects post-marketing.

**1.3 Recommendations**

**1.3.1 Approvability**

Approval is recommended.

**1.3.2 Additional Non-Clinical Recommendations**

**1.3.3 Labeling**

Applicant’s Proposed Text	Reviewer’s recommendations
8.1 Pregnancy	8.1 Pregnancy
Risk Summary	Risk Summary

<p>(b) (4)</p>	<p>Adequate and well-controlled studies with REVCOVI have not been conducted in pregnant women to inform a drug-associated risk. Animal reproduction studies have not been conducted with REVCOVI. It is not known whether REVCOVI can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. (b) (4)</p> <p>All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.</p> <p><i>[We defer assessment of the adequacy of clinical data and proposed labeling language to the clinical team]</i></p>
<p><i>Human</i> No pregnancy was reported for any patients receiving TRADE NAME. (b) (4)</p>	
<p>(b) (4)</p>	
<p>8.2 Lactation <u>Risk Summary</u> (b) (4) lactation studies have not been conducted to assess the presence of TRADE</p>	<p>8.2 Lactation <u>Risk Summary</u> Human or animal lactation studies have not been conducted to assess the presence of REVCOVI in</p>

<p>NAME in breast milk, the effects on the breastfed (b) (4) or the effects on milk production for the mother.</p> <p>The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for TRADE NAME and any potential adverse effects on the breastfed infant from TRADE NAME or from the underlying maternal condition.</p>	<p>breast milk, the effects on the breastfed (b) (4) <i>infant</i>, or the effects on milk production for the mother.</p> <p>The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for REVCovi and any potential adverse effects on the breastfed infant from TRADE NAME or from the underlying maternal condition.</p>
<p><b>10. OVERDOSAGE</b>                  There are no reports of administration of TRADE NAME in excess of the prescribed doses. The highest weekly prescribed dose administered in the clinical studies was 0.4 mg/kg. In nonclinical studies (b) (4)</p>	<p><b>10. OVERDOSAGE</b>                  There are no reports of administration of REVCovi in excess of the prescribed doses. The highest weekly prescribed dose administered in the clinical studies was 0.4 mg/kg. In nonclinical studies, there was no evidence of toxicity related to study drug at doses up to 1.8-fold the clinical dose (based on mean human AUC normalized to the dose of REVCovi administered per patient), except for a slight increase in APTT.</p>
<p><b>13 NONCLINICAL TOXICOLOGY</b></p> <p><b>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</b>                  Long-term studies in animals to evaluate carcinogenic potential or studies to evaluate mutagenic potential have not been performed with TRADE NAME.</p>	<p><b>13 NONCLINICAL TOXICOLOGY</b></p> <p><b>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</b>                  Long-term studies in animals to evaluate carcinogenic potential or studies to evaluate mutagenic potential and <i>impairment of fertility</i> have not been performed with REVCovi.</p>
<p>(b) (4)</p>	

(b) (4)

## 2 Drug Information

### 2.1 Drug

CAS number: 1709806-75-6

Generic Name: Elapegademase

Code Name: SC-PEG rADA; EZN-2279; PEG-rADA; EZ-002 ( (b) (4) project number); additional codes used in nonclinical study reports: PEG-SC-rADA, SC-rBADA-Mut, SC-rbADA-cys 75-Mut

Chemical Name: Poly (oxy-1,2-ethanediyl),  $\alpha$ -carboxy- $\omega$ -methoxyamide with adenosine deaminase (synthetic)

Molecular Formula/Molecular Weight:

- rADA:  $C_{1797}H_{2795}N_{477}O_{544}S_{12}$  (peptide monomer)/40,171  $\pm$  5 Da
- After pegylation: 113 kDa

Structure or Biochemical Description: Recombinant ADA is a monomeric protein. The amino acid sequence of rADA (C74S), comprised of 356 amino acid residues, is listed in Figure 1. (b) (4)

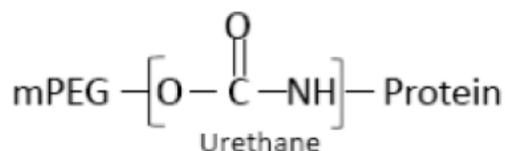
Adagen<sup>®</sup>'s active component is bovine ADA enzyme derived from bovine intestine (b) (4)

**Figure 1: Amino Acid Sequence of Recombinant Adenosine Deaminase (rADA)**

1	11	21	31	41
AQTPAFNKPK	VELHVHLDGA	IKPETILYYG	RKRGIALPAD	TPEELQNIIG
51	61	71	81	91
MDKPLSLPEF	LAKFDYYMPA	IAGSREAVKR	IAYEFVEMKA	KDGVVYVEVR
101	111	121	131	141
YSPHLLANSK	VEPIPWNQAE	GDLTPDEVVS	LVNQGLQEGE	RDFGVKVRSI
151	161	171	181	191
LCCMRHQPSW	SSEVVELCKK	YREQTVVAID	LAGDETIEGS	SLFPGHVKAY
201	211	221	231	241
AEAVKSGVHR	TVHAGEVGSA	NVVKEAVDTL	KTERLGHGYH	TLEDTTLYNR
251	261	271	281	291
LRQENMHFEV	CPWSSYLTGA	WKPDTEHPVV	RFKNDQVNYS	LNTDDPLIFK
301	311	321	331	341
STLDTDYQMT	KNEMGFTEEE	FKRLNINAAK	SSFLPEDEKK	ELLDLLYKAY
351				
GMPSA				

SC-PEG rADA (elapegademase) is produced by *E. coli* and is covalently conjugated to monomethoxypolyethylene glycol (mPEG) using a succinimidyl carbonate (SC) linker. See schematic formula in Figure 2.

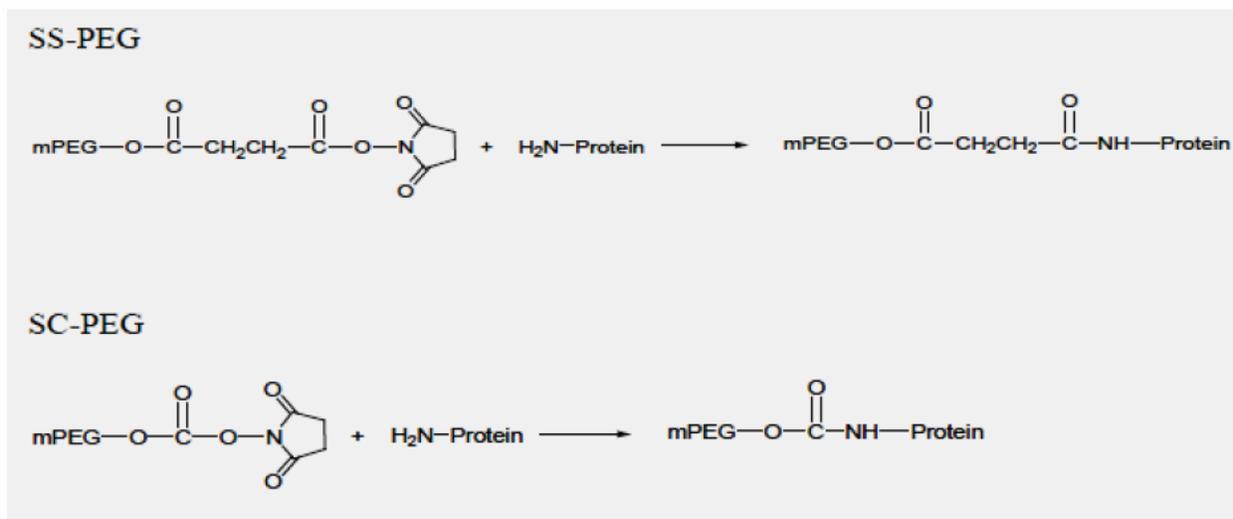
**Figure 2: Structure of SC-PEG rADA**



Therefore, SC-PEG rADA consists of three components:

- rADA Drug Substance (b) (4) derived from (b) (4) *E. coli* (b) (4) and manufactured by (b) (4)
- Monomethoxy polyethylene glycol (mPEG) molecule, approximately 5.6 kD molecular weight
  - In SC-PEG rADA, approximately 13 mPEG molecules are attached to each rADA protein molecule through lysine (K) residues. Thus, after PEGylation, the rADA modified monomer mass is approximately 113 kDa (as detected by (b) (4)).
- Carbamate linker that links the mPEG molecules to the  $\epsilon$ -amino acids of lysine and N-terminal alanine residues on the rADA enzyme
  - SC-PEG rADA uses (b) (4) succinimidyl carbamate (SC) PEG linker in place of the succinimidyl succinate (SS) PEG linker currently used in Adagen<sup>®</sup> (NDA 19-818; FDA approved on 1990).
  - (b) (4)
  - Figure 3 demonstrates the difference in the linker structure between the SC-PEG and SS-PEG protein conjugates. The linkage formed between SC-PEG and a lysine residue is a urethane (carbamate) linkage (b) (4)

**Figure 3: Structures of SS-PEG and SC-PEG Linkers in Protein Conjugates**



## Pharmacologic Class: TBD

The established pharmacological class for pegademase bovine is “bovine intestinal adenosine deaminase”. The applicant proposes (b) (4). The review team is proposing “recombinant bovine adenosine deaminase”.

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

- NDA 19818 – Adagen® (Pegademase Bovine; Leadiant Biosciences, Inc; approved 3-21-1990)
- IND 100687 – EZN-2279 (PEGylated recombinant adenosine deaminase [PEG-rADA], Leadiant Biosciences, Inc)

## 2.3 Drug Formulation

SC-PEG rADA (elapegademase) Drug Product is formulated as a sterile, isotonic, (b) (4) solution for intramuscular administration. The solution is a preservative-free, clear, colorless liquid with no visible particulates.

Each vial contains 1.5 mL of a 1.6 mg/mL solution of methoxypolyethylene glycol recombinant adenosine deaminase (SC-PEG rADA) in (b) (4) saline ((b) (4) (b) (4) pH 6.9, (b) (4) % sodium chloride). The composition of the SC-PEG rADA Drug Product, 2.4 mg/vial, is provided in Table 1.

**Table 1: Composition of SC-PEG rADA Drug Product, 2.4 mg/Vial**

Ingredient	Amount per Vial (mg)	Rationale for Use	Reference to Quality Standard
SC-PEG rADA	2.4	Active Ingredient	Meets Established cGMP Specifications
Sodium Chloride	12.75	(b) (4)	USP, Ph. Eur., BP, JP
Sodium Phosphate Dibasic Heptahydrate	12.70		USP
Sodium Phosphate Monobasic Monohydrate	3.81		USP, Ph. Eur., BP, JPE <sup>a</sup>
Water for Injection, USP, EP	(b) (4)		USP, Ph. Eur., JP

Key: USP = United States Pharmacopeia, Ph. Eur. = European Pharmacopeia, BP = British Pharmacopoeia, JP = Japanese Pharmacopoeia, JPE = Japanese Pharmaceutical Excipients, NF = National Formulary; (b) (4)

<sup>a</sup> Sodium phosphate monobasic monohydrate is not listed in the JPE. This specification is cited from the JPE monograph (b) (4)

## 2.4 Comments on Novel Excipients

There are no novel excipients in the drug product formulation.

The SS linker used to link PEG to nADA in Adagen<sup>®</sup> has been replaced by (b) (4) SC linker in SC-PEG rADA (b) (4)

To support the safety of the linker, the applicant listed the following observations:

- The SC-linker provides (b) (4) PEG-protein carbamate linkage (b) (4)
- Safety and efficacy results for SC-PEG rADA from the clinical study demonstrated that detoxification was maintained with no increase in safety events, no clinical deterioration and no increase in the development of antidrug antibodies relative to Adagen<sup>®</sup> clinical dosing.
- The safety of the SC linker has been established through its use in other approved PEGylated products (PEG-Intron, approved in 2001 for treatment of hepatitis C, and Krystexxa<sup>®</sup>, approved in 2010 for treatment of chronic gout).
  - PEG-Intron<sup>®</sup> (Peginterferon alpha-2b), a mono-PEGylated INF- $\alpha$ 2b, is synthesized using a 12-kDa succinimidyl carbonate PEG reagent (mPEG SC-12 kDa)<sup>1</sup>.
    - PEG-Intron<sup>®</sup> is indicated for chronic hepatitis C in patients  $\geq 3$  years old with compensated liver disease (weekly doses of 40  $\mu$ g to 150  $\mu$ g SC for one year).
  - Krystexxa<sup>®</sup> (Pegloticase), is a uricase tetramer where each subunit contains approximately 9 molecules of 10 kDa PEG<sup>2</sup>
    - Per information in Turecek et al<sup>2</sup>, publically available documents do not describe the PEG reagent used, but the statement of a nonproprietary name adopted by the US Adopted Name Council shows a carbamate linkage to the protein.
    - Per information in Alconsel et al<sup>3</sup>, the protein-polymer conjugate is synthesized by the use of a PEG-*p*-nitrophenyl carbonate ester. The *p*-nitrophenol is displaced by primary amine lysine side chains, forming carbamates.
    - Therefore, the mPEG-linker is not identical to REVCOVI but the resulting mPEG-protein carbamate linkage is the same.
    - Krystexxa<sup>®</sup> is indicated for treatment of chronic gout in adult patients refractory to conventional therapy (8 mg as an IV infusion every 2 weeks).

<sup>1</sup> Turecek P., Bossard M., Schoetens F., et al, 2016, PEGylation of Biopharmaceuticals: A Review of Chemistry and Nonclinical Safety Information of Approved Drugs, *J. Pharm. Sci.* 105: 460-475.

<sup>2</sup> Ibid.

<sup>3</sup> Alconsel S.N.S., Baas A.S., and Maynard H.D., 2011, FDA-Approved Poly(Ethylene Glycol)-Protein Conjugate Drugs, *Polym. Chem.* 2: 1442-1448

Nonclinical studies of 4-week duration were conducted with SC-PEG rADA. No significant toxicities were observed. Comparability of the structure and function of pegylated adenosine deaminase has been demonstrated for Adagen®, SC-PEG rADA (old plasmid/old process), and SC-PEG rADA (current plasmid/current process) (see Pharmacology Section below and CMC review). Therefore, the overall data provide support for the safety of the SC linker.

## 2.5 Comments on Impurities/Degradants of Concern

Pending Product Quality team (b) (4) ew.

## 2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is patients with ADA-SCID. The intended dosing regimens are the following:

- Patients currently receiving treatment with Adagen®: The starting weekly intramuscular (IM) dose is 0.2 mg/kg. The dose may be increased by increments of 0.033 mg/kg weekly if trough ADA activity is under 30 mmol/hr/L, deoxyadenosine nucleotides (dAXP) is above 0.02 mmol/L, and/or the immune reconstitution is judged as inadequate by the treating physician's medical assessment of the patients' clinical status. Total weekly dose administration may be divided in multiple IM administrations.
- Adagen-naïve patients: The starting weekly IM dose is 0.4 mg/kg of ideal body weight (divided into twice a week) for a minimum of 12 to 24 weeks (b) (4). Once immune reconstitution is achieved, the dose may be gradually adjusted down to maintain trough ADA activity over 30 mmol/hr/L, dAXP under 0.02 mmol/L, and to maintain adequate immune reconstitution as judged the treating physician.

## 2.7 Regulatory Background

A Pre-IND meeting was held on July 10, 2007. The briefing package was submitted on June 8, 2007. The nonclinical plan for IND submission was found acceptable. The applicant was asked to provide a justification as to why they considered that nonclinical reproductive studies were not needed (see meeting minutes filed in DARRTS on August 9, 2007).

The IND was submitted on November 3, 2009. The nonclinical team found the nonclinical data provided adequate safety information for the proposed clinical trial to proceed (see 30-day safety memo filed in DARRTS on December 7, 2009).

A pre-BLA briefing package was submitted on September 21, 2016. The FDA concurred with the overall nonclinical program for the BLA (see meeting minutes filed in

DARRTS on October 21, 2016; SD # 52 nonclinical review filed in DARRTS on October 10, 2016), pending there we no outstanding CMC issues that require nonclinical qualification. The following recommendations were conveyed:

- *The adequacy of the ongoing dose-finding embryofetal toxicity study to support this application, in conjunction with other supporting data, will be a review issue.*
- *In addition to the justification for not conducting the full battery of reproductive toxicology studies, the BLA should include pharmacovigilance evidence of successful pregnancies and lack of infertility in ADA-SCID patients treated with Adagen®.*
- *Provide all available information to support safety of the PEG SC linker.*

The pre-BLA meeting scheduled for on October 18, 2018 was canceled after the applicant received the preliminary comments. Instead, a teleconference was held with the Project Manager and Division Director to further clarify the Division's requests for information on successful pregnancies and effects on fertility and CMC information regarding the safety of the SC linker (see meeting minutes filed in DARRTS on October 21, 2016).

### **3 Studies Submitted**

#### **3.1 Studies Reviewed**

##### **Primary Pharmacology**

- Pharmacodynamics of SS nPEG-ADA, SS rPEG-ADA and SC rPEG-ADA in Adenosine Deaminase-Deficient Mice: Survival Study (Study # 0007330)
- Analysis of Recombinant Bovine PEG-ADA in Adenosine Deaminase Deficient Mice (Study # AD09005)

##### **PK/ADME**

- Comparative Pharmacokinetic (PK) Study of Pegylated Adenosine Deaminase in Rats (non-GLP) (Study # AN07001)
- SC-rBADA-cys75-Mut (Single mutant) and Adagen® Comparative Pharmacokinetic (PK) Study of Pegylated Adenosine Deaminase in Rats (non-GLP) (Study # AN07016)
- Plasma Stability of PEGylated Recombinant Human ADA and PEGylated Native Bovine ADA (Study # E1402-100)
- EZN-2242 (Adagen®), EZN-2279 (SC-PEG rADA), and PEG-SS-rADA Single-Dose Comparative Pharmacokinetic Study in Rats via Intramuscular Administration with a 14-Day Observation Period (Study # AD09004) (non-GLP)
- EZN-2242 (ADAGEN®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Rats via Intramuscular Administration with a 14-Day Observation Period (Study # 07-2018/AD07027) (non-GLP)

- EZN-2242 (Adagen®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Dogs via Intramuscular Administration with a 14-Day Observation Period (Study # 07-3265/AD07026) (non-GLP)

### **General Toxicology**

- EZN-2242 (ADAGEN®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Rats via Intramuscular Administration with a 14-Day Observation Period (Study # 07-2018/AD07027) (non-GLP)
- EZN-2242 (Adagen®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Dogs via Intramuscular Administration with a 14-Day Observation Period (Study # 07-3265/AD07026) (non-GLP)
- EZN-2279: A Toxicity and Toxicokinetic Study in Rats Following 4-Week Intramuscular Injections with a 4-Week Recovery Period (Study # 15-2483/STPI130260A) (GLP)
- Contributing Report: EZN-2279: A Local Toxicity/Pathology Study in Rats Following 4 Weeks of Intramuscular Injections with a 4-Week Recovery Period (Study # JF96NG) (GLP)
- EZN-2279: A 4-Week Intramuscular Toxicity/Toxicokinetic Study in Rats with A 4-Week Recovery Period (Study # 07-2030/AD07028) (GLP)
- EZN-2242 (Adagen®) and EZN-2279: A Comparative 4-Week Intramuscular Pharmacokinetic and Immunogenicity Study in Rats (Study # 08-2060/AD08001) (GLP)
- EZN-2279: A 4-Week Intramuscular Toxicity/Toxicokinetic Study in Dogs with A 4-Week Recovery Period (Study # 07-3280/AD07029) (GLP)

### **Embryofetal Development**

- A Dose Range-Finding Embryo-Fetal Development Study of EZN-2279 by Intramuscular Injection in Rats (Study # 20098870/STPI20098870) (GLP)

### **Special Toxicology**

- Nociceptive Effects by Test Items following Intra-plantar Injection in Mouse (Study # 5900166) (non-GLP)

## **3.2 Studies Not Reviewed**

Studies under Module 4.2.2.1. Analytical Methods and Validation Report

## **3.3 Previous Reviews Referenced**

- IND 100687 30-Day Safety Memo (Ying Mu, MD, PhD; filed in DARRTS on 12-7-2009)
- IND 100687 SD # 52 (Pre-BLA) nonclinical review (María I Rivera, PhD; filed in DARRTS on 10-12-2016)

## 4 Pharmacology

### 4.1 Primary Pharmacology

Brief summaries of these studies are given below. For further details, see Microbiology/Immunology review by Shukal Bala, PhD.

#### **Pharmacodynamics of SS nPEG-ADA, SS rPEG-ADA and SC rPEG-ADA in Adenosine Deaminase-Deficient Mice: Survival Study (Study # 0007330)**

ADA-deficient mice were injected intraperitoneally (IP) with varying dosages (0.1, 1, and 5 units) of Adagen® (SS nPEG-ADA), SS rPEG-ADA and SC rPEG-ADA (clinical form) on postnatal day 18, and survival was monitored. Results were compared to the survival of untreated ADA-deficient mice.

ADA-deficient mice treated with SC rPEG-ADA (clinical form) showed a longer life span than Adagen® (SS nPEG-ADA) or SS rPEG-ADA. This data is consistent with the extended half-life of SC rPEG-ADA relative to SS nPEG-ADA and SS rPEG-ADA. The relative differences in survival between all 3 ADA compounds were best noted at the lowest (0.5 Unit) dosage used.

#### **Analysis of Recombinant Bovine PEG-ADA in Adenosine Deaminase Deficient Mice (Study # AD09005)**

**Single Dose Treatment:** On postnatal day 18 mice were injected IP with 5 Units of Adagen® or recombinant bovine PEG-ADA (clinical form). Untreated ADA<sup>+/+</sup> and ADA<sup>-/-</sup> mice were sacrificed on postnatal day 18, while mice treated with PEG-ADA were sacrificed 72 hours (day 21) following PEG-ADA injection. ADA levels in bronchial alveolar lavage fluid (BALF) and ADA enzymatic activity in the blood were measured.

The main results (listed below) indicate that both recombinant bovine PEG-ADA behaved similarly in ADA<sup>-/-</sup> mice.

- The blood concentration of PEG-ADA in the form of either Adagen® or recombinant bovine PEG-ADA was comparable in both ADA<sup>+/+</sup> and ADA<sup>-/-</sup> mice at 72 hours post injection.
- Adenosine concentrations in the BALF fluid of ADA<sup>-/-</sup> mice were more than 7 times higher than those measured in the BALF of age matched ADA<sup>+/+</sup> littermates. Adagen® and recombinant bovine PEG-ADA worked comparably well in lowering the lung levels of endogenous adenosine in ADA<sup>+/+</sup> mice and adenosine elevations in ADA<sup>-/-</sup> mice.
- ADA<sup>-/-</sup> mice die between postnatal day 18 and 21. Per summary information, treatment of these mice with Adagen® or recombinant bovine PEG-ADA resulted

in the improvement of outward signs of respiratory distress, and all mice were still alive on postnatal day 21 when they were sacrificed for the studies described above.

**Multiple Dose Treatment:** ADA<sup>-/-</sup> and ADA<sup>+/+</sup> mice were administered 5 Units of Adagen<sup>®</sup> or PEG-rADA, intramuscularly (IM), every 4 days until postnatal Day 21, when the mice were sacrificed and tissues were harvested. Parameters evaluated consisted of body weights, thymus and spleen weights, thymocyte and splenocyte counts, thymus adenosine and deoxyadenosine levels, spleen adenosine and deoxyadenosine levels, and plasma ADA specific activity.

- Higher plasma levels of ADA activity in PEG ADA-treated ADA<sup>-/-</sup> mice correlated with statistically lower adenosine levels in the thymus compared with Adagen<sup>®</sup>, as well as a consistent trend toward a greater degree of improvement of most endpoints (body weight, survival, thymocyte and splenocyte cell number, adenosine levels in spleen) when compared with Adagen<sup>®</sup>-treated ADA<sup>-/-</sup> mice.

## 4.2 Secondary Pharmacology

No studies were conducted.

## 4.3 Safety Pharmacology

Safety pharmacology studies were not considered necessary based on the following observations:

- The lack of significant toxicological findings in the 4-week repeated-dose toxicity studies
- The structural, pharmacological, and toxicological similarity of SC-PEG rADA to Adagen<sup>®</sup>
- The 25-year successful clinical use history with Adagen<sup>®</sup>
- The safe use of the stable SC-PEG linker in chronic use medications

# 5 Pharmacokinetics/ADME/Toxicokinetics

## 5.1 PK/ADME

**Comparative Pharmacokinetic (PK) Study of Pegylated Adenosine Deaminase in Rats (non-GLP) (Study # AN07001)** – A single intravenous (IV) dose (126 U/kg) of various unpegylated and pegylated glutathione and recombinant adenosine compounds was administered to female Sprague-Dawley rats (5/group). Blood was collected at various timepoints up to 168 hours postdose.

Unpegylated glutathione and mutated recombinant ADA had a terminal elimination half-life ( $t_{1/2\beta}$ ) of 14 and 17 minutes, respectively. Pegylation of recombinant bovine ADA resulted in significant increase of their half-lives (Table 2). ANOVA analysis of the PK estimates for all pegylated conjugates and Adagen<sup>®</sup> indicated a significant difference between the  $t_{1/2\beta}$  of PEG-rBADA-Mut [clinical form] vs PEG-rBADA-GS ( $p < 0.05$ ) and between PEG-rBADA-Mut vs. Adagen<sup>®</sup> ( $p < 0.05$ ). Further, significant difference was observed between the AUC of PEG-rBADA-Mut vs. Adagen<sup>®</sup> ( $p < 0.001$ ) and between PEG-rBADA-GS vs. Adagen<sup>®</sup> ( $p < 0.001$ ).

**Table 2: Pharmacokinetic Estimates of Pegylated Native and Recombinant Bovine ADA.**

	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	C <sub>max</sub> (mIU/ml)	CL (mL/h/kg)	V <sub>ss</sub> (mL/kg)	AUC (h*mIU/mL)
SC-rBADA-Mut	6.0 ± 2.5	56 ± 7.5	3449 ± 282	0.80 ± 0.06	59 ± 2.9	158467.8 ± 11232.4
SC-rBADA-GS	4.6 ± 2.0	43 ± 3.7	3678 ± 170	0.86 ± 0.04	50 ± 2.7	147619.8 ± 7238.3
Adagen	4.9 ± 2.5	41 ± 9.4	3480 ± 137	1.16 ± 0.13	61 ± 9.2	109424.6 ± 12219.0

**SC-rBADA-cys75-Mut (Single mutant) and Adagen<sup>®</sup> Comparative Pharmacokinetic (PK) Study of Pegylated Adenosine Deaminase in Rats (non-GLP) (Study # AN07016)**

The study compared the PK profiles of SC-rBADA-cys 75-Mut (clinical form) and Adagen<sup>®</sup>, when administered as a single IV dose (126 U/kg) to female Sprague Dawley Rats (4/group). Blood was collected at various timepoints up to 168 hours postdose. The pegylated conjugate SC-rBADA-cys75-Mut and Adagen<sup>®</sup> had similar plasma PK profile; none of the PK estimates were significantly different (Table 3).

**Table 3: Pharmacokinetic Estimates of SC-rBADA-cys 75-Mut [Clinical Candidate] and Adagen<sup>®</sup>**

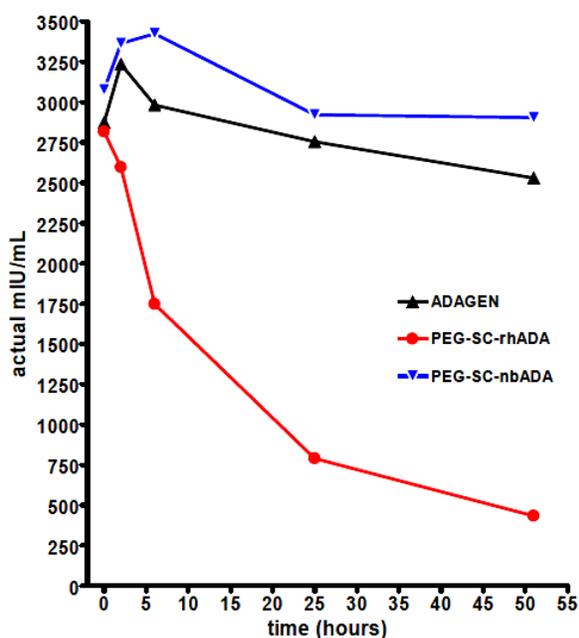
	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	C <sub>max</sub> (mIU/ml)	CL (mL/h/kg)	V <sub>ss</sub> (mL/kg)	AUC (h*mIU/mL)
SC-rBADA-cys75-Mut (Single Mutant)	3.7 ± 3.6	38 ± 12.1	4521 ± 557	0.93 ± 0.06	45 ± 7.9	136565 ± 9264
Adagen	2.7 ± 2.2	34 ± 3.4	5094 ± 504	0.9 ± 0.07	42 ± 2.1	140643 ± 10261

**Reviewer Note:** There was no explanation for the lack of a significant difference in  $t_{1/2}$  values observed in this study, in contrast to the longer value observed for the clinical form in Study # AN07001 above.

**Plasma Stability of PEGylated Recombinant Human ADA and PEGylated Native Bovine ADA (Study # E1402-100)** – The study was conducted to compare the stability of human recombinant form of ADA, which was PEGylated via the SC linker (SC-PEG-rhADA), Adagen® (SS-PEG nADA), and native bovine ADA (nbADA) that was PEGylated via the SC linker (SC-PEG nbADA). The stability of each ADA sample at various time points over a 50-hour period was examined by determining the rate of depletion of the adenosine substrate in the enzymatic reaction mixture as a result of the residual ADA activity still present in each sample.

Recombinant human SC-PEG ADA (PEG-SC-rhADA) exhibited inferior in vitro stability in human plasma compared to both bovine Adagen® and the bovine ADA enzyme with (b) (4) succinimidyl carbamate linker (SC-PEG nbADA) (Figure 4). Based on these results, SC-PEG rADA was developed (b) (4) and was manufactured using recombinant bovine DNA.

**Figure 4: Plasma Stability of Recombinant Human ADA and Native Bovine ADA**



**EZN-2242 (Adagen®), EZN-2279 (SC-PEG rADA), and PEG-SS-rADA Single-Dose Comparative Pharmacokinetic Study in Rats via Intramuscular Administration with a 14-Day Observation Period (Study # AD09004; non-GLP)**

Three groups of male and female rats (6/sex/test article) received a single IM dose (150 U/kg) of EZN-2242, EZN-2279 (clinical compound; lot # 9167) and PEG-SS-rADA on Day 1. Blood samples were collected at pre-dose, 5 min and 4, 8, 24, 48, 72, 120, 192,

264 and 336 hours postdose. The 3 compounds with their associated properties are shown below:

Compound	Adenosine deaminase	Linker
EZN-2242	Native	SS
ENZ-2279	Recombinant	SC
PEG-SS-rADA	Recombinant	SS

The clinical compound (EZN-2279) showed prolonged  $t_{1/2}$  compared to Adagen® (EZN-2242), while the  $t_{1/2}$  of Adagen® and PEG-SS-rADA (both having the SS linker) was similar. The data support that the increase in  $t_{1/2}$  of EZN-2279 compared to EZN-2242 and PEG-SS-rADA can be attributed to SC linker. The combined (male and female) mean PK parameters amongst the 3 compounds are listed in Table 4. No gender differences were observed.

**Table 4: Overall Mean Pharmacokinetic Parameters Stratified by Compound**

Compound	Mean Parameter (Unit)				
	$t_{1/2}$	AUC <sub>0-∞</sub>	Cl/F	Vz/F	C <sub>max</sub>
	(hr)	(hr*mU/mL)	(mL/hr/kg)	(mL/kg)	(mU/mL)
EZN-2242	40.2	71850	2.11	122	850
EZN-2279	61.1	98895	1.53	134	950
PEG-SS-rADA	42.1	91715	1.67	102	1029

**EZN-2242 (Adagen®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Rats via Intramuscular Administration with a 14-Day Observation Period (Study # 07-2018/AD07027; non-GLP)**

Sprague-Dawley rats (10/sex/group) received single IM injections of either Adagen® or the clinical compound, SC-PEG rADA (batch # P-E-1490-30-050707; 99.9% pure), at doses of 30 or 150 U/kg. The vehicle was phosphate-buffered saline (PBS) with 50 mM phosphate and 0.85% saline at pH 7.2 to 7.4. Blood samples for PK analyses of ADA activity were obtained at pre-dose, 5 minutes postdose, and 4, 8, 24, 48, 72, 120, 192, 264, and 336 hours postdose. The plasma PK parameters for Adagen® (EZN-2242) and SC-PEG-rADA (EZN-2279) were estimated based on individual enzyme activity-time profiles.

Systemic exposures (AUC<sub>last</sub> and C<sub>max</sub>) increased approximately proportionally with dose, suggesting linear PK over the examined dose levels of Adagen® or SC-PEG-rADA (Table 5). Based on the statistics report, SC-PEG rADA showed higher C<sub>max</sub> (12% to 23%),  $t_{1/2}$  (11% to 50%), and AUC<sub>last</sub> values compared to Adagen®. The calculated mean  $t_{1/2}$  varied from ~49 to 61 hours for SC-PEG-rADA and ~36 to 50 hours for Adagen®.

Mean PK parameters were generally slightly higher in males compared to females for both compounds.

**Table 5: Mean Plasma PK Parameters for ADA Activity in Rats Following Single Intramuscular Injection of Adagen® or SC-PEG rADA (Study # 07-2018/AD07027)**

Sex	Treatment	Dose U/kg	AUC <sub>last</sub> mU•h/mL	AUC <sub>0-336</sub> mU•h/mL	AUC <sub>0-∞</sub> mU•h/mL	C <sub>max</sub> mU/mL	C <sub>last</sub> U/mL	t <sub>max</sub> h	t <sub>last</sub> h	t <sub>1/2</sub> h
Male	Adagen	30	11900 (3660)	14200 (3850)	18200 (4350)	194 (28.9)	82.6 (20.4)	22.4 (5.06)	86.4 (23.2)	50.4 (13.1)
		150	79200 (11600)	83200 (10400)	85600 (9950)	1020 (119)	110 (68.8)	24.0 (0.00)	170 (34.8)	40.6 (3.14)
	SC-PEG rADA	30	21100 <sup>a</sup> (4020)	22700 (3830)	24400 <sup>a</sup> (3960)	237 <sup>a</sup> (33.8)	43.8 (16.1)	24.0 (0.00)	168 (36.0)	53.0 <sup>a</sup> (5.98)
		150	123000 <sup>a</sup> (15700)	124000 (15300)	127000 <sup>a</sup> (16300)	1150 (186)	39.4 (12.3)	24.0 (0.00)	322 (30.4)	61.0 <sup>a</sup> (12.0)
Female	Adagen	30	10900 (2300)	12900 (2570)	15900 (2600)	214 (46.4)	83.7 (14.4)	18.7 (8.00)	72.0 (0.00)	36.1 (3.58)
		150	66000 (13000)	72300 (13900)	75400 (14200)	951 (182)	178 (36.2)	18.7 (8.00)	120 (0.00)	36.7 (2.47)
	SC-PEG rADA	30	18700 <sup>a</sup> (4050)	20400 (3620)	22000 <sup>a</sup> (3460)	238 (32.7)	47.3 (13.1)	24.0 <sup>a</sup> (0.0)	149 (50.3)	49.4 <sup>a</sup> (8.45)
		150	103000 <sup>a</sup> (17200)	105000 (14900)	108000 <sup>a</sup> (13100)	1070 <sup>a</sup> (119)	70.7 (74.5)	22.4 <sup>a</sup> (5.06)	250 (56.8)	51.3 <sup>a</sup> (4.18)

<sup>a</sup> Statistically distinct from Adagen when comparison is done within gender using 2-sided 90% confidence interval of the ratio of the means.

**EZN-2242 (Adagen®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Dogs via Intramuscular Administration with a 14-Day Observation Period (Study # 07-3265/AD07026; non-GLP)** – Beagle dogs (5/sex/group) received single IM injections of either Adagen® or the clinical compound, SC-PEG rADA (batch # P-E-1490-30-050707; 99.9% pure), at doses of 30 or 150 U/kg. The vehicle was PBS buffer with 50 mM phosphate and 0.85% saline at pH 7.2 to 7.4. Blood samples for PK analyses of ADA activity were obtained at predose, 5 minutes postdose, and 4, 8, 24, 48, 72, 120, 192, 264, and 336 hours postdose. The plasma PK parameters for Adagen® (EZN-2242) and SC-PEG-rADA (EZN-2279) were estimated based on individual enzyme activity-time profiles.

Systemic exposure (AUC<sub>last</sub> and C<sub>max</sub>) of ADA activity increased in proportion to dose for both compounds (Table 6). Longer t<sub>1/2</sub> (1.7- to 2.1-fold) was observed for SC-PEG rADA in comparison with Adagen®, resulting in significantly greater AUC<sub>last</sub> (25% to 41%) and AUC<sub>0-∞</sub> (51% to 57%) values. C<sub>max</sub> values were comparable between both compounds. The calculated mean terminal elimination t<sub>1/2</sub> varied from ~128 to 154 hours for SC-PEG rADA and ~68 to 79 hours for Adagen®. There were no significant gender differences.

**Table 6: Mean Plasma PK Parameters for ADA Activity in Dogs Following Single Intramuscular Injection of Adagen or SC-PEG rADA (Study # 07-3265/AD07026)**

Sex	Treatment	Dose U/kg	AUC <sub>last</sub> mU•h/mL	AUC <sub>0-336</sub> mU•h/mL	AUC <sub>0-∞</sub> mU•h/mL	C <sub>max</sub> mU/mL	C <sub>last</sub> mU/mL	t <sub>max</sub> h	t <sub>last</sub> h	t <sub>1/2</sub> h
Male	Adagen	30	37200 (3110)	38600 (3230)	41300 (3570)	300 (27.4)	36.7 (3.48)	20.8 (7.16)	264 (0.00)	77.3 (1.92)
		150	191000 (6940)	191000 (6940)	197000 (8530)	1620 (108)	65.2 (15.6)	17.6 (8.76)	336 (0.00)	68.3 (4.48)
	SC-PEG rADA	30	52500 <sup>a</sup> (1690)	52500 (1690)	62200 <sup>a</sup> (3080)	316 (16.6)	51.9 (6.24)	17.6 (8.76)	336 (0.00)	128 <sup>a</sup> (20.0)
		150	252000 <sup>a</sup> (18900)	252000 (18900)	309000 <sup>a</sup> (23800)	1480 (118)	287 (30.0)	17.6 (8.76)	336 (0.00)	138 <sup>a</sup> (13.3)
Female	Adagen	30	38200 (2770)	39500 (2820)	42600 (2770)	309 (24.6)	38.2 (2.42)	11.2 (7.16)	264 (0.00)	79.0 (4.95)
		150	188000 (9530)	188000 (9530)	196000 (9860)	1460 (191)	80.0 (7.68)	17.6 (8.76)	336 (0.00)	73.9 (2.84)
	SC-PEG rADA	30	53900 <sup>a</sup> (4480)	53900 (4480)	65400 <sup>a</sup> (8180)	312 (23.0)	58.1 (12.8)	17.6 (8.76)	336 (0.00)	134 <sup>a</sup> (20.4)
		150	235000 <sup>a</sup> (27900)	235000 (27900)	298000 <sup>a</sup> (38300)	1430 (184)	280 (43.3)	17.6 (8.76)	336 (0.00)	154 <sup>a</sup> (15.2)

<sup>a</sup> Statistically distinct from Adagen when comparison is done using 2-sided 90% confidence interval of the ratio of the means.

## 5.2 Toxicokinetics

Studies submitted under Module 4.2.2.2 “Absorption” were reviewed below under each specific study.

# 6 General Toxicology

## 6.1 Single-Dose Toxicity

### EZN-2242 (ADAGEN®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Rats via Intramuscular Administration with a 14-Day Observation Period (Study # 07-2018/AD07027; non-GLP)

The study design is described under section 5.1 PK/ADME of this review. Note that the study did not include a vehicle control group as the main goal was to compare both ADA preparations. There were no mortalities, no test article-related clinical signs (as compared to predose), or differences in body weight/body weight gain and food consumption between Adagen® or SC-PEG-rADA-treated groups during the 14-day observation period following a single IM dose (30 or 150 U/kg). The NOAEL for both compounds was the high dose.

**EZN-2242 (Adagen®) and EZN-2279 (PEG-SC-rADA): A Single-Dose Comparative Pharmacokinetic Study in Dogs via Intramuscular Administration with a 14-Day Observation Period (Study # 07-3265/AD07026; non-GLP)**

The study design is described under section 5.1 PK/ADME of this review. Note that the study did not include a vehicle control group as the main goal was to compare both ADA preparations. There were no mortalities, no test article-related clinical signs (as compared to predose), or differences in body weight/body weight gain and visual food consumption estimates between Adagen® or SC-PEG-rADA-treated groups during the 14-day observation period following a single IM dose (30 or 150 U/kg). The NOAEL for both compounds was the high dose.

## 6.2 Repeat-Dose Toxicity

**Study title: EZN-2279: A Toxicity and Toxicokinetic Study in Rats Following 4-Week Intramuscular Injections with a 4-Week Recovery Period**

Study no.: STPI130260A (Leadiant); 15-2483 (b) (4)  
 Study report location: EDR Modules 4.2.3.2 and 4.2.3.7  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 23, 2015  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: EZN-2279 (PEG-rADA), lot # 5046 (code 009-10) (245 U/mL), 99.7% pure (main + late HPLC peaks), 99.5% pure by size exclusion chromatography (SEC)

EZN-2279 + (b) (4)% (b) (4) impurity, lot # 1097 (code 004-10) (231 U/mL), 89.7% pure (main + late HPLC peaks), 99.5% pure by SEC

**Notes:** Concentrations (U/mL) represent enzymatic activity determined using RP-HPLC method ACM-1617-00, divided by the conversion factor of 2.1 to match activity using the spectrophotometric assay method (See Study Report Annex 11, page 8-10, for explanation of conversion factor).

The material used in this study is the same as that used in the primary Phase 3 clinical Study

STP-2279-002 and the supporting clinical Study STM-279-301.

(b) (4) impurity is described (CMC Module 2.3.S - Characterization) as: (b) (4)

### Key Study Findings

- The study was designed to bridge the final drug product with lots used in the earlier nonclinical studies prior to the final manufacturing changes and to assess the toxicity of SC-PEG rADA with (b) (4) % (b) (4) impurity (referred to as the (b) (4) impurity), and without.
- Activated partial thromboplastin time (APTT) was slightly prolonged (30%) in groups treated with EZN-2279 and EZN-2279 spiked with (b) (4) % (b) (4) impurity, compared to control groups. The finding was shown to be reversible.
- Both test articles exhibited comparable TK and immunogenicity, under the conditions of this study.
  - The  $C_{max}$  and  $AUC_{0-72}$  values of EZN-2279 in rats were similar following administration of the formulation containing (b) (4) % (b) (4) impurity, compared to the test article without the impurity, on both Day 1 and Day 25. Females tended toward higher exposure than males. Accumulation was observed with repeated doses administered twice weekly.
  - Initial immunogenicity assessment showed formation of anti-therapeutic antibodies (ATAs) or neutralizing antibodies (Nabs) in groups treated with EZN-2279 and EZN-2279 spiked with (b) (4) % (b) (4) impurity. The presence of ATAs or Nabs did not appear to have significantly affected plasma concentrations of EZN-2279 on Day 28.
    - A confirmatory immunogenicity assay conducted by a different laboratory demonstrated fewer ATA-positive animals and no animal with Nabs.
    - No marked differences were noted in the immunogenic response by the addition of the (b) (4) % excess (b) (4) impurity.
    - The immunogenic response was milder than that observed with earlier batches of the drug product, which the applicant attributed to improvements in drug product (b) (4). However, it is not clear to this reviewer if differences in assay methodology between performing laboratories may have contributed to the differences in the degree of immunogenicity observed among studies.

## Methods

Doses: Group 1: 0 (vehicle)  
 Group 2: ENZ-2279 500 U/Kg (3.04 mg/kg)\* with levels of (b) (4) impurity below the limit of quantification (BLQ) of (b) (4) %  
 Group 3: ENZ-2279 500 U/Kg (3.22 mg/kg)\* spiked with (b) (4) % (b) (4) impurity

\*Protein concentration, per Certificate of Analysis:

Lot 5046 (EZN-2279): 1.49 mg/mL

Lot 1097 (EZN-2279 plus (b) (4) impurity): 1.49 mg/mL

\* Therefore, mg/kg were calculated as follows:

EZN-2279: 1.49 mg/mL X 2.04 mL/kg dose volume = 3.04 mg/kg

EZN-2279 plus (b) (4) impurity: 1.49 mg/mL X 2.16 mL/kg dose volume = 3.22 mg/kg

Frequency of dosing: 2x/week for 4 weeks (Days 1, 4, 8, 11, 15, 18, 22 and 25); total of 8 treatments

Route of administration: Intramuscular (IM); caudal thighs (right and left)

Dose volume: 2.16, 2.04 or 2.16 mL/kg, for Groups 1, 2 or 3, respectively

Formulation/Vehicle: Phosphate buffered saline, pH 6.9

Species/Strain: Sprague-Dawley rats [CrI:CD® (SD)BR]

Number/Sex/Group: Main study animals: 15/sex/group  
 Recovery animals: 5/sex/group

Age: 9 weeks

Weight: 232 to 286 g males; 173 to 216 g females

Satellite groups: Satellite animals were used for toxicokinetic and immunogenicity blood sampling:

- Control group: 3/sex
- ENZ-2279 500 U/Kg ± (b) (4) % (b) (4) impurity: 9/sex/group

Unique study design: Drug product stability data shows (b) (4) (b) (4) over time when the product is stored under long-term conditions (2-8 °C). EZN-2279 in the presence and absence of a (b) (4) % (b) (4) impurity excess was used to evaluate potential toxicity, the development of ATAs, and the development of Nabs. A (b) (4) % (b) (4) excess of impurity was used to give an adequate exposure margin for establishing impurity specification limits. The current specification limit for the impurity is not more than (NMT) (b) (4) %. The

“unspiked” EZN-2279 contained (b) (4) impurity below the limit of quantification, i.e., (b) (4) %.

Deviation from study protocol: Due to (b) (4) the stored/frozen toxicokinetic and immunogenicity samples were found to be compromised and it was determined that the toxicokinetic study would be repeated. Therefore, additional animals (3/sex/control group; 9/sex/ENZ-2279 500 U/Kg  $\pm$  (b) (4) % (b) (4) impurity) were added to the study.

## Observations and Results

### Mortality (2x/day)

None

### Clinical Signs (Weekly)

No test article-related signs

### Body Weights (Weekly)

No test article-related effects

### Feed Consumption (Weekly)

No test article-related effects

### Ophthalmoscopy (Pretest and Week 4; Lids, lacrimal apparatus and conjunctivae were examined visually. The cornea, anterior chamber, lens, iris, vitreous humor, retina and optic disc were examined by indirect ophthalmoscopy)

A moderate hyphema was observed in one male (out of 15) given EZN-2279 (Group 2). The veterinary ophthalmologist stated that this is an idiopathic background finding and it is not related to the test article.

### Hematology and Coagulation (At termination and end of recovery period)

It was stated in the study report that there were no test-article or impurity-related changes. However, the following coagulation parameters (Table 7) showed statistically significant changes compared to controls in both male and females in EZN-2279  $\pm$  (b) (4) % (b) (4) impurity:

**Table 7: Effects on Coagulation Parameters in Rats – Study # STPI130260A**

Males	Females
Termination	Termination

Group /Sex	Occasion Termination	PT Seconds	APTT Seconds	Group /Sex	Occasion Termination	PT Seconds	APTT Seconds
1M	Mean	16.9	16.8	1F	Mean	16.3	15.4
	SD	0.59	1.54		SD	0.57	0.98
	N	9	9		N	9	9
2M	Mean	16.5	20.9**	2F	Mean	15.6**	20.1**
	SD	0.58	2.92		SD	0.34	2.55
	N	10	10		N	9	9
3M	Mean	16.1**	22.2**	3F	Mean	15.5**	19.0**
	SD	0.35	2.26		SD	0.35	2.38
	N	10	10		N	10	10

Recovery				Recovery			
Group /Sex	Occasion Recovery	PT Seconds	APTT Seconds	Group /Sex	Occasion Recovery	PT Seconds	APTT Seconds
1M	Mean	16.7	19.2	1F	Mean	16.5	17.6
	SD	0.72	2.90		SD	0.18	2.71
	N	5	5		N	5	5
2M	Mean	17.0	19.6	2F	Mean	15.9	17.8
	SD	0.31	0.97		SD	0.69	1.78
	N	5	5		N	5	5
3M	Mean	16.7	17.5	3F	Mean	15.1**	16.6
	SD	0.62	1.08		SD	0.36	1.77
	N	5	5		N	5	5

It was stated in the study report: “Activated partial thromboplastin time (APTT) prolongations (both sexes) were not considered to be test article-related due to their small magnitude, lack of concordance with prothrombin times (PT) and because values were comparable to the study control range (values in control males and females at termination and recovery).” However, because APTT and PT measure different pathways in the coagulation cascade, this reviewer believes the increased in APTT could indicate a test-article related effect. In addition, prolonged APTT was a common finding in both species used for toxicology studies, i.e., dogs and rats (see studies below).

#### **Clinical Chemistry (At termination and end of recovery period)**

No test article-related findings

#### **Urinalysis (At termination and end of recovery period)**

No test article-related findings

#### **Gross Pathology (At termination and end of recovery period)**

No test article-related findings

### Organ Weights (Adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid, and uterus/cervix)

Statistically significant lower mean absolute (17% and 12%) and relative to body weight (16% and 11%) thyroid/parathyroid glands weights were observed in males treated with EZN-2279 with and without (b) (4) % excess of (b) (4) impurity when compared to controls, respectively. No difference was observed in recovery sacrifice animals. There was no microscopic correlate for this finding. Its relationship to treatment is uncertain.

### Histopathology (Full battery - all main study animals)

Adequate Battery - Yes

Peer Review - No

### Histological Findings

At dosing sites, minimal to moderate chronic active inflammation and/or hemorrhage were present in all dose groups (controls, EZN-2279, and EZN-2279 spiked with (b) (4) % impurity), as shown in Table 8.

**Table 8: Dosing Site Histopathological Findings – Study # STPI130260A**

Tissue/Organ and Findings	Group/Sex No. of animals	Number of animals affected					
		1M	2M	3M	1F	2F	3F
Dose Site	No. examined	10	10	10	10	10	10
Thrombus	Slight	1	0	0	0	0	0
	Total	1	0	0	0	0	0
Inflammation	Minimal	1	4	1	3	4	3
	Slight	0	2	0	0	3	0
	Moderate	1	0	0	0	0	0
	Total	2	6	1	3	7	3
Dose Site	No. examined	10	10	10	10	10	10
Hemorrhage	Minimal	1	1	0	1	0	1
	Slight	0	0	0	0	2	0
	Total	1	1	0	1	2	1

These findings were suggestive of procedure-related focal trauma from intramuscular injection. It was indicated in the Study Report that inconsistent sampling of intramuscular dosing sites precluded optimal examination and therefore test article-relatedness could not be determined. A separate study was then conducted to address findings at the injection site (Study # JF96NG; Annex 9 of the current Study Report). The study confirmed the dosing site findings were IM injection procedure-related.

### Immunogenicity (Pretest, Day 28 [end of treatment] and Day 56 [Recovery Day 29]-blood collected from TK animals)

Anti-therapeutic antibodies (ATA) were found pretest in 2 Group 3 (EZN-2279 spiked with (b) (4) % (b) (4) impurity) animals (#3556F and #3557F) with OD values of

0.104 and 0.139 (cut-point = 0.100). Both animals were negative in the Day 28 (terminal) and Day 56 (end of recovery) evaluations.

Six out of 18 animals each in Group 2 (EZN-2279) and Group 3 (EZN-2279 spiked with (b) (4) % (b) (4) impurity) were ATA-positive on Day 28. Mean OD values ranged from 0.0992 to 0.376 (Group 2) and 0.0899 to 0.211 (Group 3). Fifty percent of these values were less than twice the cut-point (cut-point between 0.086 and 0.100).

Two out of 10 recovery animals in Group 2, and 5 out of 10 recovery animals in Group 3 were found to be ATA-positive on Day 56 (Recovery Day 29). The mean OD values ranged from 0.138 and 0.181 for the 2 animals in Group 2, and 0.0969 to 0.524 for the 5 animals in Group 3.

Neutralizing ATAs (Nabs) were found in one Group 2 male and one Group 3 male on Day 28. The % inhibition in these animals was 18.4% and 6.12%, respectively. The Group 2 male was negative for Nab on Day 56 (Recovery Day 29). There was no data for the Group 3 male (not evaluated at end of recovery).

The presence of ATAs or Nabs did not appear to have significantly affected plasma concentrations of EZN-2279 on Day 28.

The applicant noted that these results of immunogenicity testing (performed at (b) (4)) showed a high number of positive samples with the to-be-marketed product (b) (4). This was contrary to the results of the (b) (4) embryofetal developmental toxicity study which used the to-be-marketed product (see Study # 20098879 below). (b) (4) did not perform a confirming assay, hence, the question remained whether the samples were true positives. Therefore, a validated two-tier confirmatory immunogenicity assay and Nab assay was performed (b) (4) (b) (4) to rule out false-positive findings (Study Report page 775). The immunogenicity in the embryofetal developmental toxicity study was also assayed (b) (4).

Results from the confirmatory assays using either formulation of SC-PEG rADA (with or without (b) (4) % (b) (4) impurity) as the coating and spiking ligands showed that only 6 animals were positive for anti-drug antibodies on Day 28 or Day 56 (Table 9). Nabs were not detected in any animals in the confirmatory assay.

Despite the differences in the number of positive samples, the results from both laboratories showed no marked differences in immunogenicity and Nab generation between SC-PEG rADA with or without (b) (4) % (b) (4) impurity.

**Table 9: Samples Confirmed Positive using either 5071 or 1097 as the Plate Coating and Spiking Ligands**

ANIMAL ID	INTERVAL	1st Tier Assay			2nd Tier Assay			
		No Spike (OD Units) Avg all points	Batch Cut point OD	Presumptive Pos/Neg	Post Spike (OD Units) Avg all points	Spike/Unspiked (OD Ratio)	% Signal Inhibition	Confirmed Pos/Neg
<b>5071 as the plate coating ligand</b>								
2028	END OF RECOVERY	0.0267	0.025	Pos	0.0177	0.663	33.75	Pos
2032	TERMINAL	0.0320	0.025	Pos	0.0200	0.625	37.50	Pos
2537	END OF RECOVERY	0.0510	0.028	Pos	0.0253	0.497	50.33	Pos
2543	TERMINAL	0.0330	0.027	Pos	0.0230	0.697	30.30	Pos
3052	TERMINAL	0.0203	0.018	Pos	0.0143	0.705	29.51	Pos
<b>1097 as the plate coating ligand</b>								
2028	END OF RECOVERY	0.0218	0.019	Pos	0.0143	0.656	34.40	Pos
3558	END OF RECOVERY	0.0230	0.022	Pos	0.0177	0.768	23.19	Pos

**Toxicokinetics (Predose, 5 minutes, 2, 6, 24, 48, and 72 [prior to Day 4 dose] hours postdose on Day 1 and Day 25; additional timepoints on Day 25, i.e., 120, 192, 264 and 335 hours postdose; levels of EZN-2279 measured by plasma ADA enzymatic activity, i.e., conversion of adenosine to inosine)**

The  $C_{max}$  and  $AUC_{0-72}$  values of EZN-2279 in rats were similar following administration of the formulation spiked with (b) (4) % (b) (4) impurity compared to the test article without the spiked impurity on both Day 1 and Day 25 (Table 10).

**Table 10: Comparison of Plasma Exposure of EZN-2279 after IM Administration to Rats in the Presence and Absence of (b) (4) % (b) (4) Impurity**

Dose level EZN-2279 500 U/kg	$C_{max}$ (mU/mL)				$AUC_{0-72}$ (mU.h/mL)			
	Day 1		Day 25		Day 1		Day 25	
	Males	Females	Males	Females	Males	Females	Males	Females
Without (b) (4) impurity <sup>a</sup>	2540	4130	6150	7960	139000	186000	383000	451000
(b) (4) % (b) (4) impurity	3660	4600	5840	8970	184000	224000	371000	481000

<sup>a</sup> Below quantification limit (b) (4) %

The  $C_{max}$  and  $AUC_{0-72}$  values of EZN-2279 ± (b) (4) % (b) (4) impurity in female rats were ~1.2 to 1.6-fold higher than those observed in males.

After repeated intramuscular doses (Day 25), the  $C_{max}$  and  $AUC_{0-72}$  values of EZN 2279 in rats were higher than those values after a single dose (Day 1). The accumulation ratios tended to be slightly lower following administration of EZN-2279 spiked with (b) (4) % (b) (4) impurity, than with the EZN-2279 without the spiked (b) (4) impurity (Table 11).

**Table 11: Accumulation Ratios of EZN-2279 after IM Administration to Rats in the Presence and Absence of (b) (4) % (b) (4) Impurity**

Dose level EZN-2279 500 U/kg	Accumulation ratio	
	Males	Females
Without (b) (4) impurity <sup>a</sup>	2.8	2.4
(b) (4) % (b) (4) impurity	2.0	2.1

<sup>a</sup> Below quantification limit 0.4%

The terminal half-life was estimated to be in the range 53.7 to 61.1 hours, and appeared to be independent of sex. The  $T_{max}$  generally occurred at 24 hours postdose.

### Dosing Solution Analysis

The test items were used "as supplied" without further processing. Therefore, analysis of the test items was not necessary to establish the strength. Per Certificate of Analysis (dated (b) (4)), the quality of lot # 5046 (normal level of impurities) and lot # 1097 ((b) (4) % (b) (4) impurity) were the following:

Test	Method #	Specification	Results	Results
			Lot 5046 (3 Months)	Lot 1097 (49 Months)
Protein Concentration	ACM-1605-03	(b) (4) mg/mL	1.49 mg/mL	1.49 mg/mL
Activity	ACM-1617-00	(b) (4) U/mL	550 U/mL	491 U/mL
Purity by SEC	ACM-1625-02	Aggregates: (b) (4) % Area Purity: (b) (4) % Area	Aggregates: 0.5 % Purity: 99.5%	Aggregates: 0.5 % Purity: 99.5%
Purity by RP-HPLC	ACM-1622-00	Early Peak: NMT (b) (4) % Main Peak: NLT (b) (4) % Late Peak: NMT (b) (4) % Main+Late Peak: NLT (b) (4) % Total Other Peaks: NMT (b) (4) %	Early Peak: 0.0% Main Peak: 86.8% Late Peak: 12.8% Main + Late: 99.7% Other: 0.4%	Early Peak: 9.6%* Main Peak: 78.5%* Late Peak: 11.2%* Main + Late: 89.7%* Other: 0.7%*

\*Lot 1097 does not have specifications for peaks analyzed under ACM-1622. At the time of lot 1097 manufacture, no specification was in place.

**Study title: EZN-2279: A 4-Week Intramuscular Toxicity/Toxicokinetic Study in Rats with A 4-Week Recovery Period**

Study no.: 07-2030/AD07028  
Study report location: EDR Module 4.2.3.2  
Conducting laboratory and location:  (b) (4)

Date of study initiation: August 2, 2007  
GLP compliance: Yes, with the following exceptions

- No homogeneity data was supplied for the test article.
- Dose confirmation and stability analysis was conducted under non-GLP conditions.
- Lot #, expiration date and purity was not provided for one of the 2 vehicles.

QA statement: Yes  
Drug, lot #, and % purity: EZN-2279 (PEG-SC-rADA), 250 U/mL (1.6 mg/mL), lot # P-E-1490-35-060407, 99.3% pure

**Note:** *There is a discrepancy in the value for enzyme activity in the Study Report; it is specified as 250 U/mL in the Analysis Report (p. 129) vs 892 U/mL in Section 2.5 Test Article (p. 16).*

**Key Study Findings**

- A slight prolongation (~ 6-seconds) in APTT was observed at the high dose in both males and females. The increase was reversible during the 4-week recovery period.
- Overall, EZN-2279 systemic exposures ( $AUC_{last}$ ) at the low-dose and mid-dose were reduced on Day 29 compared to Day 1, whereas higher exposures were noted at the high dose.
- All animals, except for one high-dose female, developed an immunogenic response.
  - A positive response was also noted in 4/10 vehicle control animals; the impact of this finding on the interpretation of the data is unknown.
- The lower EZN-2279 systemic exposure observed at Day 29 compared to Day 1 for male and female rats at the low dose (30 U/kg, 2x/week) and male rats at the mid dose (100 U/kg, 2x/week) may be secondary to the production of ATAs.
- On Day 29, systemic exposure in males was lower than females.
- Based on the slight magnitude of the effect in APTT, the NOAEL was selected as the high dose, i.e., 300 U/kg IM, 2x/week.

## Methods

Doses: 0 (vehicle), 30, 100, or 300 U/kg

(Nominal concentrations were 0, 25, 83 and 250 U/mL, respectively, per study report. Given specific activity is 156 U/mg, the concentration in mg/mL is 0.16, 0.5321 and 1.6 mg/mL at nominal concentrations).

The doses in mg/kg were not provided in the Study Report. Therefore, mg/kg were calculated by this reviewer as follows:

Per Analysis Report (page 129), specific activity is 156 U/mg (250U/mL divided by 1.6 mg/mL protein).

- $30 \text{ U/kg} \div 156 \text{ U/mg} = 0.192 \text{ mg/kg}$
- $100 \text{ U/kg} \div 156 \text{ U/mg} = 0.641 \text{ mg/kg}$
- $300 \text{ U/kg} \div 156 \text{ U/mg} = 1.92 \text{ mg/kg}$

Frequency of dosing: 2x/week for a total of 9 treatments (Days 1, 4, 7, 11, 14, 18, 21, 25 and 29)

Route of administration: IM

Dose volume: 1.2 mL/kg

**Note:** Due to the large volume delivered, the test and control articles were administered to animals by splitting each dose into 2 IM injections (right and left caudal thigh).

Formulation/Vehicle: Phosphate buffered saline, pH 7.2-7.4

Species/Strain: Sprague-Dawley rats - CrI:CD® (SD) IGS BR

Number/Sex/Group: 15

(After 9 doses, 10 animals/sex/group were euthanized; the remaining 5 animals/sex/toxicity group were euthanized at the end of the 4-week recovery period)

Age: ~ 8 weeks old

Weight: 207-297 grams for males; 182-237 grams for females

Satellite groups: An additional 12 rats/sex/test article-treated group were used for TK analysis

Unique study design: None

Deviation from study protocol: None with an impact on the interpretation of the data

## **Observations and Results**

### **Mortality (Daily)**

One mid-dose TK male (# 3016) was moribund and sacrificed on Day 16. Per Study Report, this mortality was not considered test article-related. However, no further details were given in the Study Report.

### **Clinical Signs (Daily; physical examination weekly)**

None test article-related

### **Body Weights (Weekly)**

Lower mean body weights (11%-13%; non-statistically significant) were observed in high-dose males throughout the recovery period. The Study Report acknowledges that high-dose males demonstrated a decrease (non-statistically significant) in mean body weight gain during recovery, compared to controls. Per information in the Study Report, this effect was attributed to a particularly small male (# 4011) that skewed the data. At the end of the recovery period, mean body weight gain was 12.8%, 4.3%, and 21% lower than control group at the low, mid, and high-dose, respectively. This decrease is consistent with the observed decrease in mean food consumption (see below).

Since the decrease was observed during the recovery period, did not show a dose relationship, was only observed in males (despite males having lower systemic exposure compared to females), and there were no other indications of systemic toxicity, the finding was not considered toxicologically relevant. In addition, test article exposure during the recovery period was in general below the lower limit of quantitation, further providing support for the lack of toxicological relevance for the finding.

### **Feed Consumption (Weekly)**

Mean food consumption showed a statistically significant decrease in males throughout recovery in all test article-treated groups compared to vehicle control. At the end of the recovery period (Days 22-28), mean food consumption was decreased by ~19%, 12%, and 22% at the low, mid, and high-dose, respectively. It was stated in the Study Report that feed consumption in test article-treated animals was comparable to controls.

### **Hematology and Coagulation (At termination and end of recovery period)**

There was a slight but statistically significant increase in mean platelet volume (MPV) value (3% to 7%; non-dose dependent) for male rats at all dose levels. At the high-dose, there was an approximately 6-second prolongation ( $p \leq 0.01$ ) in APTT in both males (23.9 vs. 18.2 seconds in controls) and females (24.5 vs. 18.2 seconds in controls). The findings were not observed at recovery assessment.

The increased MPV and APTT values were considered by the applicant compound related, but not adverse, because of the small magnitude of the change and lack of correlative changes. The changes in MPV were still within physiological range and therefore, may not be test article related (7.4 to 7.6 fL in test article-treated males vs. 5.0 to 8.5 fL in published historical control databases<sup>4, 5</sup>).

### **Clinical Chemistry (At termination and end of recovery period)**

No test article-related effects

### **Urinalysis (At termination and end of recovery period)**

Mean urine volume was 2.3x higher ( $p \leq 0.05$ ) in high dose females compared to control group. However, given the lack of any other indicator of kidney damage, this finding was not considered test article related.

### **Gross Pathology (Main and recovery animals)**

At the end of the dosing phase, 2 mid-dose males had red foci at the injection sites. Although not examined microscopically, per protocol, the red discolorations were presumed to be due to hemorrhages resulting from the injection procedure.

### **Organ Weights (Adrenals, brain, epididymides, heart, kidneys, liver, pituitary gland, prostate gland, spleen, testes, thymus, thyroid/parathyroid glands)**

No test article-related changes

### **Histopathology (Standard battery in main animals from control and high dose groups; only kidneys for recovery control and high-dose groups)**

Adequate Battery - Yes

Peer Review - No

Histological Findings – At the injection sites, findings included skin subacute (chronic active)/chronic inflammation, skin erosion, hemorrhage, skeletal/striated muscle atrophy/degeneration at the end of the dosing phase. These findings were observed in both control and high-dose groups and generally were of minimal to slight severity. These findings were considered to be tissue responses to the trauma of the injection procedures.

### **Immunogenicity (Day 29, predose, 2, 6, and 120 hours post last dose and at the end of recovery period)**

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<sup>4</sup> Han ZZ., Xu HD., Kim KH. et al., 2010, Reference data of the main physiological parameters in control Sprague-Dawley rats from pre-clinical toxicity studies, *Lab. Anim. Res.* 26(2), 153-164.

<sup>5</sup> <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0189837>

In test-article-treated rats at end of treatment and end of recovery, all except one high-dose female (# 4513) were considered positive for an antibody response, and 26 of the 29 positive samples exceeded the cut point by more than 2 fold.

Weak neutralization (< 2-fold the assay cut point) was evident at the end of the treatment period (120 hours postdose only) for one low-dose male rat (# 2016) and at the end of the recovery period for one high-dose female rat (# 4515).

The applicant stated (Toxicology Written Summary, p. 15) that the substantially lower systemic exposure at Day 29 compared to Day 1 for male and female rats at the low dose and male rats at the mid dose (see Toxicokinetics below) may be secondary to the production of anti-therapeutic antibodies. Although average titer data was not presented, a quick look at the individual animal listings showed the levels of anti-therapeutic antibodies at the lower doses were generally higher compared to those observed at the higher dose.

A positive antibody response was observed in 4 of the 10 vehicle-treated rats. Two of these rats had ODs (optical density values) more than 2-fold higher than the negative cut point of the assay of 0.168. The actual ODs for these rats were 0.396 for one male (# 1015) and 1.302 for one female (# 1513). It was indicated in the Study Report that the results suggest that a dosing or sample processing error may have occurred. However, a thorough investigation did not support this was the case. The impact of this finding on the interpretation of the data is unknown.

**Toxicokinetics (Day 1 predose and at 2, 6, 24, 48 and 72 hours post dose and on Day 29 predose and at 2, 6, 24, 48, 72, 96, 120 and 144 hours, and 2 and 4 weeks post dose; levels of EZN-2279 measured by plasma ADA enzymatic activity, i.e., conversion of adenosine to inosine)**

On Day 1, all male and female rats were exposed to quantifiable enzyme activity at all test article dose levels. On Day 29, not all male rats at 30 and 100 U/kg and female rats at 30 U/kg had quantifiable plasma enzyme activity, whereas all males at 300 U/kg and all females at 100 and 300 U/kg had quantifiable plasma enzyme activity.

On Day 1, systemic exposures increased dose proportionately, whereas on Day 29, exposures were generally greater than dose proportional.

On Day 1, no gender differences were observed whereas on Day 29, the systemic exposures in males were lower than females.

Overall, systemic exposure ( $AUC_{last}$ ) at the low and mid-dose was reduced (93% and 67% for the low-dose males and females, respectively, and 80% for the mid-dose males) on Day 29 compared to Day 1, whereas higher exposures were noted at the highest dose.

On Day 29 of the recovery period (672 hours after the last [9<sup>th</sup>] IM injection), no quantifiable activity was observed.

Toxicokinetic parameters (based on the mean enzyme activity data) are summarized in Table 12:

**Table 12: Mean Plasma Toxicokinetic Parameters of ADA Enzyme Activity in Male and Female Rats following Repeated Intramuscular Injection of EZN-2279 (Study # 07-2030/AD07028)**

Day	Sex	Group	Dose <sup>a</sup> (U/kg)	AUC <sub>0-72h</sub> (mU•h/mL)	AUC <sub>last</sub> (mU•h/mL)	C <sub>max</sub> (mU/mL)	t <sub>max</sub> (h)
1	Male	2	30	12200	12200	212	24
		3	100	30600	30600	545	24
		4	300	107000	107000	2260	24
	Female	2	30	12600	12600	219	24
		3	100	36000	36000	694	24
		4	300	131000	131000	2670	24
29	Male	2	30	737	846	64.6	6
		3	100	5790	6020	167	6
		4	300	154000	281000	2580	6
	Female	2	30	3210	4200	121	48
		3	100	32700	61100	619	6
		4	300	231000	416000	4560	24

<sup>a</sup>Total of 9 doses were administered intramuscularly to rats twice weekly over 4 weeks (Days 1, 4, 7, 11, 14, 18, 21, 25 and 29)

### Dosing Solution Analysis

The test article was stable in the vehicle, under storage conditions used in this study, for at least 8 hours. Dosing solution concentrations were within <sup>(b) (4)</sup> % of nominal.

**Study title: EZN-2242 (Adagen®) and EZN-2279: A Comparative 4-Week Intramuscular Pharmacokinetic and Immunogenicity Study in Rats**

Study no.: 08-2060/AD08001  
Study report location: EDR Module 4.2.3.2  
Conducting laboratory and location:  (b) (4)

Date of study initiation: April 18, 2008  
GLP compliance: The study report (p. 23) indicates the study was conducted under GLP, with the following exceptions:

- Analyses of test article EZN-2279 and all dosing solutions, and the pharmacokinetic and immunogenicity assays conducted by the applicant using scientifically valid procedures; the data underwent a quality control check

QA statement: Yes  
Drug, lot #, and % purity: EZN-2279 (SC-PEG rADA) (245 U/mL), lot # P-E-1490-89-1, 84.9% pure (HPLC)  
EZN-2242 (Adagen®) (235 U/mL), lot # NH0730, 100% pure (HPLC)

**Key Study Findings**

- The study compared some limited parameters for systemic toxicity between EZN-2242 (Adagen®) and EZN-2279 (SC-PEG rADA). No significant toxicities were observed. There was no difference in clinical signs, body weights/body weight gains, and food consumption between animals treated with a single dose or repeated doses of EZN-2242 (Adagen®) and EZN-2279 (SC-PEG rADA).
- TK and immunogenicity was not evaluated for EZN-2279 (SC-PEG rADA) because of low purity of the lot used in the study.
- EZN-2242 (Adagen®) caused an immunogenic response as early as 7 days postdose; neutralizing antibodies were observed in 2 out of 10 rats evaluated on Day 60. Low EZN-2242 levels observed in 5 animals could be related to the immunogenic response.
  - Based on the results of Study # 07-2030/AD07028 above, both Adagen® and early lots of EZN-2279 (SC-PEG rADA) were highly immunogenic in rats.

## Methods

Doses:	30 U/kg for both test articles (see Table 13)
Frequency of dosing:	Every 3 or 4 days (Groups 1 and 2) for 4 weeks (total of 9 doses*) or as a single dose (Groups 3 and 4) (see Table 13).
	*See "Note" under Table 13.
Route of administration:	IM
Dose volume:	1.2 mL/kg
	<b>Note:</b> Due to the large volume delivered, the test and control articles were administered to animals by splitting each dose into 2 IM injections (quadriceps, caudal thigh).
Formulation/Vehicle:	Phosphate buffered saline
Species/Strain:	Sprague-Dawley rats (CrI:CD <sup>®</sup> [SD] IGS BR)
Number/Sex/Group:	15 males/group (repeat dose); 10 males/group (single dose) (see Table 13)
Age:	~ 8 weeks old
Weight:	239-302 grams
Satellite groups:	10 or 15 males/ group for TK analysis or immunogenicity analysis (see Table 13)
Unique study design:	The study report indicates that only males were investigated in this study as no gender differences in ATA response were noted in Study # 07-2030/AD07028.
Deviation from study protocol:	Upon completion of dosing, the applicant notified the Testing Facility that the purity of the EZN 2279 lot used in this study was lower than expected (~85%), and a decision was made not to analyze plasma samples collected from EZN-2279-treated rats. Accordingly, this report contains pharmacokinetic and immunogenicity data for EZN-2242 (Adagen <sup>®</sup> ) and in-life data for EZN-2242 (Adagen <sup>®</sup> ) and EZN-2279.

**Table 13: Study Design – Comparative 4-Week Rat Study (Study # 08-2060/AD08001)**

Group	Compound	Dose Information <sup>a</sup>			Number of Animals		
		Dose Level (U/kg)	Dose Volume <sup>b</sup> (mL/kg)	Dose Concentration (U/mL)	Total M	Pharmacokinetics <sup>e</sup> M	Immunogenicity <sup>f</sup> M
1	EZN-2242 <sup>c</sup>	30	1.2	25	15	15	15
2	EZN-2279 <sup>d</sup>	30	1.2	25	15	15	15
3	EZN-2242	30	1.2	25	10	10	10
4	EZN-2279	30	1.2	25	10	10	10

<sup>a</sup>Doses represent enzymatic activity of the active ingredient.

<sup>b</sup>Due to the large volume delivered, the doses were administered as two equally split injections, one to each leg (quadriceps, caudal thigh) on each day of treatment.

<sup>c</sup>On Day 29, 10 rats in Group 1 continued receiving EZN-2242, the remaining 5 rats received EZN-2279 at the same dose.

<sup>d</sup>On Day 29, 10 rats in Group 2 continued receiving EZN-2279, the remaining 5 rats received EZN-2242 at the same dose.

<sup>e</sup>PK samples for determining plasma exposures (enzyme activity levels) were collected from all rats at the following time points: Groups 1 and 2: predose on Days 1, 7, 14, 21, and 28, and on Day 29 - postdose at 5 min, and 2, 6, 24, 48, 72, 120, 168, 216, 264 and 336 hours. Groups 3 and 4: predose - 4 days prior to dosing, on Day 1 - postdose at 5 min, and 2, 6, 24, 48, 72, 120, 168, 216, 264 and 336 hours.

<sup>f</sup>Blood for immunogenicity analysis was collected from all animals in Groups 1 and 2, at predose on Days 1, 7, 14, 21 and 28, from the first 10 animals in Groups 1 and 2 at 14 and 31 days after the last injection on Day 29 (Days 43 and 60), as well as from all animals in Groups 3 and 4 four days prior to study initiation.

**Note:** For Groups 1 and 2, as shown in the scheduling table below, all the animals in each group received the first 8 doses of the test article listed for that group. For the last dose on Day 29, the first 10 rats were administered the same respective test article as for the previous 8 doses. The remaining 5 rats received the opposite test article on the last day at the same dose (i.e. EZN-2242 Group 1 received EZN-2279 and EZN-2279 Group 2 received EZN-2242.).

Group	Number of Animals	Dosing Day								
		1	4	7	11	14	18	21	25	29
1	10	EZN-2242								EZN-2242
	5	EZN-2242								EZN-2279
2	10	EZN-2279								EZN-2279
	5	EZN-2279								EZN-2242

## Observations and Results

### Mortality (Daily)

None

### Clinical Signs (5-35 minutes after dosing; physical examination weekly)

No test article-related findings

### Body Weights (Pretest, prior to each dose during the study, and at the completion of the PK blood collections)

There were no differences in the body weights or body weight changes in animals dosed with EZN-2242 (Adagen®) or EZN-2279 (SC-PEG rADA).

**Feed Consumption (Weekly; Groups 1 and 2 only)**

There were no differences in food consumption in animals dosed with EZN-2242 or EZN-2279.

**Immunogenicity (Same timepoints as TK below)**

As noted under TK below, only samples from animals treated with EZN-2242 (Adagen®) were evaluated.

Rats given 30 U/kg EZN-2242 (Adagen®) exhibited ATAs starting on Day 7 and continuing through each of the weekly blood collection time points during the 29-day dosing phase (Table 14). The incidence ranged from 10-14 rats at each time point out of a total of 15 rats/time point. By Day 60 (31 days after the last dose), ATAs were present in 5 of 10 rats.

Neutralization of adenosine deaminase activity was evident for 2 of 10 rats on Day 60 (31 days after the last EZN-2242 dose).

The testing facility was Enzon Pharmaceuticals, Inc.

**Table 14: Incidence of Anti-Drug Antibodies after Treatment with Adagen® in Rats (Study # 08-2060/AD08001)**

Animal Number	Anti-Drug Antibody Results							Neutralization Results
	Pre-Dose <sup>†</sup>	Day 7 <sup>†</sup>	Day 14 <sup>†</sup>	Day 21 <sup>†</sup>	Day 28 <sup>†</sup>	Day 43 (14 days after final dose)	Day 60 (31 days after final dose)	Day 60 (31 days after final dose)
1001	-	++	-	++	-	-	-	-
1002	-	++	+	-	+	+	+	-
1003	-	-	-	-	++	++	-	-
1004	-	+	+	++	NS	+	+	+
1005	-	++	+	+	+	+	+	-
1006	-	+	+	++	+	+	+	-
1007	-	+	++	-	-	-	-	-
1008	-	+	+	+	+	+	+	+
1009	-	++	-	-	++	-	-	-
1010	-	++	-	++	++	-	-	-
1011	-	++	++	+	+	NA	NA	NA
1012	-	++	-	-	+	NA	NA	NA
1013	-	+	+	++	+	NA	NA	NA
1014	-	+	+	+	+	NA	NA	NA
1015	-	+	+	+	+	NA	NA	NA
<b>Incidence</b>	<b>0/15</b>	<b>14/15</b>	<b>10/15</b>	<b>10/15</b>	<b>12/14</b>	<b>6/10</b>	<b>5/10</b>	<b>2/10</b>

<sup>†</sup> Blood samples were collected before dosing on Days 1, 7, 14, 21, and 28.

NS = no sample.

NA = not applicable

\* = <2X cut point

**Toxicokinetics (Single dose: Pre-dose, and on Day 1 at 5 minutes postdose and 2, 6, 24, 48, 72, 120, 168, 216, 264, and 336 hours postdose; Repeat dose: Predose on Days 1, 7, 14, 21 and 28, and on Day 29 at 5 minutes postdose and 2, 6, 24, 48, 72, 120, 168, 216, 264, and 336 hours postdose; levels of EZN-2242 measured by plasma ADA enzymatic activity)**

**Note:** The purity of the SC-PEG rADA (EZN-2279) lot used in this study was substantially lower than expected (~85%). It was presumed that PK/immunogenicity results generated from the plasma samples may not reflect EZN-2279-associated effects. The decision was made to not analyze plasma samples collected from SC-PEG rADA treated rats. There are no TK/immunogenicity results for SC-PEG rADA.

Following 9 repeated doses (Day 29) and afterwards, plasma adenosine deaminase activity in 3 out of 10 Group 1 animals was below the level of quantification (50 mU/mL). Plasma adenosine deaminase activity levels in 2 additional animals were low. The effects on plasma adenosine activity in these 5 rats were likely the result of ATAs and/or neutralization.

Mean values of selected PK parameters for plasma adenosine deaminase activity for the other 5 of 10 repeat-dose rats (Group 1) from which these parameters could be evaluated and all 10 single-dose rats (Group 3) are summarized below:

**Table 15: Mean Plasma Pharmacokinetic Parameters of Enzyme Activity in Male Rats following IM Injection of 30 U/kg of EZN-2242 (Adagen®)- (Study # 08-2060/AD08001)**

Group	Day	AUC <sub>last</sub> (mU•h/mL)	AUC <sub>0-336h</sub> (mU•h/mL)	C <sub>max</sub> (mU/mL)	C <sub>last</sub> (mU/mL)	t <sub>max</sub> (h)	t <sub>last</sub> (h)	t <sub>1/2</sub> (h)
3 <sup>b</sup>	1	13300 (2510)	15100 (2150)	213 (18.7)	75.1 (20.8)	24.0 (0.00)	96.0 (25.3)	44.7 (4.69)
1 <sup>c</sup>	29	20220 (4199)	21700 (4115)	268 (29.0)	61.3 (7.0)	20.4 (8.00)	147 (94.4)	48.4 (16.90)

<sup>a</sup>Standard deviations in parentheses

<sup>b</sup>A single dose was given IM on Day 1

<sup>c</sup>Total of 9 doses were given IM on Days 1, 4, 7, 11, 14, 18, 21, 25 and 29. PK parameters for Group 1 were based on data from 5 of 10 rats. PK parameters could not be calculated properly for the remaining 5 Group 1 rats based on the available data.

The AUC values indicate some degree of accumulation was observed with repeated dosing. The finding is consistent with the accumulation observed with repeated dosing of EZN-2279 (SC-PEG-rADA) in separate studies reviewed herein.

### Dosing Solution Analysis

Samples for concentration analysis were collected from dosing solutions following completion of dosing on Days 1 and 29. All dose preparations of EZN-2242 or EZN-2279 were within <sup>(b) (4)</sup> % of nominal except the sample from Group 2 (EZN-2279) on Day 29. The purity of this sample was 80% (note that in all other areas of the Study Report it is noted that the purity of the sample was ~85%).

**Study title: EZN-2279: A 4-Week Intramuscular Toxicity/Toxicokinetic Study in Dogs with A 4-Week Recovery Period**

Study no.: 07-3280/AD07029  
Study report location: EDR Module 4.2.3.2  
Conducting laboratory and location:  (b) (4)

Date of study initiation: August 10, 2007  
GLP compliance: Yes, with the following exceptions

- No homogeneity data was supplied for the test article.
- Dose confirmation and stability analysis was conducted under non-GLP conditions.
- The immunogenicity detection method (IgG) was performed under non-GLP conditions.
- Expiration date, lot #, and purity was not provided for one or both lots of vehicle used.

QA statement: Yes  
Drug, lot #, and % purity: EZN-2279 (PEG-SC-rADA), 250 U/mL (1.6 mg/mL), lot # P-E-1490-35-060407, 99.3% pure

**Key Study Findings**

- A slight prolongation in APTT was observed at all EZN-2279 (SC-PEG rADA) dose levels in both males and females. The increased was reversible at the low and mid dose and partially reversible at the high dose during the 4-week recovery period.
- At all dose levels, EZN-2279 systemic exposure on Day 29 was greater than that on Day 1, suggesting test article accumulation after twice weekly administration. No gender differences were observed.
- An antibody response was observed at all test article doses by the end of the 4-week dosing period and was associated with a markedly decreased EZN-2279 exposure (AUC) following the last dose in one of five males at 30 U/kg and one of five females at 100 U/kg. In the latter female, the antibody response was neutralizing. However, the Study Report warns that high levels of ADA activity may have interfered with the neutralizing assay results.
- Based on the slight effect in APTT, the NOAEL was selected as the high dose, i.e., 300 U/kg IM, 2x/week.

**Methods**

Doses: 0 (vehicle), 30, 100, or 300 U/kg

(Nominal concentrations were 0, 75, 250 and 750 U/mL, respectively, per study report. Given specific activity is 156 U/mg, the concentration in mg/mL is 0.48, 1.6 and 4.8 mg/mL at nominal concentrations).

The doses in mg/kg were not provided in the Study Report. Therefore, mg/kg were calculated by this reviewer as follows:

Per Analysis Report (page 153), specific activity is 156 U/mg (250 U/mL divided by 1.6 mg/mL protein = 156.25).

- $30 \text{ U/kg} \div 156 \text{ U/mg} = 0.192 \text{ mg/kg}$
- $100 \text{ U/kg} \div 156 \text{ U/mg} = 0.641 \text{ mg/kg}$
- $300 \text{ U/kg} \div 156 \text{ U/mg} = 1.92 \text{ mg/kg}$

Frequency of dosing: 2x/week for a total of 9 treatments (Days 1, 4, 7, 11, 14, 18, 21, 25 and 29)

Route of administration: IM

Dose volume: 0.4 mL/kg

Formulation/Vehicle: Phosphate buffered saline, pH 7.3

Species/Strain: Beagle dogs

Number/Sex/Group: 5

After 9 doses, 3 animals/sex/group were euthanized; the remaining 2 animals/sex/toxicity group were euthanized at the end of the 4-week recovery period.

Age: ~5 to 6 months old

Weight: 6.8 to 87 kg for males; 4.9 to 7.0 kg for females

Satellite groups: None

Unique study design: None

Deviation from study protocol: None with an impact in the interpretation of the study

**Observations and Results****Mortality (2x/day)**

None

**Clinical Signs (2/day; physical examination weekly)**

None test article-related findings

### Body Weights (Weekly)

No test article-related effects

### Feed Consumption (Weekly; qualitative)

No test article-related effects

### Electrocardiography (ECG) (Pretest, at study termination and end of the recovery period)

Only the Summary ECG Report was included (i.e., no summary data or individual animal listing were included). Per the Summary Report, there were no test article-related findings.

### Hematology and Coagulation (Pretest, at termination, and at recovery)

At all test article-treated groups, there was a prolongation in APTT in both males and females (Table 16). At the end of the 4-week recovery period, complete reversibility was observed at the low and mid-dose and partial reversibility was observed at the high dose.

**Table 16: Effects on Coagulation Parameters in Dogs – Study # 07-3280/AD07029**

Males					Females				
Dosing					Dosing				
Dose Group		PT Seconds	APTT Seconds	FIB mg/dL	Dose Group		PT Seconds	APTT Seconds	FIB mg/dL
1	Mean	7.2	18.2	234	1	Mean	7.2	19.7	169
	SD	0.28	1.16	57.8		SD	0.25	1.13	22.7
	N	5	5	5		N	5	5	5
2	Mean	7.2	24.6**	184	2	Mean	6.8	21.6	182
	SD	0.20	3.29	19.1		SD	0.32	2.27	24.2
	N	5	5	5		N	5	5	5
3	Mean	7.2	33.3**	219	3	Mean	7.3	27.6	162
	SD	0.16	2.17	30.5		SD	0.34	6.99	2.6
	N	5	5	5		N	5	5	4
4	Mean	7.0	37.7**	225	4	Mean	6.9	33.2**	171
	SD	0.20	4.06	43.0		SD	0.13	2.72	4.4
	N	5	5	5		N	5	5	5

Recovery					Recovery				
Dose Group		PT Seconds	APTT Seconds	FIB mg/dL	Dose Group		PT Seconds	APTT Seconds	FIB mg/dL
1	Mean	7.0	18.6	202	1	Mean	7.2	19.4	175
	SD	0.14	1.41	59.4		SD	0.00	0.07	27.6
	N	2	2	2		N	2	2	2
2	Mean	7.3	20.3	192	2	Mean	6.9	19.5	162
	SD	0.35	2.62	41.0		SD	0.00	2.97	5.7
	N	2	2	2		N	2	2	2
3	Mean	7.2	20.4	214	3	Mean	7.0	18.3	173
	SD	0.35	3.68	46.7		SD	0.07	2.97	28.3
	N	2	2	2		N	2	2	2
4	Mean	7.1	28.6	208	4	Mean	7.2	21.6	150
	SD	0.21	2.33	11.3		SD	0.21	0.99	
	N	2	2	2		N	2	2	1

\*\*p≤0.01

These changes in APPT values are consistent with findings observed in rats. Therefore, they appear to be related to the test article. As noted in the Study Report, there was no clinical evidence of bleeding and no signs of hemorrhage at necropsy. Therefore, the magnitude of the change was not considered adverse.

#### Clinical Chemistry (Pretest, at termination, and end of recovery period)

There was a non-dose related, statistically significant decrease in mean serum bicarbonate values at termination of dosing for females. The Study Report indicates the decrease was attributed to slightly low values in a few individual animals. The decrease was not observed at the end of the recovery period. Based on the lack of any other sign of toxicity and lack of a similar effect in males, this toxicological relevance of the finding is unclear.

#### Urinalysis (Pretest, at termination, and end of recovery period)

No test article-related effects

#### Gross Pathology (Main and recovery animals)

No test article-related findings

#### Organ Weights (Adrenals, brain, heart, kidneys, liver, pituitary gland, prostate gland, spleen, testes, thyroid/parathyroid glands)

No test article-related changes

**Histopathology (Standard battery in main animals from control and high dose groups; kidneys for recovery control and high-dose groups)**

Adequate Battery - Yes

Peer Review - No

Histological Findings – At the injection sites, findings included dermis/subcutaneous tissue subacute inflammation with eosinophils and skeletal muscle acute/subacute inflammation. These findings were observed in both control and high-dose groups and generally were of minimal to slight severity. These findings were considered to be tissue responses to the trauma caused by the injection procedures.

**Immunogenicity (End of dosing [Day 29] and at the end of the recovery period)**

At the end of the 4-week treatment period, ATAs were present in the plasma of 3/6 dogs at 30 U/kg, 4/6 in dogs at 100 U/kg, and 6/6 dogs at 300 U/kg.

By the end of the recovery phase, ATAs were present in 1/4 dogs at 30 U/kg, 2/4 dogs at 100 U/kg, and 3/4 dogs at 300 U/kg.

Anti-therapeutic antibodies were detected in both dogs (the 30 U/kg male [# 2152M] and 100 U/kg female [# 3654F]) that had markedly decreased systemic exposures following the final EZN-2279 (SC-PEG rADA) dose (see Toxicokinetics below). However, neutralizing antibodies were only detected for the 100 U/kg female (55.6% neutralization on Day 29; 60.8% neutralization at end of recovery period). This female showed no plasma ADA activity after the 2-hour collection time point.

Regarding the neutralization assay, the Study Report warns (page 498) that the negative results may be due to interference by the high levels of ADA activity (>1000 mU/mL) in most samples at the mid and high doses on Day 29. The testing facility was Hungtingdon Life Sciences, Inc.

**Toxicokinetics (Day 1 predose and at 2, 6, 24, 48 and 72 hours after the 1<sup>st</sup> dose and on Day 29 predose and at 2, 6, and 24 hours for all animals and 48, 72, 96, 120 and 144 hours post last dose and 14 and 28 days post last dose for recovery animals; levels of EZN-2279 measured by plasma ADA enzymatic activity, i.e., conversion of adenosine to inosine)**

In general, animals were exposed to quantifiable enzyme activity after twice weekly IM administrations of 30, 100 and 300 U/kg of EZN-2279. With few exceptions, plasma enzyme activity was also quantifiable in pre-dose samples on Day 29, indicating that animals were continuously exposed to quantifiable enzyme activity over the examined dose levels of EZN 2279.

Exposure generally increased proportional to the dose from 100 to 300 U/kg, but it was higher than dose proportional from 30 to 100 U/kg (Table 17).

At all dose levels, the systemic exposure on Day 29 was greater than that on Day 1, suggesting test article accumulation after twice weekly administration.

In general, the plasma enzyme activity was quantifiable in all designated recovery animals 672 hours after the last intramuscular injection of 30, 100 and 300 U/kg of EZN-2279. The calculated mean terminal elimination half-life across groups ranged from 113 to 173 hours following the first dose and from 122-227 following repeated dosing (Day 29).

No gender differences were observed.

One male at 30 U/kg (# 2152M) showed markedly reduced plasma ADA activity after the last dose on Day 29. One female at 100 U/kg (# 3654F) had no detectable plasma ADA activity after the 2-hour collection timepoint in Day 29. As noted above, the decreased activity may be related to the formation of ATAs.

**Table 17: Mean ( $\pm$  SD) Plasma Toxicokinetic Parameters (Enzyme Activity) in Dogs – Study # 07-3280/AD07029**

Day	Sex	Dose <sup>b</sup> (U/kg)	AUC <sub>0-24h</sub> (U•h/mL)	AUC <sub>0-72h</sub> (U•h/mL)	AUC <sub>last</sub> (U•h/mL)	C <sub>max</sub> (U/mL)	t <sub>max</sub> (h)
1	Male	30	5.42 $\pm$ 1.3	18.1 $\pm$ 2.6	18.1 $\pm$ 2.6	0.30 $\pm$ 0.04	38.4 $\pm$ 21.5
		100	26.9 $\pm$ 2.16	85.5 $\pm$ 5.5	85.5 $\pm$ 5.5	1.33 $\pm$ 0.11	30.0 $\pm$ 24.7
		300	63.1 $\pm$ 11.4	195 $\pm$ 10.5	195 $\pm$ 10.5	3.18 $\pm$ 0.37	19.6 $\pm$ 9.8
	Female	30	5.69 $\pm$ 1.12	19.1 $\pm$ 2.22	19.1 $\pm$ 2.22	0.31 $\pm$ 0.03	28.8 $\pm$ 10.7
		100	26.4 $\pm$ 3.2	86.9 $\pm$ 5.8	86.9 $\pm$ 5.8	1.39 $\pm$ 0.06	28.8 $\pm$ 10.7
		300	66.6 $\pm$ 4.0	200 $\pm$ 9.6	200 $\pm$ 9.6	3.15 $\pm$ 0.19	20.4 $\pm$ 8.0
29	Male	30	17.7 $\pm$ 8.6	58.3 $\pm$ NA	107 $\pm$ 126	0.81 $\pm$ 0.37	14.4 $\pm$ 18.8
		100	92.4 $\pm$ 5.9	283 $\pm$ NA	504 $\pm$ 570	4.14 $\pm$ 0.23	20.8 $\pm$ 18.3
		300	212 $\pm$ 17.6	589 $\pm$ NA	1070 $\pm$ 1170	9.21 $\pm$ 0.71	9.6 $\pm$ 8.0
	Female	30	20.6 $\pm$ 2.4	65.5 $\pm$ NA	115 $\pm$ 130	0.91 $\pm$ 0.091	12.4 $\pm$ 10.7
		100	91.9 $\pm$ 7.0	262 $\pm$ NA	305 $\pm$ 428	4.16 $\pm$ 0.30	5.00 $\pm$ 2.0
		300	214 $\pm$ 12.9	597 $\pm$ NA	969 $\pm$ 1030	9.72 $\pm$ 0.70	6.00 $\pm$ 0.0

<sup>a</sup> NA = Not applicable.

<sup>b</sup>Total of 9 doses administered intramuscularly to dogs twice weekly over 4 weeks (Days 1, 4, 7, 11, 14, 18, 21, 25, and 29).

### Dosing Solution Analysis

Samples for concentration analysis were collected from dosing solutions following completion of dosing on Days 1 and 29. Dose preparations for Groups 2 and 4 on Day 1 were slightly lower than the (b) (4) % of nominal. All other solutions that were evaluated were within acceptable limits of nominal ((b) (4) % to (b) (4) %).

## 7 Genetic Toxicology

Studies to evaluate the mutagenic potential of elapegademase were not performed. These studies are not applicable to biotechnology-derived pharmaceuticals (ICH guidance S6).

SC-PEG rADA has a different linker compared to Adagen<sup>®</sup>. See Section 2.4 of this review for information provided by the applicant to support the safety of the linker.

## 8 Carcinogenicity

Long-term studies in animals to evaluate carcinogenic potential of elapegademase have not been performed. Per ICH S6 guidance, standard carcinogenicity studies are generally not appropriate for biotechnology-derived pharmaceuticals.

As noted by the applicant, the mechanism by which SC-PEG rADA produces its pharmacologic effect is through replacement of naturally occurring ADA (deficient in target population), which is essential for the survival of lymphocytes and to the immunity of ADA-SCID patients. In addition, SC-PEG rADA is structurally and pharmacologically similar to Adagen<sup>®</sup>. As the nonclinical and clinical safety profile of Adagen<sup>®</sup> is well-established, specific carcinogenicity studies were not conducted for SC-PEG rADA.

## 9 Reproductive and Developmental Toxicology

### 9.2 Embryonic Fetal Development

#### Study title: A Dose Range-Finding Embryo-Fetal Development Study of EZN-2279 by Intramuscular Injection in Rats

Study no.:	STPI20098870 (Leadiant); 20098870 (b) (4)
Study report location:	EDR Module 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 15, 2016
GLP compliance:	Yes (a list of exceptions was provided on page 9 of the Study Report; these are not considered to affect the integrity of the data)
QA statement:	Yes
Drug, lot #, and % purity:	EZN-2279 (556 U/mL, 1.64 mg/mL), lot # 6046 99% pure (by SEC and HPLC [late and main peak])

**Note:** The to-be-marketed product was used in this study.

## Key Study Findings

- Maternal mean body weight was lower ( $\leq 4.2\%$ ) than controls throughout the dose period in the high-dose group. The decreased mean body weight was associated with decreased (6.8%) mean body weight gain at the GD 7 to GD 8 interval.
- Based on the small magnitude of the change in mean body weight/body weight gain and lack of any other toxicities, the maternal NOAEL was selected a 500 U/kg/day, IM (high dose).
- EZN-2279 did not result in any adverse embryofetal effects.
- Immunogenicity analysis indicated that there were no gravid rats that were positive for anti-EZN-2279 or anti-PEG antibodies.
- The developmental NOAEL was stated to be 500 U/kg every 3 days (GD7 AUC of 183000 mU•hr/mL), however the study was not sufficiently powered to derive a reliable NOAEL.
  - (b) (4)  
This reviewer's calculation gives a no-effect level value of 1.48 mg/kg [2.96 mg/kg/week] using nominal dose or 1.76 mg/kg [3.52 mg/kg/week] using actual measured protein concentration - see Methods below).
- The study, however, has the following limitations:
  - Evaluation of between 16 to 20 litters per group is recommended for rodents per ICH S5A guidance. Therefore, an N of 8 litters/group is well below guidance recommendations, and the study is not considered sufficiently powered to detect rare effects. Accordingly, a reliable NOAEL cannot be calculated.
  - The safety margin is below the optimal 10-fold (see below).

The PK profile of SC-PEG rADA was evaluated in 6 patients with ADA-SCID (five adults and one pediatric) who received weekly IM injections at a dose ranging from 4.99 to 19.6 mg in two studies (page 7, Comparative Pharmacokinetic Report, Module 5.3.3.2, SD # 5, 10-24-17). (b) (4)

The dose-normalized median  $AUC_{0-168hr}$  was 5710 hr\*mmol/hr/L (range 4330 -7480) in Study 1, compared to individual results of 6270 and 8890 hr\*mmol/hr/L in Study 2. The clinical pharmacology team considered the data should be normalized by dose administered per patient. Therefore, the following mean values were calculated:

$$\text{DN } AUC_{0-168hr} ((hr*\mu\text{mol/hr/mL})/(\text{mg/kg})) = 31272.5$$

$$\text{DN } C_{max} (\mu\text{mol/hr/mL})/(\text{mg/kg}) = 219.17$$

On page 8, EDR Module 2.7.1 "Summary of Biopharmaceutic Studies and Associated Analytical Methods", it is stated that the upper and lower limits of quantitation (ULOQ and LLOQ) of the assay to determine plasma PEG-ADA activity, defined as the highest and lowest concentrations, respectively, that can be measured with acceptable precision and accuracy, were determined to be 250 mU/mL and 15 mU/mL. These values

correspond to ULOQ of 15  $\mu\text{mol/hr/mL}$  and LLOQ of 0.9  $\mu\text{mol/hr/mL}$ . Accordingly, there are 16.7 mU/mL per 1  $\mu\text{mol/hr/mL}$ .

Therefore, a human mean  $\text{AUC}_{0-168\text{hr}}$  of 31272.5 ( $\text{hr}\cdot\mu\text{mol/hr/mL}/(\text{mg/kg})$ ) is equivalent to:

$$31272.5 (\text{hr}\cdot\mu\text{mol/hr/mL})/(\text{mg/kg}) \times 16.7 (\text{mU/mL})/(\mu\text{mol/hr/mL}) = 522250 (\text{mU}\cdot\text{hr/mL})/(\text{mg/kg})$$

Exposure margin = 183000 (GD7 AUC)  $\div$  522250 (human mean AUC, as calculated above)

= 0.35-fold the mean human AUC, normalized to the dose of SC-PEG rADA administered per patient

- It is uncertain if the lack of embryofetal development findings were related to the low power of the study and/or low doses used. Therefore, the study is not considered adequate for risk assessment.

## Methods

Doses: Dose measured via RP-HPLC (dose measured via spectrophotometric assay in parentheses): 0, 100 (48), 300 (143), and 500 (238) U/kg/day

**Reviewer's note:** The study report states that protein concentration from all dose groups exceeded the acceptable limits of (b) (4) % of the nominal concentrations (0.33, 0.97, or 1.65 mg/mL) by approximately 20%. As such, actual concentrations\* were 0.40, 1.16, or 1.96 mg/mL, respectively).

The doses in mg/kg were not provided in the Study Report. Therefore, mg/kg were calculated by this reviewer as follows:

Per Section 4.1.1 in Study Report (page 15), the specific activity is 339 U/mg protein (556 U/mL divided by 1.64 mg/mL protein).

100 U/kg  $\div$  339 U/mg = 0.29 mg/kg (or 0.36 mg/kg using actual protein concentration\*)

300 U/kg  $\div$  339 U/mg = 0.88 mg/kg (or 1.04 mg/kg using actual concentration\*)

500 U/kg  $\div$  339 U/mg = 1.48 mg/kg (or 1.76 mg/kg using actual protein concentration\*)

Frequency of dosing: Once every 3 days (GDs 7, 10, 13 and 16)

Dose volume: 0.9 mL/kg

Route of administration: IM injection into the forelimb or hindlimb  
Formulation/Vehicle: Phosphate Buffered Saline, pH 6.9  
Species/Strain: Sprague Dawley (CrI:CD[SD]) female rats  
Number/Sex/Group: 8  
Satellite groups: TK animals: 3 females in control group; 6 females in all EZN-2279-treated groups  
Study design: Animals were dosed from gestation day (GD) 7 through 16. The injection site was rotated on each day of dosing (i.e., DG 7: right hindlimb, DG 10: left hindlimb, DG 13: right forelimb and DG 16: left forelimb). Animals assigned to the main study and TK study were euthanized on GD 21 and GD 19, respectively.

Note: The applicant stated that the number of animals chosen for this study was the smallest number considered necessary to provide the minimum number of pregnancies recommended by the applicable guidelines (*not specified*). However, per ICH Guidance S5A, evaluation between 16-20 litters is recommended. The design of this study (insufficiently powered) is a major study limitation, and precludes inclusion of these data in the labeling.

Deviation from study protocol: None with an impact on the interpretation of the data

## Observations and Results

### Mortality (2x/day)

None

### Clinical Signs (Daily)

None test article related

### Body Weight (Daily)

A slight decrease in maternal mean body weight was observed at the high dose throughout the study (daily decrease ranged from 2.4 to 4.2%). On Day 21, mean body weight was 4.2% lower than controls.

The decreased mean body weight was related to a decreased mean body weight gain at the GD 7 to GD 8 interval (gain of 0.9 g vs 2.5 g in controls). Afterwards, mean body weight gain in high-dose females was comparable to controls. The overall decrease, GD 7 to 21, was 6.8%.

### Feed Consumption (GDs 7, 10, 13, 16, 19, and 21)

No test article-related effects.

### Toxicokinetics (GDs 7 and 16 at 0, 2, 6, 24, 48, and 72 hours postdose)

Absorption of EZN-2279 was delayed with  $T_{max}$  observed at 24 hours postdose (Table 18). Exposure increased with increasing dose. Overall,  $AUC_{(0-t)}$  and  $C_{max}$  increased in a dose proportional manner on GD 7. On GD 16,  $AUC_{(0-t)}$  increased in a slightly greater than dose proportional manner (7-fold across a 5-fold dose range) from 100 to 500 U/kg/day, while  $C_{max}$  increased in a dose proportional manner.

It was indicated in the Study Report (page 501), that  $AUC_{inf}$  and  $t_{1/2}$  were not calculable due to limited data in the terminal elimination phase (i.e., there were < 3 measurable concentrations following  $C_{max}$ ); however,  $t_{1/2}$  estimates based on the last 2 measurable concentrations after  $C_{max}$  ranged from approximately 35 to 44 hours on GD 7 and from approximately 18 to 34 hours on GD 16.

Accumulation was not observed.

**Table 18: Toxicokinetic Parameters – Rat Dose-Range Embryofetal Development Study**

Group	Dose (U/kg)	Day of Gestation	AUC0-t (mU*h/mL)	AUC0-t/D	C <sub>max</sub> (mU/mL)	C <sub>max</sub> /D	T <sub>max</sub> (h)	Estimated T <sub>1/2</sub> <sup>1</sup> (h)	Accumulation Ratio
2	100	7	35900	359	656	6.56	24	44	NA
2	100	16	31400	314	867	8.67	6	34	0.874
3	300	7	96300	321	1830	6.09	24	35	NA
3	300	16	110000	367	2100	7.01	24	18	1.14
4	500	7	183000	366	3310	6.61	24	35	NA
4	500	16	229000	457	4540	9.08	24	18	1.25

<sup>1</sup> = T<sub>1/2</sub> was estimated using last 2 measurable concentrations following C<sub>max</sub> from each profile

NA= Not applicable

T<sub>1/2</sub> and AUC<sub>inf</sub> were not reportable due to limited data in the terminal elimination phase

Note: Units for dose normalized AUC0-t/D and C<sub>max</sub>/D are (mU\*h/mL)/(U/kg) and (mU/mL)/(U/kg), respectively

Immunogenicity analysis showed that there were no gravid rats that were positive for anti-EZN-2279 or anti-PEG antibodies.

### Dosing Solution Analysis

It was stated in the study report that dose formulation samples were collected for analysis after the 1<sup>st</sup> preparation and last preparation at all dose levels. Certificate of Analysis (CoA) dated (b) (4) and (b) (4) indicate the dosing solutions were within specifications for enzymatic activity. Protein concentration from all dose groups exceeded the acceptable limits of (b) (4) % of the nominal concentration. The percent mean bias for the 0.33, 0.97, or 1.65 mg/mL were 20%, 19% or 19% following the analysis (the actual concentration was 0.40, 1.16, or 1.96 mg/mL, respectively).

### Necropsy (Main animals on GD 21 and TK animals on GD 19)

No test article-related findings

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

There were no statistically significant changes in any test article-treated group compared to controls. There was a slight decrease/increase in the mean value of the following parameters at the high dose (Table 19). However, as the values were within historical control range ( (b) (4) June 2011- January 2015; Appendix 13 in the Study Report), the findings were considered of no toxicological relevance.

**Table 19: Summary of Selected C-Section Findings**

Sex: Female		0 U/kg Group 1	100 U/kg Group 2	300 U/kg Group 3	500 U/kg Group 4
Number of Corpora Lutea	Mean	13.5	12.8	12.0	11.9
	SD	1.7	1.5	2.1	1.7
	N	8	8	8	8
	%Diff		-5.6	-11.1	-12.0
Number of Implantations	Mean	12.8	12.4	11.9	11.3
	SD	1.8	1.6	2.4	1.7
	N	8	8	8	8
	%Diff		-2.9	-6.9	-11.8
Total Number of Fetuses	Mean	12.4	12.1	11.6	10.8
	SD	1.8	1.7	2.6	2.3
	N	8	8	8	8
	%Diff		-2.0	-6.1	-13.1
Number of Live Fetuses	Mean	12.4	12.1	11.6	10.8
	SD	1.8	1.7	2.6	2.3
	N	8	8	8	8
	%Diff		-2.0	-6.1	-13.1
% Post-implantation Loss (%)	Mean	2.96	2.08	2.60	5.06
	SD	4.16	3.86	4.95	11.69
	N	8	8	8	8
	%Diff		-29.60	-12.00	70.97

### Offspring (Malformations, Variations, etc.)

Fetal evaluations were based on 99, 97, 93 and 86 viable DG 21 Cesarean-delivered fetuses in 8, 8, 8, and 8 animals, in the 0, 100, 300, and 500 U/kg/day dose groups, respectively. All fetuses in each litter were examined for soft tissue abnormalities, skeletal abnormalities, and fetal ossification site means.

There were no EZN-2279-related fetal external, soft tissue, and skeletal malformations or variations observed at any dose level. All ossification site averages were comparable across all dose groups.

At the high dose, one fetus had small eyes. It was stated in the study report that this finding was considered unrelated to EZN-2279 because it was limited to a single

fetus. Based on historical control data, the incidence of microphthalmia is reported with a fetal and litter percent (range/study) of 0-1.9% (0-3 fetuses) and 0-12.5%, (0-3 litters) respectively. The fetal and litter incidence in this study (i.e., pooling together all 3 test article-treated groups) is within the range observed per study in the historical database (1 in 131 fetuses = 0.76% and 1 in 24 litters = 4.2%). Therefore, this reviewer agrees that the findings was unrelated to the test article.

## 10 Special Toxicology Studies

During the clinical development, severe injection site pain was experienced by the first patient dosed with SC-PEG rADA containing (b) (4). As a result, Leadiant conducted the following non-GLP study comparing the nociceptive response and swelling in a mouse model following administration of the study drug SC-PEG rADA with and without (b) (4) compared to Adagen®. (b) (4)

The study demonstrated significant pain and swelling at the injection site following administration of formulations containing (b) (4). Therefore, (b) (4) was removed from the drug product formulation.

**Nociceptive Effects by Test Items following Intra-plantar Injection in Mouse (Study # 5900166; non-GLP; EDR Module 4.2.3.6)** - Eight C57BL/6 male mice per group were treated with 5% formalin (positive control), PBS (negative control), EZN2279 (SC-PEG rADA) with (b) (4), EZN2279 without (b) (4), Adagen®, buffer without (b) (4), or buffer with high concentration (b) (4) by intra-plantar subcutaneous injection into the left hind paw. Nociceptive behaviors such as biting, licking, shaking, and flinching of the injected paw was scored in seconds over 1 minute bins for a period of 10 minutes following the intra-plantar injections. In addition, the animals were individually scored for degree of swelling and redness of the injected paw.

EZN2279 without (b) (4), Adagen® and buffer without (b) (4) did not produce significant nociceptive responses in mice compared with PBS. Time spent in nociceptive behavior was significantly higher for mice treated with 5% formalin, EZN2279 with (b) (4), (b) (4), and buffer with high concentration (b) (4) as compared to mice treated with PBS. Mice treated with EZN2279 with (b) (4) spent about four times as long on nociceptive behavior as compared to mice treated with EZN2279 without (b) (4) (significance was not provided).

Significant swelling was observed with 5% formalin as well as with EZN2279 containing (b) (4) and buffer with high concentration (b) (4), consistent with their behavioral nociceptive effects. Mean swelling score was lowest in mice treated with EZN2279 without (b) (4) (score=0). A score of ~0.85 was observed in mice treated with EZN2279 with (b) (4). Adagen® mean swelling score was approximately 0.20.

## 11 Integrated Summary and Safety Evaluation

SC-PEG rADA has been developed to replace the native bovine ADA used in the manufacture of Adagen® for the treatment of ADA-SCID. The key differences between SC-PEG rADA and Adagen® are the replacement of native bovine sourced ADA with a recombinant product and the use of (b) (4) SC linker for PEGylation. Given the 25-year successful clinical history with Adagen®, the structural and pharmacological comparability of Adagen® and SC-PEG rADA and the safe use of the stable PEG SC linker in other FDA approved biologics, a PK and PD bridging toxicology strategy was used in the nonclinical evaluations.

The nonclinical PK studies showed a longer terminal half-life and systemic exposure (AUC), as measured by ADA enzymatic activity, for SC-PEG rADA compared to Adagen®. These PK differences did not result in increased toxicity in the 4-week general toxicology studies (see below). Per applicant's statements, an approximate 2-fold higher exposure with SC-PEG rADA as compared to Adagen® was observed in primary Phase 3 clinical Study STP-2279-002, and it was not associated with additional adverse findings. SC-PEG rADA was slightly more efficacious than Adagen® in the ADA-deficient mouse model, i.e., ADA-deficient mice treated with SC rPEG-ADA showed a longer life span than those treated with Adagen®. These results likely reflect SC-PEG rADA more favorable PK.

Early nonclinical studies were not conducted with the to-be-marketed formulation. These early lots of SC-PEG rADA were well-tolerated in repeat-dose toxicity studies in rats and dogs when given every 3 to 4 days for 4 weeks (nine doses total). Drug-related findings in rats and dogs were limited to a slight increase in APTT that was reversible or partially reversible during a 4-week recovery period. The increased APTT was not associated with clinical evidence of bleeding and no signs of hemorrhage at necropsy. Therefore, the magnitude of the change was not considered adverse. The NOAEL in both rats and dogs was the highest dose, 300 U/kg 2x/week (1.92 mg/kg 2x/week) SC-PEG rADA. The NOAEL is 0.54-fold (rat) and 1.86-fold (dog) the mean human AUC normalized to the dose of SC-PEG rADA administered per patient (See Table 20).

The applicant included as reference two study reports conducted with Adagen®: 2-week study in mice (200 U/kg 2x/week, IP) and 2-month study in rats (0, 150 and 500 U/kg 1x/week, IM). No adverse findings were reported in these studies. APPT was not measured in these studies. Therefore, it is not known if Adagen® could have a similar effect on APTT in nonclinical species. However, per Adagen® label, abnormal blood clotting (thrombocytopenia, thrombocytopenia, autoimmune thrombocytopenia) have been identified as adverse reactions post-marketing. These adverse reactions have not been observed in the interim clinical trial results with SC-PEG rADA, but the applicant acknowledges that these reactions were reported from pharmacovigilance over a long post-marketing period, which is not directly comparable with clinical trial experience.

Repeat doses in both species resulted in the formation of anti-therapeutic antibodies (ATAs), including neutralizing antibodies (Nabs), that could potentially reduce

exposure to ADA activity in comparison with single-dose administration. Adagen® was also highly immunogenic in rats (Study # 08-2060/AD08001) (note: immunogenicity after Adagen® treatment was not evaluated in dogs in the present application). Immunogenicity observed in animals does not always translate into a similar effect in the clinic. The potential for immunogenicity was assessed in the clinical trials.

The product used in the 4-week repeat-dose toxicity study # 15-2483/STPI130260A and embryofetal developmental toxicity study # 20098870/STPI20098870 is the same as to the to-be-marketed product and to the product used in clinical trials. There was lower incidence or no evidence of ATA and/or Nab production in these toxicology studies. The applicant concludes that the absence of consistent ATA production in the latter toxicology studies likely reflects improvements in SC-PEG rADA

(b) (4)  
This reviewer considers this conclusion plausible. However, the performing laboratory was the same for these two studies and different from that of earlier studies. Therefore, the possibility exists that differences in assay methodology contributed to the lower immunogenicity observed in these studies.

Study 15-2483/STPI130260A was the first toxicology study that was conducted using the to-be-marketed drug product, thus serving as a bridging study between the early studies nonclinical lots and the to-be-marketed product. This study also included an arm with SC-PEG rADA spiked with (b) (4)% (b) (4) impurity excess. Drug product stability data shows (b) (4)

(b) (4) over time when the product is stored under long-term conditions (2-8 °C). A (b) (4)% (b) (4) excess of impurity was used to give an adequate exposure margin for establishing impurity specification limits. The current specification limit for the impurity is not more than (NMT) (b) (4)%. The “unspiked” SC-PEG rADA contained (b) (4) impurity below the limit of quantification, i.e., (b) (4)%.

SC-PEG rADA given every 3 or 4 days for 4 weeks (eight doses total) at a dose of 500 U/kg (with and without (b) (4)% of the (b) (4) impurity) to rats did not result in drug related adverse effects. As noted in earlier studies, a slight prolongation of APTT was observed. The NOAEL is 0.71-fold the mean human AUC normalized to the dose of SC-PEG rADA administered per patient (See Table 20).

Leadiant did not conduct fertility and early embryonic development studies to assess early embryonic safety or fertility. Leadiant provided the following information to support low infertility/early embryonic developmental risk with SC-PEG rADA treatment:

- ADA is considered important for pregnancy in part due to its potent stimulatory effects on vascular development. The activity of ADA is very high in the placenta,

relative to most other tissues, including the developing embryo itself which has low ADA activity<sup>6</sup>.

- The high ADA activity in the decidua and trophoblast reflect a physiological role for adenosine in the vascular changes that are associated with establishing the embryo in the uterus. Inhibition of ADA activity with pentostatin in mice on days 7 and 8 of pregnancy resulted in increased incidence of postimplantation resorptions (61% and 78% resorptions, respectively)<sup>7</sup>.
- There were no adverse effects in organ weight, macroscopic, and/or microscopic evaluations on male or female reproductive organs in the 4-week repeat-dose toxicity studies conducted in the rat and dogs by Leadiant.
- No reports of infertility have been received in the over 25 years of pharmacovigilance data from Adagen<sup>®</sup> patients. Two reports of successful pregnancies have been received.

The applicant conducted a dose-ranging embryofetal development study. There were no adverse maternal or embryofetal findings. The no effect level for the study was 500 U/kg/day (3.70 mg/kg/week [redacted]<sup>(b) (4)</sup>; 1.48 mg/kg/day [2.96 mg/kg/week] nominal dose or 1.76 mg/kg/day [3.52 mg/kg/week] using actual protein concentration, per this reviewer's calculations; see note). The study, however, has the following limitations:

- Evaluation of between 16 to 20 litters per group is recommended for rodents per ICH S5A guidance. Therefore, an n number of 8 litters/group is below guidance recommendations. The study was insufficiently powered to detect rare events, and a reliable NOAEL could not be calculated.
- The safety margin is below the optimal 10-fold (i.e., 0.35-fold the mean human AUC normalized to the dose of SC-PEG rADA administered per patient).

It is uncertain if the lack of embryofetal development findings were related to study design (insufficiently powered) and/or low doses used. Therefore, the study is not considered adequate for risk assessment. As such, the study results will not be included in labeling, due to concern that they may be more misleading than informative.

**Reviewer's note:** *There is a discrepancy between this reviewer's dose calculations and those of the applicant. [redacted]<sup>(b) (4)</sup> doses up to 3.70 mg/kg/week [1.85 mg/kg/day at a 2x/week dosing frequency] were administered in the dose-ranging embryo-fetal development study. This reviewer calculated a high nominal dose of 1.48 mg/kg/day and actual dose of 1.76 mg/kg/day (see calculations under Study # STPI20098870/20098870). The applicant indicated that the doses were converted from U/kg to mg/kg using the formula described in Module 5.3.5.2 Study STP-2279-002, Section 9.4.5.2. However, the formula found in the referred section is to convert Adagen<sup>®</sup>*

<sup>6</sup> Knudsen T.B., Blackburn M.R., Chinsky J.M., et al., 1991, Ontogeny of adenosine deaminase in the mouse decidua and placenta: immunolocalization and embryo transfer studies, *Biol Reprod.* 44(1): 171-184.

<sup>7</sup> Knudsen T.B., Gray M.K., Church J.K., et al., 1989, Early postimplantation embryoletality in mice following in utero inhibition of adenosine deaminase with 2'-deoxycoformycin, *Teratology* 40(6): 615-626.

*dose to the equivalent SC-PEG rADA dose, and thus, it is not considered applicable to convert SC-PEG rADA animal doses in U/kg to mg/kg.*

As noted above, two reports of successful pregnancies have been received from pharmacovigilance data with Adagen®. Given the structural and pharmacological comparability and similar toxicological profile between Adagen® and SC-PEG rADA, additional reproductive nonclinical studies are not considered necessary for approval. Pharmacovigilance data can be monitored for potential drug-related effects post-marketing.

The nonclinical studies did not include chronic dosing. The sponsor justification included the following observations, which were considered acceptable:

- EZN-2279 is structurally similar to Adagen®. Adagen® was evaluated in studies of 2-weeks in mice (200 U/kg IM) and 2 months in rats (0, 150, and 500 U/kg IM; 4-week interim). No adverse effects were observed. Extending adenosine deaminase parenteral dosing from 2 weeks to 8 weeks did not produce any additional or cumulative toxicological effects. Thus, considering the similarity between Adagen® and SC-PEG rADA, study durations of 4 weeks were considered sufficient to assess the potential toxicity of SC-PEG rADA.
- No significant adverse effects were observed in 4-week, repeat dose toxicology studies in both rats and dogs.

**Reviewer's note:** *SC-PEG rADA doses evaluated were up to 6.44 mg/kg/week in rats and 3.84 mg/kg/week in dogs, which are 0.71-fold and 1.86-fold, the mean human AUC normalized to the dose of SC-PEG rADA administered per patient (See Table 20).*

*There is a discrepancy between this reviewer's dose calculations and those of the applicant. [REDACTED] (b) (4) doses up to 6.67 mg/kg/week were administered in the 4-week repeat-dose studies. This reviewer calculated a value of 6.44 mg/kg/week for the highest dose evaluated in the rat (see calculation under Study # STPI130260A/15-2483).*

- Extensive data is available from worldwide Adagen® use in patients from varied age groups for 25 years. SC-PEG rADA has been developed [REDACTED] (b) (4) with recombinant bovine adenosine deaminase in SC-PEG rADA. Given that these enzyme replacement products are highly similar, both structurally and functionally, the abundant clinical safety data available for Adagen® can also be extended to SC-PEG rADA.

In addition, dosing for 4 weeks resulted in the development of ATAs in both rats and dogs. Longer treatments are expected to increase the occurrence of these antibodies. This might result in insufficient drug exposure and failure in gaining any useful safety information with longer treatment.

Information to support the safety of the SC-linker is presented under Section 2.4 of this review.

In conclusion, the nonclinical studies conducted established comparable pharmacological and toxicological profile for SC-PEG rADA and Adagen®. Therefore, the 25-year clinical experience with Adagen® use provide additional support for the safety of chronic treatment with SC-PEG rADA. Overall, the nonclinical data presented support the approval of the marketing application of SC-PEG rADA for the treatment of SCID due to ADA deficiency. Pharmacovigilance data can be monitored for potential drug-related effects on fertility and embryofetal development post-marketing. Approval is recommended.

**Table 20: Exposure Margins**

Toxicity	Study	Species	NOAEL		Safety Margin Based on AUC <sup>c</sup> (Dose normalized AUC) <sup>d</sup>
			Dose U/kg 2x/week (mg/kg/2x/week) <sup>a</sup>	AUC mU•hr/mL <sup>b</sup>	
Slightly increased APTT	4-week repeat-dose				
	Early lots	Rats	300 (1.92)	281,000	0.54
		Dogs	300 (1.92)	969,000	1.86
	Clinical lot	Rats	500 (3.22)	371,000	0.71
None	Embryofetal development – Dose-range finding	Rats	500 (1.76)	183,000	0.35

<sup>a</sup> See each individual study reviews under Section 6.2 “Repeat-Dose Toxicity” of this review for calculations of these values.

<sup>b</sup> The value represents the lower mean AUC<sub>last</sub> observed on Day 29 for “early lots” and AUC<sub>0-72hrs</sub> observed on Day 25 for “clinical lot”, irrespective of gender. The AUC<sub>0-t</sub> on GD 7 was used in the embryofetal development dose-range finding study.

<sup>c</sup> AUC in human:

(b) (4) The weekly dose-normalized mean AUC<sub>0-168hr</sub> is 31272.5 (hr•µmol/hr/mL)/(mg/kg). This dose was converted to (mU•hr/mL)/(mg/kg) multiplying by a conversion factor of 16.7 mU/mL per µmol/hr/mL, i.e.,

$$31272.5 \text{ (hr}\cdot\mu\text{mol/hr/mL)} / (\text{mg/kg}) \times 16.7 \text{ (mU/mL)} / (\mu\text{mol/hr/mL}) = 522250.75 \text{ (mU}\cdot\text{hr/mL)} / (\text{mg/kg})$$

See Section 9.2 “Embryonic Fetal Development” of this review for further details.

<sup>d</sup>MRHD: Maximal recommended human dose is 0.4 mg/kg/week.

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**This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.**  
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/s/  
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MARIA I RIVERA  
08/14/2018

LORI E KOTCH  
08/14/2018