Clinical Research Protocol Approval for Study STP2279-002
August 23, 2017

A Study of EZN-2279 (Polyethylene Glycol Recombinant Adenosine Deaminase [PEG-rADA]) Administered as a Weekly Intramuscular Injection in Patients with Adenosine Deaminase (ADA)-Deficient Combined Immunodeficiency

NCT01420627

CLINICAL RESEARCH PROTOCOL

Sponsor:	Leadiant Biosciences, Inc. 9841 Washingtonian Blvd. Suite 500 Gaithersburg, Maryland 20878 (800) 447-0169
IND Number:	100,687
Product Name:	EZN-2279 (PEG-rADA)
Protocol Number:	STP-2279-002
Protocol Title:	A Study of EZN-2279 (Polyethylene Glycol Recombinant Adenosine Deaminase [PEG-rADA]) Administered as a Weekly Intramuscular Injection in Patients with Adenosine Deaminase (ADA)-Deficient Combined Immunodeficiency
Version Number:	3.0
Date:	August 23, 2017

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2. STUDY SYNOPSIS

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<tr><td>Country:
USA</td><td>No. of centers:
Up to 12</td><td colspan="2">Expected Study Duration:
Approximately 48 Months</td></tr>
<tr><td colspan="4">Study Objectives:
<u>Primary Objective</u>: The primary objective of the study is to evaluate whether therapy with EZN-2279 achieves metabolic detoxification, as demonstrated by total erythrocyte dAXP concentration from a trough blood sample.
<u>Secondary Objective(s):</u> The secondary objectives of the study are to:
• Evaluate the safety and tolerability of EZN-2279
• Assess the immunogenicity to EZN-2279 and Adagen®, including binding antibodies, neutralizing antibodies and anti-PEG antibodies
• Evaluate whether therapy with EZN-2279 maintains the trough plasma ADA activity ≥15 µmol/h/mL
• Determine the PK profile of EZN-2279
• Assess the effect of EZN-2279 on immune status as determined by ALC, lymphocyte subset (B, T, and NK) analysis, and immunoglobulin (Ig) concentration (IgA, IgG, IgM),
• Compare the clinical status (infections and hospitalizations) of the patients during the EZN-2279 treatment period compared to the prior six months
• Assess the clinical growth status of the patients during the EZN-2279 treatment and maintenance periods</td></tr>
<tr><td colspan="4">Study Endpoints:
<u>Primary Endpoint:</u> The primary endpoint is the percentage of patients achieving metabolic detoxification, assessed from trough dAXP samples obtained at Weeks T-15, T-17, T-19, and T-21. Metabolic detoxification will be defined as a trough dAXP concentration equal to or below 0.02 µmol/mL.

<u>Secondary Endpoint(s):</u>
The secondary endpoints for efficacy are:
1) Trough Plasma ADA Activity - will be assessed from trough plasma ADA samples obtained at Weeks T-15, T-17, T-19, and T-21. Adequate trough ADA activity will be defined as ADA activity ≥15 µmol/h/mL.
2) Maintenance phase trough plasma dAXP and ADA activity – will be assessed from trough plasma dAXP and ADA samples obtained during the maintenance phase of the study through completion of EZN-2279 treatment.
3) Immune status will be assessed through completion of the EZN-2279 maintenance period, through assessment of:
• Absolute lymphocyte count
• B-, T-, and NK-lymphocyte subset analysis by fluorescence-activated cell sorter (FACS) with monitoring of:
o CD3+ (Mature T cells) - Percent and Absolute
o CD3+ CD8+ (Suppressor T Cells) – Percent and Absolute
o CD3+ CD4+ (Helper Cells) – Percent and Absolute
o CD (16+56)+ (Natural Killer Cells) – Percent and Absolute
o CD19+ (B Cells) – Percent and Absolute
o Absolute Lymphocytes (CD45+)</td></tr>
</table>

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- %CD4 (Helper Cells) / %CD8 (Suppressor T Cells)
- Quantitative immunoglobulin concentration (IgA, IgG, IgM)

4) Clinical Status – assessed by the following parameters:
- Infections: Clinically documented and microbiologically documented;
- Hospitalizations: Incidence and duration of hospitalizations through completion of the EZN-2279 treatment period compared to the 6-month period prior to study entry;
- Growth: Height-for-age and weight-for-age Z-scores through completion of the EZN-2279 maintenance period;
- Overall survival: will be assessed at the conclusion of the EZN-2279 treatment period though the end of EZN-2279 maintenance

The secondary endpoints for assessment of safety are:
1) Safety will be assessed through determination of adverse events (AEs), serious adverse events (SAEs), physical examinations, and laboratory evaluations through completion of the EZN-2279 maintenance period.
2) Immunogenicity of EZN-2279 and Adagen® will be assessed through the end of the EZN-2279 maintenance period by the evaluation of binding antibodies, neutralizing antibodies and anti-PEG antibodies.

The pharmacokinetic endpoint is the PK profile of EZN-2279, as assessed by plasma ADA activity. The following PK parameters will be computed from individual plasma concentrations collected during full PK sampling for Adagen® at the end of the Adagen® Lead-in period and for EZN-2279 during Week T-9 (after 9th dose of EZN-2279): C_{max}, C_{trough}, t_{max}, $AUC_{(0-t)}$, Kel, and $t_{½}$.

Study Design and Methodology:
Protocol STP-2279-002 is designed as an open-label, multicenter, single-arm, one way crossover study of EZN-2279 to determine the safety, efficacy, and PK of EZN-2279 in patients with ADA-SCID who are currently being treated with Adagen®. Each patient will serve as his or her own control with respect to assessment of study endpoints. The study will enroll enough patients to allow for up to six evaluable patients with ADA-deficient combined immunodeficiency and stable clinical status while currently receiving therapy with Adagen® who meet all eligibility criteria and written informed consent/assent is obtained. The initial three patients will be enrolled and complete through full EZN-2279 pharmacokinetic sampling (Week T-9). An independent data and safety monitoring committee (DSMC) will review safety, PK (ADA activity), and erythrocyte dAXP data for the first three patients who complete through Week T-9. If no concerns are noted, enrollment will continue for the remaining patients. A similar DSMC review will occur after all patients complete through 9-weeks of EZN-2279 dosing.

Patients for whom written informed consent/assent is obtained will be assigned a screening number and undergo screening procedures. Patients completing screening assessments and meeting all eligibility criteria will be enrolled in the study. There are three periods to study treatment: the Adagen® lead-in period, the EZN-2279 treatment period (Weeks T-1 through T-21) and the EZN-2279 maintenance period. Patients enrolled in the study will enter the Adagen® lead in phase of the study. In this phase of the study, patients will receive a single i.m. dose of Adagen® weekly and be assessed weekly for dAXP levels and ADA activity. Patients receiving Adagen® more frequently than once a week will have his/her dose consolidated to a once a week dose regimen during the Adagen Lead-in Phase of the study. Adagen® dose adjustments will be made if the patient does not meet criteria for trough dAXP levels (≤0.02 μmol/mL) and trough ADA activity (≥15 μmol/h/mL) specified in the inclusion criteria. Once the patient meets protocol

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criteria for trough dAXP and ADA activity, he/she will have a full pharmacokinetic assessment performed on Adagen®. Prior to receiving the dose of Adagen® for pharmacokinetic assessment patients will have the following baseline procedures performed: physical examination, vital signs, growth assessment, performance status, safety laboratory assessments (hematology, chemistry, urinalysis), and have blood samples obtained for assessment of lymphocyte subset analysis, quantitative immunoglobulins and Adagen® antibody titers (for neutralizing and binding antibodies) and anti-PEG antibodies. Following Adagen® dosing, plasma ADA samples for full PK evaluation will be collected for 7 consecutive days following dosing. After completing Adagen® lead-in treatment and Pharmacokinetic assessments, patients will enter the EZN-2279 treatment phase (Weeks T-1 through T-21) of the study.

Adagen® will be discontinued and therapy with an equivalent dose of EZN-2279 will be initiated. In the EZN-2279 treatment phase of the study, patients will receive a single i.m. dose of EZN-2279 weekly for 21 consecutive weeks. Prior to receiving the initial dose of EZN-2279, patients will have the following procedures performed: physical examination, vital signs, growth assessment, performance status, safety laboratory assessments (hematology, chemistry, urinalysis), and have blood samples obtained for assessment of lymphocyte subset analysis, quantitative immunoglobulins, EZN-2279 and Adagen® antibody titers (neutralizing and binding antibodies) and anti-PEG antibodies. Trough total erythrocyte dAXP concentration and trough plasma ADA activity will be measured prior to dosing at weeks T-1, T-3, T-5, T-7, T-8 and every other week from Week T-9 through Week T-21. At week T-8 the patient will have dAXP levels and ADA activity assessed to assure the dose of EZN-2279 administered maintains dAXP and ADA activity at protocol specified levels. If these levels are not met, the patient will undergo a dose adjustment and be reassessed prior to entering the EZN-2279 full PK sampling week (Week T-9). Plasma ADA samples for full PK evaluation will be collected at Week T-9 (Days 58-63). Physical examination and safety laboratory assessments will be performed every 4 weeks (Weeks T-5, T-9, T-13, T-17 and T-21). Blood samples obtained for assessment of lymphocyte subset analysis, quantitative immunoglobulins, EZN-2279 antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies will be obtained at selected timepoints during the EZN-2279 treatment period.

Following completion of the EZN-2279 treatment period, patients may enter the EZN-2279 maintenance period. During this phase of the study patients will continue to receive single i.m. doses of EZN-2279 weekly. The following assessments will be performed during study visits every 3 months: physical examination, vital signs, growth assessment, performance status, safety laboratory assessments (hematology, chemistry, urinalysis), and have blood samples obtained for assessment of trough ADA and dAXP levels, lymphocyte subset analysis, quantitative immunoglobulins, antibody titers for EZN-2279 (i.e., neutralizing and binding antibodies), anti-PEG antibodies and PEG. The maintenance phase of the study will continue until the study is concluded (i.e., full regulatory approval of EZN-2279 or early study termination).

Throughout the duration of the study (i.e., Adagen® lead-in, EZN-2279 treatment and EZN-2279 maintenance) patients will continually be assessed for adverse events, infectious complications, hospitalizations, concomitant medications changes and concomitant procedures.

The analysis of study endpoint data will occur after all patients complete the EZN-2279 treatment period (i.e., the primary endpoint analysis), at 6 months after the last patient enters the EZN-2279 maintenance phase, and annually thereafter.

Sample Size:
The study will enroll a sufficient number of patients with ADA-deficient combined immunodeficiency who

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meet all study entry criteria described to assure six (6) patients are evaluable.

Subject Selection Criteria:

Inclusion Criteria

1. Diagnosis of ADA-deficient combined immunodeficiency
2. Stable clinical status while receiving therapy with Adagen®. Patients previously receiving gene therapy or undergoing hematopoietic stem cell transplantation who still require Adagen® treatment are eligible. The dose of Adagen® must be stable for at least 6 months prior to study entry.
3. Have both of the following during the Adagen® Lead-in phase of the study in order to proceed with EZN-2279 dosing:
   a. Trough plasma ADA activity ≥15 μmol/h/mL while receiving Adagen® and
   b. Total erythrocyte dAXP ≤0.02 μmol/mL from a trough blood sample
4. Patients or parent/guardian must be capable of understanding the protocol requirements and risks and providing written informed assent/consent.

Exclusion Criteria

1. Autoimmunity requiring immunosuppressive treatment.
2. Patients with neutralizing anti-Adagen® antibodies at screening evaluation.
3. Severe thrombocytopenia (platelet count $<50 \times 10^9$/L).
4. Current participation in other therapeutic protocols for ADA-deficient combined immunodeficiency.
5. Current or prior participation in another clinical study with an investigational agent and/or use of an investigational drug in the 30 days before study entry.
6. Known planned participation in a gene-therapy study for the planned duration of this study.
7. Any condition that, in the opinion of the PI or Leadiant Biosciences, makes the patient unsuitable for the study.
8. Inability or unwillingness to administer Adagen® or EZN-2279 on a one time per week regimen.
9. Inability to comply with the study protocol.
10. Female patients who are pregnant or lactating.
11. Female patients who are breast-feeding.

Study Duration:

All patients will receive a minimum of a 3-week Adagen® lead in followed by 21-weeks of study treatment with EZN-2279. Patients will be given the opportunity to remain on EZN-2279 maintenance until the study is concluded (full regulatory approval of EZN-2279 or early study termination). Assuming a 12 month enrollment period and 18 months for the submission of data and regulatory approval of EZN-2279, the initial patient enrolled will receive study treatment for approximately 3 years and the last patient enrolled will receive study treatment for approximately 2 years. Patient participation will be approximately 8-weeks longer than the treatment duration (4 week screening period and a 30-day post treatment safety follow-up of patients who discontinue study drug early).

Reference Therapy, Dose Route of Administration and Duration of Treatment:

Adagen® by weekly i.m. injection; patients will be maintained on the same weekly Adagen® dose he/she was taking prior to study entry. Adagen® dose adjustments will be made during the Adagen® Lead-in phase of the study if the patient does not meet criteria for dAXP levels (≤0.02 μmol/mL) and ADA activity (≥15 μmol/h/mL) specified in the inclusion criteria.

Study Drug, Dose, Route of Administration, and Duration of Treatment:

EZN-2279 by weekly i.m. injection; patients will be transitioned to receive a dose of EZN-2279 equivalent to the Adagen® dose received during the Adagen® Lead-in phase of the study. As 1 mg of EZN-2279 contains ADA enzymatic activity equivalent to 150 U of Adagen®, the equivalent dose will be calculated as follows:

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Adagen® dose (U/kg) x 1mg EZN-2279/150U Adagen® = EZN-2279 dose (mg/kg)		

Criteria for Evaluation:

Safety Variables: AEs, SAEs, clinical signs and symptoms from physical examination, laboratory evaluations, and immunogenicity.

Efficacy Variables: Total erythrocyte dAXP from a trough blood sample; trough plasma ADA activity, immune status (B-, T-, and NK-lymphocyte subset analysis, quantitative immunoglobulin concentration [IgA, IgG, IgM]), infections (clinically documented and microbiologically documented), incidence and duration of hospitalizations, growth (height-for-age and weight-for-age Z-scores), and overall survival.

Pharmacokinetic Variables: Adagen® and EZN-2279 pharmacokinetics via assessment of plasma ADA activity. The following PK parameters will be computed: C_{max}, C_{trough}, $t_{max,}$ $AUC_{(0-t)}$, Kel, and $t_{½}$.

Statistical Methodology:

Each patient will serve as his/her own control based on the baseline assessment of each endpoint as appropriate.

Safety Analysis:

- Adverse events will be coded according to MedDRA version 10.0 or later and will be summarized in frequency tables displaying counts and percentage by body system, preferred term, and treatment group. In addition, AEs will be summarized by relationship to study drug and by severity. All Serious AEs will also be summarized in a frequency table.
- Laboratory parameters (Chemistry, Hematology, and Urinalysis) will be summarized descriptively at each timepoint and by treatment group. Out-of-normal range values will be listed.
- Immunogenicity - the results of antibodies (binding and neutralizing) against EZN-2279, Adagen® and anti-PEG antibodies will be summarized. The relationships between immunogenicity, PK, and the clinical toxicities will be examined as appropriate.

Efficacy Analysis:

The primary efficacy endpoint is the percentage of patients achieving metabolic detoxification, defined as achieving and maintaining the target trough total erythrocyte dAXP concentration of ≤0.02 µmol/mL while receiving EZN-2279.

Analyses of the following secondary efficacy endpoints will be primarily descriptive, with data listings, graphical presentations, frequency tabulations, and summary statistics as appropriate: B-, T-, and NK-lymphocyte subset analysis; Quantitative immunoglobulin concentration; infection incidence; incidence and duration of hospitalizations ; growth; and overall survival.

Pharmacokinetic Analysis:

PK parameters (C_{max}, C_{trough}, $t_{max,}$ $AUC_{(0-t)}$, Kel, and $t_{½}$) and the plasma level-versus-time curves will be determined for each evaluable patient. Descriptive statistics including mean, standard deviation, coefficient of variation, median, minimum, maximum, mean and median plasma level graphs will be provided as appropriate.

3. LIST OF ABBREVIATIONS

Abbreviation	Term
Ab	Antibody
ADA	adenosine deaminase
ADA+/+ mice	mice heterozygous for the null Ada allele; ADA control
ADA-/- mice	mice homozygous for the null Ada allele; ADA-deficient mice
Adagen®	pegademase bovine, SS-PEG-ADA, PEG-ADA, PEGylated ADA
ADA-SCID	ADA-deficient SCID
ADR	adverse drug reaction
AE	adverse event/experience
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the drug concentration-time curve
AUC(0-t)	AUC from time zero to time t
BMT	bone marrow transplantation
BUN	blood urea nitrogen
C	Celsius/Centigrade
C74S	Cys74ser mutation
CBC	complete blood count
CFR	Code of Federal Regulations
Cmax	maximum observed drug concentration.
cont'd	Continued
CRF	case report form
Ct	last measurable concentration
Ctrough	concentration minimum before repeat dosing
CVs	curriculum vitae
Cys74	cysteine at position 74
d-adenosine	2′-deoxyadenosine
d-ATP	2'-deoxyadenosine 5'-triphosphate
dAXP	deoxyadenosine nucleotide
dCydK	deoxycytidine kinase
d-inosine	2′-deoxyinosine
DLT	dose limiting toxicity
DNA	deoxyribonucleic acid
DSMC	Data and Safety Monitoring Committee
EDTA	ethylenediaminetetraacetic acid
EZN-2279	PEG-rADA; SC-PEG-rbADA-C74S
F	Fahrenheit
FACS	fluorescence-activated cell sorter
FDA	Food and Drug Administration
GCP	Good Clinical Practices
HIPPA	Health Insurance Portability and Accountability Act
HLA	human leukocyte antigen
HPLC	high-performance liquid chromatography
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IgA	immunoglobulin A
IgG	immunoglobulin G

Abbreviation	Term
IgM	immunoglobulin M
h	Hour
i.m.	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
i.v.	Intravenous
kg	Kilogram
Lead PI	Lead Principal Investigator (study)
LFTs	liver function tests
LLN	lower limit of normal range
ln	natural logarithm
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
mmHg	millimeter of mercury
NA	not applicable
NOAEL	no-observed-adverse-effect level
PEG	polyethylene glycol
PEG-ADA	PEGylated ADA, Adagen®
PEG-rADA	PEGylated recombinant ADA, EZN-2279
pH	negative logarithm of hydrogen ion concentration (“power of hydrogen”)
PHI	Protected Health Information
PI	Principal Investigator (site)
PK	pharmacokinetic(s)
rADA	recombinant ADA
RBC	red blood cell
SAE	serious adverse event/experience
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Systems
SCID	severe combined immunodeficiency
SC linker	succinimidyl carbamate linker
ser	Serine
SS linker	succinimidyl succinate linker
t1/2	time of elimination half-life calculated as ln(2)/Kel
tmax	time of the maximum drug concentration (obtained without interpolation)
TSE	transmissible spongiform encephalopathy
U	Unit; amount of adenosine deaminase that converts 1 μM of adenosine to inosine per minute at 25°C and pH 7.4
μL	Microliter
μmol	Micromole
U.S.	United States (of America)
WBC	white blood cell

4. GENERAL STUDY INFORMATION

Sponsor Name:	Leadiant Biosciences, Inc.
Sponsor Address:	9841 Washingtonian Blvd. Suite 500 Gaithersburg, Maryland 20878 (800) 447-0169
Sponsors Authorized Representative(s):	[redacted] Vice President, Clinical Affairs Leadiant Biosciences, Inc. 9841 Washingtonian Blvd. Suite 500 Gaithersburg, Maryland 20878 (301) 670-1532
Lead PI/Coordinating Investigator:	[redacted]
Medical Monitor:	[redacted] Vice President, Clinical Affairs Leadiant Biosciences, Inc. 9841 Washingtonian Blvd. Suite 500 Gaithersburg, Maryland 20878 (301) 670-1532

The following study information is contained in a study specific reference manual for this study protocol:

- Participating site Principal Investigators
- Contract Research Organization(s)
- Central Laboratories

5. INTRODUCTION

Leadiant Biosciences, Inc. is developing EZN-2279 (Polyethylene Glycol Recombinant Adenosine Deaminase [PEG-rADA]) for the treatment of patients with ADA- deficient combined immunodeficiency (ADA-SCID).

5.1. Disease Background

Adenosine deaminase-deficient combined immunodeficiency is a rare, inherited, and often fatal disease. It is characterized by severe and recurrent opportunistic infection, failure to thrive, profound lymphopenia with absent or severely impaired cellular and humoral immune function, and metabolic abnormalities [1,2]. These patients are lymphopenic at birth and predisposed to recurrent illnesses caused by pathogens and opportunistic organisms that often begin within a few weeks. The average age at diagnosis for patients with adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID) is 4.4 months [3,4].

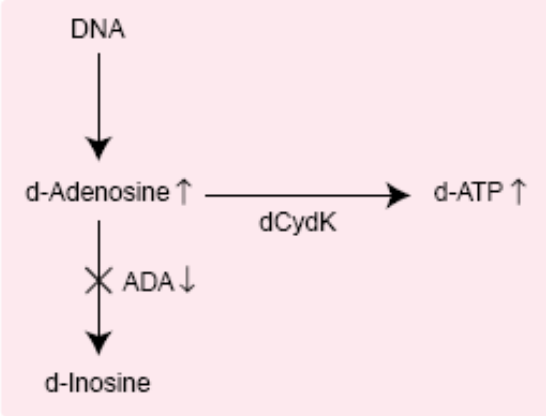
The incidence of ADA deficiency is estimated to be between 1:200,000 and 1:1,000,000 live births [2,4]. ADA deficiency can be categorized into four phenotypes [1]:

- ADA-SCID: The largest number of patients with ADA deficiency (about 85% to 95%) present with ADA-SCID, which is the most severe form of the immunodeficiency.
- Delayed onset ADA deficiency has been identified in 10% to 15% of patients with ADA deficiency which presents after the first year of life and is most likely related to the specific ADA mutation that in such cases results in some residual ADA activity and less profound lymphopenia and immune function.
- Late (or adult) onset ADA deficiency has been identified in a handful of individuals.
- Partial ADA deficiency can be an incidental finding in a healthy individual who has normal immune function and abnormal ADA expression in erythrocytes. Partial ADA deficiency is extremely rare.

Patients with ADA-deficient combined immunodeficiency are unable to produce the ADA enzyme in their cells because of mutations in the *ADA* gene on chromosome 20q. ADA deficiency is a disorder of purine salvage [5]. In the absence of this enzyme, the purine substrates adenosine, 2′-deoxyadenosine, and their metabolites reach unusually high levels in cells and are toxic to lymphocytes (see Figure 1) [6]. Thus, deficiency of the ADA enzyme is the underlying defect that leads to the buildup of toxic metabolites, which in turn affect different organ systems, most notably the immune system [2]. It is still not entirely clear whether ADA-deficient patients have intrinsically abnormal lymphocytes or whether the defects seen are secondary to the effect of accumulation of 2′-deoxydenosine and adenosine [2]. In addition, accumulation of toxic metabolites also may interfere with thymic stroma development, maturation, and function, resulting in impaired ability to support T-cell development [2].

Absence of the enzyme ADA allows accumulation of toxic metabolites, resulting in complete or partial deficiency of both cell-mediated and humoral immunity [1].

Figure 1. Biochemical defects in ADA deficiency



ADA is expressed in all tissues of the body. In normal cells, DNA turnover is mediated by ADA catalyzing the deamination of d-adenosine to d-inosine. In ADA-deficient cells, there is an accumulation of d-adenosine, which is then converted by dCydK to d-ATP. The build-up of these two metabolites has profound effects on lymphocyte development and function – through effects on DNA synthesis, impaired cell division, and apoptosis – causing immunological defects.

Source: Qasim et al 2004 [6].

Key: ADA = adenosine deaminase; d-adenosine = 2′-deoxyadenosine; d-ATP = 2′-deoxyadenosine 5′-triphosphate; dCydK = deoxycytidine kinase; d-inosine = 2′-deoxyinosine; DNA = deoxyribonucleic acid.

5.1.1. Treatment of ADA-Deficient Combined Immunodeficiency

Without treatment, ADA-deficient combined immunodeficiency is fatal in the first years of life, and therefore early intervention is required [2]. Enzyme delivery is key to the successful detoxification of metabolic substrates and promotion of immune recovery in these patients [2]. The ADA enzyme can be delivered in the form of allogeneic wild-type cells, gene-modified autologous cells, or exogenous direct enzyme replacement therapy [2].

5.1.1.1. Hematopoietic Stem Cell Transplantation (HSCT)

Transplanting hematopoietic stem cells (HSCs) from a human leukocyte antigen (HLA)-identical sibling donor is well tolerated and results in long-term correction of the immunodeficiency in ADA deficiency; thus, HSCT is the treatment of choice for ADA-SCID [2,4]. If a genotypically matched family donor is available, bone marrow transplantation (BMT) is a highly successful procedure, with recent results indicating a long-term survival rate of approximately 90% for all forms of severe combined immunodeficiency (SCID) [7].

Since the first transplantations were performed nearly 40 years ago, an increasing number of procedures have been performed with significant improvement in survival rates over time [8]. In 2004, Buckley published the results of 132 transplants performed over 21.3 years at Duke University for various genetic types of SCID [9]. Twenty-two of the 132 patients had ADA-SCID. Eighteen of the ADA-deficient patients received T-cell–depleted haploidentical marrows, and 4 underwent HLA-identical transplantation. The 22 ADA-deficient patients did not receive pre-transplant chemotherapy or post-transplant prophylaxis for graft-versus-host disease. Seventeen (77%) of the ADA-deficient patients were reported as alive at 0.3-18.6 years after transplantation. Five of the recipients of T-cell–depleted haploidentical marrows died. One ADA-deficient patient, who was previously treated with Adagen® for 18 years, died of pulmonary

hypertension. Hematopoietic chimerism (T cell) was evident in 10 of the ADA-deficient patients. The number and function of natural killer cells normalized after transplantation. However, in most cases, the B cells were of recipient origin.

Transplantation of parental or unrelated allogeneic hematopoietic stem cells in the approximately 80% of infants with SCID who lack an HLA-matched sibling donor has success rates of 50% to 85%, with a considerable number of patients dying from various complications [10].

Although haploidentical transplantation is a significant medical advance, it is not without complications. BMT from mismatched related donors has been associated with significantly delayed or incomplete immune reconstitution [7,11]. The delay in T-cell immune recovery may be 6 months or more. During this period after mismatched related donor HSCT, infections remain a major cause of morbidity and mortality.

Furthermore, neurologic abnormalities, mostly in association with infection or after bone marrow transplantation, occur in some ADA-deficient patients [12]; bilateral sensorineural deafness also has been reported [13]. Hönig et al reported a high rate (50%) of neurologic abnormalities observed in six patients with ADA deficiency surviving after HSCT [14]. The authors conclude that this high rate indicates that HSCT commonly fails to control central nervous system complications in this metabolic disease. Recent results from a large study of 105 patients confirmed that children with severe immunodeficiency treated with HSCT have an increased risk of long-term cognitive difficulties and associated emotional and behavioral outcomes [8].

In summary, in patients with ADA-SCID use of HSCT from a matched related donor is associated with long-term survival and effective immune recovery in the majority of the patients, but does not prevent non immune complications of the disease [2]. However, in the absence of a suitable matched donor, parental haploidentical transplants are associated with greater complications and sometimes poorer long-term immune recovery [7]. The clinical status influences the risk of morbidity and mortality in the pre-transplant period, before effective immune reconstitution. This risk is greater when preconditioning is used to improve the chances of engraftment. In the absence of preconditioning there is less transplant-associated morbidity but a high rate of non-engraftment. Overall, the success of non-HLA identical transplantation is much lower for patients with ADA-SCID than for patients with SCID due to other gene defects. Patients whose transplants are successful demonstrate good immunologic recovery, with normal cellular and humoral function in most cases [5]. Completing a successful BMT depends upon finding a matched donor, but the probability of this is low. ADA enzyme therapy is thus considered for patients lacking HLA-matched bone marrow donors and for patients whose clinical status places them at high risk for transplant-associated morbidity [15].

5.1.1.2. Gene Therapy

Gene therapy for the treatment of ADA-SCID commonly involves gamma-retroviral–mediated introduction of the human ADA gene into autologous bone marrow progenitors and subsequent infusion of cells back to the patient following mild non-myeloablative chemotherapy [2]. Ex vivo gene therapy of hematopoietic cells with ADA does not correct the underlying ADA mutation in other lineages, such as neuronal cells [5]. To date, more than 25 ADA-deficient patients have been treated with gene therapy. The treatment protocols vary between the different sites with respect to the specific retroviral constructs used for gene transfer and the conditioning protocols used.

Trials of gene therapy for ADA-SCID were conducted starting in the early 1990s [16-21]. All of these studies used retroviral vectors to transfer normal human ADA deoxyribonucleic acid (DNA) and targeted peripheral blood T cells or CD34+ hematopoietic progenitor cells from bone marrow

or cord blood. No serious adverse events were reported in these trials, but there also were no significant clinical benefits achieved [21]. While pilot trials have shown the safety and feasibility of gene therapy in patients with SCID due to ADA deficiency [16,17,22], all patients required maintenance with polyethylene glycol (PEG)-ADA (see Section 5.1.1.3), and the ADA-transduced stem cells were unable to reconstitute the recipient's immune system [23].

Starting in the late 1990s, a second generation of clinical trials evaluated gene therapy in patients with ADA-SCID or the X-linked form of SCID [10,24-26]. Although 18 of the 20 treated infants with X-linked SCID are alive and well with restored immunity, the use of gene therapy has been associated with the development of T-cell leukemia in five patients with X-linked SCID due to insertional mutagenesis caused by the retroviral vector [7,10,23,27-31]. Molecular studies indicated that three of the four leukemias in the French study [29] were caused by inadvertent activation of the proto-oncogene *LMO2* due to vector integration in or around this gene [32]. Preliminary studies suggest a similar molecular etiology for the development of leukemia in the patient from the UK study [32]. The development of leukemia in patients with X-linked SCID is a concern for use of retroviruses in other types of HSC-directed gene transfer, including ADA-SCID [33]. A recent commentary in *Human Gene Therapy* concluded that SCID-X1 gene therapy using murine leukemia virus vectors with fully functional retroviral promoter/enhancer carries a high risk of leukemia insertion [34].

Since the design of the first gene-therapy trials, HSCs have been considered the optimal target cells for long-term, full correction of the ADA-SCID defect [35]. Aiuti *et al* first reported the restoration of immune function and correction of the metabolic defect by stem-cell gene therapy in two children with ADA-SCID [25,35]. Unlike all previous studies, these patients were not concurrently treated with Adagen® and received low-dose conditioning. Aiuti *et al* recently published results from the long-term follow-up of these two patients, as well as an additional eight patients treated with the same regimen [23]. All patients are alive after a median follow-up of 4 years (range = 1.8 to 8.0 years). Nine patients had immune reconstitution with increases in T-cell counts (median count at 3 years = 1.07×10^7/L) and normalization of T-cell function. No adverse events could be attributed to the ADA-transduced cells. The authors report that the use of nonmyeloablative conditioning and withdrawal of enzyme replacement therapy were crucial factors in the successful outcome of the trial; they conclude that gene therapy, combined with reduced-intensity conditioning, is a safe and effective option to be considered for SCID in patients with ADA deficiency who lack an HLA-identical sibling donor [23].

The sharp dichotomy between the absence of the T-cell lymphoproliferative syndrome in the patients with ADA-SCID [23] and its occurrence in 25% of the patients with X-linked SCID is important to understand if the therapeutic efficacy of gene therapy is to be retained while minimizing its risks [10]. The time to immune recovery in the patients with ADA-SCID is markedly slower (6 to 12 months) than the rapid development (3 to 6 months) of T cells in the patients with X-linked SCID who received gene therapy. This difference may reflect important biologic differences between the corrected hematopoietic stem cells in X-linked SCID and ADA- SCID [10]. The long-term follow-up reported by Aiuti [23] and the results of other trials of ADA- SCID [21,22,36,37] did not reveal the leukemic proliferation complications observed in the patients with X-linked SCID [23].

In summary, gene therapy with nonmyeloablative conditioning is associated with clinical benefit and is now an option to be considered for patients with ADA-SCID who lack an HLA-identical sibling [2]. In recent trials in Milan, London, and the United States, at least 20 patients who received gene therapy for ADA-SCID have been followed for longer than 1 year; all patients have survived, which compares favorably with HSCT data, and the majority of patients have near or full recovery of immune function [2]. Importantly, none of the patients showed clonal proliferation or

adverse events related to gene transfer, despite observation of integration of vector sequences into known oncogenes [2]. Results of molecular studies indicate that the retroviral integration profile after successful gene therapy did not cause selection or expansion of malignant cell clones in vivo [31,38]. In general, gene therapy poses minimal toxicity within the first year with a good chance of immune recovery; however, using current vector technology, the potential for long-term toxicity is retained [2]. The benefits and risks of gene therapy should be weighed carefully against the risks and efficacy of existing treatment [7,21,39]. Ongoing and upcoming clinical trials will use safer designs of retroviral vectors, newer types of vectors for viral gene delivery, and emerging methods for direct in situ gene repair [10].

5.1.1.3. Enzyme Replacement Therapy with Adagen®

Adagen® (pegadamase bovine, PEG-ADA) provides specific and direct replacement of the ADA enzyme [40]. The active ingredient in Adagen® is a PEGylated adenosine deaminase. The enzyme in Adagen® is purified from the bovine intestine. The drug is then PEGylated. The PEGylation of the molecule is critical to the pharmacologic activity of Adagen® because ADA without PEGylation does not have a sufficient half-life to be efficacious.

Adagen® was approved in 1990 as enzyme replacement therapy for ADA deficiency in patients with SCID who are not suitable candidates for, or who have failed, bone marrow transplantation [40]. Since its approval in 1990, approximately 170 patients worldwide have been treated with Adagen® [Leadiant Biosciencesdata on file], and approximately 98 patients are currently receiving treatment with Adagen® [2]. PEGylated ADA is a life-saving product for these patients. Overall, 70% of patients treated with PEG-ADA had SCID and began enzyme replacement therapy at <1 year of age (50% were <6 months old); of the remaining patients, half started treatment at 1 to 3 years of age, and half started treatment at 3 to 34 years of age [2]. Studies have shown that upon initiation of Adagen® therapy, the absolute numbers of circulating T and B lymphocytes and NK cells increase, and protective immunity function develops [41]. Approximately two thirds of the patients who are currently receiving Adagen® have been taking the drug for more than 5 years, and 20% have received the drug for 15 to 22 years [2]. The overall probability of surviving 20 years while receiving enzyme replacement therapy is estimated to be 78%, and a patient who is alive 6 months after starting Adagen® has about a 90% probability of surviving the next 12 years [2].

The mechanism of action of Adagen® is to correct the metabolic abnormalities associated with adenosine accumulation due to the absence of ADA and to detoxify the cells. Improvement in immune function and diminished frequency of opportunistic infections, compared with the natural history of ADA-SCID, occurs only after correction of the metabolic abnormalities. There is a lag between the correction of the metabolic abnormalities and improvement in immune function. This period of time for Adagen® to improve immune function is usually less than 6 months [40].

The rapid metabolic detoxification afforded by enzyme replacement allows clinical stabilization of patients and provides longer term treatment options when no suitable donor is available [1].

For patients scheduled to undergo HSCT, enzyme replacement may be important in stabilizing the child through metabolic detoxification before the transplant [1]. If a matched family donor is not available, most centers would start PEG-ADA to stabilize the child and to improve metabolic and immune recovery while initiating a search for an unrelated donor.

Adagen® has been given as an adjunctive therapy in prior gene therapy trials, but it is recognized that enzyme replacement may blunt the survival advantage of gene-corrected cells [1]. Therefore, patients have received PEG-ADA as maintenance therapy until the time of gene therapy, at which point the enzyme replacement has been discontinued. The use of enzyme replacement therapy may

be important before gene therapy in the maintenance of a healthy hematopoietic stem cell pool from which sufficient cells can be harvested for the transduction procedure [1].

The effectiveness of PEG-ADA in correcting metabolic and immunologic parameters and, more importantly, in promoting clinical well-being in patients makes it an important option in the care of patients with ADA-deficient combined immunodeficiency [1].

It is highly desirable to replace the bovine-derived components in PEG-ADA with a recombinant protein, which will eliminate the risk of exposure of patients to transmissible spongiform encephalopathy (TSE) and potential adventitious viruses from bovine-derived components as well as guarantee a more consistent, stable, and indefinite supply of drug for this critical patient population.

5.2. Description of Investigational Product

EZN-2279 (PEG-rADA) is a PEGylated recombinant ADA that is a well-characterized therapeutic biological product. EZN-2279 has been developed to replace the native ADA in Adagen® with recombinant ADA.

[redacted]

The bovine sequence of ADA is used to manufacture EZN-2279. [redacted] The human sequence of ADA has been found not to result in a stable pharmaceutical product and is not used as the basis for the design and synthesis of EZN-2279. Thus, EZN-2279 is manufactured using DNA recombinant technology, with a more stable linker.

A detailed description of the nonclinical results for EZN-2279 is provided in the Investigator's Brochure. Brief summaries of these results are provided below.

5.3. Investigational Product Background Information

EZN-2279 provides specific and direct replacement of the ADA enzyme. As for Adagen®, the mechanism of action of EZN-2279 is to correct the metabolic abnormalities associated with adenosine accumulation due to the absence of ADA and to detoxify the cells.

5.3.1. Non-Clinical Studies

The PD, PK, and toxicologic properties of EZN-2279 were evaluated in animal models [Leadiant Biosciences data on file]. Where appropriate, comparisons were made with Adagen®. The ability of the drug to generate antibodies that bind and neutralize EZN-2279 was studied together with the PK analysis.

5.3.1.1. Pharmacology

Administration of Adagen® to $ADA^{-/-}$ mice (i.e., ADA-deficient mice homozygous for the null Ada allele) has been shown to correct the purine metabolism defect, restore thymus and spleen

weights, correct immune deficiencies, resolve pulmonary abnormalities, and markedly extend the lifespan of such ADA-deficient mice. For example, $ADA^{-/-}$ mice have been shown to accumulate adenosine in their lungs by postnatal Day 18. In such mice, administration of a single i.m. injection of 2.5 U of Adagen® on Day 18 led to approximately a 10-fold reduction in lung adenosine levels by Day 21 (which was close to levels found in normal mice), as well as improvement in the lung phenotype and survival of these mice. Therefore, $ADA^{-/-}$ mice are a relevant animal model to compare the efficacy of Adagen® and EZN-2279.

In these experiments, $ADA^{-/-}$ mice were generated and genotyped. $ADA^{-/-}$ mice were injected i.m. or intraperitoneally (i.p.) in several dosing regimens with selected doses of Adagen® or EZN-2279. Compounds were given on the same schedule (once every 4 days × 5 starting on postnatal Day 1 until postnatal Day 21 followed, if needed, once each week until postnatal Day 42) and equivalent doses (5 U/mouse injected i.m. or i.p.). Doses and schedules of Adagen® or EZN-2279 were chosen such that the peak and trough levels of ADA in the plasma were similar to what has been achieved in patients given therapeutic doses of Adagen®. In each experiment, groups of mice, including littermates, were left untreated for comparisons. Both untreated $ADA^{+/+}$ and $ADA^{-/-}$ mice as well as $ADA^{-/-}$ mice treated with EZN-2279 or Adagen® were sacrificed 18 days to 6 weeks following injection. Bronchial alveolar lavage (BAL) fluid, thymus, and spleen were collected for the analysis of adenosine concentrations using RP-HPLC as well as for total cell counts and cellular differentials. Blood was obtained from the circulation or thoracic cavity at the time of sacrifice to measure ADA enzymatic activity using zymogram or spectrophotometric analysis, respectively. Survival and body weight also were assessed.

Results of the pharmacology and PD studies performed in the $ADA^{-/-}$ mouse model suggest EZN-2279 behaves very similarly to Adagen® and both compounds are highly effective. Consistent with this, when Adagen® and EZN-2279 were given at equivalent doses and schedules, both PEG-ADA compounds 1) were delivered to the circulatory system with equal efficiency in both $ADA^{+/+}$ and $ADA^{-/-}$ mice; 2) increased ADA plasma levels to similar, clinically relevant levels; 3) decreased abnormally high adenosine levels found in the lung, spleen, or thymus to a similar extent; 4) maintained thymus and spleen cellularity, including total cell numbers and T- and B-cell populations; and 5) promoted the survival of $ADA^{-/-}$ mice. There is a suggestion that EZN-2279 may be more efficacious than Adagen® in the $ADA^{-/-}$ mouse model. The results tended to show EZN-2279-treated $ADA^{-/-}$ mice having statistically lower adenosine levels in the thymus compared with Adagen®, as well as a consistent trend toward better improvement of most PD endpoints (body weight, survival, and thymocyte and splenocyte cell number, as well as adenosine levels in spleen) when compared with Adagen®. These effects correlated with 35% higher plasma levels of ADA activity derived from EZN-2279 compared with Adagen® when analyzed either 24 or 72 hours after the last dose; the higher plasma levels of ADA activity are likely due to the longer half-life of EZN-2279.

5.3.1.2. Pharmacokinetics

EZN-2279 demonstrated improved *in vitro* stability in human plasma in comparison with Adagen®. Two single-dose intravenous (i.v). studies in rats were conducted. In these studies, PK parameters for ADA activity were markedly increased for EZN-2279 as compared with unPEGylated EZN-2279 and were equivalent to or slightly different than those for Adagen® (i.e., in one experiment, EZN-2279 had lower clearance, longer half-life, and higher AUC compared to Adagen®).

Single i.m. doses of EZN-2279 or Adagen® were given to rats or dogs at two dose levels (30 and 150 U/kg). AUC and C_{max} values for ADA activity increased in proportion to dose for both rats and dogs. The AUC values for EZN-2279 were statistically significantly higher (approximately

45% in rats, 55% in dogs) compared with Adagen®. Terminal elimination half-lives ($t_{1/2}$) also were increased approximately 30% in rats and 80% in dogs when EZN-2279 was compared with Adagen®. The calculated mean $t_{1/2}$ in rats after a single dose was 49 to 61 hours for EZN-2279 and 36 to 50 hours for Adagen®. In dogs, these values were 128 to 154 hours for EZN-2279 and 68 to 79 hours for Adagen®.

In multiple-dose studies, EZN-2279 was given every 3 to 4 days for 4 weeks (total of 9 doses) at three dose levels (30, 100, and 300 U/kg). These studies were conducted under GLP guidance. AUC and C_{max} values for ADA activity were higher on Day 29 as compared with Day 1 for rats that received 300 U/kg EZN-2279 and dogs that received 30, 100, or 300 U/kg EZN-2279. In contrast, likely due to detectable anti-drug antibodies, marked reductions in Day 29 exposure values for ADA activity occurred for male rats that received 30 U/kg EZN-2279 or 30 U/kg Adagen®, female rats that received 30 U/kg EZN-2279, and male rats that received 100 U/kg EZN-2279. In dogs, Day 29 AUC values were markedly reduced for 1 of 5 males that received 30 U/kg EZN-2279 and 1 of 5 females that received 100 U/kg EZN-2279. Anti-drug binding antibodies were present in the plasma of rats that received EZN-2279 or Adagen® and in most dogs that received EZN-2279 at the end of the 4-week treatment periods and/or recovery periods. In rare cases, neutralizing antibodies were detected that reduced the enzymatic activity of ADA in experimental assays.

In conclusion, the PK (ADA activity) following single-dose i.m. administration to rats and dogs demonstrate that that AUC and $t_{1/2}$ were significantly higher for EZN-2279 compared to Adagen®. Repeat doses of 30 U/kg EZN-2279 or 30 U/kg Adagen® in rats, as well as 30 U/kg or 100 U/kg in dogs, resulted in the formation of anti-drug antibodies in some animals and markedly reduced exposure to ADA activity in comparison with single-dose administration. Despite the presence of anti-drug binding antibodies in rats and dogs in the repeat-dose studies, there was no evidence for clinical anaphylactic reactions to EZN-2279 or Adagen® in either species.

5.3.1.3. Toxicology

The single-dose studies of EZN-2279 and Adagen® in rats and dogs were conducted primarily for PK assessment. EZN-2279 and Adagen® were dosed intramuscularly. Toxicology evaluations including clinical signs, body weight, and food consumptions were done for up to 14 days after injection, after which animals were sacrificed. The experiments were performed under non-GLP conditions.

The toxicity profile of EZN-2279 also was evaluated in Sprague-Dawley CD® rats and Beagle dogs in 4-week repeat-dose i.m. GLP toxicology studies. Animals were administered 30, 100, or 300 U/kg of EZN-2279 every 3 or 4 days for 4 weeks (9 doses total) with a 4-week recovery period. Microscopic pathology evaluations were performed on approximately 40 tissues/animals.

EZN-2279 given i.m. was well tolerated in rats and dogs in single- and repeat-dose studies. No mortality was observed in any of these studies, and no adverse effects on clinical condition, body weight, food consumption, electrocardiograms (dogs), or microscopic pathology were evident in either species at any dose. Adagen® was similarly well tolerated in rats and dogs at single doses of 30 and 150 U/kg and in rats at 30 U/kg given every 3 or 4 days for 4 weeks as part of a PK study.

Compound-related findings were seen in the repeat-dose i.m. studies after 4 weeks of treatment (9 doses) and were limited to slight increases in MPV values (3% to 7%) for male rats that received 30, 100, or 300 U/kg EZN-2279; slightly prolonged mean values for aPTT for male and female rats that received 300 U/kg EZN-2279 (5 to 6 seconds); slightly prolonged aPTT for male dogs that received 30, 100, and 300 U/kg EZN-2279 (6 to 20 seconds) and female dogs that

received 100 and 300 U/kg EZN-2279 (8 and 14 seconds); and red foci at the injection sites for 2 of 10 male rats that received 100 U/kg EZN-2279. The increased MPV and aPTT values were considered compound related but not adverse because of the small magnitude of change and lack of correlative changes. Partial or complete recovery was evident in both species for all of the changes after 4 weeks of recovery. These clinical pathology changes could not be compared to effects associated with Adagen® because Adagen® was not administered in these studies.

The NOAELs were the highest doses given in each of the i.m. studies, which were 150 U/kg EZN-2279 in the single-dose rat and dog studies and 300 U/kg EZN-2279 in the repeat-dose rat and dog studies. Despite the presence of anti-drug binding antibodies, which were present in the rat plasma following repeated doses of EZN-2279 or Adagen® and in most dogs that received repeated doses of EZN-2279, no clinical observations were suggestive of an anaphylactoid response in any of the studies. The Day 29 $AUC_{0-\infty}$ values at the NOAEL doses of 300 U/kg in the repeat-dose studies were 1.7- to 2.0-fold higher in rats and 3.2-fold higher in dogs than the corresponding Day 1 values.

5.3.1.4. Conclusions

The nonclinical data demonstrates that EZN-2279 was highly efficacious in a mouse knockout model that mimics human ADA-SCID. In such ADA-deficient mice, compared with Adagen®, EZN-2279 was at least as effective in its ability to:

- Increase ADA plasma levels to clinically relevant levels.
- Decrease abnormally high adenosine levels found in the lung, spleen or thymus to a similar extent.
- Maintain thymus and spleen cellularity, including total cell numbers and T- and B-cell populations.
- Promote the survival of mice.

EZN-2279 was well tolerated in rats and dogs at doses that are physiologically relevant.

5.3.2. Clinical Studies

No clinical studies of EZN-2279 have been performed.

5.4. Target Population and Study Rationale

PEGylated bovine adenosine deaminase (PEG-ADA, Adagen®) is life saving for individuals with ADA deficiency. Adagen® results in metabolic detoxification by providing the ADA enzymatic activity lacking in these patients. The ADA in Adagen® is bovine derived.

EZN-2279 is a PEGylated recombinant adenosine deaminase that has been developed to replace the bovine-derived components in PEG-ADA with a recombinant protein, which will eliminate any risk of transmission of TSE or potential adventitious viruses from bovine-derived components to patients.

EZN-2279 has been developed to be similar to Adagen®. [redacted] The more stable linker for EZN-2279 imparts the potential for reduced immunogenicity due to improved shielding of the rADA and subsequently may decrease the incidence of neutralizing antibodies, and in animal models imparts a longer half-life compared to Adagen®. The enzyme used for manufacturing EZN-2279 is recombinant, and based on the same as Adagen®, [redacted].

Single i.m. doses of EZN-2279 or Adagen® were given to rats or dogs. Area under the drug concentration-time curve (AUC) and maximum observed drug concentration (C_{max}) values for ADA activity increased in proportion to dose for both rats and dogs. The AUC values for EZN-2279 were statistically significantly higher (approximately 45% in rats, 55% in dogs) compared with Adagen®. Terminal elimination half-lives ($t_{1/2}$) also were increased approximately 30% in rats and 80% in dogs when EZN-2279 was compared with Adagen®. The calculated mean $t_{1/2}$ in rats after a single dose was 49 to 61 hours for EZN-2279 and 36 to 50 hours for Adagen®. In dogs, these values were 128 to 154 hours for EZN-2279 and 68 to 79 hours for Adagen®. Thus, administration of doses of EZN-2279 and Adagen® with the same initial activity may result in slightly higher exposure to ADA.

Results of the pharmacology and PD studies performed in the $ADA^{-/-}$ mouse model suggest EZN-2279 behaves very similarly to Adagen® and both compounds are highly effective. Consistent with this, when Adagen® and EZN-2279 were given at equivalent doses and schedules, both PEG-ADA compounds 1) were delivered to the circulatory system with equal efficiency in both $ADA^{+/+}$ and $ADA^{-/-}$ mice; 2) increased ADA plasma levels to similar, clinically relevant levels; 3) decreased abnormally high adenosine levels found in the lung, spleen, or thymus to a similar extent; 4) maintained thymus and spleen cellularity, including total cell numbers and T- and B-cell populations; and 5) promoted the survival of $ADA^{-/-}$ mice. There is a suggestion that EZN-2279 may be more efficacious than Adagen® in the $ADA^{-/-}$ mouse model. The results tended to show EZN-2279-treated $ADA^{-/-}$ mice having statistically lower adenosine levels in the thymus compared with Adagen®, as well as a consistent trend toward better improvement of most PD endpoints (body weight, survival, and thymocyte and splenocyte cell number, as well as adenosine levels in spleen) when compared with Adagen®. These effects correlated with 35% higher plasma levels of ADA activity derived from EZN-2279 compared with Adagen® when analyzed either 24 or 72 hours after the last dose; the higher plasma levels of ADA activity are likely due to the longer half-life of EZN-2279.

The rarity of patients with ADA-deficient combined immunodeficiency precludes a randomized controlled study; however, as described, each patient will serve as his or her own control. Monitored treatment of patients with Adagen® before EZN-2279 allows a direct comparison of an equivalent starting dose in these patients regarding changes in body weight as percentile on the growth curve, metabolic detoxification, lymphocyte subsets, antibody formation, etc.

5.5. Potential Risks and Benefits

EZN-2279 has been developed to be similar to Adagen®. [redacted] The more stable linker for EZN-2279 imparts the potential for reduced immunogenicity due to improved shielding of the rADA and subsequently may decrease the incidence of neutralizing antibodies, and in animal models imparts a longer half-life compared to Adagen®. The enzyme used for manufacturing EZN-2279 is recombinant, and based on the same bovine sequence as Adagen®, [redacted]

In addition to monitoring adverse events/experiences (AEs) as required in all clinical trials, particular attention will be paid to AEs that have been observed with Adagen®. It is possible that EZN-2279 will have side effects and DLTs similar to those of Adagen® [40].

Clinical trial experience with Adagen® is based on the patient population for this orphan drug, estimated to be approximately 100 patients worldwide [Leadiant Biosciences data on file]. Based on six

patients, the following adverse reactions are reported in the Adagen® product label: pain at the injection site (two patients) and headache (one patient) [40].

In addition, during post-approval use of Adagen®, the following adverse reactions have been identified, regardless of relationship with the drug or the disease [40, Leadiant Biosciences data on file]:

- At least 5% of cases: Respiratory failure, failure to thrive, drug level decreased, respiratory tract infection, thrombocythemia, adverse reactions pertaining to an immune reaction (such as hemolytic anemia and neutralizing antibodies), infection, pneumonia, and death due to an unknown cause
- 3% to 4% of cases: Autoimmune hemolytic anemia, drug-specific antibody present, injection-site erythema, urticaria, adenovirus infection, bovine tuberculosis, brain abscess, convulsion, developmental delay, drug ineffective, diarrhea, encephalitis, Epstein-Barr virus infection, erythema, hearing impairment, hepatoblastoma, Hodgkin's disease, interstitial lung disease, lung disorder, lymphocyte count decreased, nervous system disorder, sepsis, thrombocytopenia, and viral pneumonia

Because Adagen® is administered over extended periods, including life-time administration, and these reactions are reported voluntarily from a small population, it is not always possible to accurately estimate their frequency or establish a definitive causal relationship to drug exposure.

In addition, the assessment of immune system function, which is part of the assessment of efficacy in this clinical study, also represents an important aspect of the assessment of safety.

Refer to the EZN-2279 Investigator's Brochure for additional information.

6. STUDY PURPOSE, OBJECTIVES AND ENDPOINTS

6.1. Purpose

The purpose of this clinical study is to evaluate the safety and efficacy of EZN-2279 in patients with ADA-deficient combined immunodeficiency, with the goal of replacing PEGylated native bovine ADA (Adagen®) with PEGylated rADA (EZN-2279). The EZN-2279 product is manufactured using deoxyribonucleic acid (DNA) recombinant technology, with a more stable linker, which may potentially reduce immunogenicity and which will replace bovine protein with recombinant protein in patients with ADA-deficient combined immunodeficiency.

6.2. Objectives

6.2.1. Primary Objectives

The primary objective of this study is to evaluate whether therapy with EZN-2279 achieves metabolic detoxification, as demonstrated by total erythrocyte dAXP concentration from a trough blood sample.

6.2.2. Secondary Objectives

The secondary objectives of this study are to:

- Evaluate the safety and tolerability of EZN-2279
- Assess the immunogenicity to EZN-2279 and Adagen®, including binding antibodies, neutralizing antibodies and anti-PEG antibodies
- Evaluate whether therapy with EZN-2279 maintains trough plasma ADA activity ≥15 μmol/h/mL

- Determine the PK profile of EZN-2279
- Assess the effect of EZN-2279 on immune status as determined by absolute lymphocyte count, lymphocyte subset (B, T, and NK) analysis, and immunoglobulin (Ig) concentration (IgG, IgA, IgM)
- Compare the clinical status (infections and hospitalizations) of the patients during the ENZ-2279 treatment period compared to the prior six months
- Assess the clinical growth status of the patients during the EZN-2279 treatment and maintenance periods

6.3. Endpoints

EZN-2279 treatment will occur in 2 distinct study phases, an EZN-2279 treatment phase (i.e., the initial 21 weeks of EZN-2279 treatment) and a long term EZN-2279 maintenance phase (see Section 7.1.1).

The procedures used to collect study data for the assessment of study endpoints are described in Section 10.

The analysis of study endpoint data will occur after all patients complete the EZN-2279 treatment period (i.e., the primary endpoint analysis), at 6 months after the last patient enters the EZN-2279 maintenance phase and annually thereafter (see Section 13.2.3).

6.3.1. Primary Endpoint

The primary endpoint is the percentage of patients achieving metabolic detoxification**,** assessed from trough dAXP samples obtained at Weeks T-15, T-17, T-19, and T-21. Metabolic detoxification will be defined as a trough dAXP concentration equal to or below 0.02 μmol/mL.

6.3.2. Secondary Endpoints

The secondary endpoints for efficacy are:

- Trough Plasma ADA Activity - will be assessed from trough plasma ADA samples obtained at Weeks T-15, T-17, T-19, and T-21. Adequate trough ADA activity will be defined as ADA activity ≥15 μmol/h/mL.
- Maintenance phase trough plasma dAXP and ADA activity - will be assessed from trough plasma dAXP and ADA samples obtained during the maintenance phase of the study through completion of EZN-2279 treatment
- Immune Status – will be assessed through completion of the EZN-2279 maintenance period, through assessment of:
  - Absolute lymphocyte count
  - B-, T-, and NK-lymphocyte subset analysis: The number of cells for each subset will be determined by FACS using the following panel:
    - CD3+ (Mature T cells)
      - Percent and Absolute
    - CD3+ CD8+ (Suppressor T Cells)
      - Percent and Absolute
    - CD3+ CD4+ (Helper Cells)
      - Percent and Absolute
    - CD (16+56)+ (Natural Killer Cells)
      - Percent and Absolute
    - CD19+ (B Cells)
      - Percent and Absolute

    - Absolute Lymphocytes (CD45+)
    - %CD4 (Helper Cells) / %CD8 (Suppressor T Cells)
  - Quantitative immunoglobulin concentration (IgG, IgA, IgM)
- Clinical status – will be assessed through determination of the following parameters
  - Infections through completion of the EZN-2279 treatment period will be determined and defined as either:
    - Clinically documented – patients with documented signs and symptoms of infection without positive microbiologic cultures
    - Microbiologically documented – patients with documented signs and symptoms of infection and with positive viral or bacterial cultures
  - Hospitalizations – Incidence and duration of hospitalizations through completion of the EZN-2279 treatment period compared to the 6-month period prior to study entry
  - Growth – height, weight and growth curve determinations through completion of the EZN-2279 maintenance period
  - Overall survival – will be assessed at the conclusion of the EZN-2279 treatment period through the end of EZN-2279 maintenance

The secondary endpoints for safety are:

- Safety – will be assessed through determination of adverse events (AEs), serious adverse events (SAEs), physical examinations, laboratory evaluations, and immunogenicity through completion of the EZN-2279 maintenance period.
- Immunogenicity of EZN-2279 and Adagen® will be assessed through the end of the EZN-2279 maintenance period by the evaluation of binding antibodies, neutralizing antibodies and anti-PEG antibodies.

6.3.3. Pharmacokinetic Endpoints

Pharmacokinetics of EZN-2279 and Adagen® will be based on plasma concentrations of ADA activity. The concentration of ADA activity in all collected samples will be determined by a validated assay. The following PK parameters will be computed from individual plasma concentrations collected during full PK sampling for Adagen® at the end of the Adagen® Lead-in period and for EZN-2279 during Week 13 (after 9th dose of EZN-2279): C_{max}, C_{trough}, t_{max}, $AUC_{(0-t)}$, Kel, and $t_{½}$.

For pediatric patients < 10 years of age, full PK sampling will not be done in an effort to minimize blood collection volumes to ensure the safety of the patients.

7. STUDY DESIGN

7.1. Overview of Study Design

Protocol STP-2279-002 is designed as an open-label, multicenter, single-arm, one way crossover study of EZN-2279 to determine the safety, efficacy, and PK of EZN-2279 in patients with ADA-SCID who are currently being treated with Adagen®. Each patient will serve as his or her own control with respect to assessment of study endpoints.

The study will enroll enough patients to ensure six evaluable patients with ADA-deficient combined immunodeficiency and stable clinical status while currently receiving therapy with Adagen® who meet all eligibility criteria and written informed consent/assent is obtained. The initial three patients will be enrolled and complete through full EZN-2279 pharmacokinetic sampling (Week T-9). An independent data and safety monitoring committee (DSMC) will review safety, PK (ADA activity), and erythrocyte dAXP data. If no concerns are noted, enrollment will

continue for the remaining patients. A similar DSMC review will occur after all six patients complete through 9-weeks of EZN-2279 dosing.

Patients for whom written informed consent/assent is obtained will be assigned a screening number and undergo screening procedures. Patients completing screening assessments and meeting all eligibility criteria will be enrolled in the study. There are three periods to study treatment: the Adagen® lead-in period, the EZN-2279 treatment period (Weeks T-1 through T-21) and the EZN-2279 maintenance period. Patients enrolled in the study will enter the Adagen® lead- in phase of the study. In this phase of the study, patients will receive a single i.m. dose of Adagen® weekly and be assessed weekly for dAXP levels and ADA activity. Patients receiving Adagen® more frequently than once a week will have his/her dose consolidated to a once a week dose regimen during the Adagen Lead-in Phase of the study. Adagen® dose adjustments will be made if the patient does not meet criteria for trough dAXP levels (≤0.02 μmol/mL) and trough ADA activity (≥15 μmol/h/mL) specified in the inclusion criteria. Once the patient meets protocol criteria for trough dAXP and ADA activity, and is considered to be fully detoxified, he/she will have a full pharmacokinetic assessment performed if equal to or older than 10 years of age. Prior to receiving the dose of Adagen® for pharmacokinetic assessment, patients will have the following baseline procedures performed: physical examination, vital signs, growth assessment, performance status, safety laboratory assessments (hematology, chemistry, urinalysis), and have blood samples obtained for assessment of lymphocyte subset analysis, quantitative immunoglobulins and Adagen® antibody titers (for neutralizing and binding antibodies) and anti-PEG antibodies. Following Adagen® dosing, plasma ADA samples for full PK evaluation will be collected for 7 consecutive days following dosing. After completing Adagen® lead-in treatment and pharmacokinetic assessments as indicated, patients will enter the EZN-2279 treatment phase (Weeks T-1 through T-21) of the study.

Adagen® will be discontinued and therapy with an equivalent dose of EZN-2279 will be initiated. In the EZN-2279 treatment phase of the study, patients will receive a single i.m. dose of EZN-2279 weekly for 21 consecutive weeks. Prior to receiving the initial dose of EZN-2279, patients will have the following procedures performed: physical examination, vital signs, growth assessment, performance status, safety laboratory assessments (hematology, chemistry, urinalysis), and have blood samples obtained for assessment of lymphocyte subset analysis, quantitative immunoglobulins, EZN-2279 and Adagen® antibody titers (neutralizing and binding antibodies) and anti-PEG antibodies. Trough total erythrocyte dAXP concentration and trough plasma ADA activity will be measured prior to dosing at weeks T-1, T-3, T-5, T-7, T-8 and every other week from Week T-9 through Week T-21. At week T-8 the patient will have dAXP levels and ADA activity assessed to assure the dose of EZN-2279 administered maintains dAXP and ADA activity at protocol specified levels. If these levels are not met and the patient is not considered fully detoxified, the patient will undergo a dose adjustment and be reassessed prior to entering the EZN-2279 full PK sampling week (Week T-9). During study week T-9, plasma ADA samples for full PK evaluation will be collected at Week T-9 (Days 58-63). Physical examination and safety laboratory assessments will be performed every 4 weeks (Weeks T-5, T-9, T-13, T-17 and T-21). Blood samples obtained for assessment of lymphocyte subset analysis, quantitative immunoglobulins, EZN-2279 antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies will be obtained at selected timepoints during the EZN-2279 treatment period.

Following completion of the EZN-2279 treatment period, patients may enter the EZN-2279 maintenance period. During this phase of the study patients will continue to receive single i.m. doses of EZN-2279 weekly. The following assessments will be performed during study visits every 3 months: physical examination, vital signs, growth assessment, performance status, safety laboratory assessments (hematology, chemistry, urinalysis), and have blood samples obtained for

assessment of trough ADA and dAXP levels, lymphocyte subset analysis, quantitative immunoglobulins, EZN-2279 antibody titers (for neutralizing and binding antibodies), and anti-PEG antibodies. The maintenance phase of the study will continue until the study is concluded (i.e., full regulatory approval of EZN-2279 or early study termination).

Throughout the duration of the study (i.e., Adagen® lead-in, EZN-2279 treatment and EZN-2279 maintenance) patients will continually be assessed for adverse events, infectious complications, hospitalizations, concomitant medications changes and concomitant procedures.

The analysis of study endpoint data will occur after all patients complete the EZN-2279 treatment period (i.e., the primary endpoint analysis), at 6 months after the last patient enters the EZN-2279 maintenance phase, and annually thereafter (see Section 13.2.3).

7.1.1. Study Periods

Patient participation in the study will be defined by the four distinct study periods defined below:

- Screening (up to 28-days prior to start of Adagen® Lead in dosing) – This period starts at the time of the initial screening procedure through administration of the first dose of Adagen® in the lead in period. Note: patients will continue to receive his/her prescribed dose of Adagen® during this period.
- Adagen® Lead-in – This period starts with the initial dose of study-required Adagen® dosing through the start of EZN-2279 study treatment. This period will be a minimum of 3 weeks.
- EZN-2279 Study Treatment – This period starts with the initial dose of EZN-2279 through completion of at least 21 weeks of EZN-2279 dosing.
- EZN-2279 Maintenance – This period begins after completion of the EZN-2279 study period and continues until the end of the study (full regulatory approval of EZN-2279 or early study termination).

7.1.2. Recruitment and Enrollment

It is anticipated that patients will be enrolled in the study within approximately 12-months.

7.1.2.1. Recruitment Definitions

Screening Failure – Patients who provide informed consent and do not proceed to study treatment (e.g., subject withdrawals consent, does not meet entry criteria). Screening data will be documented on the study specific Screening Log. Reasons for screen failure will be documented in the subject's source documents and on the Screening Log.

Enrolled Subject – Patients who pass pre-study (screening) evaluations and are assigned to study treatment.

Treated Subject – Patients who are enrolled and receive at least one dose of Adagen® during the Adagen Lead-In period.

Discontinued Subject – Patients who are treated but fail to complete through the end of the 21-week EZN-2279 treatment period.

Completed Subject – Patients who are treated and complete through the end of the 21-week EZN-2279 treatment period.

7.2. Study Treatments

7.2.1. Rationale for Dose Selection and Choice of Control Groups

Patients enrolled in this study will have ADA-SCID and be well-controlled on a weekly dose regimen of Adagen®. Patients will be maintained on the same weekly Adagen® dose during the Adagen® lead-in period but will be switched to a once weekly dosing regimen to permit assessment of Adagen® and EZN-2279 pharmacokinetics (only for patients ≥ 10 years of age) and trough levels. During the Adagen Lead-in Phase, Adagen® dose adjustments will be made if the patient does not meet criteria for dAXP levels (≤0.02 μmol/mL) and ADA activity (≥15 μmol/h/mL) specified in the inclusion criteria and the patient is considered to not be fully detoxified.

Following the Adagen® lead in phase, patients will be transitioned to receive an equivalent dose of EZN-2279. As 1 mg of EZN-2279 contains ADA enzymatic activity equivalent to 150 U of Adagen®, the equivalent dose will be calculated as follows:

Adagen® dose (U/kg) x 1mg EZN-2279/150U Adagen® = EZN-2279 dose (mg/kg)

7.3. Assignment of Patients to Treatment

This is a non-randomized study. Each patient who completes the study screening assessments, meets all eligibility criteria, and is accepted for study participation will be assigned a unique patient number and begin Adagen® lead-in treatment followed by EZN-2279 treatment and maintenance periods.

7.4. Study Duration

The primary analysis of data for efficacy, PK, immunogenicity, clinical status, and safety will be performed when the Week T-21 (at least 21 weeks of EZN-2279 treatment) data is available for the last patient enrolled in the study.

7.4.1. Duration of Treatment and Patient Participation

All patients will receive a minimum of a 3-week Adagen® lead-in followed by 21-weeks of study treatment with EZN-2279. Patients will be given the opportunity to remain on EZN-2279 maintenance until the study is concluded (full regulatory approval of EZN-2279 or early study termination). Assuming a 12 month enrollment period and 18 months for the submission of data and regulatory approval of EZN-2279, the initial patient enrolled will receive study treatment for approximately 3 years and the last patient enrolled will receive study treatment for approximately 2 years.

Patient participation will be approximately 8-weeks longer than the treatment duration (4 week screening period and a 30-day post treatment safety follow-up of patients who discontinue study drug early).

7.5. Methods Used to Minimize Bias

Due to the single-arm, one way crossover study design used, blinding is not possible.

Laboratory assessments performed for pharmacokinetics (only for patients ≥ 10 years of age), safety and efficacy are objective data provided by central laboratories and are not subject to bias.

Physical examination and adverse event assessments are subjective assessments that are subject to bias due to the inability to blind study staff to the identity of study treatments administered.

7.6. Appropriateness of Study Measurements

In this study, for patients ≥ 10 years of age, pharmacokinetics will be assessed by repeated sampling for ADA activity following a dose and pharmacodynamics will be assessed by monitoring dAXP levels. These are assessments that are commonly used to monitor patients treated with Adagen®.

Safety in this study will be assessed by monitoring adverse events, laboratory parameters (serum chemistry, hematology, and urinalysis), physical examination changes and vital signs. These are standard assessments for monitoring safety in clinical trials. In addition, monitoring of binding and neutralizing antibodies is commonly used to monitor patients treated with Adagen®.

In addition to pharmacodynamics, efficacy will be assessed by monitoring trough ADA activity, immune status (ALC, lymphocyte subset analysis, quantitative immunoglobulins) and clinical evaluations (growth, incidence of infections and hospitalizations). These are assessments that are commonly used to monitor patients treated with Adagen®.

7.7. Study Monitoring Committees

An independent Data and Safety Monitoring Committee (DSMC) will be set up to monitor the conduct of the study. The DSMC will review the safety information after the initial three patients have been treated with weekly administrations of EZN-2279 through completion of the full PK sampling assessments (Week T-9), after all 6 patients have been treated with weekly administrations of EZN-2279 through completion of Week T-9, after all patients complete the EZN-2279 Treatment Phase (i.e., Week T-21) and when all patients completed 1 year (i.e, 2 maintenance cycles) of treatment with EZN-2279. At minimum, the DSMC will review safety, PK (ADA activity), and erythrocyte dAXP data during the study.

A Charter for the DSMC will be written as an independent document.

8. STUDY DRUG AND TREATMENTS

8.1. Description of Study Drug(s)

EZN-2279 and Adagen® are isotonic, pyrogen-free, sterile solutions in phosphate-buffered saline. Adagen pH is 7.2 to 7.4. EZN-2279 pH is 6.7 to 7.1., both solutions are clear and colorless, contain no preservatives and designed for i.m. injection only.

Both drug products (EZN-2279 and Adagen®) are manufactured by Leadiant Biosciences, Inc., Indianapolis, IN.

8.2. Supply and Labeling

8.2.1. How Supplied

Leadiant Biosciences will supply sufficient quantities of EZN-2279 and lead-in Adagen® for the study.

8.2.1.1. EZN-2279

EZN-2279 will be supplied in 1.5 mL single-use vials containing 2.4 mg of EZN-2279 (1.6 mg/mL). Vials of EZN-2279 will be boxed individually.

8.2.1.2. Adagen®

Adagen® will be supplied in boxes of four 1.5 mL single-use vials (NDC-57665-001-01). Each vial contains 375 units of Adagen® (250 units/mL).

8.2.2. Labeling

8.2.2.1. EZN-2279

EZN-2279 will be labeled in accordance with FDA requirements for a clinical investigational study.

Each vial of EZN-2279 provided to the study site will contain a label with the wording as noted in the example below on both the box and vial; however, the layout of the wording and the size of the label affixed to the drug vial may be different.

Protocol STP-2279-002
Contents: **EZN-2279** Sterile–For intramuscular use only. Each 1.5-mL single-dose vial contains 2.4 mg (1.6 mg/mL).
Storage: +2ºC to +8ºC (36ºF to 46ºF) Protect from light **REFRIGERATE - DO NOT FREEZE**
Caution: New Drug - Limited by Federal (United States) law to investigational use.
Lot No.:
Manufactured [redacted]
Manufactured for: Leadiant Biosciences, Inc., Gaithersburg, MD 20878

8.2.2.2. Adagen®

Adagen® will be labeled with the standard labeling of the commercially available product.

For inventory control purposes, a secondary label will be added to each box of Adagen® to designate Adagen® supplied by Leadiant Biosciences only for study use.

A sample label is provided below.

Protocol STP-2279-002
Only for use for patients enrolled in Protocol STP-2279-002

8.3. Storage Conditions

8.3.1. EZN-2279

EZN-2279 should be stored refrigerated at +2°C to +8ºC (36°F to 46°F). Do not freeze. EZN-2279 should not be stored at room temperature. EZN-2279 should not be administered if it has been shaken or vigorously agitated. EZN-2279 should not be used if there are any indications that it may have been frozen.

EZN-2279 should be protected from light. Avoid excessive or prolonged light exposure when storing EZN-2279.

8.3.2. Adagen®

Adagen® should be stored refrigerated at +2°C to +8ºC (36°F to 46°F). Do not freeze. Adagen® should not be stored at room temperature. Adagen® should not be administered if it has been shaken or vigorously agitated. Adagen® should not be used if there are any indications that it may have been frozen.

8.4. Preparation for Administration

8.4.1. EZN-2279

As with all parenteral drug products, aseptic technique should be used during administration of EZN-2279. EZN-2279 should not be shaken or vigorously agitated. EZN-2279 should not be administered if there are any indications that it may have been frozen.

Before administration of EZN-2279, the solution should be inspected visually for particulate matter and discoloration. The solution should be clear and free of particulates. EZN-2279 should not be diluted nor mixed with any other drug before administration.

Use only one dose per vial; do not re-enter the vial. Discard unused portions. Do not save unused drug for later administration.

Note: All vials of EZN-2279 used for one dose must be from the same lot.

8.4.2. Adagen®

As with all parenteral drug products, aseptic technique should be used during administration of Adagen®. Adagen® should not be shaken or vigorously agitated. Adagen® should not be administered if there are any indications that it may have been frozen.

Before administration of Adagen®, the solution should be inspected visually for particulate matter and discoloration. The solution should be clear and free of particulates. Adagen® should not be diluted nor mixed with any other drug before administration.

Use only one dose per vial; do not re-enter the vial. Discard unused portions. Do not save unused drug for later administration.

Note: All vials of Adagen® used for one dose must be from the same lot.

8.5. Study Drug Accountability

The Food and Drug Administration requires accounting for the disposition of all study drugs (active or placebo). The Investigator at each study site is responsible for ensuring that a current record of study drug disposition is maintained and dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product disposition as required by federal law consist of the date received, date dispensed/administered, quantity dispensed/administered, and the subject to whom the drug was dispensed/administered.

The Investigator or designee authorized by the Investigator will be responsible for maintaining accurate records of the shipment, dispensing/return of the study drug by the study site, and return of used/undispensed study drug to Leadiant Biosciences. Drug accountability records must be available for inspection by Leadiant Biosciences or its representative and is subject to inspection by a regulatory agency (e.g., FDA, EMEA) at any time. Copies of the records will be provided to Leadiant Biosciences at the conclusion of the study. Product accountability records will be reviewed by the Leadiant Biosciences Monitor.

During the study, all opened and partially used investigational product and containers should be disposed of by the investigational site or designee in accordance with their institutional policies. The Leadiant Biosciences monitor or designee will verify drug inventory and dispensing/accountability records. At the end of the study, all investigational product distributed to the Investigator,

including unassigned, unused, and partially used investigational product and containers, must be removed from the study site or destroyed as directed by Leadiant Biosciences. The disposal of the investigational product must be in accordance with institutional, local, and federal guidelines.

A written explanation will be required for any product not returned to Leadiant Biosciences or documented as destroyed by the site(s). All Clinical supply return, destruction, and accountability forms must be maintained in either the pharmacy or in another approved area.

The lot numbers of EZN-2279 and Adagen® used in this study will be documented in the site's study record (e.g., the Drug Accountability Log or similar record). Details regarding the receipt and dispensing of the study drug will be recorded in the study records. At the conclusion of the study, any unused EZN-2279 or Adagen® will be returned to Leadiant Biosciences or its designee. If no EZN-2279 or Adagen® supplies remain, this fact will be indicated on the Drug Accountability Log.

9. STUDY POPULATION

9.1. Number of Patients

The study will enroll a sufficient number of patients who meet all study entry criteria described in Sections 9.2 and 9.3, respectively to assure 6 evaluable patients. More than 6 patients may be enrolled to achieve the required number of evaluable patients. It is expected that up to 12 study centers will participate in the study. There are no restrictions on the number of patients a single study center may enroll.

9.2. Inclusion Criteria

Patients must meet <u>all</u> of the following criteria to be eligible for enrollment into the study.

1. Diagnosis of ADA-deficient combined immunodeficiency
2. Stable clinical status while receiving therapy with Adagen®. Patients previously receiving gene therapy or undergoing hematopoietic stem cell transplantation who still require Adagen® treatment are eligible. The dose of Adagen® must be stable for at least 6 months prior to study entry.
3. Have both of the following during the Adagen® Lead-in phase of the study prior to EZN-2279 transition:
   a. Trough plasma ADA activity ≥15 μmol/h/mL while receiving Adagen® and
   b. Total erythrocyte dAXP ≤0.02 μmol/mL from a trough blood sample
4. Patients or parent/guardian must be capable of understanding the protocol requirements and risks and providing written informed assent/consent.

9.3. Exclusion Criteria

Patients meeting <u>any</u> of the following exclusion criteria will not be eligible for enrollment.

1. Autoimmunity requiring immunosuppressive treatment.
2. Patients with detectable neutralizing anti-Adagen® antibodies at screening evaluation.
3. Severe thrombocytopenia (platelet count $<50 \times 10^9$/L).
4. Current participation in other therapeutic protocols for ADA-deficient combined immunodeficiency.

5. Current or prior participation in another clinical study with an investigational agent and/or use of an investigational drug in the 30 days before study entry.
6. Known planned participation in a gene-therapy study for the planned duration of this study.
7. Any condition that, in the opinion of the PI, makes the patient unsuitable for the study.
8. Inability or unwillingness to administer Adagen® or EZN-2279 on a one time per week regimen.
9. Inability to comply with the study protocol.
10. Female patients who are pregnant or lactating.
11. Female patients who are breast-feeding.
12. Female patients of childbearing potential who are not using an FDA approved birth control method.

9.4. Post-Enrollment Restrictions

9.4.1. Concomitant Medications

There are no known drug interactions with EZN-2279 or Adagen®. However, vidarabine is a substrate for ADA, and 2'-deoxycoformycin is a potent inhibitor of ADA. Thus, the activities of these drugs and EZN-2279 or Adagen® could be substantially altered if they are used in combination with one another.

Investigational agents, including those intended to treat the patient's disease, are disallowed concomitant to study conduct.

9.4.2. Concurrent Treatments

Concurrent treatments intended to directly treat the patient's disease are disallowed.

Concurrent treatments intended to prevent or treat complications associated with ADA-SCID (e.g., antibiotics, antifungal agents, intravenous immunoglobulins) are permitted.

9.4.3. Dietary Restrictions

There are no dietary restrictions for patients enrolled in the study.

9.5. Withdrawal of Enrolled Patients

In the absence of a medical contraindication or significant protocol violation, every effort will be made by the Principal Investigator to keep subject in the study. The primary reason for a subject withdrawing prematurely should be selected from the following standard categories:

- Adverse Event – clinical or laboratory events that in the judgment of the Principal Investigator require discontinuation of study medication in the best interests of subject.
- Death – death of the subject, whether study related or not.
- Withdrawal of Consent – subject desires to withdraw from further participation in the study in the absence of a medical need to withdraw determined by the Principal Investigator.
- Lost to follow-up – the subject did not return for one or more follow-up visit(s) following the start of study treatment.

- Protocol Non-compliance – the subject's participation in the study failed to meet protocol requirements which had a direct impact on subject safety and evaluability of the subject's data. The violation necessitated premature termination from the study.
- Subject meets withdrawal criteria – the subject's condition meets the criteria for withdrawal from the study (Section 9.5.1).
- Other – causes of premature termination from the study other than the above (e.g., termination of study by Leadiant Biosciences, subject relocated).

9.5.1. Withdrawal Criteria

Reasons for patient withdrawal or completion of the maintenance period may include the following:

- Adverse event requiring the discontinuation of EZN-2279 treatment
- Death
- Protocol violation requiring the discontinuation of EZN-2279 treatment
- Loss to follow-up
- Withdrawal of consent
- Investigator's decision, e.g., PI believes it is no longer in the patient's best interest to remain in the study
- Sponsor's decision to discontinue the study
- Lack of efficacy
- Study completion (only applies to patients in the maintenance period)
- Other reason - causes of premature termination from the study other than the above (e.g., subject relocated)

The reason for study discontinuation must be documented in the patient's source documents and in the CRF.

The study will end upon full regulatory approval of EZN-2279 or early study termination of the study by Leadiant Biosciences.

As required, a patient will be followed up after the day of withdrawal, as described below in this section, in Section 11.5, Section 11.5.1 and Section 10.4.4.

Patients are free to withdraw from the study at any time for any reason. Leadiant Biosciences reserves the right to discontinue this study at any time.

The reasons for patient withdrawal or study completion, as well as details relevant to the patient withdrawal or study completion, shall be recorded in the CRF. Patients withdrawn before their completion of the study will undergo all procedures scheduled for study completion as outlined in Section 11.5.

Any patient withdrawn due to any AE (whether serious or non-serious) or clinically significant abnormal laboratory test values will be evaluated by the PI or his/her designee, or a monitoring physician, and will be treated and/or followed up until the symptoms or values return to normal or acceptable levels, as judged by the Investigator (see Section 10.4.4).

9.5.2. Withdrawal Procedures

Under any circumstance, a final physical examination of each patient must be performed at the time of study discontinuation. See Section 11.5 for procedures related to the discontinuation of treatment, the completion of maintenance, or the end of the study.

Should a subject be withdrawn from the study, all efforts will be made to complete and report the observations as thoroughly as possible, including a complete final evaluation at the time of the patient's withdrawal with an explanation of the reason subject was withdrawn from the study.

9.5.3. Replacement of Discontinued Patients

Patients who do not complete through EZN-2279 Week T-9 will be replaced. Additionally, any patient not completing the EZN-2279 treatment phase of the protocol (i.e. completion of Week T-21) will be considered unevaluable and will need to be replaced to achieve 6 evaluable patients.

10. DESCRIPTION OF STUDY PROCEDURES

10.1. Informed Consent

Patients or the legally authorized representative of eligible patients who express interest in participating in the study must sign an IRB/IEC-approved Informed Consent form that conforms to the Elements of the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule Authorization (see Sections 14.3.3 and 14.3.4) before initiation of any study activities. Documentation of consent must be completed by the Investigator or his/her designee.

The PI and other members of the study site's treating team will review the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits, and alternative therapies including best supportive care. Patients must be informed that participation in the study is voluntary, he/she may withdraw from the study at any time, and withdrawal from the study will not affect his/her subsequent medical treatment or relationship with the treating physician. Patients will also be informed of any financial costs that the patient will or may incur as a result of participation in the study, and that their study record and medical records/documents that pertain directly to the study will be reviewed and possibly copied by Leadiant Biosciences or its designee, or a governmental agency (such as the FDA), and that every effort will be made to maintain patient confidentiality.

The Informed Consent must be witnessed and dated by the PI or his/her designee, and the original retained by the PI/Study Site as part of the patient's study record.

For patients under 18 years of age, Assent of the minor child must be obtained in accordance with Good Clinical Practices and the requirements of the reviewing Institutional Review Board (IRB)

A copy of the fully executed/signed Informed Consent form and Assent form (if applicable) must be given to the patient. In the event the patient is re-screened, the patient is not required to sign another Informed Consent form unless the patient is re-screened more than 30 days from the previous Informed Consent form's signature date.

10.2. Screening and Enrollment

The Investigator or his/her designee will obtain a signed Informed Consent and Assent (if required) before initiating any study-specific procedures. Each patient that is consented will be given a 3-digit sequential patient number, beginning with 001 at each study site.

Leadiant Biosciences will track enrollment and notify participating study sites when the target total enrollment has been attained and enrollment closed.

10.3. History and Baseline Characteristics

The following history and demographic data will be collected:

- Demographics – age, gender, race, and ethnicity
- Disease background and history:
  - Date of diagnosis
  - Prior treatments for SCID, including dates and results
  - Adagen® treatment dates and current dose and regimen of Adagen®
  - Adagen® toxicities experienced – including the name of the toxicity, the date it last occurred, and status (i.e., resolved or ongoing)
  - History of hospitalizations and infectious complications over the past year
  - Other significant disease-related medical history
- Medical History – in addition to the disease background and history, a complete evaluation of history of disorders of the following systems – cardiovascular, pulmonary, gastrointestinal, urologic, hematologic, neurologic, ophthalmologic, otolaryngeal, musculoskeletal, endocrine, and psychosocial; Note: any medications being taken for active conditions and include in medication history; prior surgical procedures; allergies to medications, including medication and reaction.
- Medication History – all prescription and non-prescription medications (including blood products, use of vitamins, supplements and herbal remedies) taken within 60 days of enrollment (i.e., start of Adagen® lead-in period) will be recorded. Information recorded includes the medication name, dose, dose regimen, route of administration, start and stop (if applicable) dates, and indication for use. Note if a product is being taken for the prevention of a condition, "prophylaxis for" or "prevention of" should be included in the description of indication.

10.4. Safety Procedures

10.4.1. Physical Examination

Physical examination will include an examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and nervous system, and include a performance status evaluation (see Appendix 3).

A physical examination will be performed as indicated in Sections 11.1 through 11.5 and Appendix 1.

Height and weight will be collected as part of growth assessments as indicated in Sections 10.5.3.3, and 11.1 through 11.5 and Appendix 1.

Changes in physical examination findings (e.g., new findings, changes in status of previous findings) will be documented. Findings made after the start of study drug administration that meet the definition of an AE (see Section 12.2.1) must be recorded on the Adverse Event CRF.

10.4.2. Vital Signs

Vital signs will include sitting systolic and diastolic blood pressure, pulse rate, respirations, and temperature. Blood pressure will be measured and recorded to the nearest millimeter of mercury (mmHg).

Clinically meaningful changes in vital signs made after the start of study drug, which meet the definition of an AE, must be recorded on the Adverse Event CRF.

10.4.3. Laboratory Assessments

The following laboratory evaluations will be performed by the site's local laboratory as indicated in Sections 11.1 through 11.5 and Appendix 1.

- Hematology: Hemoglobin, hematocrit, WBC count, differential (includes at least: neutrophils [include bands], lymphocytes, monocytes, eosinophils, and basophils), and platelet count.
- Serum chemistry: Bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, magnesium, phosphate, potassium, sodium, and total protein, albumin, alkaline phosphatase, ALT, AST, and total bilirubin.
- Urinalysis: Macroscopic (bilirubin, blood, glucose, ketones, leukocyte esterase, nitrite, pH, protein, specific gravity, and urobilinogen). Microscopic examination will include red blood cells (RBCs), WBCs, bacteria, and casts.
- Serum or urine pregnancy test: Only for female patients of child-bearing potential.

If a laboratory assessment has been performed within the specified protocol window as part of standard of care by a laboratory listed on the FDA 1572 form, it does not need to be repeated for the purpose of the protocol. The laboratory assessment result will be included on the CRF for the study.

Following the start of study treatment clinically meaningful laboratory abnormalities will be reported as an adverse event.

10.4.3.1. Immunogenicity

Venous blood samples for determination of anti-EZN-2279 and anti-Adagen® binding and neutralizing antibodies, and anti-PEG antibodies will be collected in 2mL ethylenediaminetetraacetic acid (EDTA) tubes at the timepoints listed in Appendix 2.

If the results of ADA activity indicate an unexpected reduction in activity from previous sampling timepoints, and if there is an adequate amount of sample remaining for analysis, assay for binding, neutralizing, and anti-PEG antibodies will be performed

Samples will be collected, processed and shipped to the central laboratory for analysis as described in the laboratory manual for the study.

10.4.4. Adverse Event Assessment

The patients will be instructed to inform the PI or his/her designee of any AEs occurring during the study. All AEs whether observed by the PI or his/her designee, elicited by the PI or his/her designee (e.g., via physical examination findings or review of laboratory results), or spontaneously reported by the patient will be documented in the patient's medical record/chart.

Patients will be questioned in an open manner (e.g., "how do you feel?", "any changes since your last visit?") regarding any new adverse events that may have occurred. Patients will also be specifically questioned regarding the status of ongoing adverse events. For each adverse event the following information will be recorded:

- Onset date and time
- Resolution date and time
- Severity/intensity (see section 12.2.5)
- Relationship to study drug (see section 12.2.4)
- Serious or non-serious (see section 12.2.3)

- Actions taken to manage/treat the event
- Outcome of the event - resolved, resolved with sequalae, ongoing, death

All AEs should be followed until they are resolved or until a stable clinical endpoint is reached. Any

SAE should be reported to Leadiant Biosciences Drug Safety Surveillance (see Study

Reference
Manual for contact information).

Any patient withdrawn due to any AE (whether serious or non-serious) or clinically significant abnormal laboratory test value will be evaluated by the PI or his/her designee, or a monitoring physician, and will be treated and/or followed up until the symptom(s) or value(s) return to normal/Baseline or acceptable levels, as judged by the PI.

10.5. Efficacy Procedures

10.5.1. Trough Plasma ADA Activity

Venous blood samples for the determination of ADA activity will be collected in a 2mL EDTA collection tube at the timepoints listed in Appendix 2. Samples will be collected, processed and shipped to the central laboratory for analysis as described in the laboratory manual for the study.

10.5.2. Immune Status

10.5.2.1. Lymphocyte Subset Analysis

Blood samples (4 mL) will be collected into a lavender-top collection tube containing EDTA for lymphocyte subset analysis as described below.

Samples will be collected, processed and shipped to the central laboratory for analysis as described in the laboratory manual for the study. The blood sample must be kept at room temperature; do not freeze or refrigerate.

- CD3+ (Mature T cells)
  - Percent and Absolute
- CD3+ CD8+ (Suppressor T Cells)
  - Percent and Absolute
- CD3+ CD4+ (Helper Cells)
  - Percent and Absolute
- CD (16+56)+ (Natural Killer Cells)
  - Percent and Absolute
- CD19+ (B Cells)
  - Percent and Absolute
- Absolute Lymphocytes (CD45+)
- %CD4 (Helper Cells) / %CD8 (Suppressor T Cells)

10.5.2.2. Quantitative immunoglobulin concentration

Blood for assessments of serum IgG, IgA, and IgM will be collected in 3.5mL gold top serum separator tube (SST) at the timepoints listed in Appendix 2. Blood samples will be collected, processed and shipped to the central laboratory for analysis as described in the laboratory manual for the study.

10.5.3. Clinical Status

10.5.3.1. Infections

Patients suspected of having an infection will have signs and symptoms of the suspected infection evaluated. Appropriate cultures will be obtained based upon clinical presentation.

All infections will be classified by the PI as clinically or microbiologically documented as described below:

- Clinically documented – patients with documented signs and symptoms of infection without positive cultures
- Microbiologically documented – patients with documented signs and symptoms of infection and with positive cultures

10.5.3.2. Hospitalizations

If a patient is hospitalized, the reason for and the duration of each hospitalization will be recorded.

All hospitalizations should be reported to Leadiant Biosciences Drug Safety Surveillance (see Study
Reference Manual for contact information).

10.5.3.3. Growth

Height (in cm) and weight (in kg) will be obtained for determination of height-for-age and weight-for-age Z-scores.

10.5.3.4. Overall Survival

Patient survival will be recorded throughout the patient's participation in the study. If death is reported during the study, the date of death and reason for the patient death will be documented and recorded.

All patient deaths should be reported immediately (see Section 12.4) to Leadiant Biosciences Drug Safety
Surveillance (see Study Reference Manual for contact information).

10.6. Pharmacokinetic and Pharmacodynamic Assessments

10.6.1. Pharmacokinetic Assessments

Only patients ≥ 10 years of age will undergo full PK assessments due to blood volume restrictions in pediatric patients. All patients < 10 years of age will have the same assessments and in lieu of full PK sampling will have limited PK sampling done. Only trough samples along with 48 hours post dose during Adagen Lead In Week T-5 and EZN-2279 Week T-9 will be collected.

Venous blood samples (EDTA tube, up to 2 mL per timepoint) for the determination of the PK profile of EZN-2279 will be collected at Week L-4, after the Adagen® lead-in, and Week T-9 after initiation of EZN-2279 therapy (see Appendix 2). Note: If the dose of EZN-2279 is altered, the full PK profile should be repeated not earlier than 4 weeks after adjustment of the dose of EZN-2279.

Note: The site should calculate sample collection dates and times in advance of scheduling a patient's first day of administration, so as to avoid sample collections during non-routine times such as evenings, holidays, etc.

It is extremely important to collect all samples at the times specified. Both the scheduled and actual times of sample collection should appear on the sample collection record used in the clinic. In addition, the actual times at which the infusion is started and stopped must be recorded.

10.6.2. Pharmacodynamic Assessments

10.6.2.1. dAXP

Venous blood samples for the determination of total erythrocyte dAXP (EDTA tube, 2 mL per timepoint) will be collected at the timepoints listed in Appendix 2. Samples will be collected, processed and shipped to the central laboratory for analysis as described in the laboratory manual for the study.

10.6.2.2. Maximum Total Blood Collection Volume

During a patient's study participation, the maximum amounts of venous blood that will be collected are listed in Appendix 2. Note: there is limited PK sampling for patients < 10 years of age.

10.7. Study Medication Administration and Compliance

10.7.1. Dosage and Administration

10.7.1.1. Adagen®

Adagen® will be administered during the Adagen® Lead-in phase of the study. Patients will remain on his/her weekly Adagen® dose. The total weekly dosage will be consolidated to a once weekly dose regimen. This phase of the study will continue until the patient meets protocol criteria for dAXP levels and ADA activity.

For example, a 50kg patient on a dose of 15U/kg twice a week will have his/her dose consolidated to 30U/kg once weekly. The dose volume will be calculated as follows:

50 kg x 30U/kg x 1 mL/250 U = 6.0 mL (1500 U)

The date and time of each dose administered will be recorded.

10.7.1.2. EZN-2279

EZN-2279 will be administered starting after completion of the Adagen® Lead-in phase. Patients will be transitioned to receive an equivalent dose of EZN-2279 as a weekly i.m. injection. Based upon 1 mg EZN-2279 containing ADA enzymatic activity of 150 U of Adagen®, the equivalent dose of EZN-2279 will be calculated as follows:

Adagen® dose (U/kg) x 1mg EZN-2279/150U Adagen® = EZN-2279 dose (mg/kg)

For example, for a 50 kg patient on a dose of 30 U/kg of Adagen®:

$$\textbf{Adagen® 30 U/kg x } \frac{\textbf{1 mg EZN-2279}}{\textbf{150 U Adagen®}} \textbf{ = 0.2 mg/kg EZN-2279}$$

50 kg x 0.2mg/kg x 1 mL/1.6 mg = 6.25 mL (10 mg)

The date and time of each dose administered will be recorded.

All patients will be observed for at least 1 hour after administration of EZN-2279 for doses administered at the study site through study Week T-9.

10.7.2. Dose Adjustments

Adjustments of the dose and/or schedule of EZN-2279 or Adagen® may be required if a patient's trough plasma ADA activity is less than 15 μmol/h/mL and/or the trough dAXP level is greater than 0.02 μmol/mL for two or more consecutive assessments and compliance with the study drug regimen is confirmed and the patient is not considered fully detoxified. The Investigator, the sponsor, and Lead PI must agree upon all modifications in the dosing regimen for each patient before any changes are instituted.

The dose of Adagen® should not be adjusted more than once every 2 weeks and the dose EZN-2279 should not be adjusted more than once every 4 weeks to allow the effect of these changes on trough level of ADA to be manifested.

10.7.3. Treatment Delays

EZN-2279 or Adagen® will be administered weekly ± 1 day. The PI, after upon consultation with Leadiant Biosciences and Lead PI, may elect to delay or withhold a dose if the clinical condition of the patient warrants such action. The PI, Leadiant Biosciences, and Lead PI must agree upon all modifications in the dosing regimen for each patient before any changes are instituted.

10.7.4. Assessment of Study Medication Compliance

Compliance will be assured by having the study drug administered under the supervision of the PI and/or designated site staff members while the patient is at the clinic/study site during the visit(s). Administration of the study drug must be recorded in source documents. For EZN-2279 Treatment Week 12, the patient is permitted to self dose at home, but enough drug will be dispensed along with diary cards which become part of the patient source documentation.

10.8. Recording of Concomitant Medications

All changes in prior and new prescription and non-prescription medications (including i.v. immunoglobulins, blood products, use of vitamins, supplements and herbal remedies) from the start of screening through the final study visit are to be recorded. For each change in prior medication and new medication the following will be recorded:

- Name of the medication
- Total daily dose
- Route of administration
- The start and stop dates
- The indication for use
  - If a product is being taken for the prevention of a condition, "prophylaxis for" or "prevention of "should be included in the description of indication
  - If a product is being taken for an active condition, a corresponding medical condition must be included in the medical history

10.9. Compliance

This study is to be conducted according to:

- United States (U.S.) and international standards of Good Clinical Practices (GCP) as defined in the U.S. Food and Drug Administration (FDA) Code of Federal Regulations (CFR), and International Conference on Harmonization (ICH) guidances,
- Applicable government regulations,

- Institutional Review Board(s) (IRB)/Independent Ethics Committee(s) (IEC), and
- Institutional (study site) research policies and procedures.

The Principal Investigator and the study personnel will verify patient compliance with the study requirements, procedures and schedule of events. The Principal Investigator or physician Sub-Investigator (listed on Form FDA 1572) will be present for the initial dose administration of Adagen® during the Lead-In phase and EZN-2279 (Week T-1) and for the post-dose monitoring and evaluation for all patients. The Principal Investigator or physician sub-investigator will be available and accessible at all other times throughout the study for the management of the patients enrolled in this clinical trial. Non-compliance with any study evaluations/procedures will be documented in the patient's source documents and case report forms (CRFs).

11. SCHEDULE OF STUDY PROCEDURES

Study activities are listed in Appendix 1.

11.1. Screening

Patients who fulfill all of the inclusion criteria and none of the exclusion criteria may be entered into the study.

The following evaluations are to be performed within 28 days before start of the Adagen® Lead-in period unless otherwise indicated.

- Informed assent/consent
- Obtain patient history and baseline characteristics
  - Demographics
  - Disease background and history
  - Assessment of AEs/toxicities the patient has experienced while on Adagen® treatment
  - Assessment of infectious complications and hospitalizations within the past 12 months
  - Medical history
  - Medication history
- Physical examination
- Vital signs
- Height and weight for growth assessment
- Performance status score
- Urine or serum pregnancy test for females of childbearing potential must be performed within 7-days before starting Adagen® Lead-in.
- Safety Lab
  - Hematology
  - Serum chemistry
  - Urinalysis
- Immunogenicity - Adagen® antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies

Patients passing screening assessment will enter the Adagen Lead-in phase of the study.

For patients currently maintained with Adagen® on a 1 time per week dosage regimen, the following additional laboratory assessments will be performed to determine whether an Adagen® dosage adjustment when entering the Adagen® Lead-in phase is required:

- Total erythrocyte dAXP from a trough blood sample
- Trough plasma ADA activity

11.2. Adagen® Lead -in

11.2.1. Adagen® Dosage and Schedule Adjustments

Patients entering the Lead-in phase will have Adagen® dose/dose schedule changes implemented prior to the first Adagen lead-in dose as described below:

- Patients on once weekly Adagen® dosing and meet entry criteria for dAXP level and ADA activity – these patients will enter Adagen® Lead-in on the dose and regimen he/she was taking prior to study entry
- Patients on once weekly Adagen® dosing and do not meet entry criteria for dAXP levels and ADA activity – these patients should have his/her dosage adjusted (see Section 10.7.2)
- Patients on two or more weekly doses of Adagen® – these patients will have his/her dose regimen of Adagen® consolidated into a single weekly dose. For example, a patient receiving 20U/kg twice weekly will have his/her dose regimen consolidated to a dose of 40U/kg once weekly.

11.2.2. Weekly Adagen Dosing

Patients will have weekly study visits (± 1 day). For each visit, the patient will return to the study site have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit
- Trough blood samples will be obtained 15 to 30 minutes prior to Adagen® dosing for:
  - dAXP
  - ADA activity

The patient will receive his/her dose of Adagen®.

Patients will be followed weekly for dAXP and ADA activity.

- If the results of these tests indicate a dAXP level ≤ 0.02 μmol/mL and a trough ADA activity level ≥ 15 μmol/h/mL for two consecutive weekly measurements and all other section criteria are still met the patient will be able to proceed to the Adagen® Pharmacokinetic assessment (see Section 11.2.3).
- If the dAXP and trough ADA activity levels do not meet protocol selection criteria for two consecutive weeks and the patient is not considered fully detoxified, the patient should have his/her dosage re-adjusted (see Section 10.7.2). This process may be repeated until the patient meets the ADA activity and dAXP levels and is considered fully detoxified for study inclusion.

A schematic diagram of procedures for dose adjustment and consolidation is provided in Appendix 4.

11.2.3. Adagen® Pharmacokinetics

11.2.3.1. Day 1

Day 1 is the day of the first administration of Adagen® on study. Prior to administration of Adagen® the following baseline procedures will be performed:

- Update medical history
- Physical examination
- Height and weight for growth assessment

- Performance status
- Vital Signs
- Safety Lab
  - Hematology
  - Chemistry
  - Urinalysis
- Other labs – blood samples will be obtained 15 to 30 minutes prior to dosing for:
  - dAXP
  - Trough ADA activity
  - B-, T-, and NK-lymphocyte subset analysis
  - Quantitative immunoglobulins
  - Adagen® antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies
- Assessment of concomitant medications and procedures since last study visit

Following completion of the above procedures, the patient will receive Adagen® at his/her current weekly dose administered as a single weekly injection.

11.2.3.2. Days 2 through 8

Blood samples for ADA activity will be obtained at 24, 48, 72, 96, 120, 144, and 168 hours following Day 1 PK dose. All blood samples will be obtained ± 4 hours of the scheduled collection time. Note: the 168 hour post-dose sample will be obtained immediately prior to the initial dose of EZN-2279.

For patients < 10 years of age, PK sampling will be limited to trough samples and at 48 hours post dose for monitoring of ADA activity and C_{max} timepoint.

11.3. EZN-2279 Study Treatment Period

11.3.1. Day 1 (Week T-1)

Prior to administration of EZN-2279 the following procedures will be performed:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology
  - Chemistry
  - Urinalysis
- Other labs – blood samples will be obtained 15 to 30 minutes prior to dosing for:
  - dAXP
  - Trough ADA activity (168 hours following Day 1 Adagen® PK dose)
  - B-, T-, and NK-lymphocyte subset analysis
  - Quantitative immunoglobulins
  - EZN-2279 and Adagen® antibody titers (neutralizing and binding antibodies) and anti-PEG antibodies
- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Following completion of the above procedures, the patient will receive EZN-2279. The equivalent dose of EZN-2279 will be calculated as described in Sections 7.2.1 and 10.7.1.

11.3.2. Day 8 (Week T-2)

On Day 8 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

The patient will receive his/her dose of EZN-2279 after all procedures have been completed.

11.3.3. Day 15 (Week T-3)

On Day 15 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity
- EZN-2279 antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

11.3.4. Day 22 (Week T-4)

On Day 22 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

The patient will receive his/her dose of EZN-2279 after all procedures have been completed.

11.3.5. Day 29 (Week T-5)

On Day 29 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology
  - Chemistry
- Adverse event assessment

- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

11.3.6. Day 36 (Week T-6)

On Day 36 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

11.3.7. Day 43 (Week T-7)

On Day 43 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

11.3.8. Day 50 (Week T-8)

On Day 50 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

11.3.8.1. EZN-2279 Dose Evaluation for Adjustment

The results from trough ADA activity and dAXP will be reviewed to assess whether patients have a dAXP level ≤ 0.02 μmol/mL and a trough ADA activity level ≥ 15 μmol/h/mL.

Based upon this review:

- If the Week T-7 and T-8 values for ADA activity and dAXP levels meet the established values, the patient will proceed to Day 57 (Week T-9) and begin full PK sampling
- If the Week T-7 and T-8 values for ADA activity and dAXP levels **DO NOT** meet the established values and the patient is not considered fully detoxified:
  - The patient will have his/her dosage of EZN-2279 adjusted. Dose adjustments will be based upon dAXP and ADA values and agreed upon by the PI, Sponsor and Lead PI
  - Return to Week T-5 (Section 11.3.5) and continue through weekly dosing for 4 weeks (T-5 through T-8) and be re-assessed as described in this section

11.3.9. Day 57 (Week T-9)

On Day 57 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology
  - Chemistry
- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity
- B-, T-, and NK-lymphocyte subset analysis
- Quantitative immunoglobulins

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

This is the first day of the full PK sampling for EZN-2279. Note: If the dose or schedule of EZN-2279 is altered, the full PK profile should be repeated not earlier than 9 weeks after adjustment of the dose/schedule of EZN-2279.

11.3.10. Days 58 through 63 (Week T-9)

The patient will return to the study site each day for blood sampling (only for patients ≥ 10 years of age). Blood samples for ADA activity will be obtained at 24, 48, 72, 96, 120, 144, and 168 hours following Day 29 dose. All blood samples will be obtained ± 4 hours of the scheduled collection time. Note: the 168 hour post-dose sample will be obtained immediately prior to the Week T-10 dose of EZN-2279.

For patients < 10 years of age, PK sampling will be limited to trough samples and at 48 hours post dose for monitoring of ADA activity and C_{max} timepoint.

11.3.11. Day 64 (Week T-10)

On Day 64 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity (168 hours following Day 57 EZN-2279 dose)
- EZN-2279 antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

11.3.12. Day 71 (Week T-11)

On Day 71 (± 1 day) the patient will receive EZN-2279 at a dose of 0.2 mg/kg and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

If the patient will self-administer the Week T-12 dose of EZN-2279, the dose will be dispensed with a dosing diary card and administration instructions.

11.3.13. Day 78 (Week T-12)

On Day 78 (± 1 day) the patient will receive his/her dose of EZN-2279. The dose may be given at the study site or self-administered by the patient.

11.3.14. Day 85 (Week T-13)

On Day 85 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
    - Hematology

  - Chemistry
- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

If the patient will self-administer the Week T-14 dose of EZN-2279, the dose will be dispensed with a dosing diary card and administration instructions.

11.3.15. Day 92 (Week T-14)

On Day 92 (± 1 day) the patient will receive his/her dose of EZN-2279. The dose may be given at the study site or self-administered by the patient.

11.3.16. Day 99 (Week T-15)

On Day 99 (± 1 day) the patient will return to the study site and have the following procedures performed prior:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

If the patient will self-administer the Week T-16 dose of EZN-2279, the dose will be dispensed with a dosing diary card and administration instructions.

11.3.17. Day 106 (Week T-16)

On Day 106 (± 1 day) the patient will receive his/her dose of EZN-2279. The dose may be given at the study site or self-administered by the patient.

11.3.18. Day 113 (Week T-17)

On Day 113 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology

  - Chemistry
- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

If the patient will self-administer the Week T-18 dose of EZN-2279, the dose will be dispensed with a dosing diary card and administration instructions.

11.3.19. Day 120 (Week T-18)

On Day 120 (± 1 day) the patient will receive his/her dose of EZN-2279. The dose may be given at the study site or self-administered by the patient.

11.3.20. Day 127 (Week T-19)

On Day 127 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit

Blood samples will be obtained 15 to 30 minutes prior to administration of EZN-2279 for:

- dAXP
- Trough ADA activity

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

If the patient will self-administer the Week T-20 dose of EZN-2279, the dose will be dispensed with a dosing diary card and administration instructions.

11.3.21. Day 134 (Week T-20)

On Day 134 (± 1 day) the patient will receive his/her dose of EZN-2279. The dose may be given at the study site or self-administered by the patient.

11.3.22. Day 141 (Week T-21)

On Day 141 (± 1 day) the patient will return to the study site and have the following procedures performed:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology

  - Chemistry
  - Urinalysis
- Other labs – obtained 15 to 30 minutes prior to dosing
  - dAXP
  - Trough ADA activity
  - B-, T-, and NK-lymphocyte subset analysis
  - Quantitative immunoglobulins
  - EZN-2279 antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies
- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit
- Assessment of overall survival

The patient will receive his/her dose of EZN-2279 after all procedures and laboratory blood draws have been completed.

Following completion of assessments and dosing at Week T-21 the patient will enter the EZN-2279 maintenance period.

11.4. EZN-2279 Maintenance Period

As part of standard clinical care, the following evaluations will be performed during visits approximately every 3 months (13 weeks ±3 weeks) until study completion. For example, based upon the start of EZN-2279 treatment (Day 1, Week T-1), maintenance visits would be scheduled to occur on Weeks 34 (Cycle 1), 47 (Cycle 2), 60 (Cycle 3), 73 (Cycle 4), 86 (Cycle 5), 99 (Cycle 6), 112 (Cycle 7), 125 (Cycle 8), 138 (Cycle 9), 151 (Cycle 10), 164 (Cycle 11), 177 (Cycle 12), 190 (Cycle 13) ±3 weeks from start of EZN-2279 treatment.

The patient will have the following procedures performed at each maintenance visit prior to EZN-2279 dosing:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology
  - Chemistry
  - Urinalysis
- Other labs – blood samples will be obtained 15 to 30 minutes prior to dosing for:
  - dAXP
  - Trough ADA activity
  - B-, T-, and NK-lymphocyte subset analysis
  - Quantitative immunoglobulins
  - EZN-2279 antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies
- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Review of EZN-2279 administration diary card and returned vials for assessment of treatment compliance
- Assessment of concomitant medications and procedures since last study visit

- Assessment of overall survival

An appropriate supply of EZN-2279 will be dispensed along with a medication compliance diary card. Note that it is not necessary to dispense a full 13 week supply during a maintenance visit; quantities of EZN-2279 dispensed will be at the discretion of the Investigator based upon the patient's proximity to the study site and medication compliance.

11.5. Study Completion: End-of Treatment, Completion of Maintenance or End of Study

At the time of study discontinuation, the following evaluations should be repeated:

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology
  - Chemistry
  - Urinalysis
- Other labs – blood samples will be obtained for:
  - dAXP
  - Trough ADA activity
  - B-, T-, and NK-lymphocyte subset analysis
  - Quantitative immunoglobulins
  - EZN-2279 antibody titers (i.e., neutralizing and binding antibodies) and anti-PEG antibodies
- Adverse event assessment
- Assessment of infectious complications and hospitalizations
- Assessment of concomitant medications and procedures since last study visit
- Assessment of overall survival

All patients, regardless of the reason for study discontinuation (see Section 9.5.1), should have the above evaluations at the time of study discontinuation, whenever possible. All reasons for discontinuation of study treatment must be documented.

11.5.1. 30-Day Early Discontinuation Follow-up

For patients who discontinue treatment and the study prior to study completion, the following evaluations for safety will occur at least 30 days after the last dose of study drug (EZN-2279 or Adagen®):

- Physical examination
- Height and weight for growth assessment
- Performance status
- Vital Signs
- Safety Lab
  - Hematology
  - Chemistry
  - Urinalysis
- Adverse event assessment
- Assessment of concomitant medications

Any ongoing drug-related toxicities noted will be followed until a stable clinical endpoint is reached (see Section 10.4.4).

12. ADVERSE EVENTS

12.1. Assessment Period

The AE reporting period for this study begins with the first lead-in dose of Adagen® and ends 30 days after the final dose of EZN-2279 (or, for patients who are withdrawn before switching treatment to EZN-2279, 30 days after the final dose of Adagen®).

12.2. Definitions

12.2.1. Adverse Event

An *adverse event* is any untoward medical event associated with the use of a drug in humans, whether or not considered drug related. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a drug and does not imply any judgment about causality.
An untoward medical event which occurs outside the period of follow-up as defined in the protocol will not be considered an adverse event. Worsening of a medical condition for which the efficacy of the study drug is being evaluated will not be considered an adverse event.

12.2.2. Unexpected Adverse Event

An unexpected adverse event is one that is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

12.2.3. Serious Adverse Event

- A *serious adverse event* is an adverse event that in the view of either the investigator or sponsor, results in any of the following outcomes: Results in death
- Is life-threatening (an event that in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death; it does not include an event that had it occurred in a more severe form, might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly or birth defect
- Is another important medical event that may not be immediately life-threatening or result in death or hospitalization but based upon appropriate medical judgment, may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room for

allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

The term "severe" is often used to describe the intensity (severity) of an event; the event itself may be of relatively minor medical significance (such as a severe headache). This is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning.

12.2.4. Relationship to Study Drug

The Investigator must attempt to determine if an adverse event is in some way related to the use of the study drug. This relationship should be described based upon the following definitions:

- ***Unrelated:*** The adverse event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the patient's clinical state, environmental factors, the effect of other concomitant medications or treatments administered.
- ***Unlikely:*** The adverse event does not follow a reasonable temporal sequence from administration of the study drug, does not follow a known response pattern to the study drug, and could readily have been due to other causes such as the patient's clinical state, environmental factors, the effect of other concomitant medications or treatments administered.
- ***Possible:*** The adverse event follows a reasonable temporal sequence from administration of the study drug and follows a known response pattern to the study drug, *BUT,* the adverse event could readily have been produced by the patient's clinical state, environmental factors, the effect of other concomitant medications or treatments administered.
- ***Probable:*** The adverse event follows a reasonable temporal sequence from administration of the study drug and follows a known response pattern to the study drug, *AND* cannot be reasonably explained the patient's clinical state, environmental factors, the effect of other concomitant medications or treatments administered. The event improves upon discontinuation of the study drug.
- ***Definite:*** The adverse event follows a reasonable temporal sequence from administration of the study drug and follows a known response pattern to the study drug. Based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug. The adverse event improves upon discontinuation of the study drug and reappears upon repeat exposure.

12.2.5. Severity (Intensity)

The Investigator will assess the severity (intensity) of all AEs according to the following grading system:

- Mild AE- awareness of the event but is easily tolerated
- Moderate AE – interferes with activities of daily living
- Severe AE – incapacitating and causes inability to perform activities of daily living

12.3. Reporting Adverse Events

All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur must be captured. The Investigator or his/her staff should elicit information regarding the occurrence of adverse events through information volunteered by the patient, open-ended questioning of the patient, physical examination results and review of laboratory results.

Information to be recorded for each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded)
- The date and time of onset of the event
- The date and time of resolution of the event
- Assessments of severity, causal relationship, and seriousness of the adverse event (see definitions in Section 12.2.1 through 12.2.4)
- Action(s) taken (if any) for management of the adverse event, including but not limited to change in the study drug administration (e.g., temporary interruption in dosing, dose reduction); drug treatment; non-drug treatment; diagnostic procedures performed
- Outcome of the adverse event: e.g., patient recovered without sequelae; patient recovered with sequelae; event ongoing; patient died

If a medical condition is known to have caused the injury or accident (e.g., a fall secondary to dizziness), the medical condition (dizziness) and the medical condition resultant to the fall (hip fracture) should be reported as two separate AEs. The act of falling (i.e., fall) should be recorded as part of the event term in the CRF.

All adverse events will be followed until resolution, stabilization, or for 30 days after last dose of study drug, whichever occurs first.

12.4. Reporting Serious Adverse Events

All serious adverse events (SAEs), regardless of relationship to study drug, must be reported to Leadiant Biosciences as described below.

- Serious adverse events that are fatal or life–threatening must be reported to Leadiant Biosciences or their designee by telephone ***immediately*** after site personnel first become aware of the event. Within 24 hours, the Leadiant Biosciences Serious Adverse Event Form must be faxed to the medical monitor or designee regardless of whether full information regarding the event is known or not. If full information is not known, additional follow-up by the Investigator will be required.

- All other serious adverse events must be reported to the Leadiant Biosciences medical monitor or designee within 24 hours by phone, e-mail or fax after becoming aware of the event. Within 48 hours, the Leadiant Biosciences Serious Adverse Event Form must be faxed to the medical monitor or designee regardless of whether full information regarding the event is known or not. If full information is not known, additional follow-up by the Investigator will be required.

Report all SAEs to the Leadiant Biosciences safety representative listed in the Study Reference Manual.

All SAEs will be evaluated by the Leadiant Biosciences Medical Monitor or designee. If meeting the requirements for expedited reporting (i.e., serious, unexpected and related), Leadiant Biosciences will so report the adverse event to all regulatory authorities with jurisdiction over ongoing trials with the study drug and to all other investigators involved in clinical trials with EZN-2279.

The Investigator must report all SAEs reported by Leadiant Biosciences to regulatory authorities in an expedited manner to the reviewing IRB. For all other SAEs, the Investigator must report all serious adverse events in accordance with the requirements of the reviewing IRB.

Leadiant Biosciences will forward all reports of SAEs to the DSMC and the Lead PI.

12.4.1. Reporting of Other Non-serious Events

In addition to reporting of SAEs, the following are specific conditions defined by Leadiant Biosciences for this study, which may or may not be SAEs (i.e., as defined above [Section 12.2.3). These conditions, whether serious or not, are to be reported to Leadiant Biosciences within 48-hours using the Leadiant Biosciences Serious Adverse Event Form:

- All signs and symptoms of an allergic reaction that may not necessarily be deemed serious. For example: urticaria, facial swelling, lip swelling, orbital swelling, rash, pruritus, nasal congestion, injection site reaction, etc., should be reported to Leadiant Biosciences as "Special AE Report." Note: An anaphylactic reaction meets the definition of "serious" and would, therefore, be reported as an SAE.

12.5. Reporting of Pregnancy

If a patient becomes pregnant or is found to be pregnant while on the study, the Leadiant Biosciences medical monitor or designee will be contacted to discuss the management of the patient, which may include discontinuation of study drug.

Although pregnancy is not a serious adverse event all pregnancies occurring during this study will be reported to the Leadiant Biosciences Drug Safety within 48 hours of becoming aware of the pregnancy and complete and submit a pregnancy report form.

All pregnancies will be followed until delivery or pregnancy termination for maternal and fetal outcomes.

13. STATISTICS

13.1. Sample Size Determination

The sample size of six patients accounts for more than 5% of the entire eligible patient population worldwide and 10% of the entire eligible patient population in the United States.

The study will enroll a sufficient number of patients with ADA-SCID who meet all study entry criteria to assure six (6) patients are evaluable, where evaluable is defined as completing the EZN-2279 treatment period and providing primary study endpoint data.

The patients enrolled in this study all will have achieved metabolic detoxification with Adagen® prior to initiating EZN-2279 treatment. Patients are expected to maintain the target trough total erythrocyte dAXP concentration with EZN-2279 at a planned success rate of 100%. Therefore, the usual statistical sample size calculations using precision analysis, power analysis, or probability are not applicable due to zero variability.

13.2. Statistical Methodology

13.2.1. Level of Significance

If applicable, hypotheses will be tested at a two-sided statistical significance level of 0.05.

13.2.2. Analysis Populations

The as-treated population, defined as all patients who are enrolled and receive at least one dose of

study drug (EZN-2279 or Adagen®) will be the primary analysis set in evaluating patient characteristics, treatment administration/compliance and safety.

The primary efficacy endpoint, the percentage of patients achieving metabolic detoxification, will be determined for patients who achieve or who do not achieve metabolic detoxification while receiving EZN-2279 per protocol. Metabolic detoxification is defined as achieving and maintaining the target trough total erythrocyte dAXP concentration of ≤0.020 μmol/mL while receiving EZN-2279.

The PK evaluable population, defined as all patients who have received EZN-2279 for at least 9 weeks (i.e., through at least Week 13) and who have sufficient PK data at Weeks 5 and 13 (or later, if the dose or schedule of EZN-2279 was altered before Week 13) to indicate exposure, will be the analysis set for evaluating PK parameters.

Patients < 10 years of age will not have full PK sampling done due to the blood volume requirements and will only have limited sampling done.

13.2.3. Data Analysis

Statistical analysis will be conducted by Leadiant Biosciences, or its designee. The primary analysis of study data will be performed after the last patient has completed through the EZN-2279 treatment phase (Study Week T-21). Once the last patient enters the EZN-2279 maintenance phase, data will be analyzed after all patients complete 6-months of EZN-2279 maintenance (EZN-2279 treatment week 47) and annually thereafter. The analysis of study data will be repeated upon study completion.

Efficacy and safety analyses will be performed using Statistical Analysis Systems® (SAS®) Version 9 or later (SAS Institute, Cary, NC), or comparable software.

A complete description of data handling rules and planned statistical analyses is detailed in a separate Statistical Analysis Plan (SAP). Unless otherwise specified in this document or in the SAP, baseline is defined as the last measurement for a variable before the initial dose of study drug (EZN-2279 or Adagen®), and missing data will generally not be imputed. If applicable, hypotheses will be tested at a two-sided statistical significance level of 0.05.

Analyses will be primarily descriptive, with data listings, graphical presentations, frequency tabulations, and summary statistics as appropriate.

Each patient will serve as his/her own control based on the baseline assessment of each endpoint as appropriate, including changes in body weight as percentile on the growth curve, antibody formation, etc.

Additional statistical analyses, other than those described in this section, may be performed if deemed appropriate to further explore the study data.

13.2.3.1. Study Conduct and Patient Disposition

The number of patients treated will be tabulated. Patients not meeting the eligibility criteria will be documented. Protocol violations will be reviewed. Reasons for study discontinuation and time of withdrawal from study will be summarized.

13.2.3.2. Baseline Characteristics

Patient characteristics at study entry will be summarized in frequency tables, and descriptive statistics will be provided for quantitative variables.

13.2.3.3. Study Drug Administration

Study drug administration will be summarized for EZN-2279 and Adagen®. The total number of doses administered; the median (range) of doses administered; the median (range) treatment duration; dose modifications, dose delays, and dose omissions; and reasons for deviations from planned therapy will be described.

13.2.3.4. Efficacy Data Analysis

- Primary Efficacy Endpoint – the primary efficacy endpoint is the percentage of patients achieving metabolic detoxification, defined as achieving and maintaining the target trough total erythrocyte dAXP concentration of ≤0.020 μmol/mL while receiving EZN-2279. Total erythrocyte dAXP from a trough blood sample will be evaluated at various timepoints (see Appendix 2 for collection schedule).

- Secondary Efficacy Endpoints – Analyses of the following secondary efficacy endpoints will be primarily descriptive, with data listings, graphical presentations, frequency tabulations, and summary statistics as appropriate.
  - Trough plasma ADA activity: the percentage of patients achieving and maintaining the target trough plasma ADA activity of ≥15 μmol/h/mL while receiving EZN-2279
  - Immune function status:
    - Absolute Lymphocyte count
    - B-, T-, and NK-lymphocyte subset analysis: the number of cells for each subset will be determined by FACS. The following subsets will be assessed:
      - CD3+ (Mature T cells) - Percent and Absolute
      - CD3+ CD8+ (Suppressor T Cells) - Percent and Absolute
      - CD3+ CD4+ (Helper Cells) - Percent and Absolute
      - CD (16+56)+ (Natural Killer Cells) - Percent and Absolute
      - CD19+ (B Cells) - Percent and Absolute
      - Absolute Lymphocytes (CD45+)
      - %CD4 (Helper Cells) / %CD8 (Suppressor T Cells)
    - Quantitative immunoglobulin concentration (IgA, IgG, IgM)
  - Infections: Clinically documented and microbiologically documented
  - Incidence and duration of hospitalizations
  - Growth: Height-for-age and weight-for-age Z-scores
  - Overall survival

13.2.3.5. Safety Data Analysis

Adverse Events and Serious Adverse Events – AEs will be categorized using Medical Dictionary for Regulatory Activities (MedDRA) Version 10.0 or later. The intensity of toxicities will be graded by the Investigator as mild, moderate, or severe as described in Section 12.2.5. Serious Adverse Events (SAEs) will be a subset of the overall AE analysis and may be analyzed in a similar manner.

The calculation of AE incidence will be based on the number of patients per AE category. For each patient who has multiple AEs classified to the same category, that patient will be tabulated under the maximum toxicity intensity for that AE category. The incidence of AEs will be tabulated by MedDRA system organ class and preferred term, as well as by treatment group.

An AE onset between the date of the first dose of study drug and 30 days after the last dose of study drug is considered as a treatment-emergent AE.

- Laboratory Safety Data – Hematology, chemistry, and urinalysis data will be summarized by timepoint of collection and by treatment group. In addition, out-of-normal range values will be listed.

- Other Safety Data – the results of antibodies against EZN-2279, antibodies against Adagen®, and antibodies against PEG will be summarized. The relationships between immunogenicity, PK, and the clinical toxicities will be examined as appropriate.

Concomitant medications and findings in physical examinations will be described textually as recorded in the CRF.

Descriptive statistics of vital signs measurements as well as changes from baseline will be tabulated.

13.2.3.6. Pharmacokinetic Data Analysis

Pharmacokinetics of EZN-2279 and Adagen® will be based on plasma concentrations of ADA activity. The concentration of ADA activity in all collected samples will be determined by a validated assay. The following PK parameters (secondary endpoints) will be computed from individual plasma concentrations using appropriate validated PK software.

- C_{max} Maximum observed drug concentration
- C_{trough} Concentration minimum before repeat dosing
- t_{max} Time of the maximum drug concentration (obtained without interpolation)
- $AUC_{(0-t)}$ AUC calculated using linear trapezoidal summation from time zero to time t, where t is the time of the last measurable concentration (C_t)
- Kel Terminal elimination rate constant calculated by linear regression of the terminal linear portion of the log concentration versus time curve
- $t_{1/2}$ Apparent terminal half-life calculated as ln(2)/Kel

PK parameters and the plasma level-versus-time curves will be determined for each evaluable patient. Descriptive statistics including mean, standard deviation, coefficient of variation, median, minimum, maximum, mean and median plasma level graphs will be provided as appropriate.

13.2.3.7. Linkage Between Pharmacologic Effects and Clinical Effectiveness

The linkage between the pharmacologic effects of EZN-2279 and any measured or anticipated evidence of clinical effectiveness will be explored and summarized.

13.2.4. Handling Missing, Repeated, Unused and Spurious Data

The handling of missing, repeated, unused and spurious data will be described in the statistical analysis plan for the study.

13.2.5. Reporting of Deviations to Statistical Methodology

The methods for documenting deviations to the statistical methodology will be described in the statistical analysis plan for the study. These deviations will be described in the clinical study report.

13.3. Interim Analysis

An interim analysis is planned to occur for all available data after the third patient completes the primary endpoint. The interim analysis will include all data available at the time, but at a minimum the first three patients data completing through 21 weeks of EZN-2279 dosing.

13.4. Statistical Criteria for Termination of the Study

There are no statistical criteria for the termination of the study.

14. ADMINISTRATIVE

14.1. Changes to Study Protocol

14.1.1. Protocol Amendments

Protocol changes must be in the form of a written amendment approved by Leadiant Biosciences.

Protocol amendments and necessary revisions to the informed consent form must be submitted by the Investigator to the local IRB and such amendments will only be implemented after written approval of the requisite IRB. Protocol changes to eliminate an immediate hazard to a study patient may, at the direction of Leadiant Biosciences, be implemented immediately by the Investigator. The Investigator must then immediately inform the IRB and obtain required approvals.

If a protocol amendment requires revision to the informed consent form, the revised IRB-approved form must be used to re-consent patients currently enrolled in the study and the new form must be used to obtain consent from new patients prior to enrollment.

All amendments will be submitted to local regulatory authorities by Leadiant Biosciences as required by local regulation.

14.1.2. Protocol Deviations

Deviations to the protocol will not be permitted without the prior approval of Leadiant Biosciences. All departures from this protocol will be recorded as protocol deviations, independent of whether the deviation was approved by Leadiant Biosciences. If Leadiant Biosciences approves a protocol deviation, a written waiver will be provided to the Investigator. The original waiver will be filed in the site regulatory files and a copy filed with the study records for the patient.

Departures from the protocol involving informed consent, eligibility criteria, study drug administration and the administration of prohibited treatments will require written IRB notification by the Investigator.

14.2. Study Termination

Leadiant Biosciences reserves the right to temporarily or permanently discontinue the study at any site and at any time. Reasons for study discontinuation may include, but are not limited to, the following:

- Investigator non-compliance with the protocol, Good Clinical Practice guidelines or regulatory requirements
- Insufficient enrollment to complete the study within the prescribed timeframe
- Safety concerns
- Drug supply issues
- Discontinuation of the study protocol and/or all studies with EZN-2279

- Request to discontinue the study by a regulatory or health authority

Leadiant Biosciences will promptly inform all Investigators and the requisite regulatory authorities if the study is suspended or terminated for safety reasons. In the case of such suspension or termination, Leadiant Biosciences will provide the Investigator with instructions regarding the disposition of patients (e.g., termination of treatment, patient follow-up) currently on the study. The Investigator will promptly notify the IRB and implement patient disposition instructions.

Should the study be terminated prematurely, all unused study drug(s), unused case report forms and any other investigational study material will be returned to the Sponsor.

14.3. Ethics

14.3.1. Compliance Statement

This study will be conducted in accordance the principles of the Declaration of Helsinki, the International Conference on Harmonization Guidance on Good Clinical Practice and the requirements of federal regulatory authorities regarding the conduct of clinical trials and the protection of human subjects.

14.3.2. Institutional Review Board/Ethics Committee

The Investigator will submit the protocol and subsequent amendments, the Investigator's Brochure and subsequent revisions, the informed consent and any other material used to inform patients/subjects about the study to the local IRB for approval prior to enrolling any patient/subject into the study. The IRB should be duly constituted according to applicable regulatory requirements. Approval must be in the form of a letter signed by the Chairperson of the IRB or the Chairperson's designee, must be on IRB stationary and must include the protocol by name and/or designated number. IRB approval of the informed consent form must be clearly indicated in the IRB approval letter (indicating version/date of the version approved) or by other means utilized by the IRB (e.g., IRB approval stamp on the approved version of the form). If an Investigator is a member of the IRB, the approval letter must stipulate that the Investigator did not participate in the final vote, although the Investigator may participate in the discussion of the study.

The Investigator will also report the progress of the study to the IRB on an annual basis or more frequently as required by the IRB. The Investigator will also promptly inform the IRB of:

- Serious adverse events that the Sponsor reports to regulatory authorities in an expedited manner
- All changes in research activity
- Protocol deviations, as required by Leadiant Biosciences or the IRB
- Other reports as required by the IRB
- The completion, termination, or discontinuation of the study, and
- A final summary of the final results at the conclusion of the study as required by the IRB

Copies of all correspondence between the Investigator and the IRB will be provided to Leadiant

Biosciences.

14.3.3. Informed Consent

The Investigator will obtain written informed consent from each patient or the patient's legal representative prior to performing any study-related procedures. The consent form used to

document informed consent from study participants must contain the elements of informed consent as described in 21 CFR, Part 50.

The study records (i.e., patient source documents and applicable study logs) will document that informed consent was obtained prior to patient participation in the study.

14.3.4. Health Insurance Portability and Accountability Act

The Investigator must obtain authorization from the patient to use and/or disclose protected heath information (PHI) in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). HIPAA authorization may be included in the informed consent form or a separate document.

HIPAA authorization may be obtained as part of the informed consent form or in a separate document and will include:

- Identification of the parties that can use and disclose PHI
- Identification of the parties to whom PHI may be disclosed
- A description of the PHI
- A description of the purpose for use and disclosure
- Information pertaining to the patient's rights related to authorization
- Information about the expiration of the authorization and how to revoke authorization
- A statement about what may happen if authorization is not provided
- A statement that once information has been disclosed, it may be disclosed again without further authorization.

14.3.5. Confidentiality of Subject Records

It is the responsibility of the Investigator to insure that the confidentiality of all patients participating in the study and all of their medical information is maintained. Case report forms (CRFs) and other documents submitted to Leadiant Biosciences must not contain the name of a study participant. Each patient in the study will be identified by a unique identifier that will be used on all CRF's and any other material submitted to Leadiant Biosciences. All CRFs and any identifying information must be kept in a secure location with access limited to the study staff directly participating in the study.

Personal medical information may be reviewed by representatives of Leadiant Biosciences, of the IRB or of regulatory authorities in the course of monitoring the progress of the study. Every reasonable effort will be made to maintain such information as confidential.

The results of the study may be presented in reports, published in scientific journals or presented at medical meetings; however, patient names will never be used in any reports about the study.

14.3.6. Conflict of Interest

The Investigator shall acknowledge, by signing the Investigator's Statement/ Signature Page (Section 15), that the participation in this clinical study by the Investigator and his/her sub-investigators presents no conflict of interest with the study.

14.4. Investigator Obligations

The Investigator is responsible to meet the obligations of clinical investigators as described in ICH GCP guidelines, the Declaration of Helsinki and U.S. federal regulations as defined in 21 CFR Parts 50, 54, 56, and 312. These obligations include, but are not limited to:

- Protect the rights, safety and welfare of patients under the Investigator's care
- Conduct the study in accordance with the approved study protocol
- Conduct the study in accordance with GCP guidelines and applicable federal, state and local regulations and laws
- Ensure that the staff involved with the conduct of the study are knowledgeable on the study agents used, the study protocol, study procedures, and reporting requirements
- Properly obtain informed consent and HIPAA authorization from all patients enrolled using the current IRB-approved forms
- Supply study drug only to those patients who are participating in the study and are under the direct supervision of the Investigator or an authorized sub-investigator. The Investigator must not supply the study drug to any person not authorized to receive it.
- Prepare and maintain accurate and complete case histories for all patients participating in this trail that document all study procedures performed and record all data required for this study protocol
- Report all serious adverse events to the sponsor and IRB (as necessary) within the timeframes described in this protocol
- Report any changes in research activity and unanticipated problems involving risk to study participants promptly to the IRB
- Report all protocol deviations promptly to Leadiant Biosciences. Significant deviations will require prompt notification to the IRB.
- Provide the IRB with copies of reports of serious adverse events submitted by Leadiant Biosciences to regulatory authorities in an expedited manner (e.g., IND Safety Reports)
- Provide Leadiant Biosciences with complete and accurate financial information to allow submission of complete and accurate certification and disclosure statements to FDA as required.

The Investigator, or a licensed physician sub-investigator to which the Investigator has delegated responsibility, is required to administer or oversee the care of study patients/subjects and review study data (e.g., adverse events, laboratory data, treatment response data, etc.) in a timely manner.

14.5. Financial Disclosure

All Investigators and sub-investigators listed on any FDA 1572 form supplied by the Investigator will disclose the following information as required by 21 CFR, Part 54:

- Any financial arrangement entered into between Leadiant Biosciences and the Investigator/sub- investigator whereby the value of the compensation to the Investigator/sub-investigator for conducting the study could be influenced by the outcome of the study.
- Any significant payments totaling more than $25,000 USD, exclusive of the costs of conducting this or other clinical studies, from Leadiant Biosciences, such as a grant to fund ongoing
  research, compensation in the form of equipment, retainer for ongoing consultation, or honoraria.
- Any proprietary interest in the test product.
- Any significant equity interest in Leadiant Biosciences in excess of $50,000 USD.

The Investigator/sub-investigator shall promptly update financial disclosure information if any changes occur during the course of the study and for 1-year following completion of this study. This financial disclosure requirement includes the spouse and the dependent children of the Investigator/sub-investigator.

14.6. Quality Control and Quality Assurance of Study Data

14.6.1. Monitoring and Audits

During the course of the study, a clinical monitor assigned by Leadiant Biosciences will make regularly scheduled visits to the investigational site to review the progress of the study. The frequency of monitoring visits will depend on the enrollment rate and performance at each site. During each visit, the monitor will review various aspects of the study including, but not limited to:

- Compliance with the protocol
- Compliance with the principles of Good Clinical Practice and regulatory requirements
- Review of written informed consent forms for patients enrolled
- Comparison of source documentation to data recorded on case report forms to assure the completeness and accuracy of data collected
- Continued acceptability of facilities and staff
- Assessment of proper study drug accountability and storage

During scheduled monitoring visits, the Investigator and the investigational site staff must be available to meet with the study monitor in order to discuss the progress of the study, make necessary corrections to case report form entries, respond to data clarification requests and respond to any other study-related inquiries of the monitor.

In addition to the above, representatives of Leadiant Biosciences' auditing staff or government inspectors may review the conduct/results of the study at the investigational site. The Investigator must promptly notify Leadiant Biosciences of any audit requests by regulatory authorities. The Investigator will cooperate with the auditor(s), make available to the auditor all requested documentation, and ensure that issues detected during the course of these audits are satisfactorily resolved. The Investigator will supply Leadiant Biosciences with copies of all documentation and correspondence related to regulatory agency audits as outlined in the Clinical Study Agreement between Leadiant Biosciences and the Investigator and/or Institution. If the results of the audit result in an FDA-483 (or similar document from another regulatory agency), the Investigator will promptly provide a copy to Leadiant Biosciences and will provide a copy of the draft response to Leadiant Biosciences prior to submission to the regulatory agency.

14.6.2. Data Processing and Data Quality Assurance

Case report form/electronic case report form entries will be reviewed for correctness against source document data by Leadiant Biosciences' monitor. If any entries into the CRF are incorrect, incomplete or illegible, the monitor will request the Investigator or the study site staff to make appropriate corrections. Once the CRF/eCRF page is complete, it will be processed as described in the data management plan (DMP) for the study. Data edit and consistency checks will be performed to review for missing, out of range, or inconsistent data.

A quality control (QC) audit of the database will be performed to assure accuracy of the electronic database prior to database lock. Discrepancies noted during the audit will be corrected. Error rates determined during the QC audit must be within the acceptable error rate defined by the DMP prior to lock of the database.

14.7. Study Records

The Investigator is responsible for preparing and maintaining adequate records to enable the conduct of the study to be documented. Study records include, but are not limited to, regulatory documentation (Section 14.7.1) and patient records (Sections 14.7.2 and 14.7.3).

14.7.1. Regulatory Documentation

Prior to initiating the study, the Investigator will provide Leadiant Biosciences the following documents:

- A signed FDA Form 1572
- A current curriculum vitae for the Principal Investigator and each sub-investigator listed on the FDA Form 1572
- Copy of the current medical licenses for the Investigator and physician sub-investigators
- Written IRB approval of the protocol, informed consent form and any other material provided to potential study participants with information about the study (e.g., advertisements)
- A copy of the IRB-approved informed consent document and HIPAA authorization
- Current IRB membership list for the reviewing IRB and/or multiple project assurance number or an IRB organization number under the Federal Wide Assurance program (www.ohrp.osophs.dhhs.gov)
- A signed Investigator Protocol Agreement (Section 15 of study protocol)
- Completed financial disclosure form for the Investigator and all sub-investigators
- Local reference laboratory documentation, including current laboratory certification, current laboratory normal values, and directors CV

During the course of the study, the Investigator will maintain current records to document regulatory compliance with the study including: the study protocol and amendments, all versions of the Investigators Brochure in effect during study conduct, signed Investigator Agreement protocol page(s), FDA 1572 forms, curricula vitae of the Investigator and sub-investigators, medical licenses of the Investigator and physician sub-investigators, financial disclosure of the Investigator and sub-investigators, IRB approvals of the protocol, protocol amendment(s), informed consent form(s), IRB membership list, IRB-approved informed consent form(s), IRB correspondence, protocol deviations, study logs (as provided by Leadiant Biosciences), drug dispensing and accountability records, safety reports, and all correspondence pertaining to the conduct of the study. Regulatory documentation will be reviewed by Leadiant Biosciences or its representatives during monitoring visits to assure regulatory compliance.

14.7.2. Source Documents

The Investigator will maintain records separate from the case report forms in the form of clinical charts, medical records, original laboratory, radiology and pathology reports, pharmacy records, patient diary cards, etc. The Investigator will document in the clinic chart or medical record the name and number of the study and the date on which the patient signed informed consent prior to the patient's participation in the study. Source documents must completely reflect the nature and extent of the patient's medical care, and must be available for verification against case report form entries when the Leadiant Biosciences or its representatives visit the investigational site. All information obtained from source documents will be kept in strict confidentiality.

14.7.3. Case Report Forms

All site-generated study data will be entered either onto case report forms (CRFs) supplied by Leadiant Biosciences or into an electronic Case Report Form (eCRF) via an electronic data capture (EDC) system. The CRFs/eCRFs are not to be used as the primary method for collection of study data (unless otherwise described in this section of the study protocol) and CRF/eCRF entries must be supported by source documents maintained by the Investigator. Only those site staff so authorized at the initiation of the study may enter data onto the case report forms or into an EDC system.

For those studies using paper CRFs, all entries must be legible and made in black pen. If an entry error is made, a single line will be placed through the incorrect entry. The correct entry will then

be made, with the correction dated and initialed by the person making the entry. Any corrections to data entered into the CRF must be made in such a way that the original entry is not obscured. Resolutions to data clarification forms (DCFs) issued by Leadiant Biosciences or its designated data management contractor will be maintained with the CRFs for each patient.

The Investigator is responsible for the completeness and accuracy of all CRF/eCRF data as certified by the Investigator's dated signature on designated CRF/eCRF pages.

14.7.4. Access to Study Records

The Investigator will make available all records pertaining to the conduct of this study to Leadiant Biosciences and its representatives, and auditors from domestic and foreign regulatory authorities to facilitate monitoring visits and study audits.

14.7.5. Records Retention

The Investigator will retain the records of the study for 15 years, or for 2 years following the date that a marketing application for the study drug is approved, or if no marketing application is filed, or if such an application is not approved, for 2 years after the IND has been closed. Leadiant Biosciences will notify Investigators when retention of study records is no longer required. All study records must be maintained in a safe and secure location that allows for timely retrieval, if needed.

Study records that must be retained include copies of case report forms, signed informed consents, regulatory documentation, source documents, clinic charts, medical records, laboratory results, radiographic reports, and other study-specific documentation.

Should the Investigator relocate or retire, or should there be any changes in the archival arrangements for the study records, Leadiant Biosciences must be notified. The responsibility for maintaining the study records may be transferred to another suitable individual, but Leadiant Biosciences must be notified of the identity of the individual assuming responsibility for maintaining the study records and the location of their storage. If no other individual at the investigational site is willing to assume this responsibility, Leadiant Biosciences will assume responsibility for maintaining the study records.

14.8. Publication Policy

Publication of study data is addressed in the Clinical Study Agreement between Leadiant Biosciences and the Investigator and/or Institution.

14.9. Financing and Insurance

Financing and Insurance are addressed in the Clinical Study Agreement between Leadiant Biosciences and the Investigator and/or Institution.

15. INVESTIGATOR AGREEMENT

I have reviewed Leadiant Biosciences' Protocol STP-2279-002, entitled "A Study of EZN-2279 (Polyethylene Glycol Recombinant Adenosine Deaminase [PEG-rADA]) Administered as a Weekly Intramuscular Injection in Patients with Adenosine Deaminase (ADA)-Deficient Combined Immunodeficiency" and agree that it contains all the information necessary to conduct the study as required. I will conduct the trial in accordance with the principles of ICH Good Clinical Practice and the Declaration of Helsinki.

I will maintain as confidential all written and verbal information provided to me by Leadiant Biosciences, including but not limited to, the protocol, case report forms, Investigators' Brochure, material supplied at investigator meetings, minutes of teleconferences, etc. Such material will only be provided as necessary to site personnel involved in the conduct of the trial, the IRB or IEC, or local regulatory authorities.

I will obtain written informed consent from each prospective trial patient or each prospective trial patient's legal representative prior to conducting any protocol-specified procedures. The consent form used will have the approval of the local IRB or IEC.

I will maintain adequate source documents and record all observations, treatments and procedures pertinent to trial patients in their medical records. I will accurately complete the case report forms supplied by Leadiant Biosciences in a timely manner. I will ensure that my facilities and records will be available for inspection by representatives of Leadiant Biosciences, the local IRB or IEC or local regulatory authorities. I will ensure that I and my staff are available to meet with representatives of Leadiant Biosciences during regularly scheduled monitoring visits.

I will notify Leadiant Biosciences, or its designated representative, within 24 hours of any serious adverse events. Following this notification, a written report describing the serious adverse event will be provided to Leadiant Biosciences, or its designated representative, as soon as possible, but no later than three days following the initial notification.

My signature below indicates that my participation in this clinical study presents no conflict of interest for me or my sub-investigators with the study.

_____

Investigator's Name (Print)

_____ _____

Investigator's Signature **Date**

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Appendix 1 Study Activities

Procedure	Screening	Adagen® Lead-In Period										
						Adagen® Pharmacokinetics						
		Week 1	Week 2	Week 3	Weeks 4+	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Informed assent/consent	X											
Demographics	X											
Disease background/history (Prior Treatments, Adagen® Dose Adagen® AEs, Infectious complications, and Hospitalizations)	X					X						
Medical history	X					X						
Medication history	X											
Physical examination	X					X						
Vital signs	X					X						
Performance status	X					X						
Height, weight, growth curve	X					X						
Pregnancy test	X											
Serum chemistry	X					X						
Hematology	X					X						
Urinalysis	X					X						
Total trough erythrocyte dAXP	X[a]	X	X	X	X	X						
Trough ADA activity	X[a]	X	X	X	X	X						
Full PK profile[j]							X	X	X	X	X	X
B-/T-/NK-lymphocyte subset						X						
Quantitative immunoglobulins						X						
Antibody titers (binding, neutralizing), anti-PEG antibodies	X	X				X						
Clinical status Infectious complications Hospitalizations						X						
Adverse Events	◄	———	———	———	———	———	———	———	———	———	———	►
Concomitant medications/procedures		X				X						
Adagen® administration (± 1 day)	X	X	X	X		X						
Adagen® dose/regimen adjustment	X	X[b]		X[c]	X[c]							
EZN-2279 administration (± 1 day)												

a – if necessary, only for patients maintained on a once weekly dose regimen; b – if necessary, only patients on a twice weekly or greater dose regimen; c – if necessary based upon meeting inclusion criteria for trough dAXP and ADA activity; patient requires trough dAXP ≤ 0.02 µmol/mL and trough ADA activity ≥15 µmol/h/mL on 2 consecutive weekly measurements to proceed to Adagen ®pharmacokinetics; j- full PK profile conducted only on patients ≥ 10 years of age. Patients < 10 years of age will have limited PK sampling of trough and 48 hour post dosing only.

Procedure	EZN-2279 Treatment Period (Weeks 1 through 8)								
	Week T-1 (Day1)	Week T-2 (Day 8)	Week T-3 (Day 15)	Week T-4 (Day 22)	Week T-5 (Day 29)	Week T-6 (Day 36)	Week T-7 (Day 43)	Week T-8 (Day 50)	EZN-2279 Dose Adjustment[e]
Informed assent/consent									
Demographics									
Disease background/history Prior Treatments Adagen® Dose Adagen® AEs Infectious complications Hospitalizations									
Medical history									
Medication history									
Physical examination	X				X				
Vital signs	X				X				
Performance status	X				X				
Height, weight, growth curve	X				X				
Pregnancy test									
Serum chemistry	X				X				
Hematology	X				X				
Urinalysis	X								
Total trough erythrocyte dAXP	X		X		X		X	X	
Trough ADA activity	X[d]		X		X		X	X	
Full PK profile[j]									
B-/T-/NK-lymphocyte subset	X								
Quantitative immunoglobulins	X								
Antibody titers (binding, neutralizing), anti-PEG antibodies	X		X						
Clinical status Infectious complications Hospitalizations	X	X	X	X	X	X	X	X	
Adverse Events	←								→
Concomitant medications/procedures	X	X	X	X	X	X	X	X	
Adagen® administration (± 1 day)									
EZN-2279 administration (± 1 day)	X	X	X	X	X	X	X	X	X

d – trough sample for ADA activity is also the 168 hour full PK sample
e – prior to week T-9 if necessary based upon dAXP and ADA results from weeks T-7 and T-8; if adjustment made, patients return to week T-5

Procedure	EZN-2279 Treatment Period (Weeks 9 through 13)										
	Week T-9							Week T-10	Week T-11	Week T-12	Week T-13
	Day 57	Day 58	Day 59	Day 60	Day 61	Day 62	Day 63	Day 64	Day 71	Day 78	Day 85
Informed assent/consent											
Demographics											
Disease background/history Prior Treatments Adagen® Dose Adagen® AEs Infectious complications Hospitalizations											
Medical history											
Medication history											
Physical examination	X										X
Vital signs	X										X
Performance status	X										X
Height, weight, growth curve	X										X
Pregnancy test											
Serum chemistry	X										X
Hematology	X										X
Urinalysis											
Total trough erythrocyte dAXP	X							X	X		X
Trough ADA activity	X							X[f]	X		X
Full PK profile[j]	X	X	X	X	X	X	X				
B-/T-/NK-lymphocyte subset	X										
Quantitative immunoglobulins	X										
Antibody titers (binding, neutralizing), anti-PEG antibodies								X			
Clinical status Infectious complications Hospitalizations	X							X	X		X
Adverse Events	◄										►
Concomitant medications/procedures	X							X	X		X
Adagen® administration (± 1 day)											
EZN-2279 administration (± 1 day)	X							X	X	X	X

f – trough sample for ADA activity is also the 168 hour full PK sample
j – Full PK profile conducted only on patients ≥ 10 years of age. Patients < 10 years of age will have limited PK sampling of trough and 48 hour post dosing only.

Procedure	EZN-2279 Treatment Period (Weeks 14 through 21)								EZN-2279 Maintenance[g]	End of Study/Early Discontinuation	30-Day Early Discontinuation Follow-up[i]
	Week T-14 Day 92	Week T-15 Day 99	Week T-16 Day 106	Week T-17 Day 113	Week T-18 Day 120	Week T-19 Day 127	Week T-20 Day 134	Week T-21 Day 141			
Informed assent/consent											
Demographics											
Disease background/history Prior Treatments Adagen® Dose Adagen® AEs Infectious complications Hospitalizations											
Medical history											
Medication history											
Physical examination				X				X	X	X	X
Vital signs				X				X	X	X	X
Performance status				X				X	X	X	X
Height, weight, growth curve				X				X	X	X	X
Pregnancy test											
Serum chemistry				X				X	X	X	X
Hematology				X				X	X	X	X
Urinalysis								X	X	X	X
Total trough erythrocyte dAXP		X		X		X		X	X	X	
Trough ADA activity		X		X		X		X	X	X	
Full PK profile[j]											
B-/T-/NK-lymphocyte subset								X	X	X	
Quantitative immunoglobulins								X	X	X	
Antibody titers (binding, neutralizing), anti-PEG antibodies								X	X	X	
Clinical status Infectious complications Hospitalizations		X		X		X		X	X	X	
Overall Survival								X	X	X	
Adverse Events	←----------										----------→
Concomitant medications/procedures		X		X		X		X	X	X	X
Adagen® administration (± 1 day)											
EZN-2279 administration (± 1 day)	X	X	X	X	X	X	X	X	X[h]		

g - assessments every 13 weeks ± 3 weeks; h – dosing of EZN -2279 continues once a week ± 1 day; i – only for patients who discontinue the study early

Appendix 2. Blood Sampling Schedule and Collection Volumes

	Routine Safety		Study Assessments						Total*	
Test:	Hematology (incl. ALC)	Chemistry	dAXP*	Trough ADA*	Full PK ADA	Immunogenicity*	Quant. Ig	Lymph subset		
Volume per Sample (mL)	3	4	2	2	2	2	3.5	4	Patients ≥ 10 years of age	Patients < 10 years of age
Tube Type	EDTA (lavender)	Gel separator (tiger top)	EDTA (lavender)	EDTA (lavender)	EDTA (lavender)	EDTA (lavender)	Gold top SST	EDTA (lavender)		
Study Days										
Screening	3	4				2			9	9
Adagen® Patients on 1x week dosing only			*2*	*2*					*4*	*4*
Adagen® Lead-in Week -1			2	2					4	4
Adagen® Lead-in Week -2			2	2					4	4
Adagen® Dose Adjustment (if necessary)									0	0
Adagen® Lead-in Week -3 *(if necessary)*			2	2					4	4
Adagen® Lead-in Week -4 *(if necessary)*			2	2					4	4
Adagen® PK - Day 1 (0 hrs)	3	4	2	2		2	3.5	4	20.5	20.5
Adagen® PK - Day 2 (24 hrs)					2				2	0
Adagen® PK - Day 3 (48 hrs)					2				2	2
Adagen® PK - Day 4 (72 hrs)					2				2	0
Adagen® PK - Day 5(96 hrs)					2				2	0
Adagen® PK - Day 6 (120 hrs)					2				2	0
Adagen® PK - Day 7 (144 hrs)					2				2	0
Week T-1 (168 hrs)	3	4	2	2		2	3.5	4	20.5	20.5

	Routine Safety		Study Assessments						Total*	
Test:	Hematology (incl. ALC)	Chemistry	dAXP*	Trough ADA*	Full PK ADA	Immunogenicity*	Quant. Ig	Lymph subset		
Volume per Sample (mL)	3	4	2	2	2	2	3.5	4	Patients ≥ 10 years of age	Patients < 10 years of age
Tube Type	EDTA (lavender)	Gel separator (tiger top)	EDTA (lavender)	EDTA (lavender)	EDTA (lavender)	EDTA (lavender)	Gold top SST	EDTA (lavender)		
Week T-2									0	0
Week T-3			2	2		2			6	6
Week T-4									0	0
Week T-5	3	4	2	2					11	11
Week T-6									0	0
Week T-7			2	2					4	4
Week T-8			2	2					4	4
EZN-2279 Dose Adjustment (if necessary)			*Repeat Weeks T-5 through T-8 sampling*							
Week T-9 - Day 57 (0 hrs)	3	4	2	2			3.5	4	18.5	18.5
Week T-9 - Day 58 (24 hrs)					2				2	0
Week T-9 - Day 59 (48 hrs)					2				2	2
Week T-9 - Day 60 (72 hrs)					2				2	0
Week T-9- Day 61 (96 hrs)					2				2	0
Week T-9 - Day 62 (120 hrs)					2				2	0
Week T-9 - Day 63 (144 hrs)					2				2	0
Week T-10 (168 hrs)			2	2		2			6	6
Week T-11			2	2					4	4
Week T-12									0	0
Week T-13	3	4	2	2					11	11

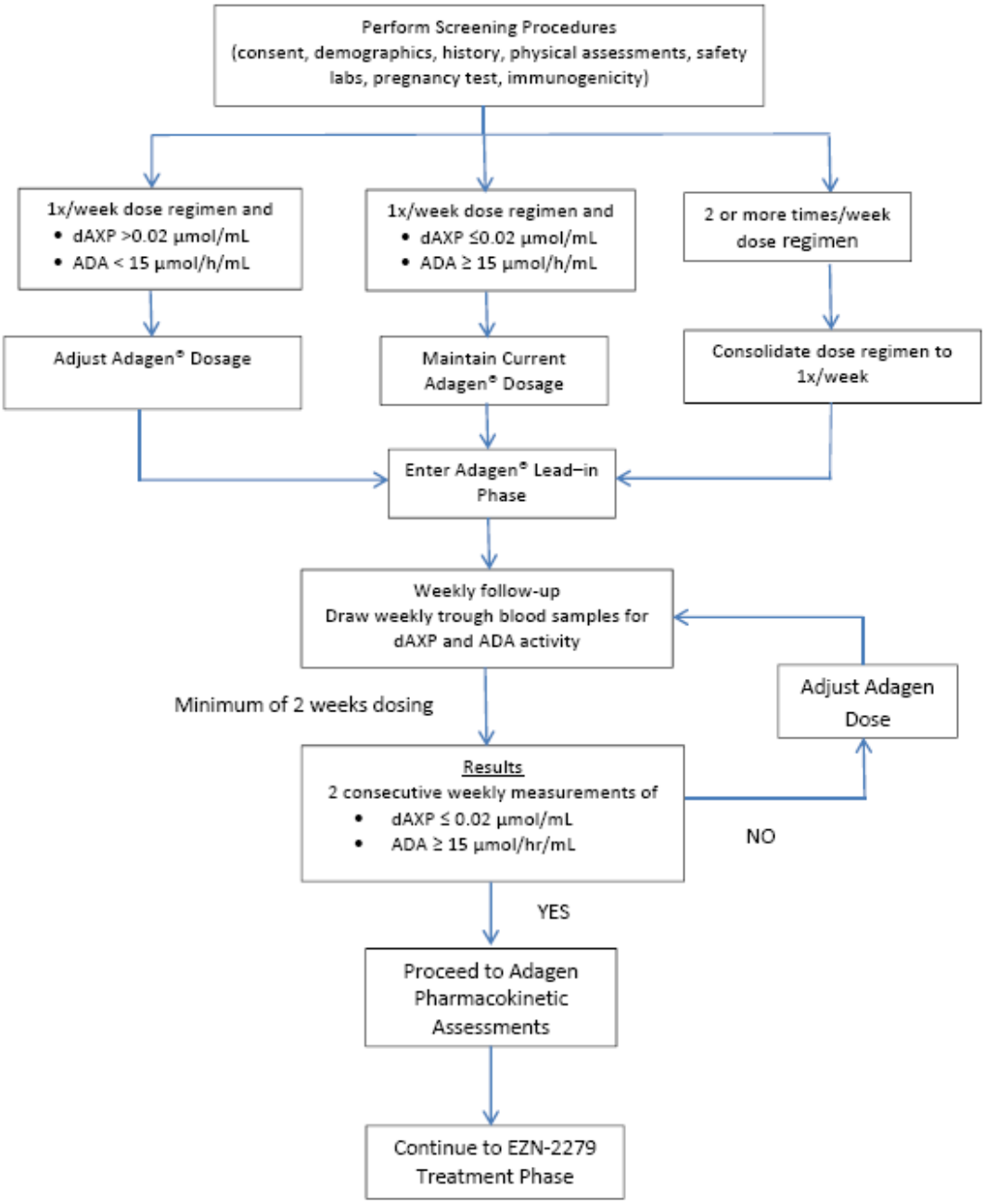
	Routine Safety		Study Assessments						Total*	
Test:	Hematology (incl. ALC)	Chemistry	dAXP*	Trough ADA*	Full PK ADA	Immunogenicity*	Quant. Ig	Lymph subset		
Volume per Sample (mL)	3	4	2	2	2	2	3.5	4	Patients ≥ 10 years of age	Patients < 10 years of age
Tube Type	EDTA (lavender)	Gel separator (tiger top)	EDTA (lavender)	EDTA (lavender)	EDTA (lavender)	EDTA (lavender)	Gold top SST	EDTA (lavender)		
Week T-14									0	0
Week T-15			2	2					4	4
Week T-16									0	0
Week T-17	3	4	2	2					11	11
Week T-18									0	0
Week T-19			2	2					4	4
Week T-20									0	0
Week T-21	3	4	2	2		2	3.5	4	20.5	20.5
TOTAL									244	178
Maintenance (Every 13 weeks)	3	4	2	2		2	3.5	4	20.5	20.5

* at timepoints when immunogenicity, dAXP and ADA activity sampling is performed, only 2 x 2mL EDTA tubes will need to be collected

Appendix 3 Performance Status Criteria

Karnofsky (≥16 years old)		Lansky (<16 years old)	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal
90	Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
80	Normal activity with effort; some signs or symptoms of disease	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work	70	Both greater restriction of and less time spent in play activity
60	Required occasional assistance, but is able to care for most of his/her needs	60	Up and around, but minimal active play; keeps busy with quieter activities
50	Requires considerable assistance and frequent medical care	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities
40	Disabled, requires special care and assistance	40	Mostly in bed; participates in quiet activities
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play
20	Very sick, hospitalization indicated; death not imminent	20	Often sleeping; play entirely limited to very passive activities
10	Moribund, fatal processes progressing rapidly	10	No play; does not get out of bed
0	Dead	0	Dead

Appendix 4 Adagen® Lead-in Dose Consolidation/Adjustment Schematic



Appendix 5 EZN-2279 Dose Adjustment Schematic