Amended Version: 30Nov2021

# CLINICAL STUDY PROTOCOL

## Expanded Access Protocol:

## Thymus Transplantation for Immunodeficiency, Hematologic Malignancies, and Autoimmune Disease Related to Poor Thymic Function

## Protocol No. 51692

| | |
|---|---|
| **Development Phase:** | I/II, IND#9836<br>Open label treatment |
| **Protocol date** | 30Nov2021 |
| **Sponsor representative:** | [redacted] |
| **Sponsor:** | Enzyvant Therapeutics GmbH<br>Viaduktstrasse 8<br>Basel, Switzerland 4051 |

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## SPONSOR SIGNATURE PAGE

**Study Title**: Expanded Access Protocol Thymus Transplantation for Immunodeficiency, Hematologic Malignancies, and Autoimmune Disease Related to Poor Thymic Function

**Protocol Number**: 51692

This protocol has been reviewed and approved by Enzyvant Therapeutics GmbH. The following signatures document this approval.

[redacted]

Signature Date

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Signature Date

## TABLE OF CONTENTS

## PROTOCOL SYNOPSIS

**Study Design:** The primary purpose is to provide expanded access to cultured thymus tissue implantation for patients who have immunodeficiency, or severe autoimmune disease related to poor thymic function. The patients enrolled have a high likelihood of death if they do not receive a thymus implant because of lack of thymus function. As there are many types of patients who may be enrolled, study results will not have statistical significance.

**Objective:** The objective of this study is to make cultured thymus tissue implantation available on an expanded access basis. Data will be collected on survival, naïve T cell development, T cell chimerism, and implant related toxicities, as well as unexpected serious adverse events related to the study.

**Eligibility:** Patients must have immunodeficiency or severe autoimmune disease related to poor thymic function.

**Treatment Description:** Subjects will undergo cultured thymus tissue implantation (CTTI). The Thymus Transplantation Data and Safety Monitoring Board (DSMB) will review and approve the plan for each subject prior to initiation of that plan. Immune suppression may be given depending on the patient's immune status and clinical condition.

**SUMMARY OF CHANGES: AMENDMENT VERSION 30NOV2021.**

This amendment was created at the time of transfer of sponsorship of this IND (#9836) from M. Louise Markert MD, PhD to Enzyvant Therapeutics. Since the previous version the investigational product administered in this protocol (Allogeneic processed thymus tissue-agdc [RVT-802; also referred to in this protocol as cultured thymus tissue for transplantation]) was approved by the FDA for immune reconstitution of pediatric patients with congenital athymia, with the proprietary name of RETHYMIC®. Given this FDA approval, the roto ol has ee closed to e rollment of new pat e ts. Thus, this amendment will only apply to follow-up of two patients who are currently enrolled and have been treated under the protocol within the past 2 years; the amendment is principally administrative. The administrative updates to the protocol include changes to the IND sponsor, the principal investigator, and the safety reporting procedures that are utilized by Enzyvant Therapeutics. References to the prior sponsor/Investigator have been removed or clarified and formatting errors have been corrected. With agreement of the Principal Investigator the Data Safety Monitoring Board (DSMB) will be disbanded and safety review will be conducted by the Sponsor. Thus, references to the DSMB are removed in this amendment. In lieu of an Investigator Brochure the amended protocol is supplemented with the US Prescribing Information for RETHYMIC, which includes the safety and efficacy information available at the time of this protocol amendment.

The appendices have been updated and renumbered.
Summary of changes:

- Section 1 Sponsor updated
- Section 1.1 Reference to US Prescribing Information for RETHYMIC added
- Section 2.2 Comment on current status of the study added
- Section 3.1 Comment on current status of the study added
- Section 4.5 Sponsor and site responsibilities added, reporting to the DSMB removed and reference made to USPI in identifying unexpected adverse events
- Section 4.6 DSMB description removed
- Section 4.7 now becomes Section 4.6 and sponsor replaced DSMB in responsibilities
- Section 4.8 now becomes Section 4.7
- Appendices 5.1 and 5.2 changed to Appendix 1 and Appendix 2. New Appendix 3 is the RETHYMIC USPI, new Appendix 4 is persons responsible for conducting the study and new Appendix 5 is the Investigator Agreement

## 1 BACKGROUND AND RATIONALE

Data accumulated over the last 20 years has shown that cultured thymus tissue implantation leads to survival and naïve T cell development in young children with athymia. An integrated database for all patients treated under IND 9836 with data available through August 2020 has been prepared by Enzyvant Therapeutics, Inc. (Enzyvant), the US authorized representative of Enzyvant Therapeutics GmbH (now the IND holder). Enzyvant was the licensing partner of Duke University responsible for the development and commercialization of the product under BLA 125685. Adverse events in this dataset were coded using MedDRA version 19.1.

The database includes data from a total of 105 subjects (complete DiGeorge anomaly and other immunodeficient conditions). Analyses have been conducted for 3 populations. Efficacy analysis sets included 1) patients with complete DiGeorge anomaly (EAS-cDGA; n=93) and 2) patients with cDGA and FOXN1 mutations (EAS; n=95). These patients were all treated under thymus implantation protocols. The survival of patients in both EAS populations has been evaluated using Kaplan-Meier estimated survival rates. The survival rates at year 1 and year 2 after implantation for congenital athymia (complete DiGeorge anomaly and FOXN1 deficiency (EAS) were 77% and 76%, respectively.
The patients in the Full Analysis Set (FAS) population included the 95 patients with complete DiGeorge anomaly and FOXN1 mutation patients and an additional 10 subjects (n=105). These 10 subjects had prior transplants, or severe combined immunodeficiency (SCID), or unknown causes of immune dysfunction. Of these 10 patients, seven are alive, 1 died and 2 were withdrawn.

Patients similar to these 10 subjects and other subjects who are not eligible for Protocol 00025966 Safety and Efficacy may be treated under this protocol.

Three examples (unpublished) of patients who may enrolled in this protocol follows:

1. A pediatric patient with Foxn1 deficiency[1] who was initially treated with hematopoietic cell transplantation. The patient developed pulmonary and renal failure with 2 months on the ventilator and dialysis. Less than 100 T cells developed and no naïve T cells. A cultured thymus tissue implant (CTTI; RVT-802) was given and on day 406 post CTTI, the patient had 219/$mm^3$ naïve T cells. This patient is alive over 2.5 years post-CTTI.

2. A pediatric patient who was initially thought to have severe combined immunodeficiency (SCID). That patient received an ablative bone marrow transplant and then peripheral blood from the donor but did not develop naïve T cells. On approximately day 1000, the patient was given a CTTI. On approximately day 306 post CTTI, the naïve T cell number was 140/$mm^3$ and the number increased to 650/$mm^3$ by the end of the 2$^{nd}$ year. The patient is over 8.2 years post CTTI.

3. A pediatric patient was born with no T cells, hypoparathyroidism, and a small ventricular septal defect. As the patient did not have a 22q11.2 deletion, the patient was given a cord blood transplant. When the cord blood transplant was not successful, the patient was given a myeloablative transplant, but again, no T cells developed. The patient was referred for CTTI. The patient was treated with pre-implant rabbit anti-thymocyte globulin (RATGAM) and received CTTI at approximately 2.5 years of age. The patient's initial post-operative course was uneventful. However, a few months after returning home he developed pancytopenias. The bone marrow showed multiple non-necrotizing granulomas. The patient was not given steroids because of the concern for mycobacterial disease. The cytopenias were so severe that eventually immunosuppression was given. The spleen also had to be removed. With time the cytopenias were controlled and the patient has subsequently done well with follow up of 5 years.

The above cases demonstrate some of the diversity of patients who might benefit from CTTI under this protocol. The patients listed above all met the enrollment criteria: immunodeficiency or severe autoimmune disease related to poor thymic function. The patients had very low naïve T cells thought to be secondary to thymus hypoplasia or thymus dysfunction.

### 1.1 Risk/Benefit Analysis and Adverse Events

The potential benefits of CTTI (RVT-802) include development of functional T cells and the ability of these cells to protect against infection and severe autoimmune disease. RETHYMIC (RVT-802) was approved for the treatment of pediatric congenital athymia in October 2021. The major risks and benefits relevant to the treatment of congenital athymia is in the US prescribing information (Appendix 3).

The consent document thoroughly discusses risks and adverse events that the sponsor has seen in CTTI recipients, and the risks associated with immune suppression.

As there have been few patients in the Expanded Access protocol, to assess adverse events, we are reporting here on the safety of all subjects who received a cultured thymus tissue implantation (FAS, n=105).

As of August 2020 an analysis of all adverse events reported in all subjects (FAS, n=105) that have received cultured thymus tissue implant treatment under IND 9836 found that 44 (42%) subjects out of 105 subjects that were treated experienced 87 events that were considered by the Sponsor/Investigator to be possibly related to cultured thymus tissue implantation.

Overall, the related events generally were distributed into three main categories: autoimmune diseases, complications associated with the implantation procedure itself, and events considered specifically related to T cells (e.g. rash and GVHD).

The most severe post-CTTI adverse events have been i.) autologous GVHD, ii.) maternal GVHD, iii.) transverse myelitis, iv.) autoimmune hepatitis, and v.) autoimmune cytopenias.

i) Autologous GVHD: Many patients with complete DiGeorge anomaly have pre-existing recipient T cells causing rash, potential organ damage, and other symptoms, a condition which the sponsor refers to as ‘autologous GVHD’. Autologous GVHD from pre-existing recipient T cells is a risk for these patients, before or in the initial months after CTTI.

A patient with complete DiGeorge Anomaly, developed autologous GVHD post CTTI manifested as enterocolitis. The sponsor thought the autologous GVHD might have been associated with the high thymus dose given. The patient developed host T cells that did not express naïve T cell markers and did not have detectable T regulatory cells. Despite attempts to treat the autologous GVHD, the patient died from disseminated candidal disease.

ii.) Maternal GVHD:

A patient with severe combined immunodeficiency (SCID) who received CTTI developed GVHD post-thymus and maternal stem cell transplant. The SCID patient had received a paternal Bone Marrow Transplant (BMT) at approximately age 6 months and a maternal BMT at approximately 1 year. When neither BMT worked, it was thought that the patient might have both a bone marrow defect and a thymus defect. A BMT from the mother was attempted again along with a CTTI. Five months later genetically maternal T cells appeared in the child. The maternal T cells attacked the patient, similar to what happens in GVHD. The patient was withdrawn. The genetic defect of that patient is unknown. The reason for the GVHD is not well understood.

Another patient with complete DiGeorge anomaly presented with maternal GVHD pre-implantation. The patient developed acute Epstein Barr Virus

(EBV) infection post CTTI. The maternal T cells expanded and fought the EBV infection. When the EBV was under control, the maternal T cells attacked the child. The maternal GVHD led to severe skin and gut disease. Two years post implantation, the patient had no signs of GVHD or EBV infection. As of day 783 post CTTI, the patient had only 13.8% maternal cells, the rest were recipient T cells. As of day 2340 post CTTI, the patient had 487/$mm^3$ naïve CD4T cells.

iii.) Transverse Myelitis: Post-CTTI, transverse myelitis developed in one patient who developed Human Herpesvirus 6 (HHV6) infection after CTTI. There was no evidence that HHV6 contributed to the transverse myelitis. The transverse myelitis was accompanied by demyelination of white matter in the brain. The patient could not stand up or walk during this illness. The patient was found to carry a mutation in CD45. That mutation is associated with multiple sclerosis.[2] It is unknown if the CD45 mutation was associated with the demyelination or if the demyelination was secondary to immune dysregulation after cultured thymus tissue implantation. The etiology could be the latter (immune dysregulation) as the demyelination developed at 9 months post CTTI, when T cells were developing and the T cell receptor repertoire is usually still oligoclonal. The patient has improved and is now ambulatory.

iv.) Autoimmune Hepatitis: Autoimmune hepatitis developed in two patients post implantation.

In one patient, the autoimmune hepatitis was confirmed on day 245 after CTTI. Per reports from the local physicians, the patient never developed more than 50/$mm^3$ naïve T cells. The sponsor's assessment is that the donor thymus tissue may have been damaged after the patient returned to the local physicians and had a cardiorespiratory arrest possibly secondary to hypocalcemia. Thus, autoimmunity likely was secondary to the lack of function of the donor thymus. The autoimmune hepatitis resolved with treatment.

Another patient also developed autoimmune hepatitis. On day 409 after CTTI, the patient developed 112/$mm^3$ naïve CD4 T cells. The patient developed autoimmune hepatitis on day 949 after CTTI. The patient was treated with immunosuppression. The autoimmune hepatitis resolved by Day 1116 after CTTI. Per report from local physician, at 8.1 years after CTTI, the patient had 280/$mm^3$ $CD4^+RA^+$ T cells.

v.) Autoimmune Cytopenias: As of August 2020 in the population of all subjects treated with cultured thymus tissue implantation (CTTI) (FAS, n=105) 21 subjects reported 32 autoimmune cytopenias which were considered possibly related to CTTI. Twelve subjects experienced thrombocytopenia (11%), 8 subjects experienced neutropenia (8%), and 9 subjects experienced anemia (includes anemia, Coombs positive hemolytic anemia, hemolytic anemia, and hemolysis (9%). The cytopenias usually occurred in the first 2 years after CTTI, suggesting

that the immaturity of the immune system soon after CTTI may increase the risk of cytopenias.

### 1.2 Description of Subject Population

For this expanded access protocol, patients with immunodeficiency and/or severe autoimmune disease related to poor thymus function are given cultured thymus tissue implantation. In some cases, patients have been given prior therapies such as bone marrow transplantation and/or myeloablation.

Examples of immunodeficiency include:

- patients with complete DiGeorge anomaly or other primary immunodeficiency who have very low naïve T cells despite
  - bone marrow transplants
  - peripheral blood infusions
  - cord blood transplants
  - cultured thymus tissue implants
- patients with primary immunodeficiency who have low naïve T cells but greater than 50/$mm^3$.

## 2 STUDY DESIGN

### 2.1 Study Design

The primary purpose of the study is to provide expanded access of cultured thymus tissue for the treatment of immunodeficiency and autoimmune disorders.

The cultured thymus tissue implant is intended to result in development of naïve T cells, T cell subsets, and T regulatory cells such that T cell function will normalize.

### 2.2 Eligibility

Note that at the time of this amendment (November 2021) the clinical study is closed to enrollment.

The purpose of the protocol is to provide access to patients who otherwise would not have access to cultured thymus tissue implantation. The protocol design does not specify an enrollment number because the sponsor cannot predict the exact number of subjects who may present and enroll in the protocol. The sponsor anticipates two to four cultured thymus tissue implantations per year might be conducted under this protocol.

2.2.1 Cultured Thymus Tissue Implant Recipient Inclusion Criteria
Patients with poor thymus function will be eligible for this protocol.

Eligibility screening for cultured thymus tissue implantation includes the PI's review of medical testing, laboratory studies, and physical examinations. The PI uses the data, examinations, and consultations to determine whether the patient is clinically stable and will potentially benefit from cultured thymus tissue implantation. The PI reviews each patient with the DSMB prior to enrollment.

To be eligible for cultured thymus tissue implantation, patients must:

- Have an immunodeficiency or severe autoimmunity for which development of naïve T cells would be expected to lead to clinical improvement. Examples of underlying conditions may include the following:
  - An immunodeficiency secondary to primary or acquired thymus deficiency
  - An autoimmune disease likely secondary to primary or acquired thymus deficiency
- Have given written consent (or consent of parent/legal guardian as applicable).

2.2.2 Cultured Thymus Tissue Implant Recipient Exclusion Criteria
Any one of the following items excludes a patient from enrollment or implantation:

- Unrepaired cyanotic congenital heart disease
- Uncontrolled infections. "Uncontrolled" defined as requiring a ventilator, dialysis, or vasopressor support or anticipated as requiring such support within 6 months.
- Pregnancy
  - For females of child-bearing potential, a serum pregnancy test is done after consent, at the same time another blood draw is done if possible.
  - Females of child-bearing potential must agree to contraceptive measures as indicated in the consent form.
  - A second serum pregnancy test is done within 48 hours prior to administration of study interventions involving FDA pregnancy class D drugs, chemotherapy drugs, or other drugs or interventions known to pose risks to a potential fetus.
- History of malignancy
- CMV Infection
  - For subjects receiving immunosuppression as part of the implantation protocol, CMV infection as documented by >500 copies/ml in blood by PCR on two consecutive assays is an exclusion
- HIV Positive

**2.3 Consent Process**
The consent process may begin at recognition of subject's possible eligibility. Consent is requested and obtained per institutional practices before study interventions are initiated. All subjects and/or the legal guardian must sign a consent document consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and the possible side effects and risks. Subjects (parent/guardian) are informed that no efficacy is guaranteed, and that unforeseen toxicities may occur. Subjects may withdraw from this protocol at any time. Confidentiality of subjects and subject records are protected to the full extent possible according to federal, state, and

institutional guidelines. The consent document reviews different forms of immunosuppression and associated risks. During the consent process, the subject/parent/guardian is told which medications are likely to be used. The subject/parent/guardian is told that the immunosuppression plan may be altered and other drugs added, depending on changes in the subject's clinical and immunological status.

# 3 CULTURED THYMUS TISSUE IMPLANTATION PLAN

## 3.1 General

Note that at the time of this amendment (November 2021) the clinical study is closed to enrollment. Patients currently remaining in the trial are in the post-transplantation period and have returned to the care of the referring/local physician.

Adjunctive therapies are allowed. Adjunctive therapies may include bone marrow transplantation with myeloablation and cytotoxic lymphocyte infusions for viral infections. Subjects may receive immunosuppression depending on the immune status of the subject. These regimens will be based on standard of care practices, patient condition, and history of other chemotherapy administration. The DSMB will review the plan for each subject prior to enrollment, including administration of immunosuppression drugs/chemotherapy and cultured thymus tissue implantation.

The entire cultured thymus tissue implantation procedure will be carried out under the supervision of the Duke Thymus Transplant Center, including but not limited to the following:

- pre-transplant work-up,
- selection of cultured thymus tissue for implantation,
- administration of immunosuppression if necessary,
- administration of medications to treat the immunodeficiency or autoimmune symptoms,
- inpatient care during the immediate post-implantation period, and
- inpatient or outpatient follow-up post-implant until the subject is stable enough to return to care of the referring physician

After cultured thymus tissue implantation, standard blood counts should be done at least once a week for 2 months, or for as long as the recipient subject is on immunosuppression. After 2 months and after immunosuppression is stopped, standard blood counts should be done once a month for the first year after implantation.

Blood samples for flow cytometry immune testing, including naïve T cell numbers, will be obtained at 3, 6, 9 and 12 months post cultured thymus tissue implantation. Immune tests may be done more frequently if medically necessary. Yearly follow-up clinic visits are strongly encouraged.

At 2 to 3 months after cultured thymus tissue implantation, blood for T cell chimerism will be obtained. Chimerism may be repeated if necessary. If thymus donor T cells are

present, chimerism testing should be repeated at 6 months after cultured thymus tissue implantation, and may be repeated at intervals, until the donor T cells disappear.

After the subject leaves the Duke area, all recommended medications, monitoring, and testing is entirely dependent upon the subject, parent/guardian, and/or local physicians.

### 3.2 Thymus Procurement

Thymus tissue, which would otherwise be discarded, will be obtained from pediatric heart surgery cases in which the heart surgery patient is less than or equal to 9 months of age. Consent for use of thymus is obtained from the parent/guardian of the thymus donor under an IRB approved protocol.

### 3.3 Thymus Processing

Thymus processing occurs as detailed in IND 9836. The thymus tissue must meet lot release criteria.

### 3.4 Thymus Donor Screening

Screening of the donor and the donor's birth mother is performed as detailed in the IRB approved thymus donor protocol and under IND 9836.

### 3.5 Dose

The thymus tissue dose used in implantation is 4,500 to 22,000$mm^2$ of thymus tissue per recipient BSA in $m^2$.

### 3.6 Implantation Procedure

The cultured thymus tissue implantation is performed in the operating room by a general surgeon. The cultured thymus tissue slices are inserted into pockets made in the quadriceps muscles in one or both legs depending on the amount of tissue to be implanted and the size of the recipient.

### 3.7 Toxicities of Cultured Thymus Tissue Implantation Procedure & Thymus Tissue Biopsy

Risks based on the safety experience of 105 patients (FAS) who received a cultured thymus tissue implant are detailed in the consent document. The procedure risks are detailed in the consent document.

See Appendix 1 for adverse events related to treatment by system organ class and preferred term. See Appendix 2 for adverse events possibly related to RVT-802 by system organ class and preferred term.

### 3.8 Venous Access

All subjects require venous access (central or peripheral) for cultured thymus tissue implantation surgery. Central venous access is required for subjects receiving rabbit anti-thymocyte globulin (RATGAM) and other chemotherapy. The type of venous access is dependent on the medical condition for each individual subject.

### 3.9 Cultured Thymus Tissue Implant Preparative Regimen

Subjects will receive immunosuppression depending on the immune status of the subject. These regimens will be based on standard of care practices, patient condition, and history of other chemotherapy administration. The DSMB will review and approve the plan for each subject prior to initiation of that plan.

### 3.10 Skin Biopsy

A skin biopsy may be done pre and/or post cultured thymus tissue implantation. If skin rashes develop, the skin biopsies may be examined for the presence of T cells.

### 3.11 Thymus Tissue Biopsy

A thymus tissue biopsy may be obtained approximately 2 months after cultured thymus tissue implantation. Biopsies are done at the discretion of the PI and if the subject is medically stable. The graft biopsy, if done, is evaluated for thymopoiesis by immunohistochemistry.

### 3.12 Graft Versus Host Disease (GVHD)

GVHD prophylaxis will not be included, as GVHD from thymus donor cells has not been seen to date in any thymus tissue recipient. Standard of care management of GVHD will be initiated if GVHD occurs. The medications may include cyclosporine or tacrolimus, steroids, and/or mycophenylate mofetil (MMF) depending on the severity and organs affected by the GVHD.

### 3.13 Prophylactic Medications

Prophylactic medications will be given as clinically indicated to protect against infection.

### 3.14 Supportive Care

Subjects will receive supportive care such as blood components, antibiotics, immunoglobulin replacement, and other interventions as appropriate for medical care.

## 4 SUBJECT MONITORING AND SAFETY EVALUATION

### 4.1 Subject Monitoring

While at Duke, subjects will receive standard-of-care monitoring. The exact schedule will be tailored to the subject, and the subject's underlying problems.

### 4.2 Study Duration

Subjects will be followed for approximately two years post-implantation. Recipients of cultured thymus tissue implants are encouraged to have annual follow-up appointments for ongoing medical care and evaluation.

### 4.3 Study Endpoints

This is an expanded access descriptive study. Survival of subjects at one-year post-implantation is the primary safety and efficacy endpoint.

We will record survival and collect data on CD3, CD4, CD8, and naïve T cells at 3, 6, and 9 months and 1 year post implantation. Chimerism will be assessed at 2 to 3 months post implantation.

### 4.4 Statistical Considerations

This is an expanded access study so there is no formal sample size or statistical analysis. Post cultured thymus tissue implantation data will be reviewed on the naïve T cell count at 3, 6, and 9 months and 1 year (engraftment), T cell chimerism at 2 to 3 months, and unexpected, study related serious adverse events. After the subject leaves Duke, data collection is dependent upon the subject/parent/guardian and local physicians.

### 4.5 Adverse Event Recording and Reporting

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. All adverse events that meet the criteria for serious (see Definitions below) and deaths are to be entered into the clinical database and to be forwarded to the SAE contact. Review of SAEs and any that require expedited reporting will be conducted according to the procedures at Enzyvant and responsible safety vendor (Appendix 4).

The criteria listed in the NCI Common Terminology Criteria for Adverse Events v 4.0 (CTCAE) will be used in grading toxicity.

US Regulations require that an IND sponsor report Serious Adverse Events (SAEs) occurring with use of its product in a clinical trial if it is unexpected, and felt to be likely related to use of the investigational agent. In this study, all SAEs that occur will be evaluated by the PI and sponsor for assessment regarding the need for reporting to the FDA, and the IRB. After an Adverse Event is identified, the classification and relationship to the study procedures will be determined and assessed by the investigator and sponsor using the definitions that follow:

***Definitions***

An **Adverse Event** is any undesirable experience occurring to a subject during the clinical trial, whether or not the event is considered related to the study interventions. Pre-existing conditions are not recorded as Adverse Events.

A **Serious Adverse Event** is any untoward medical occurrence that is fatal, life-threatening, disabling, or results in inpatient hospitalization or prolongation of hospitalization. Occurrence of malignancy and adverse events resulting from overdose are considered serious adverse events. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Note: The term "life-threatening" in the definition of "Serious Adverse Event" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

An **Unexpected Adverse Event** is an event not listed in this document, in the consent form, in the US prescribing information (Appendix 3) or not associated with expected events given a subject's underlying condition.

Expected adverse events are those generally associated with CTTI and/or chemotherapy/radiation therapy. Subjects undergoing high dose chemotherapy are anticipated to experience anorexia, nausea, vomiting, mucositis; pancytopenia and associated infections while neutropenic. Some may develop organ toxicities from high dose therapy.

**Causality or "Relationship to Cultured Thymus Tissue Implantation"** is a determination of whether there is reasonable possibility that the investigational product may have caused or contributed to an adverse event. It includes assessing temporal relationships, dechallenge/rechallenge information, association (or lack of association) with underlying diseases, and the presence (or absence) or a lack of one or more likely causes. The assessment of the relationship of an adverse event to implantation (possible, probable, or definitely related) is a clinical decision based on all available information.

### 4.6 Stopping Rules

All serious adverse events (SAEs) that are unanticipated but related to the investigational interventions are reviewed by the IRB, sponsor, and FDA. SAEs do not stop enrollment or interventions unless requested by the PI, Sponsor, FDA, or IRB. Examples of SAEs are GVHD and cancer.

### 4.7 References

1. Markert ML, Marques JG, Neven B, et al. First use of thymus transplantation therapy for FOXN1 deficiency (nude/SCID): a report of 2 cases. *Blood* 2011; **117**(2): 688-96.
2. Jacobsen M, Schweer D, Ziegler A, et al. A point mutation in PTPRC is associated with the development of multiple sclerosis. *Nat Genet* 2000; **26**(4): 495-9.

# APPENDICES

## Appendix 1 Adverse Events Related to Treatment

| System Organ Class (SOC)<br>Preferred Term (PT) | FAS (N=105)<br>n | (%) | E |
|---|---|---|---|
| **Number of Related Adverse Events[1]** | 82 | ( 78.1) | 264 |
| **Blood and lymphatic system disorders** | 25 | ( 23.8) | 40 |
| Thrombocytopenia | 12 | ( 11.4) | 15 |
| Neutropenia | 9 | ( 8.6) | 9 |
| Coombs positive haemolytic anaemia | 3 | ( 2.9) | 3 |
| Autoimmune haemolytic anaemia | 2 | ( 1.9) | 3 |
| Anaemia | 2 | ( 1.9) | 2 |
| Haemolysis | 2 | ( 1.9) | 2 |
| Haemolytic anaemia | 2 | ( 1.9) | 2 |
| Lymphadenopathy | 2 | ( 1.9) | 2 |
| Immune thrombocytopenic purpura | 1 | ( 1.0) | 1 |
| Splenomegaly | 1 | ( 1.0) | 1 |
| **Skin and subcutaneous tissue disorders** | 25 | ( 23.8) | 29 |
| Rash | 11 | ( 10.5) | 11 |
| Urticaria | 5 | ( 4.8) | 6 |
| Alopecia | 4 | ( 3.8) | 4 |
| Dermatitis atopic | 1 | ( 1.0) | 1 |
| Erythema | 1 | ( 1.0) | 1 |
| Granuloma skin | 1 | ( 1.0) | 1 |
| Pigmentation disorder | 1 | ( 1.0) | 1 |
| Psoriasis | 1 | ( 1.0) | 1 |
| Rash papular | 1 | ( 1.0) | 1 |
| Skin mass | 1 | ( 1.0) | 1 |
| Stevens Johnson Syndrome | 1 | ( 1.0) | 1 |
| **Immune system disorders** | 23 | ( 21.9) | 26 |
| Cytokine release syndrome | 19 | ( 18.1) | 20 |
| Graft versus Host Disease | 2 | ( 1.9) | 2 |
| Graft versus Host Disease in gastrointestinal tract | 2 | ( 1.9) | 2 |
| Graft versus Host Disease in skin | 1 | ( 1.0) | 1 |
| Hypersensitivity | 1 | ( 1.0) | 1 |
| **Metabolism and nutrition disorders** | 22 | ( 21.0) | 32 |
| Hypomagnesaemia | 17 | ( 16.2) | 17 |
| Hyperglycaemia | 4 | ( 3.8) | 4 |
| Acidosis | 3 | ( 2.9) | 3 |
| Hypoalbuminaemia | 2 | ( 1.9) | 2 |
| Decreased appetite | 1 | ( 1.0) | 1 |
| Fluid retention | 1 | ( 1.0) | 1 |
| Hyperkalaemia | 1 | ( 1.0) | 1 |
| Hypokalaemia | 1 | ( 1.0) | 1 |
| Hyponatraemia | 1 | ( 1.0) | 1 |
| Lactase deficiency | 1 | ( 1.0) | 1 |
| **Vascular disorders** | 21 | ( 20.0) | 24 |
| Hypertension | 20 | ( 19.0) | 21 |
| Thrombosis | 2 | ( 1.9) | 2 |
| Haematoma | 1 | ( 1.0) | 1 |

| System Organ Class (SOC)<br>Preferred Term (PT) | FAS (N=105)<br>n | (%) | E |
|---|---|---|---|
| **Investigations** | 17 | ( 16.2) | 25 |
| Alanine aminotransferase increased | 4 | ( 3.8) | 4 |
| Aspartate aminotransferase increased | 4 | ( 3.8) | 4 |
| Blood bicarbonate decreased | 3 | ( 2.9) | 3 |
| Blood creatinine increased | 3 | ( 2.9) | 3 |
| Lymphocyte morphology abnormal | 1 | ( 1.0) | 2 |
| Blood alkaline phosphatase increased | 1 | ( 1.0) | 1 |
| Blood chloride decreased | 1 | ( 1.0) | 1 |
| Blood cortisol decreased | 1 | ( 1.0) | 1 |
| Blood immunoglobulin E increased | 1 | ( 1.0) | 1 |
| Blood magnesium decreased | 1 | ( 1.0) | 1 |
| Lipase increased | 1 | ( 1.0) | 1 |
| Lymphocyte count abnormal | 1 | ( 1.0) | 1 |
| Weight decreased | 1 | ( 1.0) | 1 |
| White blood cell count decreased | 1 | ( 1.0) | 1 |
| **Renal and urinary disorders** | 11 | ( 10.5) | 16 |
| Proteinuria | 7 | ( 6.7) | 7 |
| Acute kidney injury | 3 | ( 2.9) | 3 |
| Renal failure | 3 | ( 2.9) | 3 |
| Glomerulonephritis minimal lesion | 1 | ( 1.0) | 1 |
| Glycosuria | 1 | ( 1.0) | 1 |
| Renal tubular acidosis | 1 | ( 1.0) | 1 |
| **Gastrointestinal disorders** | 11 | ( 10.5) | 14 |
| Diarrhoea | 4 | ( 3.8) | 4 |
| Pancreatitis | 3 | ( 2.9) | 3 |
| Abdominal distension | 1 | ( 1.0) | 1 |
| Diarrhoea haemorrhagic | 1 | ( 1.0) | 1 |
| Enteritis | 1 | ( 1.0) | 1 |
| Gastrointestinal haemorrhage | 1 | ( 1.0) | 1 |
| Gingival hypertrophy | 1 | ( 1.0) | 1 |
| Ileus | 1 | ( 1.0) | 1 |
| Nausea | 1 | ( 1.0) | 1 |
| **Respiratory, thoracic and mediastinal disorders** | 11 | ( 10.5) | 11 |
| Hypoxia | 5 | ( 4.8) | 5 |
| Respiratory failure | 2 | ( 1.9) | 2 |
| Respiratory distress | 1 | ( 1.0) | 1 |
| Sleep apnoea syndrome | 1 | ( 1.0) | 1 |
| Stridor | 1 | ( 1.0) | 1 |
| Tachypnoea | 1 | ( 1.0) | 1 |
| **General disorders and administration site conditions** | 8 | ( 7.6) | 9 |
| Pyrexia | 6 | ( 5.7) | 7 |
| Face oedema | 1 | ( 1.0) | 1 |
| Oedema peripheral | 1 | ( 1.0) | 1 |
| **Infections and infestations** | 8 | ( 7.6) | 8 |
| Cytomegalovirus infection | 2 | ( 1.9) | 2 |
| Cellulitis staphylococcal | 1 | ( 1.0) | 1 |
| Device related infection | 1 | ( 1.0) | 1 |
| Staphylococcal skin infection | 1 | ( 1.0) | 1 |
| Stitch abscess | 1 | ( 1.0) | 1 |
| Varicella zoster virus infection | 1 | ( 1.0) | 1 |
| Wound infection staphylococcal | 1 | ( 1.0) | 1 |

| System Organ Class (SOC)<br>Preferred Term (PT) | FAS (N=105)<br>n | (%) | E |
|---|---|---|---|
| **Injury, poisoning and procedural complications** | 6 | ( 5.7) | 6 |
| Wound dehiscence | 4 | ( 3.8) | 4 |
| Graft haemorrhage | 1 | ( 1.0) | 1 |
| Venous injury | 1 | ( 1.0) | 1 |
| **Nervous system disorders** | 6 | ( 5.7) | 6 |
| Seizure | 3 | ( 2.9) | 3 |
| Febrile convulsion | 1 | ( 1.0) | 1 |
| Infantile spasms | 1 | ( 1.0) | 1 |
| Myelitis transverse | 1 | ( 1.0) | 1 |
| **Hepatobiliary disorders** | 4 | ( 3.8) | 4 |
| Autoimmune Hepatitis | 2 | ( 1.9) | 2 |
| Hepatomegaly | 1 | ( 1.0) | 1 |
| Hepatosplenomegaly | 1 | ( 1.0) | 1 |
| **Endocrine disorders** | 3 | ( 2.9) | 5 |
| Hypothyroidism | 2 | ( 1.9) | 2 |
| Adrenal insufficiency | 1 | ( 1.0) | 1 |
| Basedow's disease | 1 | ( 1.0) | 1 |
| Hyperthyroidism | 1 | ( 1.0) | 1 |
| **Musculoskeletal and connective tissue disorders** | 3 | ( 2.9) | 3 |
| Growth retardation | 1 | ( 1.0) | 1 |
| Juvenile idiopathic arthritis | 1 | ( 1.0) | 1 |
| Psoriatic arthropathy | 1 | ( 1.0) | 1 |
| **Cardiac disorders** | 2 | ( 1.9) | 2 |
| Sinus Tachycardia | 2 | ( 1.9) | 2 |
| **Congenital, familial and genetic disorders** | 1 | ( 1.0) | 1 |
| Albinism | 1 | ( 1.0) | 1 |
| **Neoplasms benign, malignant and unspecified (inc cysts and polyps)** | ..1 | ( 1.0) | 1 |
| Epstein-Barr virus associated lymphoma | 1 | ( 1.0) | 1 |
| **Psychiatric disorders** | 1 | ( 1.0) | 1 |
| Irritability | 1 | ( 1.0) | 1 |
| **Reproductive system and breast disorders** | 1 | ( 1.0) | 1 |
| Ovarian failure | 1 | ( 1.0) | 1 |

Abbreviations: AE = adverse event; E = number of events; FAS = full analysis set; MedDRA = Medical Dictionary for Regulatory Activities; N = number of subjects included in the analysis set; n = number of subjects with events; PT = preferred term; SOC = system organ class

Note: If a subject had multiple occurrences of an AE, the subject was presented only once in the subject count for a given SOC and PT. Adverse events were coded using MedDRA version 19.1.

[1]Related events were defined as events that were definitely, probably or possibly related to RVT-802 or study procedures or with an unknown relationship based on investigator review.

Source: Table 14.3.1.8.1 Adverse Events Related to Treatment by System Organ Class and Preferred Term

## Appendix 2 Adverse Events Possibly Related to RVT-802

| | FAS (N=105) | | |
|---|---|---|---|
| **System Organ Class (SOC)** Preferred Term (PT) | n | (%) | E |
| **Number of Related Adverse Events[1]** | 44 | ( 41.9) | 87 |
| **Blood and lymphatic system disorders** | 21 | ( 20.0) | 32 |
| Thrombocytopenia | 11 | ( 10.5) | 13 |
| Neutropenia | 8 | ( 7.6) | 8 |
| Coombs positive haemolytic anaemia | 3 | ( 2.9) | 3 |
| Autoimmune haemolytic anaemia | 2 | ( 1.9) | 3 |
| Haemolysis | 2 | ( 1.9) | 2 |
| Haemolytic anaemia | 2 | ( 1.9) | 2 |
| Immune thrombocytopenic purpura | 1 | ( 1.0) | 1 |
| **Skin and subcutaneous tissue disorders** | 9 | ( 8.6) | 10 |
| Alopecia | 4 | ( 3.8) | 4 |
| Rash | 2 | ( 1.9) | 2 |
| Granuloma skin | 1 | ( 1.0) | 1 |
| Psoriasis | 1 | ( 1.0) | 1 |
| Skin mass | 1 | ( 1.0) | 1 |
| Stephens-Johnson syndrome | 1 | ( 1.0) | 1 |
| **General disorders and administration site conditions** | 6 | ( 5.7) | 6 |
| Pyrexia | 5 | ( 4.8) | 5 |
| Oedema peripheral | 1 | ( 1.0) | 1 |
| **Renal and urinary disorders** | 5 | ( 4.8) | 6 |
| Proteinuria | 5 | ( 4.8) | 5 |
| Glomerulonephritis minimal lesion | 1 | ( 1.0) | 1 |
| **Gastrointestinal disorders** | 5 | ( 4.8) | 5 |
| Diarrhoea | 3 | ( 2.9) | 3 |
| Enteritis | 1 | ( 1.0) | 1 |
| Ileus | 1 | ( 1.0) | 1 |
| **Injury, poisoning and procedural complications** | 5 | ( 4.8) | 5 |
| Wound dehiscence | 4 | ( 3.8) | 4 |
| Graft haemorrhage | 1 | ( 1.0) | 1 |
| **Investigations** | 4 | ( 3.8) | 6 |
| Blood bicarbonate decreased | 2 | ( 1.9) | 2 |
| Lymphocyte morphology abnormal | 1 | ( 1.0) | 2 |
| Blood immunoglobulin E increased | 1 | ( 1.0) | 1 |
| Lymphocyte count abnormal | 1 | ( 1.0) | 1 |
| **Infections and infestations** | 4 | ( 3.8) | 4 |
| Cytomegalovirus infection | 1 | ( 1.0) | 1 |
| Staphylococcal skin infection | 1 | ( 1.0) | 1 |
| Stitch abscess | 1 | ( 1.0) | 1 |
| Wound infection staphylococcal | 1 | ( 1.0) | 1 |
| **Immune system disorders** | 2 | ( 1.9) | 3 |
| Graft versus Host Disease | 1 | ( 1.0) | 1 |
| Graft versus Host Disease in gastrointestinal tract | 1 | ( 1.0) | 1 |
| Graft versus Host Disease in skin | 1 | ( 1.0) | 1 |
| **Hepatobiliary disorders** | 2 | ( 1.9) | 2 |
| Autoimmune hepatitis | 2 | ( 1.9) | 2 |
| **Musculoskeletal and connective tissue disorders** | 2 | ( 1.9) | 2 |
| Juvenile idiopathic arthritis | 1 | ( 1.0) | 1 |
| Psoriatic arthropathy | 1 | ( 1.0) | 1 |

| System Organ Class (SOC)<br>Preferred Term (PT) | FAS (N=105)<br>n | (%) | E |
|---|---|---|---|
| **Endocrine disorders** | 1 | ( 1.0) | 2 |
| Basedow's disease | 1 | ( 1.0) | 1 |
| Hyperthyroidism | 1 | ( 1.0) | 1 |
| **Congenital, familial and genetic disorders** | 1 | ( 1.0) | 1 |
| Albinism | 1 | ( 1.0) | 1 |
| **Nervous system disorders** | 1 | ( 1.0) | 1 |
| Myelitis transverse | 1 | ( 1.0) | 1 |
| **Reproductive system and breast disorders** | 1 | ( 1.0) | 1 |
| Ovarian failure | 1 | ( 1.0) | 1 |
| **Vascular disorders** | 1 | ( 1.0) | 1 |
| Haematoma | 1 | ( 1.0) | 1 |

Abbreviations: AE = adverse event; E = number of events; FAS = full analysis set; MedDRA = Medical Dictionary for Regulatory Activities; N = number of subjects included in the analysis set; n = number of subjects with events; PT = preferred term; SOC = system organ class

Note: If a subject had multiple occurrences of an AE, the subject was presented only once in the subject count for a given SOC and PT. Adverse events were coded using MedDRA version 19.1.

[1]Related events were defined as events that were definitely, probably or possibly related to RVT-802 or study procedures or with an unknown relationship based on investigator review.

Source: Table 14.3.1.8.9 Adverse Events Possibly Related to RVT-802 by System Organ Class and Preferred Term

## Appendix 3 RETHYMIC US Prescribing Information

Amended Version: 30Nov2021

**HIGHLIGHTS OF PRESCRIBING INFORMATION**
**These highlights do not include all the information needed to use RETHYMIC safely and effectively. See full prescribing information for RETHYMIC.**

**RETHYMIC (Allogeneic processed thymus tissue–agdc)**
**For surgical implantation**
**Initial U.S. Approval: 2021**

**INDICATIONS AND USAGE**

RETHYMIC is indicated for immune reconstitution in pediatric patients with congenital athymia. (1)

Limitations of Use:

- RETHYMIC is not indicated for the treatment of patients with severe combined immunodeficiency (SCID).

**DOSAGE AND ADMINISTRATION**

RETHYMIC is administered by a surgical procedure. The recommended dose range is 5,000 to 22,000 $mm^2$ of RETHYMIC/$m^2$ recipient body surface area (BSA). (2) Immunosuppressive therapy is recommended for patients receiving RETHYMIC based on disease phenotype and PHA levels. (14)

**DOSAGE FORMS AND STRENGTHS**

RETHYMIC consists of yellow to brown slices of processed tissue with varying thickness and shape. The dosage is determined by the surface area of the RETHYMIC slices and recipient BSA. (3)

**CONTRAINDICATIONS**

None.

**WARNINGS AND PRECAUTIONS**

- Immune reconstitution sufficient to protect from infection is unlikely to develop prior to 6 to 12 months after treatment with RETHYMIC. Given the immunocompromised condition of athymic patients, infection control measures should be followed until the development of thymic function can be established. (5.1)
- Monitor and treat patients at risk for the development of graft versus host disease (GVHD). (5.2)
- Monitor for the development of autoimmune disorders, including complete blood counts with differential, liver enzymes, serum creatinine, urinalysis, and thyroid function. (5.3)
- Pre-existing renal impairment is a risk factor for death. (5.4)
- Pre-existing cytomegalovirus infection may result in death prior to the development of thymic function. (5.5)
- Monitor for the development of lymphoproliferative disorder (blood cancer). (5.6)
- Transmission of infectious diseases may occur because RETHYMIC is derived from human tissue. (5.7)
- Immunizations should not be administered in patients who have received RETHYMIC until immune-function criteria have been met. (5.8)
- Patients should be tested for anti-HLA antibodies prior to treatment. (5.9)

**ADVERSE REACTIONS**

The most common (>10%) adverse events related to RETHYMIC included: hypertension (high blood pressure, 19%), cytokine release syndrome (18%), rash (15%), hypomagnesemia (low magnesium, 16%), renal impairment / failure (decrease of kidney function, 12%), thrombocytopenia (low platelets, 12%), and graft versus host disease, (10%). (6.1)

**To report SUSPECTED ADVERSE REACTIONS, contact Enzyvant at 833-369-9868 or FDA at 1-800-FDA-1088 or https://www.fda.gov/safety/medwatch-fda-safety-information-and-adverse-event-reporting-program.**

**See 17 for PATIENT COUNSELING INFORMATION.**

**Revised: 10/2021**

**FULL PRESCRIBING INFORMATION: CONTENTS***



* Sections or subsections omitted from the full prescribing information are not listed.

## FULL PRESCRIBING INFORMATION

### 1 INDICATIONS AND USAGE

RETHYMIC® is indicated for immune reconstitution in pediatric patients with congenital athymia.

Limitations of Use

- RETHYMIC is not indicated for the treatment of patients with severe combined immunodeficiency (SCID).

### 2 DOSAGE AND ADMINISTRATION

#### 2.1 Dosage

RETHYMIC is administered by a surgical procedure. The dosage is determined by the total surface area of the RETHYMIC slices and recipient body surface area (BSA). A RETHYMIC slice is defined as the contents on a single filter membrane; the RETHYMIC slices are variable in size and shape. The recommended dose range is 5,000 to 22,000 $mm^2$ of RETHYMIC surface area/$m^2$ recipient BSA. The manufacturer calculates the dose in advance for the specific patient; the amount of product provided is adjusted at the manufacturing facility to ensure the maximum dose for the patient cannot be exceeded. Up to 42 cultured RETHYMIC slices will be provided for each patient. At the time of surgery, the manufacturing personnel communicate to the surgical team the portion of the product that represents the minimum dose. Patients with evidence of maternal engraftment or an elevated response to phytohemagglutinin (PHA) should receive RETHYMIC with immunosuppressive medications (Table 2).

#### 2.2 Administration Instructions

Surgical implantation of RETHYMIC should be done by a qualified surgical team in a single surgical session at a qualified hospital. RETHYMIC should be implanted in the quadriceps muscle in accordance with the instructions provided below. Implantation of RETHYMIC into the quadriceps requires a healthy bed of muscle tissue.

*Preparation for the Implantation Procedure:*

1. Operating room culture dishes (sterile 100 mm tissue culture dishes) and saline for injection are supplied by the operating room; a sufficient supply of operating room culture dishes and saline must be provided by the hospital for use in the implantation procedure.
2. The product is delivered to the operating room by manufacturing personnel. The recommended dose is determined based on the patient's BSA. The manufacturer calculates the dose in advance for the specific patient. Manufacturing personnel and the operating room staff confirm that the lot delivered is for the intended recipient.
3. Manufacturing personnel communicate to the surgical team the minimum number of RETHYMIC slices to be implanted to achieve the minimum dose. The product expiration date and time for the entire lot is labeled on each polystyrene dish (drug product dish).
4. Always handle RETHYMIC slices aseptically. Do not use if there is evidence of contamination.

5. Outside the sterile field, manufacturing personnel unpack RETHYMIC from the shipping box. One drug product dish at a time is removed from the drug product box and shipping box. Manufacturing personnel inspect the drug product box and each drug product dish for signs of contamination, damage, spills, or leakage. If damage to the drug product dishes, leaks, spillage or evidence of contamination is noted, manufacturing personnel will notify the surgical team that the lot cannot be implanted.
6. When the surgical team is ready, manufacturing personnel and surgical staff begin the transfer of drug product to the sterile operative field. Manufacturing personnel carry one drug product dish, which contains up to 4 RETHYMIC slices on up to 2 surgical sponges, with each RETHYMIC slice on a filter membrane, to the surgical staff near the sterile field. Manufacturing personnel open the drug product dish to expose the RETHYMIC slices.
7. The surgical staff team member uses a pair of forceps to remove individual RETHYMIC slices with their filter membranes from the drug product dish (Figure 1). The surgical team member places each RETHYMIC slice with its filter membrane into a sterile 100 mm tissue culture dish ("operating room culture dish") containing approximately 2 mL preservative-free saline that resides in the sterile field on the instrument table. This is repeated to transfer all RETHYMIC slices from the first drug product dish into a sterile operating room culture dish. After the first set of RETHYMIC slices has been prepared for surgical implantation and provided to the surgeon, another drug product dish with RETHYMIC slices is passed to the surgical staff member for removal from their filter membranes as described above.
8. Using 2 pairs of sterile forceps, the surgical staff team member should use one pair of forceps to hold the filter in place while using the other forceps to scrape and loosen the RETHYMIC slice from the filter membrane (Figure 1). Then, while using one pair of forceps to hold the filter in place, the surgical staff team member uses the other forceps to lift the RETHYMIC slice away from the filter membrane by pulling the tissue up. The surgical staff team member places each RETHYMIC slice separately into the saline-containing operating room culture dish in the sterile field on top of its original filter membrane. The RETHYMIC slice will change from a flatter slice to a condensed, lumpy shape at this stage of the procedure. The surgeon then implants the first set of RETHYMIC slices. The surgical team staff member should process the next set of up to 4 RETHYMIC slices from the next drug product dish into a second operating room culture dish in the same manner while the surgeon continues implanting the first set of up to 4 slices. When the surgeon finishes implanting the first set of RETHYMIC slices, the surgical staff team member transfers and prepares the next operating room culture dish on the surgical field. Continue this cycle until all the desired tissue is transferred during the implantation procedure.

**Figure 1: Preparing for the Implantation Procedure**

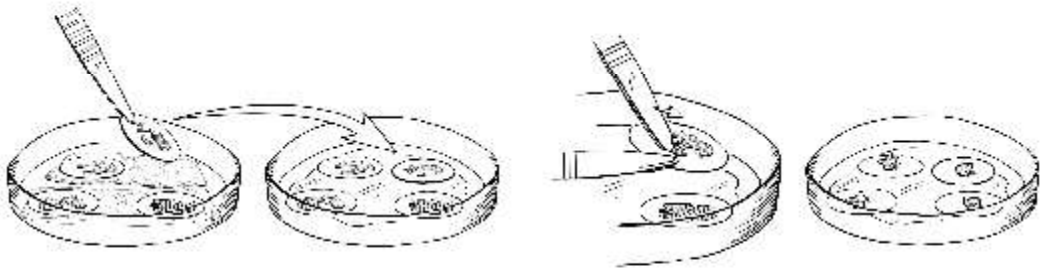
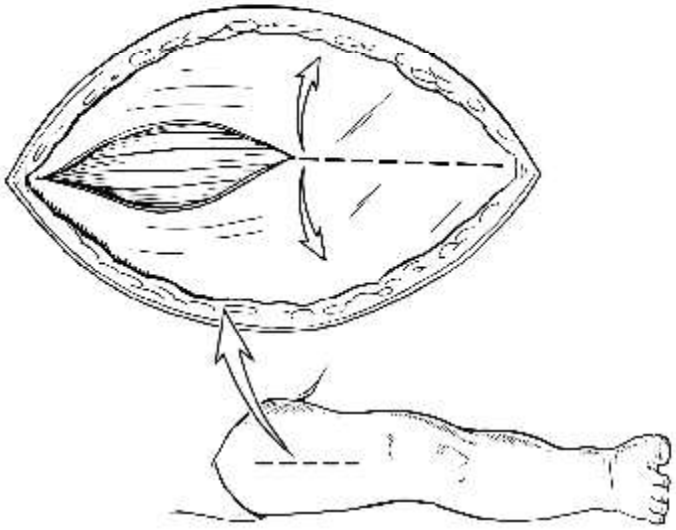


Figure 1: Within the sterile field, forceps are used to move individual RETHYMIC slices with their filter membranes from the drug product dish to the operating room culture dish (left images). A pair of forceps is used to gently scrape and lift the RETHYMIC slice off the filter membrane in the operating room culture dish in preparation for easy removal prior to implantation (right images).

*Implantation of RETHYMIC:*

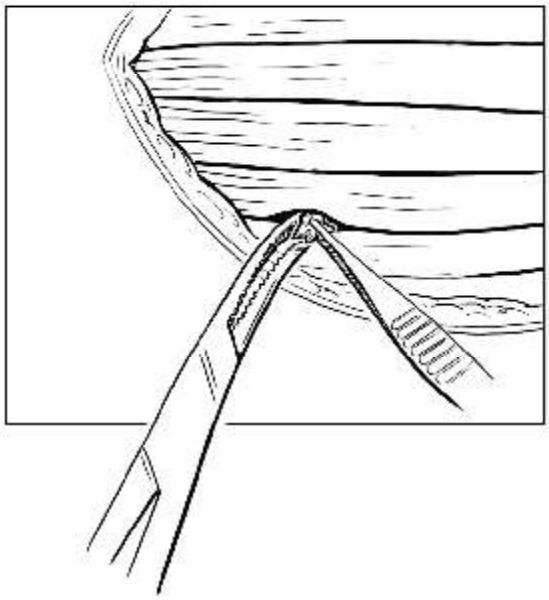
1. After induction of general anesthesia, a cranial-caudal skin incision (typically ~5 cm in length; Figure 2) should be made over the anterior thigh compartment. The size of the incision and the use of one or both legs for the implantation procedure is determined by the size of the patient, his/her muscle mass, and the amount of tissue to be implanted. If all or nearly all of the tissue can be implanted in one leg, then only one leg should be used.

**Figure 2: Surgical Incision and Opening of Fascia**



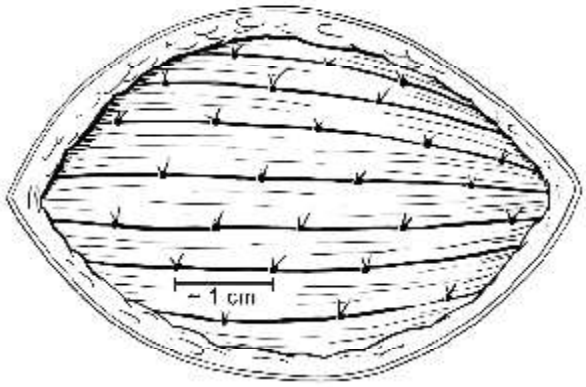
2. Open fascia to expose the anterior compartment muscles (Figure 2).
3. Create a pocket in between the muscle fibers using a tonsil clamp or similar instrument. Each pocket should be made along the natural furrows throughout the quadriceps muscle group.
4. Individual RETHYMIC slices should be implanted approximately 1 cm in depth and approximately 1 cm apart into the pockets between the muscle fibers in the quadriceps muscle (Figure 3).

**Figure 3: Implant Individual RETHYMIC Slices**



5. A large or thick RETHYMIC slice may be cut in half, at the surgeon's discretion, to ensure the slice is surrounded by muscle tissue once implanted. Implant as many RETHYMIC slices as possible within the recommended dose range of 5,000 to 22,000 $mm^2$ of processed thymus tissue/$m^2$ recipient BSA. During the procedure, the surgeon uses their judgment to balance the benefit of implanting additional RETHYMIC slices against the risk(s) that may be associated with implantation into limited muscle mass, number of implantations sites and other patient considerations.
6. Once each RETHYMIC slice has been implanted, it should be fully covered by muscle tissue. Then a single absorbable suture should be used to close the pocket where the RETHYMIC slice was implanted (Figure 4).

**Figure 4: Close the Site of Implantation**




7. Once the intended dose has been implanted, confirm hemostasis. Close the skin incision with 2 layers of absorbable sutures and apply a standard dressing, such as wound closure strips or skin glue. Leave the fascia open to allow room for muscle compartment swelling. An occlusive dressing may be used to prevent contamination.

## 3 DOSAGE FORMS AND STRENGTHS

RETHYMIC consists of yellow to brown slices of processed thymus tissue with varying thickness and shape. Each drug product dish contains up to 4 RETHYMIC slices that adhere to circular filter membranes on top of surgical sponges in 5 mL of medium. The RETHYMIC slices are variable in size and shape; a RETHYMIC slice is defined as the contents of a single filter membrane. The dosage is based on the total surface area of the RETHYMIC slices, and the amount administered is calculated based on recipient BSA. The surgeon should implant as many RETHYMIC slices as possible within the recommended dose range of 5,000 to 22,000 $mm^2$ of RETHYMIC/$m^2$ recipient BSA. The manufacturer calculates the dose in advance for the specific patient; the amount of product provided is adjusted at the manufacturing facility to ensure the maximum dose for the patient cannot be exceeded. Up to 42 RETHYMIC slices will be provided for each patient. At the time of surgery, the manufacturing personnel will inform the surgical team of the portion of the product that represents the minimum dose.

## 4 CONTRAINDICATIONS

None.

## 5 WARNINGS AND PRECAUTIONS

### 5.1 Infection Control and Immunoprophylaxis

Immune reconstitution sufficient to protect from infection is unlikely to develop prior to 6-12 months after treatment with RETHYMIC. Given the immunocompromised condition of athymic patients, follow infection control measures until the development of thymic function is established as measured through flow cytometry. This should include counseling patients and their caregivers on good handwashing practices and minimizing exposure to visitors. Monitor patients closely for signs of infection, including fever. If a fever develops, assess the patient by blood and other cultures and treat with antimicrobials as clinically indicated.

Patients should be maintained on immunoglobulin replacement therapy until all of the following criteria are met:

- No longer on immunosuppression (at least 10% of $CD3^+$ T cells are naïve in phenotype).
- At least 9 months post-treatment.
- Phytohemagglutinin (PHA) response within normal limits.
- Normal serum IgA is also desirable but not required.

Two months after stopping immunoglobulin replacement therapy, the IgG trough level should be checked.

- If the IgG trough level is in the normal range for age, the patient can remain off of immunoglobulin replacement.
- If the IgG trough level is lower than the normal range for age, immunoglobulin replacement therapy should be restarted and continued for a year before being retested using the above guidelines.

Prior to and after treatment with RETHYMIC, patients should be maintained on Pneumocystis jiroveci pneumonia prophylaxis until all of the following criteria are met:

- No longer on immunosuppression (at least 10% of CD3+ T cells are naïve in phenotype).
- At least 9 months post-treatment.
- PHA response within normal limits.
- CD4+ T cell count > 200 cells/$mm^3$.

### 5.2 Graft versus Host Disease

In clinical studies with RETHYMIC, GVHD occurred in 11 (10%) RETHYMIC-treated patients of whom 6 (55%) died. RETHYMIC may cause or exacerbate pre-existing GVHD. Seven patients (7%) experienced autologous GVHD, 3 patients (3%) experienced GVHD due to maternal cells and 1 patient (1%) experienced GVHD due to cells from a prior hematopoietic cell transplant (HCT). Risk factors for GVHD include atypical complete DiGeorge anomaly phenotype, prior HCT and maternal engraftment. GVHD may manifest as fever, rash, lymphadenopathy, elevated bilirubin and liver enzymes, enteritis, and/or diarrhea. Patients with elevated baseline T cell proliferative response to PHA > 5,000 cpm or > 20-fold over background should receive immunosuppressive therapies to decrease the risk of GVHD (Table 2 and Table 3). Development of GVHD symptoms should be closely monitored and promptly treated.

### 5.3 Autoimmune Disorders

Thirty-seven patients (35%) in the RETHYMIC clinical program experienced autoimmune-related adverse reactions. These events included: thrombocytopenia (including idiopathic thrombocytopenic purpura) in 13 patients (12%), neutropenia in 9 patients (9%), proteinuria in 7 patients (7%), hemolytic anemia in 7 patients (7%), alopecia in 4 patients (4%), hypothyroidism in 2 patients (2%), autoimmune hepatitis in 2 patients (2%), and autoimmune arthritis (juvenile idiopathic and psoriatic arthritis) in 2 patients (2%). One patient (1%) each experienced transverse myelitis, albinism, hyperthyroidism, and ovarian failure. The onset of autoimmune related events ranged from the three days before the surgical implantation procedure until 16 years post-treatment. Most events occurred within the first year after treatment.

Monitor complete blood counts with differential weekly for the first 2 months post-treatment and then monthly through 12 months post-treatment. Liver enzymes including aspartate aminotransferase and alanine aminotransferase, serum creatinine levels, and urinalysis should be performed monthly for 3 months and then every 3 months through 12 months post-treatment. Thyroid function studies should be performed prior to treatment and then at 6 months and 12 months post-treatment. After 12 months, testing should be performed annually.

### 5.4 Renal Impairment

Ten patients with renal impairment (elevated serum creatinine at baseline) were treated in studies with RETHYMIC. Five of these patients died within 1 year and a sixth patient died 3 years after treatment with RETHYMIC. Renal impairment at baseline is considered a risk factor for death.

### 5.5 Cytomegalovirus Infection

In clinical studies with RETHYMIC, 3 out of 4 patients with preexisting CMV infection prior to treatment with RETHYMIC died. The benefits/risks of treatment should be considered prior to treating patients with pre-existing CMV infection.

### 5.6 Malignancy

Because of the underlying immune deficiency, patients who receive RETHYMIC may be at risk of developing post-treatment lymphoproliferative disorder (blood cancer). The infant tissue donor is screened for Epstein-Barr virus (EBV) and cytomegalovirus (CMV), but patients should be tested for EBV and CMV using PCR prior to and 3 months following treatment with RETHYMIC, or after any exposure to or suspected infection with CMV or EBV.

### 5.7 Transmission of Serious Infections and Transmissible Infectious Diseases

Transmission of infectious disease may occur because RETHYMIC is derived from human tissue. Disease may be caused by known or unknown infectious agents. Donors are screened for increased risk of infection with human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV), hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum, Trypanosoma cruzi*, West Nile virus (WNV), transmissible spongiform encephalopathy (TSE) agents, vaccinia and Zika virus. Donors are also screened for clinical evidence of sepsis, and communicable disease risks associated with xenotransplantation. Blood samples (from the infant tissue donor or the birth mother, as applicable) are tested for HIV types 1, 2, and O, HTLV types I and II, HBV, HCV, *T. pallidum*, WNV, and *T. cruzi*. Blood from the infant tissue donor is also tested for *Toxoplasma gondii*, Epstein-Barr virus (EBV) and CMV. RETHYMIC is tested for sterility, endotoxin, and mycoplasma. These measures do not eliminate the risk of transmitting these or other infectious diseases and disease agents.

Testing of maternal and infant donor blood is also performed for evidence of donor infection due to cytomegalovirus (CMV).

Product manufacturing includes porcine- and bovine-derived reagents. While all animal-derived reagents are tested for animal viruses, bacteria, fungi, and mycoplasma before use, these measures do not eliminate the risk of transmitting these or other transmissible infectious diseases and disease agents.

Final sterility and mycoplasma test results are not available at the time of use, but manufacturing personnel will communicate any positive results from sterility testing to the physician. Report the occurrence of transmitted infection to Enzyvant at 833-369-9868.

### 5.8 Vaccine Administration

Immunizations should not be administered in patients who have received RETHYMIC until immune-function criteria have been met.

*Inactivated vaccines:*

Inactivated vaccines may be administered once all of the following criteria are met:

- Immunosuppressive therapies have been discontinued.
- Immunoglobulin (IgG) replacement therapy has been discontinued.
- The total $CD4^+$ T cell count is > 200 cells/$mm^3$ and there are more $CD4^+$ T cells than $CD8^+$ T cells ($CD4^+ > CD8^+$).

It is recommended that no more than 2 inactivated vaccines be given per month.

*Live Vaccines:*

Live virus vaccines should not be administered until patients have met the criteria for inactivated vaccines and received vaccinations with inactivated agents (e.g., tetanus toxoid). No additional vaccines (live or inactivated), except the inactivated influenza vaccine, should be given within 6 months after vaccination with a measles-containing vaccine or within 2 months after the varicella vaccine. Consider verifying response to vaccination with appropriate testing, in particular varicella and measles.

### 5.9 Anti-HLA Antibodies

All patients should be screened for anti-HLA antibodies prior to receiving RETHYMIC. Patients testing positive for anti-HLA antibodies should receive RETHYMIC from a donor who does not express those HLA alleles.

### 5.10 HLA Typing

HLA matching is required in patients who have received a prior hematopoietic cell transplantation (HCT) or a solid organ transplant. Patients who have received a prior HCT are at increased risk of developing GVHD after RETHYMIC if the HCT donor did not fully match the recipient. To minimize this risk, HLA matching of RETHYMIC to recipient alleles that were not expressed in the HCT donor is recommended.

## 6 ADVERSE REACTIONS

The most common adverse reactions (incidence in at least 10% of patients) reported following administration of RETHYMIC were hypertension (high blood pressure), cytokine release syndrome, rash, hypomagnesemia (low magnesium), renal impairment / failure (decrease of kidney function), thrombocytopenia (low platelets), and graft versus host disease.

### 6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety data described in this section are derived from 10 prospective, single-center, open-label studies, and include 105 patients who were treated with RETHYMIC in these studies and who had at least one year of follow-up. Table 1 lists the adverse reactions occurring in 105 patients who were treated with RETHYMIC in these studies.

**Table 1: Adverse Reactions Occurring in at least 5% of Patients Treated with RETHYMIC During Clinical Studies**

| System Organ Class<br>Preferred Term | RETHYMIC<br>(N=105)<br>n (%) |
|---|---|
| **Number of Patients with Adverse Reactions[1]** | **80 (76)** |
| Hypertension (high blood pressure) | 20 (19) |
| Cytokine release syndrome[2] | 19 (18) |
| Hypomagnesemia (low magnesium) | 17 (16) |
| Rash[3] | 16 (15) |
| Renal impairment / failure[4] (decrease of kidney function) | 13 (12) |
| Thrombocytopenia[5] (low platelets) | 13 (12) |
| Graft versus host disease[6] | 11 (10) |
| Hemolytic anemia[7] (low red bloods cells) | 9 (9) |
| Neutropenia (low white blood cells) | 9 (9) |
| Respiratory distress[8] (difficulty breathing) | 8 (8) |
| Proteinuria (protein in urine) | 7 (7) |
| Pyrexia (fever) | 6 (6) |
| Acidosis[9] | 6 (6) |
| Diarrhea[10] | 5 (5) |
| Seizure[11] | 5 (5) |

1. Reactions which occurred in the 2 years after treatment.

2. All events (19/19) of cytokine release syndrome occurred in association with ATG-R treatment.

3. Rash includes rash, granuloma skin, rash popular, urticaria.

4. Renal impairment / failure includes renal failure and acute kidney injury, proteinuria and blood creatinine increased.

5. Thrombocytopenia includes thrombocytopenia and Immune thrombocytopenic purpura.

6. GVHD includes GVHD, GVHD-gut, GVHD-skin, Omenn syndrome.

7. Hemolytic anemia includes autoimmune hemolytic anemia, Coombs-positive hemolytic anemia, hemolysis, hemolytic anemia.

8. Respiratory distress includes respiratory distress, hypoxia, respiratory failure.

9. Acidosis includes acidosis, renal tubular acidosis and blood bicarbonate decreased.

10. Diarrhea includes diarrhea and hemorrhagic diarrhea.

11. Seizures include infantile spasms, seizures and febrile convulsion.

Of the 105 patients, 29 patients died after receiving RETHYMIC, including 23 deaths in the first year (<365 days) after treatment with RETHYMIC. Causes of death in the first year included 13 deaths due to infection or complications due to infection, 5 deaths due to respiratory failure / hypoxia, 3 deaths due to hemorrhage-related events, and 2 deaths due to cardiorespiratory arrest. Of the 6 patients who died more than 1 year after treatment with RETHYMIC, the deaths were considered unrelated to study treatment: 2 died due to respiratory failure and 1 died due to each of the following: cardiopulmonary arrest, intracranial hemorrhage, infection, and unknown cause.

*Severe combined immunodeficiency (SCID) Patients*

Two patients with SCID were treated in the RETHYMIC clinical program. One patient died two years after receiving RETHYMIC, and the other patient died three years after receiving RETHYMIC.

*Patients with Prior hematopoietic cell transplant*

Six patients with a prior hematopoietic cell transplant (HCT) were treated in the RETHYMIC clinical program. Two patients died within the first 2 years after receiving RETHYMIC.

## 7 DRUG INTERACTIONS

No drug interaction studies have been conducted with RETHYMIC. If possible, prolonged use of immunosuppressive therapies, including high-dose corticosteroids, should be avoided.

## 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

Risk Summary

There are no clinical data with RETHYMIC in pregnant women. No animal reproductive and developmental toxicity studies have been conducted with RETHYMIC. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

### 8.2 Lactation

Risk Summary

There is no information regarding the presence of cellular components of RETHYMIC in human milk, the effect breastfeeding may have on RETHYMIC, the effect of being breastfed from a mother who received RETHYMIC as a child, or the effects of RETHYMIC on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for RETHYMIC and potential adverse effects on the breastfed infant from RETHYMIC.

### 8.3 Females and Males of Reproductive Potential

No nonclinical or clinical studies were performed to evaluate the effects of RETHYMIC on fertility.

### 8.4 Pediatric Use

The efficacy and safety of RETHYMIC have been established in pediatric patients with congenital athymia. The efficacy of RETHYMIC has been established in 95 pediatric patients (median age 9 months [range: 33 days to 3 years], including 65 patients age <1 year, 24 patients age 1 to <2 years, and 6 patients age 2 to <3 years at time of treatment) who were treated with RETHYMIC and included in the analysis of efficacy [see *Clinical Studies (14)*]. The safety of RETHYMIC has been established in 105 pediatric patients (median age 9 months [range: 33 days to 16.9 years] at time of treatment) with congenital athymia who were evaluated for safety following RETHYMIC administration. The safety population included 65 patients age <1 year, 27 patients age 1 to <2 years, 9 patients age 2 to <3 years, 1 patient age 3 to <6 years, and 3 patients age 13 to 17 years at time of treatment. Within the safety population, survival was similar across age groups. Adverse reactions were reported at similar frequencies across the age groups and were generally of similar types and severities.

### 8.6 Renal Impairment

In the clinical studies with RETHYMIC, 10 of 105 patients had impaired renal function at baseline based on elevated screening creatinine *[see Warnings and Precautions (5.4)]*. Baseline renal function should be considered when selecting immunosuppressants. Ensure appropriate involvement of a nephrologist in care of patients with renal impairment.

## 10 OVERDOSAGE

The maximum recommended dose is 22,000 $mm^2$ of RETHYMIC/$m^2$ recipient body surface area (BSA). Standard clinical care is recommended for patients receiving a dose > 22,000 $mm^2$ of RETHYMIC/$m^2$ recipient BSA. The product, as provided, has been adjusted at the manufacturing facility to not exceed the maximum dose based on the patient body surface area.

During clinical development one patient received a dose higher (23,755 $mm^2/m^2$) than the maximum recommended dose. This patient developed enteritis. A biopsy showed T cell, B cell, and neutrophil infiltration of the gut which resolved after treatment with immunosuppression, 5 months after treatment with RETHYMIC. The enteritis may have been related to the high dose of RETHYMIC.

## 11 DESCRIPTION

RETHYMIC consists of yellow to brown slices of allogeneic processed thymus tissue for administration by surgical implantation. Three to 11 drug product containers, with a total of 10 to 42 RETHYMIC slices, are provided for each patient. Each drug product container provides up to 4 RETHYMIC slices of variable size. The total dose, based on the number of slices administered to the patient, is 5,000 to 22,000 $mm^2$ of RETHYMIC/$m^2$ recipient BSA.

Thymus tissue is obtained from donors less than or equal to 9 months of age undergoing cardiac surgery. This thymus tissue is aseptically processed and cultured for 12 to 21 days to produce RETHYMIC slices. Each product lot is manufactured from a single unrelated donor and one product lot treats a single patient. The manufacturing process preserves the thymic epithelial cells and tissue structure and depletes most of the donor thymocytes from the tissue. These RETHYMIC slices are then surgically implanted into patients with congenital athymia.

The product manufacture uses reagents derived from animal materials. The surgical sponge used during culturing is porcine-derived. Fetal bovine serum is a component in the culture medium used to culture the thymus slices and RETHYMIC is formulated in media that is supplemented with fetal bovine serum. Therefore, bovine- and porcine-derived proteins will be present in RETHYMIC. These animal-derived reagents are tested for animal viruses, retroviruses, bacteria, fungi, yeast, and mycoplasma before use.

## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

RETHYMIC is intended to reconstitute immunity in patients who are athymic. The proposed mechanism of action involves the migration of recipient T cell progenitors from the bone marrow to the implanted RETHYMIC slices, where they develop into naïve immunocompetent recipient T cells. Evidence of thymic function can be observed with the development of naïve T cells in the peripheral blood; this is unlikely to be observed prior to 6-12 months after treatment with RETHYMIC.

### 12.2 Pharmacodynamics

The pharmacodynamic effects of RETHYMIC are not known.

### 12.3 Pharmacokinetics

The pharmacokinetic effects of RETHYMIC are not known.

## 14 CLINICAL STUDIES

The efficacy of RETHYMIC was evaluated in 10 prospective, single-center, open-label studies that enrolled a total of 105 patients, including 95 patients in the primary efficacy analysis. The demographics and baseline characteristics of the patients enrolled in the clinical studies were similar across studies. Across the efficacy population, 59% were male; 70% were White, 22% were Black, 4% were Asian/Pacific Islander; 2% were American Indian/Alaskan Native; and 2% were multi-race. The median (range) age at the time of treatment was 9 months (1-36). The diagnosis of congenital athymia was based on flow cytometry documenting fewer than 50 naïve T cells/$mm^3$ ($CD45RA^+$, $CD62L^+$) in the peripheral blood or less than 5% of total T cells being naïve in phenotype in 91/95 patients (range 0-98 naïve T cells/$mm^3$). In addition to congenital athymia, patients also had complete DiGeorge syndrome (cDGS; also referred to as complete DiGeorge anomaly (cDGA)) if they also met at least one of the following criteria: congenital heart defect, hypoparathyroidism (or hypocalcemia requiring calcium replacement), 22q11 hemizygosity, 10p13 hemizygosity, CHARGE (coloboma, heart defect, choanal atresia, growth and development retardation, genital hypoplasia, ear defects including deafness) syndrome, or CHD7 mutation. Across the efficacy population, 93 patients (98%) were diagnosed with cDGS, and the most common DiGeorge gene mutations or syndromic associations were Chromosome 22q11.2 deletion (36 patients; 38%) and CHARGE syndrome (23 patients; 24%). There were 35 patients with missing or no identified genetic mutations. Two (2%) patients had FOXN1 deficiency, and 1 patient (1%) had a TBX variant. There were 50 (53%) patients with typical cDGS; these patients had congenital athymia with the absence of a T cell-related rash. There were 42 (44%) patients diagnosed with atypical cDGS; these patients may have had a rash, lymphadenopathy, or oligoclonal T cells. Patients who did not have congenital athymia (e.g. SCID) and patients with prior transplants, including thymus and HCT, were excluded from the efficacy analysis population. The baseline demographics and disease characteristics were similar in the safety population.

Patients with heart surgery anticipated within 4 weeks prior to, or 3 months after, the planned RETHYMIC treatment date, patients with human immunodeficiency virus (HIV) infection, and patients who were not considered good surgical candidates were excluded from study participation.

Patients in the efficacy population received RETHYMIC in a single surgical procedure at a dose of 4,900 to 24,000 $mm^2$ of RETHYMIC / recipient BSA in $m^2$. Patients were assigned to receive immunosuppressive therapy prior to and/or after treatment according to their disease phenotype and pre-RETHYMIC PHA response. Table 2 summarizes the criteria used to administer immunosuppression. Table 3 summarizes the specific immunosuppressant dosing used in RETHYMIC clinical studies. No patients were retreated with RETHYMIC.

**Table 2: Summary of Treatment Assignment to Immunosuppression During Clinical Studies**

| Complete DiGeorge Anomaly Phenotype | Phytohemagglutinin (PHA) Response[1] | Immunosuppression Used During Clinical Studies with RETHYMIC |
|---|---|---|
| Typical | < 5,000 cpm<br>or<br>< 20-fold response to PHA over background | None |
| Typical | ≥ 5,000 cpm and < 50,000 cpm<br>or<br>Evidence of maternal engraftment | • ATG-R<br>• Methylprednisolone |
| Typical | ≥ 50,000 cpm | • ATG-R<br>• Methylprednisolone<br>• Cyclosporine[2] |
| Atypical | < 40,000 cpm on immunosuppression<br>or<br>< 75,000 cpm when not on immunosuppression | • ATG-R<br>• Methylprednisolone<br>• Cyclosporine[2] |
| Atypical | ≥ 40,000 cpm on immunosuppression<br>or<br>≥ 75,000 cpm when not on immunosuppression<br>or<br>Evidence of maternal engraftment | • ATG-R<br>• Methylprednisolone<br>• Cyclosporine[2]<br>• Basiliximab[3]<br>• MMF[4] |

Abbreviations: ATG-R: anti-thymocyte globulin [rabbit] (Thymoglobulin); cpm: counts per minute; MMF: mycophenylate mofetil; PHA: phytohemagglutinin

1. Values for PHA response are reported from Duke University Medical Center and may not be comparable to values reported at other clinical laboratories. A patient background value (cells without stimulus) of less than 5,000 cpm was required to consider PHA test results valid. A normal control value of > 75,000 cpm was also required during clinical studies.
2. If the patient could not tolerate cyclosporine due to adverse events (AEs), then the immunosuppression could have been changed to tacrolimus.
3. Basiliximab could have been given 24 hours prior to RETHYMIC administration for activated T cells (> 200 cells/mm$^3$ or > 50% T cells expressing CD25$^+$) persisting after ATG-R administration. Post-implantation, if the T cell count was > 2000 cells/mm$^3$ and > 50% of T cells were expressing CD25$^+$, a single dose of basiliximab could be given if not previously administered.
4. MMF could have been given if T cells remained elevated 5 days after ATG-R administration. MMF was stopped after 35 days if there was no extensive rash and if the aspartate aminotransferase and alanine aminotransferase were less than 3x the upper limit of normal and if T cells were < 5,000 cells/mm$^3$. If these criteria were not met, MMF could have been continued for up to 6 months.

**Table 3: Summary of Immunosuppressant Dosing During Clinical Studies**

| Immunosuppressant | Dose of Immunosuppressant |
|---|---|
| ATG-R | • 2 mg/kg IV administered once per day for 3 consecutive days pre-implantation (3 total doses)<br>• Administered over ~12 hrs starting at 0.125 ml/kg/hr into a central line for 1 hr, then 0.25 mL/kg/hr x 1 hr, then 0.35 mg/kg/hr for remainder of the infusion<br>• RETHYMIC implantation occurred within 7 days of last dose of ATG-R<br>o If the implant occurred more than 7 days after the last dose of ATG-R, a T cell count was repeated:<br>– If the T cell count was <50/mm³, no more ATG-R was administered<br>– If the T cell count was >50/mm³, ATG-R was repeated at the same schedule and dose as the initial infusion.<br>• Administration was planned for Days -5, -4, and -3 pre-implantation, followed by 2 days of rest prior to implantation. |
| Methylprednisolone[1,2] | • 2 mg/kg IV x 1 dose 4 hrs prior to ATG-R, then 0.5 mg/kg IV every 6 hrs until 24 hrs after the end of the ATG-R dosing |
| Cyclosporine[3,4,5] | • Target trough level of 180 to 220 ng/mL |
| Basiliximab | • A single dose of 5 mg/kg IV |
| MMF | • 15 mg/kg/dose q 8 hrs IV or PO |
| Alemtuzumab[6] | • 0.25 mg/kg daily, infused over 2 hours x 4 days IV |

Abbreviations: ATG-R: anti-thymocyte globulin [rabbit] (Thymoglobulin); IV: intravenous; MMF: mycophenylate mofetil; PO: oral

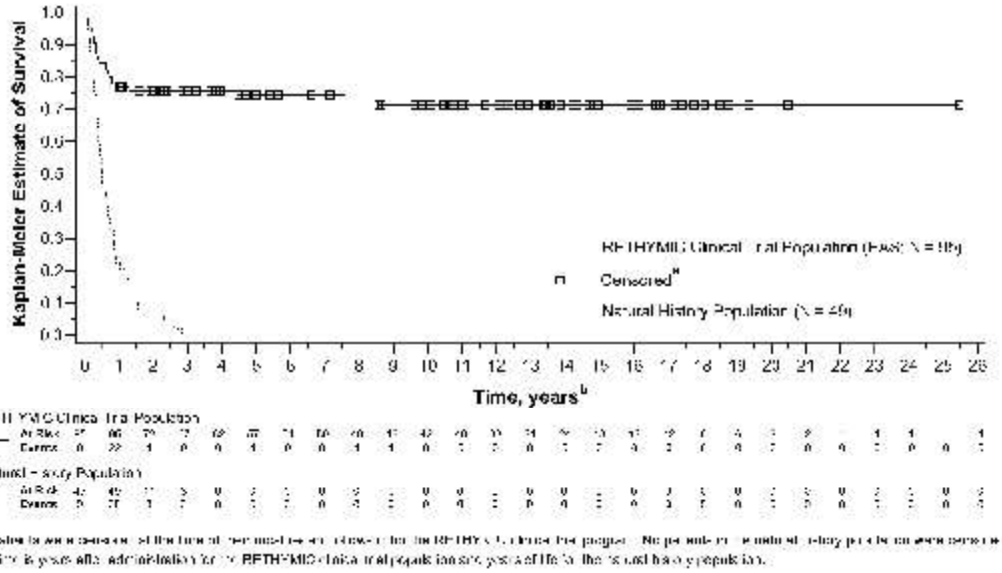
1. Additional pre-implantation corticosteroids (methylprednisolone) were used for atypical patients if pre-implantation CD3⁺ T cell numbers or the absolute lymphocyte count (ALC) was greater than 4,000 cells/mm³. A starting dose of 1 mg/kg/day was used if the T cell count or ALC was between 4,000 and 10,000 cells/mm³. A dose of 2 mg/kg/day was used if the T cell count was > 10,000 cells/mm³.
2. Corticosteroids (methylprednisolone or prednisolone) were initiated as soon as the diagnosis was confirmed in patients with evidence of maternal engraftment or with atypical cDGS and a PHA response of > 40,000 cpm on immunosuppression or > 75,000 cpm when not on immunosuppression. The steroid was weaned as soon as possible when the rash and other symptoms were brought under control.
3. Cyclosporine was initiated as soon as the diagnosis was confirmed and at least 7 days prior to ATG-R administration. If the CD3⁺ T cells fell and remained below 50/mm³, cyclosporine was weaned to have a cyclosporine trough level of 100 to 150 ng/mL. If the T cell count remained over 50/mm³, cyclosporine was maintained until the naive T cells were 10% of CD3⁺ T cells. Cyclosporine was then weaned over 10 weeks. To preserve renal function, the initiation of cyclosporine may have been delayed prior to implantation. Renal function was monitored according to the cyclosporine or tacrolimus prescribing information.
4. A higher target trough concentration of 250 to 300 ng/mL was used in patients with evidence of maternal engraftment or with atypical cDGS and a PHA response of > 40,000 cpm on immunosuppression or > 75,000 cpm when not on immunosuppression.
5. If the patient could not tolerate cyclosporine due to adverse events (AEs), then the immunosuppression could have been changed to tacrolimus (target trough concentration of 7 to 10 ng/mL). In patients with evidence of maternal engraftment or with atypical cDGS and a PHA response of > 40,000 cpm on immunosuppression or > 75,000 cpm when not on immunosuppression, the tacrolimus target trough level was 10 to 15 ng/mL.
6. Premedications given 30 minutes prior to alemtuzumab include methylprednisolone (1 mg/kg IV), acetaminophen (10 mg/kg IV), and diphenhydramine, (0.5 mg/kg IV).

The Kaplan-Meier estimated survival rates were 77% (95% CI [0.670, 0.841]) at 1 year and 76% (95% CI [0.658, 0.832]) at 2 years. For patients who were alive at 1 year after treatment with RETHYMIC, the survival rate was 94% at a median follow-up of 10.7 years.

Without treatment, congenital athymia is fatal in childhood. In a natural history population observed from 1991 through 2017, 49 patients diagnosed with congenital athymia received supportive care only. The 2-year survival rate was 6%, with all patients dying by 3 years of age. This population included 33 (67%) males. The most common cause of death was infection in 26 (53%) patients. Other common causes (≥10%) included support withdrawn in 7 (14%) patients, respiratory arrest in 5 (10%) patients, and cardiac arrest in 5 (10%) patients.

The Kaplan-Meier estimated survival rates for the RETHYMIC clinical trial population and the natural history population are shown in Figure 5. Four patients with >50 naïve T cells/mm$^3$ (CD45RA$^+$, CD62L$^+$) at time of RETHYMIC administration have been treated; 2 (50%) were alive with follow-up less than 2 years.

**Figure 5: Kaplan-Meier Survival by Year (RETHYMIC Efficacy Analysis Population and Natural History Population)**



RETHYMIC significantly reduced the number of infections over time. In the first year after treatment with RETHYMIC, the number of patients with an infection event onset 6 to ≤ 12 months after treatment decreased by 38% (from 63 to 39) relative to the number of patients with an infection event onset in the first 6 months post-treatment. A two-year analysis showed a decrease in both the number of patients with an infection event and the mean number of infection events per patient, with an onset in the first 12 months post-treatment as compared to 12 to ≤ 24 months after treatment. There was a mean difference of 2.9 events ($p<0.001$) per patient.

Naïve $CD4^+$ and $CD8^+$ T cells reconstituted over the first year, with a durable increase through Year 2. Median (minimum, maximum) naïve $CD4^+$ T cells/$mm^3$ increased from a baseline of 1 (0, 38) to values of 42 (0, 653), 212 (1, 751), and 275 (33, 858) at 6, 12, and 24 months after treatment with RETHYMIC, respectively. Median naïve $CD8^+$ T cells/$mm^3$ increased from a baseline of 0 (0, 46) to values of 9 (0, 163), 58 (0, 304), and 86 (6, 275) at 6, 12, and 24 months after treatment with RETHYMIC, respectively. This was accompanied by functional improvements based on T cell proliferative responses to PHA.

## 16 HOW SUPPLIED/STORAGE AND HANDLING

How Supplied

- RETHYMIC, NDC 72359-001-01, contains a single-dose unit, supplied ready for use as slices of processed thymus tissue, in sterile, polystyrene dishes (drug product dishes). Each drug product dish contains up to 4 RETHYMIC slices, adhered to circular filter membranes on top of surgical sponges in 5 mL of medium containing fetal bovine serum.
- Up to 42 RETHYMIC slices are supplied in a single-dose unit according to the dosage calculated in advance by the manufacturer for the specific patient. The dosage is determined by the total surface area of the RETHYMIC slices and recipient body surface area (BSA). The recommended dose range is 5,000 to 22,000 $mm^2$ of RETHYMIC surface area/$m^2$ recipient BSA. At the time of surgery, the manufacturing personnel communicate to the surgical team the portion of the product that represents the minimum dose.
- All drug product dishes are supplied in a polycarbonate container in an insulated shipping box.

Storage and Handling

- Use RETHYMIC prior to the time and date of expiration printed on the polycarbonate container.
- Store RETHYMIC at room temperature in the polycarbonate container in the insulated shipping box until ready for use. Do not refrigerate, freeze, agitate, or sterilize RETHYMIC.
- In the operating room, manufacturing personnel inspect the drug product containers as they are removed from the shipping box. If damage to the drug product dishes, leaks, spillage or evidence of contamination is noted, manufacturing personnel will notify the surgical team that the lot cannot be implanted.
- Match the patient's identity with the patient identifiers on the patient label on the polycarbonate container. Do not remove the drug product containers from the polycarbonate container if the information on the patient label does not match the intended patient.

- Manufacturing personnel record which RETHYMIC slices are used during the surgery. If any RETHYMIC slices are not administered to the patient, manufacturing personnel return this tissue to the manufacturing facility and dispose of this tissue as biohazardous waste in accordance with local requirements. Manufacturing personnel calculate the total dose that was administered to the patient.

## 17 PATIENT COUNSELING INFORMATION

Advise patients and/or their caregivers that:

- Immune reconstitution sufficient to protect from infection usually develops between 6-12 months after treatment with RETHYMIC, but for some patients elevated naïve T cell numbers are not observed until 2 years after treatment. Strict infection control measures should be observed until the healthcare provider confirms that immune function has been reconstituted through the evaluation of blood using flow cytometry and the criteria for the discontinuation of immunoglobulin replacement therapy and *Pneumocystis jiroveci* pneumonia prophylaxis have been met. Patients and caregivers should follow good handwashing practices, minimize contact with others, and immediately report signs and symptoms of infection to their healthcare provider *[see Warnings and Precautions (5.1)]*.
- Congenital athymia alters the immune response to vaccines. Instruct patients and/or their caregivers to notify their healthcare professional to evaluate the immune status of RETHYMIC recipients prior to receiving vaccinations *[see Warnings and Precautions (5.8)]*.
- Immunosuppression should be administered in patients with elevated T cell response, maternal engraftment, or oligoclonal T cell expansion and autoreactive T cells manifested by rash, lymphadenopathy and/or diarrhea. Inform patients and/or their caregivers on risks associated with short-term and long-term use of immunosuppression and refer them to review the risks of the specific immunosuppressants prescribed with their physician.
- Congenital athymia is associated with a wide spectrum of genetic anomalies. Instruct patients and/or their caregiver to consult with a clinical geneticist prior to receiving RETHYMIC.

Advise patients and/or their caregivers of the following risks:

- Graft versus Host Disease *[see Warnings and Precautions (5.2)]*
- Autoimmune Disorders (patient's immune (defense) system mistakenly attacks patient's body) *[see Warnings and Precautions (5.3)]*
- Renal Impairment (decrease of kidney function) *[see Warnings and Precautions (5.4)]*
- Cytomegalovirus Infection *[see Warnings and Precautions (5.5)]*
- Malignancy (*Cancer*) *[see Warnings and Precautions (5.6)]*
- Transmission of Serious Infections and Transmissible Infectious Diseases *[see Warnings and Precautions (5.7)]*

Manufactured for:

Enzyvant Therapeutics, Inc.
Cambridge MA 02142

## Appendix 4 Persons responsible for conducting the study

| Study Role | Name | Daytime Phone Number and Email Address |
|---|---|---|
| Primary Medical Monitor | [redacted] | [redacted] |
| Clinical Program Lead | [redacted] | [redacted] |
| SAE Contact Information | [redacted] | [redacted] |
| Principal Investigator | [redacted] | [redacted] |
| Sponsor Contact | [redacted] | [redacted] |

## Appendix 5 Investigator Statement of Agreement.

- I agree to conduct the study in compliance with the protocol.
- I agree that I am aware of and will comply with international ethical and quality standards of Good Clinical Practice (GCP).
- I agree not to make changes to the protocol without prior agreement from Enzyvant and documentation that the Institutional Review Board (IRB) or its equivalent has reviewed and approved these changes.
- I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.
- I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about and fulfil their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

| Principal Investigator Name (Printed) | Principal Investigator Signature |
|---|---|
| [redacted] | |
| Date | Site |