CLINICAL STUDY PROTOCOL

Safety and Efficacy of Thymus Transplantation in Complete DiGeorge Anomaly

Protocol No. 25966

Development Phase:	I/II, IND#9836 Open label treatment
Protocol date	Amendment version 15Apr2022
Sponsor representative:	[redacted]
Sponsor:	Enzyvant Therapeutics GmbH Viaduktstrasse 8 Basel, Switzerland 4051

SPONSOR SIGNATURE PAGE

Study Title: **Safety and Efficacy of Thymus Transplantation in Complete DiGeorge Anomaly**

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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

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1. General Information

1.1. Title: Safety and Efficacy of Thymus Transplantation in Complete DiGeorge Anomaly, IND #9836

1.2. Identifying number: Pro00025966

1.3. Date: Amendment version 15Apr2022

1.4 Sponsor: Enzyvant Therapeutics GmbH
Address of Sponsor: Viaduktstrasse 8
Basel, Switzerland 4051

1.5 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 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 protocol has been closed to enrollment of new patients. Thus, this amendment will only apply to follow-up of three 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.

1.5.1 Detailed Summary of changes:

- Title page with sponsor information added
- Sponsor Signature Page added
- Section 1 updated with sponsor information
- Section 1.5 Amendment version and Summary of changes added
- Section 2.2.1 Reference to sponsor investigator corrected and updated to Enzyvant Therapeutics GmbH
- Section 2.3 Updated to also refer to RETHYMIC US Prescribing Information for most recent risk and benefit background
- Section 4.2.1 Reference to Sponsor/investigator removed
- Section 4.6 Note added that study is closed to enrollment.
- Section 4.6.1 Sponsor updated, DSMB removed
- Section 4.6.2 DSMB removed
- Section 4.6.3 DSMB removed
- Section 5 Note added regarding the status of the trial at time of amendment

- Section 6. Note added regarding the status of the trial and participants at the time of amendment.
- Section 6.2.1.9.2.1 Clarification added that adverse events are to be reported per protocol section 8.3
- Section 6.4.1.1 Collection of information after discharge from Duke Medical Center linked to information on data collection in 6.1.3.2.4.
- Section7.2.1.1 Reference to Appendix corrected.
- Section 7.2.1.1 Removed section referring to DSMB review
- Section 8.2.1 DSMB removed
- Section 8.3.2.2 Serious adverse event reporting procedures updated to those of the sponsor, Enzyvant Therapeutics. Reporting to funding agencies was deleted as this is not applicable.
- Section 8.3.2.4 Updated to Enzyvant Therapeutics procedure.
- Section 8.3.3.3 Updated to include USPI as source for evaluation of unexpected adverse event
- Section 8.3.4.1 Related clarified to encompass possibly, probably or definitely related. Reporting to funding agencies was deleted as this is not applicable.
- Section 8.3.5.1 Reporting to funding agencies was deleted as this is not applicable.
- Section 9.1 Reference to Appendix corrected
- Section 9.1.3.2 DSMB replaced with sponsor
- Section 9.3.1 Reference to Appendix corrected
- Section 9.4 DSMB removed
- Section 14 Financing and Insurance deleted as not relevant to the follow-up of continuing patients with Enzyvant as sponsor.
- Section 15 Publication Policy was deleted as this is covered in a separate agreement.
- Appendix B Updated to reflect document on file at Enzyvant Therapeutics
- Appendix C Added to provide current USPI for RETHYMIC
- Appendix D Added to provide contact information for Study Personnel
- Appendix E Added to provide for Investigator Agreement for study conduct

1.6 Summary of Changes: Amendment version 15Apr2022 was created to update Sponsor Information

1.6.1 Detailed Summary of changes:

- Title page with sponsor information updated
- Sponsor Signature Page updated
- Section 1 Version date updated
- Section 1.6 Amendment version and Summary of changes added
- Appendix D (responsible persons), the Medical Monitor and the sponsor contact updated.

2. Background Information.
   2.1. Name and description of the investigational product.
      2.1.1. Allogeneic processed thymus tissue-agdc (RVT-802; also referred to in this protocol as thymus tissue for transplantation).
   2.2. Summary of findings from clinical trials that are relevant to the trial.
      2.2.1. Through 2010, the original IND sponsor, M. Louise Markert MD, had published 10

papers on thymus transplantation for pediatric patients with complete DiGeorge anomaly (cDGA) (Markert et al 1997; Markert et al 1999; Markert et al 2003, Markert et al 2004b, Markert et al 2007, Markert et al 2008a, Markert et al 2008b, Markert et al 2009, Markert et al 2010, Rice et al 2004). These papers present the first 60 pediatric patients with complete DiGeorge anomaly who were treated with thymus transplantation through 2010. Additional subjects have been treated since 2010 and an integrated database for all subjects treated under the IND with data available through August 2020 has been prepared. This integrated database and accompanying summaries were 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 development and commercialization of the product under BLA 125685. Adverse events in this dataset were coded using MedDRA (version 19.1). This database includes data from a total of 105 subjects (cDGA and other conditions) treated with thymus transplantation. Analyses in the integrated database were performed for three population subsets: full analysis set (FAS, n=105), efficacy analysis set (EAS, n=95) and cDGA-efficacy analysis set (EAS-cDGA, n=93). The data and analyses are on file at Enzyvant and have been submitted to the FDA through a clinical update to BLA 125685.

2.2.2. As of August 2020, 97 subjects with complete DiGeorge anomaly have received thymus transplants (RVT-802).

2.2.2.1. Ninety-three of these subjects comprise the EAS-cDGA.

2.2.2.1.1. Four subjects with cDGA with prior attempts at immune reconstitution were excluded from the EAS-cDGA.

2.2.2.2. In the EAS-cDGA, 32 of 93 athymic subjects did not receive any immunosuppression.

2.2.2.3. Sixty-one subjects received some type of immunosuppression medication pre and/or post thymus transplantation.

2.2.2.3.1. Fifty-five of the 61 subjects were treated with pre-transplantation rabbit anti thymocyte globulin. Subgroups were given additional immunosuppression with steroids and cyclosporine or tacrolimus.

2.2.2.3.1.1. The 55 subjects who received rabbit anti-thymocyte globulin included a group of 5 subjects with complete DiGeorge anomaly who were enrolled in a protocol to receive thymus plus parathyroid transplants (through 11/27/06). Four of the 5 subjects received both a thymus and parathyroid transplant.

2.2.2.3.2. Two of the 61 subjects who were treated with pre-transplantation rabbit anti thymocyte globulin and cyclosporine had maternal T cell engraftment.

2.2.2.4. Eight additional subjects received thymus transplantation and are not counted in the 97 subjects with EAS-cDGA mentioned above.

2.2.2.4.1. Three pediatric patients with the nude phenotype (*Foxn1* deficiency) received thymus transplants. One of these subjects was previously treated with hematopoietic cell transplantation.

2.2.2.4.2. Two subjects with severe combined immunodeficiency received thymus transplants.

2.2.2.4.3. One subject had a TBX1 point mutation but no other potentially linked genetic abnormality.

2.2.2.4.4. One subject was initially diagnosed with partial DiGeorge anomaly and was excluded from the EAS-cDGA but is now thought to have atypical cDGA.

2.2.2.4.5. One subject had an unknown form of athymia and had received 2 previous hematopoietic cell transplants.

2.2.3. Overall survival of athymic pediatric patients with cDGA after thymus transplantation is 73% (68 survivors of 93 transplanted subjects in the EAS-cDGA [through August 2020]). See Figure 1. Most deaths (n=21) occurred prior to 1 year post-transplantation.

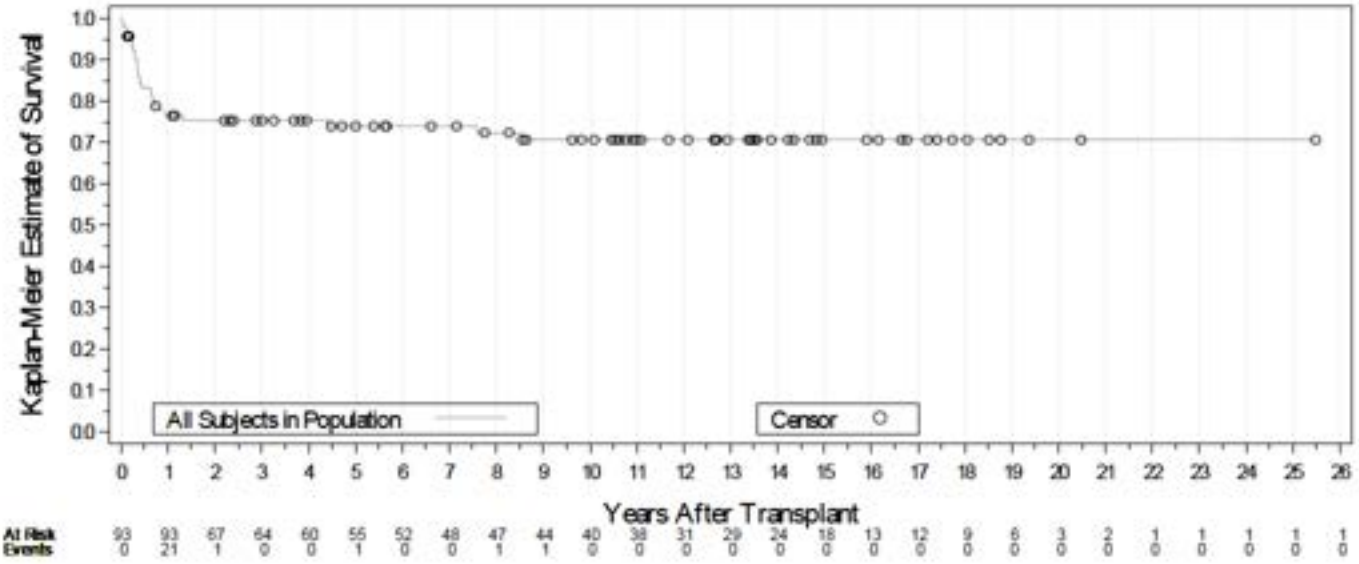

Figure 1. Kaplan Meier analysis of survival after thymus transplantation in 93 pediatric patients with complete DiGeorge anomaly.

2.2.4. Detailed analysis of the immune findings in a subset of 60 subjects with cDGA showed striking improvement in all survivors (Markert et al 2003; Markert et al 2004b, Markert et al 2007, Markert et al 2010). Figure 2 shows the data for naïve CD4 counts in those subjects who survived past 1 year post-transplantation.

- T cell numbers increase to levels at or slightly lower than the tenth percentile for age (based on normal values of Shearer et al 2003).
- T cell proliferative responses to mitogens normalize in the first year after transplantation. Of 41 subjects who survived over 2 years from transplantation, 38 subjects have been tested for proliferative responses to tetanus toxoid. Of the 38 subjects, 32 subjects (84%) have developed a greater than 10-fold *in vitro* proliferative responses to the antigen tetanus toxoid.
- The T cell receptor (TCR) beta variable repertoire (TCRBV) has become polyclonal in 41 of 43 surviving subjects who are over one year from thymus transplantation. One additional subject was lost to follow up at 15 months after thymus transplantation prior to developing T cells.
- Naive T cells as assessed by co-expression of CD45RA and CD62L increase to low adult levels during the first 2 years after transplantation.
- T cell receptor rearrangement excision circles (TRECs) increase in the first few years as well.
- The ability to make antibodies to tetanus toxoid and/or pneumococcal antigens has developed in 28 subjects tested after 2 years post thymus transplantation out of a total 41subjects surviving past 2 years.
  - Seven of the 41 subjects who survived to 2 years from transplantation were not tested or are in the process of being tested.
  - Six of the 41 subjects are on immunoglobulin replacement therapy and cannot be tested until the immunoglobulin replacement therapy is stopped.
  - One of the 28 responders was tested by the local physician for antibody response to the Prevnar conjugated pneumococcal vaccine and this was normal. This subject was not tested for antibodies to tetanus toxoid.

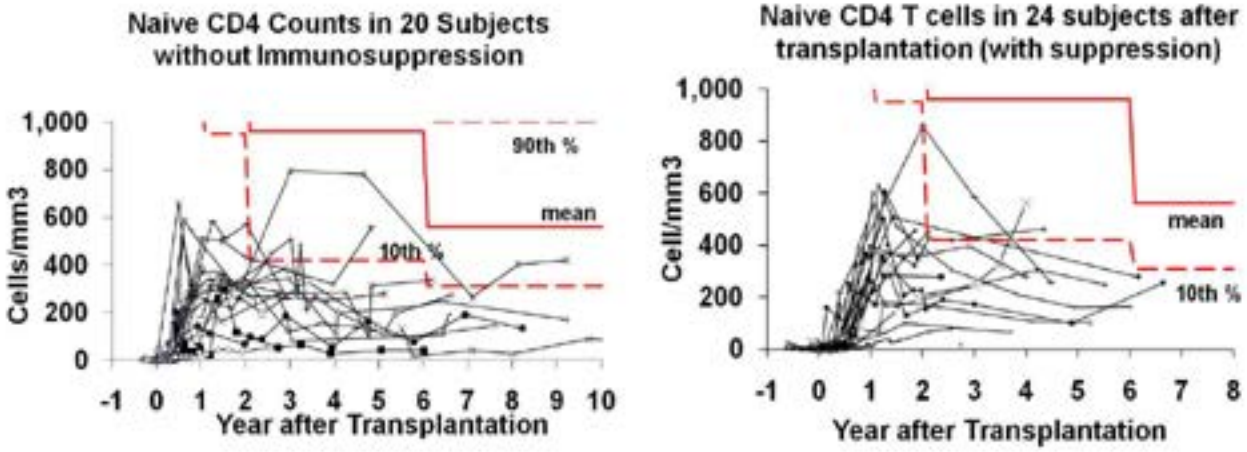

Figure 2. The development and persistence of naïve CD4 T cells in complete DiGeorge anomaly subjects after thymus transplantation. Data for 44 subjects who have survived past 1 year post transplantation are shown.

2.2.5. The frequency of infection in these pediatric patients decreased beginning approximately 6 months after thymus transplantation in a population of 44 subjects transplanted up to Dec 2006 (Markert et al. 2007). In a more recent evaluation, using EAS-cDGA in the integrated dataset, the Wilcoxon signed-rank test was used to compare the number of infection-related adverse events which occurred in the first six months after transplantation to the number of events between 6 and 12 months after transplantation for subjects that had at least 12 months of follow-up and had any infection-related AE in the first 12 months after transplantation. The same approach was used to evaluate the number of infection-related AEs that occurred in the first year after transplantation compared to the number of events that occurred in between 12 and 24 months after transplantation for subjects that had at least 24 months of follow-up and had any infection-related AE in the 24 months after transplantation. Table 1 shows the results of these analyses for the EAS-cDGA population. There was a significantly lower frequency of infection-related AEs between 6 and 12 months than in the first 6 months after transplantation and there was a lower frequency of events between 12 and 24 months than in the first 12 months after transplantation.

Table 1. Decreased infections 6 to 12 months compared with 0 to 6 months, and 12 to 24 months compared with less than 12 months for EAS-cDGA.

	First Year Analysis			Two Year Analysis		
	AE Onset within 6 Months	AE Onset 6 to ≤12 Months	Difference Between Periods	AE Onset within 12 Months	AE Onset 12 to ≤24 Months	Difference Between Periods
Number of Subjects Analyzed	N=65[1]			N=63[2]		
Number of Subjects with Event During the Period	60	36		63	32	
Mean Number of Events per Subject (SD)	3.4 (2.60)	2.3 (2.42)	1.8 (3.08)[3]	4.3 (3.66)	2.8 (3.08)	2.9 (4.00)[3]
Median Number of Events per Subject	2.5	1.5	1[4]	3	2	2[4]
p-value[5]			<0.001			<0.001

[1] To be included in the first year analysis, subjects must have been alive and on follow-up for at least 365 days after implantation, and experienced at least one infection-related AE during one or both onset periods.
[2] To be included in the second year analysis, subjects must have been alive and on follow-up for at least 790 days after implantation, and experienced at least one infection-related AE during one or both onset periods.
[3] The mean and SD of the per patient difference in infection-related AEs that occurred during the specified onset periods is presented.
[4] The median of the per patient difference in infection-related AEs that occurred during the specified onset periods is presented.
[5] 2-sided p-value based on a Wilcoxon Signed-Rank test of the difference in the number of infection-related AEs per subject between the first and second periods (null hypothesis defined as zero for difference).

2.2.6. The effect of dose on outcomes has been studied (see Figure 3). The outcomes evaluated have included CD4 count, naïve CD4 T cell number, the T cell proliferation response to the mitogen phytohemagglutinin (PHA), and the T cell receptor beta variable (TCRBV) diversity at one year. [Note: TCRBV variability is measured using spectratyping and is reported as the Kullback-Leibler divergence (D_{KL}) score. The lower the D_{KL}, the more normal the diversity. High D_{KL}s reflect limited diversity. Normal adults have D_{KL}s around 0.1. Abnormal D_{KL} values can be >1.0.] The graphs below show the one-year results from previous subjects for whom we have measured dose data who are beyond 1 year after thymus transplantation. The T cell number and PHA data reflect the mean of the first two values beyond year one. The D_{KL} data are a single time point at approximately year one. The correlation coefficients are poor and none approach significance. The lack of significance holds if the data are analyzed separately for the groups with suppression and without suppression.

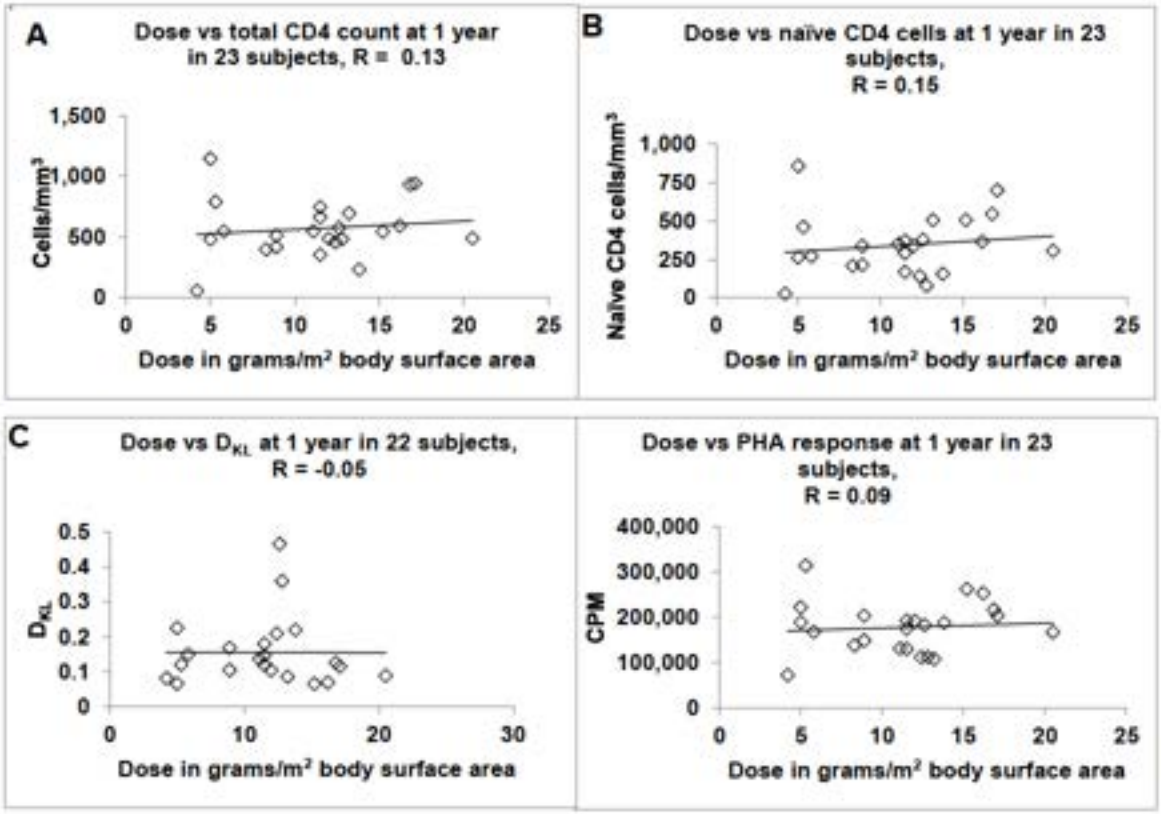

Figure 3. Lack of effect of dose on immune outcomes. A) Dose plotted versus the CD4 count at 1 year. B) The effect of dose on naïve CD4 cells at one year. C) Dose plotted versus the statistical measure of TCRBV diversity, D_{KL}, at one year. D) Dose versus the PHA response at one year. Trend lines are shown. (Markert et al, 2008b Am J Transplantation)

2.2.7. The relationship between matching at HLA-DR and immune outcomes has been tested. No significance has been found. (Markert et al, 2008b Am J Transplantation)

2.2.8. Rationale for use of immunosuppression.

2.2.8.1. To understand the use of immunosuppression in some subjects with complete DiGeorge anomaly, it is important to appreciate the clinical and immunological presentation of atypical complete DiGeorge anomaly (Markert et al 2004a). The word "atypical" refers to the development of rash characterized by infiltration of T cells in the skin. The pathologic diagnosis of the skin is usually spongiotic dermatitis. The rash can be a serious problem. It can clinically resemble severe atopic dermatitis. The rash is associated with oligoclonal T cells in the blood.

Lymphadenopathy develops at some point after the rash develops. If biopsied, the nodes are characterized as having dermatopathic lymphadenopathy.

Oligoclonal T cells may also infiltrate the liver as well as the skin and gut

resulting in elevations of liver enzymes, hepatomegaly, and diarrhea respectively.

The oligoclonal T cells have the phenotype of activated T cells with high expression of CD69, CD71, HLA-DR, and/or CD25. The numbers of T cells can rise to over 50,000/mm^3. The T cell proliferative response to phytohemagglutinin (PHA) can rise to over 100,000 counts per minute (cpm). Clinical experience has shown that the longer these T cells are allowed to expand without suppression, the more difficult they are to suppress.

2.2.8.2. In the conduct of clinical protocol 884 (eIRB Pro00013734), it became clear that there was a great variability in the types and activation of T cells in the subjects being managed under this protocol.

2.2.8.2.1. The use of pre-transplantation rabbit anti-thymocyte globulin alone was insufficient to suppress the oligoclonal T cells found in atypical DiGeorge anomaly subjects.

2.2.8.2.2. If only pre-transplantation rabbit anti-thymocyte globulin (RATGAM) was used, the oligoclonal T cell caused pre- and post-transplantation morbidity (rash, hepatomegaly, and diarrhea). In addition, pre-transplantation cyclosporine helped decrease morbidity of the RATGAM by suppressing activated T cells.

2.2.8.2.3. For two subjects, very high levels of activated T cells remained after treatment with rabbit anti-thymocyte globulin, pre-transplantation cyclosporine and steroids. These two subjects were given anti-CD25 (daclizumab) prior to transplantation.

- Daclizumab decreased the numbers of oligoclonal activated T cells.

2.2.8.2.3.1. In one of these two subjects, persistent activated T cells were treated with a course of post-transplantation mycophenolate mofetil (MMF).

2.2.8.2.4. The thymus transplantation Data and Safety Monitoring Board recommended that different treatment regimens be established for subjects with different phenotypes and criteria be established for the use of rescue immunosuppressive medications. Thus, clinical protocol #950 was created and had three treatment groups based on immune characteristics, target goals for cyclosporine levels, and criteria for the use of 2 rescue medications. Under protocol 950, fourteen subjects were treated with a standard regime of immunosuppression. Ten subjects survive. One subject met the criteria for the higher doses of immunosuppression/and rescue immunosuppressive medications.

2.2.9. Therapies used in the past for complete DiGeorge anomaly with relevance to this patient population.

2.2.9.1. Immune reconstitution, Alternative Therapies.

2.2.9.1.1. A number of different therapies have been tried for pediatric patients with complete DiGeorge anomaly.

2.2.9.1.2. Janda et al (2010) published a survey of results for alternative therapies for 17 complete DiGeorge anomaly patients. The alternative therapies included bone marrow, unmobilized peripheral blood, and cord blood transplants. In the discussion section of the manuscript, Janda et al included information on previously published data from 9 patients (the data on the 9 subjects was included in a supplemental figure to this publication) with DiGeorge anomaly who received the same alternative therapies.

- From the cohort of 17 patients
  - The overall survival was 41% with a median follow up of 5.8 (4-11.5) years.

2.2.9.1.3. Janda et al reported the immune reconstitution data on the long term survivors with evaluable data (n=5) measured at last follow up. The median (range) for CD4 was 348 (225-782) cells/mm^3.

2.2.9.1.4. The data from Janda et al was extracted and the following information is based on our analysis of the data.

- *Survival:* (For our analysis, the survival data from the 17 patients and the previously published data on 9 patients [included in a supplemental figure to this publication] were combined for a total of 26 patients.)
  - Thirteen patients had HLA matched-related sibling bone marrow or peripheral blood donors. The survival after HLA matched–related sibling bone marrow or peripheral blood transplants was 8 of 13 (62%).
  - Eight patients had HLA matched-unrelated bone marrow or peripheral donors. The survival after HLA matched-unrelated bone marrow or peripheral transplants was 3 of 8 (38%).
  - Three patients had HLA matched-unrelated cord blood transplants. The survival rate was 1 of 3 (33%).
  - Two patients had parental HLA partially-matched bone marrow or peripheral blood transplants. Both patients died.

- *Immune Reconstitution*: (Our analysis of the Janda immune reconstitution data on 16 patients with available CD4 data published in this article.)
  - The mean CD4 count for survivors of sibling bone marrow or peripheral blood transplants (n=4) was 350 cells/mm^3.
  - The mean CD4 count for survivors of non-sibling bone marrow or peripheral blood transplants (n=6) was 418 cells/mm^3.
  - The overall mean CD4 count for survivors (n=10) was 390 cells/mm^3.

2.2.9.1.5. In contrast to these approaches, the thymus transplantation data from EAS-cDGA follow.

- *Survival:*
  - After thymus transplantation, survival is 73%
- *Immune Reconstitution:*
  - The mean CD4 count in all subjects (typical and atypical cDGA) after 1 year is 578 cells/mm^3 with data available from 54 subjects. This represents a mean increase of 406 cells/mm^{3}with data available from 48 subjects.

2.3. Summary of the known and potential benefits and risks, if any, to human subjects.
RETHYMIC (RVT-802) was approved by the US FDA for the treatment of pediatric congenital athymia in October 2021. The major risks and benefits relevant to the population treated in this study is in the US prescribing information (Appendix C).

2.3.1. Benefits and risks related to thymus transplantation.

2.3.1.1. Benefits.

2.3.1.1.1. Benefits of thymus transplantation are stated in 2.2 above and most importantly include an overall survival rate of 73% in the EAS-cDGA population compared to an expected 100% mortality without therapy. The median follow-up time for the EAS-cDGA population was 7.15 years (2612 days).

2.3.1.1.2. The benefits of thymus transplantation include the potential development of functional T cells and the ability of these cells to protect against infection.

2.3.1.2. Risks.

2.3.1.2.1. There are several known risks to subjects with complete DiGeorge anomaly even without therapy.

2.3.1.2.2. Development of rash, lymphadenopathy, and oligoclonal T cells prior to the development of normal T cells. These oligoclonal T cells can affect other organs, for example, the liver, resulting in elevated enzymes or serum bilirubin (Markert et al 2009). This is a risk prior to transplantation and continues for the initial months after transplantation (Davis et al 1997; Markert et al 2004a, 2004b, Markert et al, 2007). Approximately half of complete DiGeorge subjects who did not have a rash at the time of transplantation have developed a rash (that could be brief) at some point after transplantation. Lymphadenopathy and oligoclonal T cells have developed in those subjects who began with those problems prior to and after transplantation. The rash resolved when naïve T cells developed. The circulating T cells in all cases of pre-transplantation rash have been host in origin with two exceptions in subjects with maternal engraftment. Pediatric patients with rashes can have marked lymphadenopathy. Lymph nodes from three subjects who developed rashes prior to transplantation were examined and did not contain tumor. The rash and lymphadenopathy resolved in all survivors.

2.3.1.2.3. Autoimmunity is a risk for pediatric patients with complete DiGeorge anomaly after thymus transplantation. Autoimmunity is also seen in

partial DiGeorge anomaly. In particular, 20% of adults with partial DiGeorge anomaly due to 22q11 hemizygosity have thyroid disease (Bassett et al 2005). Animal studies suggest a role for the gene *Tbx1* in thyroid disease (Fagman et al 2007).

2.3.1.2.4. Hospitalizations for infections after discharge from DUMC but prior to development of functional T cells: These infections may include rotavirus and varicella and mycobacterium.

- It is important to note that many subjects have cleared infections they had at DUMC ***after*** normal T cells developed. These infections included RSV, parainfluenza virus, adenovirus, rotavirus, and mycobacterium.

2.3.1.2.5. Hospitalizations for infections after discharge from DUMC and after T cells developed: These infections have included RSV, respiratory infections partially related to aspiration, Herpes simplex virus, respiratory infection, varicella, and H1N1 swine flu. (In some cases, the hospitalizations were a precaution.)

2.3.1.2.6. Hospitalizations because of i) infections secondary to aspiration, ii) hypocalcemia (associated with minor infections), or iii) central line infections: These hospitalizations would have occurred even in children with no T cell problems and thus are considered separately.

2.3.1.2.7. There are other potential risks of thymus transplantation. These include:

- Infection from the thymus donor.
  - CMV infection occurred after thymus transplantation in one atypical subject. All the pre-transplantation testing of the thymus donor was negative for CMV. At 6 weeks after transplantation, the thymus donor had a detectable CMV PCR in the blood but a negative CMV urine culture. The level of CMV in the blood was too low to amplify for sequencing. It is unclear if the CMV infection came from the thymus donor, but this is possible.
- Infection from contamination of the tissue allograft during the culturing process. This is not known to have occurred to date.
- Graft versus host disease from donor T cells that were in the allograft when the tissue was transplanted. GVHD from thymus donor T cells has not been documented in any subject with complete DiGeorge anomaly who did not have prior attempts at immune reconstitution.
- Virus-associated lymphoproliferative process could develop associated with Epstein Barr virus (EBV) or cytomegalovirus (CMV) (Borzy et al 1979; Dictor et al 1984; Dimery et al 1988). This is not known to have occurred to date.

2.3.1.2.8. AEs in the EAS-cDGA have been evaluated for their relationship to thymus transplant and/or to the transplantation procedure. Table 2

presents the related AEs in the EAS-cDGA population. A total of 70 subjects reported 212 events that were considered related to the thymus transplant and/or the transplant procedure. The related events generally were distributed into 3 main categories: autoimmune events/diseases, complications of the transplant procedure itself, and events considered specifically related to T cells.

Table 2. Summary of Adverse Events Related to Treatment by System Organ Class

	EAS-cDGA (N=93)		
System Organ Class (SOC) Preferred Term (PT)	n	(%)	E
Number of Related Adverse Events [1]	70	(75.3)	212
Blood and lymphatic system disorders, n (%)	21	(22.6)	30
Thrombocytopenia	11	(11.8)	14
Neutropenia	6	(6.5)	6
Coombs positive haemolytic anaemia	2	(2.2)	2
Autoimmune haemolytic anaemia	1	(1.1)	1
Anaemia	2	(2.2)	2
Haemolysis	1	(1.1)	1
Haemolytic anaemia	2	(2.2)	2
Lymphadenopathy	1	(1.1)	1
Immune thrombocytopenic purpura	1	(1.1)	1
Skin and subcutaneous tissue disorders, n (%)	22	(23.7)	26
Rash	10	(10.8)	10
Urticaria	4	(4.3)	5
Alopecia	4	(4.3)	4
Dermatitis atopic	1	(1.1)	1
Erythema	1	(1.1)	1
Granuloma skin	1	(1.1)	1
Pigmentation disorder	1	(1.1)	1
Psoriasis	1	(1.1)	1
Rash papular	1	(1.1)	1
Skin mass	1	(1.1)	1
Immune system disorders, n (%)	18	(19.4)	20
Cytokine release syndrome	18	(19.4)	19
Hypersensitivity	1	(1.1)	1
Metabolism and nutrition disorders, n (%)	17	(18.3)	24
Hypomagnesaemia	13	(14.0)	13
Hyperglycaemia	3	(3.2)	3
Acidosis	2	(2.2)	2
Hypoalbuminaemia	2	(2.2)	2
Decreased appetite	1	(1.1)	1
Fluid retention	1	(1.1)	1
Hyperkalaemia	1	(1.1)	1
Hyponatraemia	1	(1.1)	1
Vascular disorders, n (%)	16	(17.2)	19
Hypertension	15	(16.1)	16
Thrombosis	2	(2.2)	2
Haematoma	1	(1.1)	1
Laboratory Investigations, n (%)	14	(15.1)	21
Alanine aminotransferase increased	4	(4.3)	4
Aspartate aminotransferase increased	4	(4.3)	4
Blood bicarbonate decreased	2	(2.2)	2
Blood creatinine increased	2	(2.2)	2

	EAS-cDGA (N=93)		
System Organ Class (SOC) Preferred Term (PT)	n	(%)	E
Lymphocyte morphology abnormal	1	(1.1)	2
Blood alkaline phosphatase increased	1	(1.1)	1
Blood chloride decreased	1	(1.1)	1
Blood cortisol decreased	1	(1.1)	1
Blood immunoglobulin E increased	1	(1.1)	1
Blood magnesium decreased	1	(1.1)	1
Lipase increased	1	(1.1)	1
White blood cell count decreased	1	(1.1)	1
Renal and urinary disorders, n (%)	10	(10.8)	15
Proteinuria	7	(7.5)	7
Acute kidney injury	3	(3.2)	3
Renal failure	3	(3.2)	3
Glomerulonephritis minimal lesion	1	(1.1)	1
Renal tubular acidosis	1	(1.1)	1
Gastrointestinal disorders, n (%)	6	(6.5)	9
Diarrhoea	1	(1.1)	1
Pancreatitis	3	(3.2)	3
Abdominal distension	1	(1.1)	1
Diarrhoea haemorrhagic	1	(1.1)	1
Enteritis	1	(1.1)	1
Gastrointestinal haemorrhage	1	(1.1)	1
Ileus	1	(1.1)	1
Respiratory, thoracic and mediastinal disorders, n (%)	11	(11.8)	11
Hypoxia	5	(5.4)	5
Respiratory failure	2	(2.2)	2
Respiratory distress	1	(1.1)	1
Sleep apnoea syndrome	1	(1.1)	1
Stridor	1	(1.1)	1
Tachypnoea	1	(1.1)	1
General disorders and administration site conditions, n (%)	5	(5.4)	5
Pyrexia	3	(3.2)	3
Face oedema	1	(1.1)	1
Oedema peripheral	1	(1.1)	1
Infections and infestations, n (%)	7	(7.5)	7
Cytomegalovirus infection	2	(2.2)	2
Cellulitis staphylococcal	1	(1.1)	1
Device related infection	1	(1.1)	1
Staphylococcal skin infection	1	(1.1)	1
Stitch abscess	1	(1.1)	1
Wound infection staphylococcal	1	(1.1)	1
Injury, poisoning and procedural complications, n (%)	5	(5.4)	5
Wound dehiscence	4	(4.3)	4
Venous injury	1	(1.1)	1
Nervous system disorders, n (%)	5	(5.4)	5
Seizure	2	(2.2)	2
Febrile convulsion	1	(1.1)	1
Infantile spasms	1	(1.1)	1
Myelitis transverse	1	(1.1)	1
Hepatobiliary disorders, n (%)	2	(2.2)	2
Autoimmune hepatitis	1	(1.1)	1
Hepatosplenomegaly	1	(1.1)	1
Endocrine disorders, n (%)	3	(3.2)	5
Hypothyroidism	2	(2.2)	2

	EAS-cDGA (N=93)		
System Organ Class (SOC) Preferred Term (PT)	n	(%)	E
Adrenal insufficiency	1	(1.1)	1
Basedow's disease	1	(1.1)	1
Hyperthyroidism	1	(1.1)	1
Musculoskeletal and connective tissue disorders, n (%)	3	(3.2)	3
Growth retardation	1	(1.1)	1
Juvenile idiopathic arthritis	1	(1.1)	1
Psoriatic arthropathy	1	(1.1)	1
Cardiac disorders, n (%)	2	(2.2)	2
Sinus tachycardia	2	(2.2)	2

Abbreviations: AE = adverse event; E = number of events; EAS-cDGA = efficacy analysis set for complete DiGeorge anomaly subjects; 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. 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

2.4. Description of and justification for the route of administration, dosage, dosage regimen, and treatment periods.

2.4.1. Thymus.

2.4.1.1. The thymus tissue for transplantation is implanted into furrows in the quadriceps muscles in one or both legs. This site was chosen since it is one of the larger muscles in pediatric patients. As muscles have a good blood supply, this allows quick development of capillaries into the tissue graft. In addition, this site is easily accessed for biopsy. Thymus transplantation currently has a dose range of 4500 to 22000 mm^2 per meter squared (m^2) of recipient body surface area (BSA). The tissue must fit into the quadriceps muscle. As of August 2020, in 93 pediatric patients (EAS-cDGA) receiving measured thymus tissue, the mean dose has been 13087 mm^2 per m^2 BSA (SD 4392 mm^2 per m^2 BSA).

2.4.2. For typical subjects with a proliferative response to PHA of >5000 cpm and atypical subjects, three doses of rabbit anti-thymocyte globulin (2 mg/kg/dose IV) are administered to the subject prior to thymus transplantation in Groups 2, 3, and 4.

- This is usually administered on days -5, -4, and -3 followed by two days of rest and the transplant is done. The actual timing of the doses of rabbit anti-thymocyte globulin depends on the medical condition of the subject and other logistical issues.
- The rationale for this dose and schedule is based on experience with 55 subjects.

2.4.3. Cyclosporine is started as soon as the diagnosis of atypical complete DiGeorge anomaly is made and at least 7 days prior to rabbit anti-thymocyte globulin administration for Groups 3 and 4. Depending on the Group that the subject is in, the cyclosporine trough level range of approximately 180 to 300 ng/ml is the goal.

- If the levels are outside of the target range, dosing will be modified appropriately (see details below).
- If the T cells fall and remain below 50/mm^3, the cyclosporine will then be weaned to have a cyclosporine trough level of 100 to 150 ng/ml.
- If the T cell count remains over 50/mm^3, the cyclosporine is maintained until the naive T cells are 10% of CD3 T cells. The cyclosporine will then be weaned over 10 weeks.
- The rationale for the use of cyclosporine is based on the experience with subjects with atypical complete DiGeorge anomaly. Survivors with atypical DiGeorge anomaly who received cyclosporine developed normal T cell function after transplantation.

2.4.4. If the subject cannot tolerate cyclosporine secondary to adverse events, then cyclosporine may be changed to treatment with tacrolimus (FK506).

2.4.4.1. The target tacrolimus trough level is 7 to 10 ng/ml. When trough levels are outside of the target range, dosing will be modified appropriately.

2.4.5. The rationale for the use of mycophenolate mofetil for subjects in Group 4 is that it is a fairly low risk immunosuppressive agent that may add to the immunosuppressive effects of cyclosporine and steroids.

2.4.6. Some atypical complete DiGeorge anomaly subjects develop oligoclonal T cells post implantation while on cyclosporine. Addition of steroids in this situation usually controls expansion of the oligoclonal T cells.

2.4.7. Because of the morbidity associated with oligoclonal T cells in subjects with atypical complete DiGeorge anomaly, cyclosporine and steroids may be started prior to arrival at Duke.

2.5. The trial will be conducted in compliance with the protocol, GCP and the applicable regulatory requirements such as current Good Tissue Practice, and FDA guidelines for suitability for tissue donors.

2.6. Description of the population to be studied.

2.6.1. Immune Dysfunction.

2.6.1.1. DiGeorge anomaly is a congenital disorder characterized by T cell deficiency secondary to thymic hypoplasia, hypoparathyroidism, and cardiac defects (DiGeorge 1965; Conley et al 1979). Approximately 1% of patients with DiGeorge anomaly have athymia and are considered to have "complete" DiGeorge anomaly (Ryan et al 1997; Markert et al 1998; Bastian et al 1989). The evidence for athymia is the absence (<50/mm^3 or < 5%) of peripheral T cells co-expressing CD45RA and CD62L, markers of recent thymic emigrants (Picker et al 1993). Other evidence for athymia is the very low level (<100/100,000 T cells) of T cell receptor rearrangement excision circles (TRECs). TRECs are episomes which form when T cell receptor genes rearrange in the thymus and are detectable in recent thymic emigrants in peripheral blood. Patients with athymia fall into two phenotypes that we describe as "typical" and "atypical" (Markert et al 2004a;

Markert et al 2007; Markert et al 2010). See section 2.6.1.4 and 2.6.1.5 for definitions of typical and atypical complete DiGeorge anomaly.

2.6.1.2. Many patients with DiGeorge anomaly are hemizygous for 22q11 (Driscoll et al 1992, Carey et al 1992). A small number are hemizygous for 10p13 (Daw et al 1996). DiGeorge anomaly is also found in conjunction with CHARGE association (coloboma, heart defect, choanal atresia, growth and development retardation, genitourinary defects, ear defects including deafness) (Pagon et al 1981; Davenport et al 1986; Metlay et al 1987; Lin et al 1987; de Lonlay-Debeney et al 1997; Black et al 1998). A significant percentage of patients with CHARGE and cleft palate have mutations in CHD7 (Vissers et al 2004). Another group of patients are infants of diabetic mothers (Gosseye et al 1982; Wang et al 2002). Rare children have been described with mutations in *Tbx1* and UFD1L (Yamagishi et al 1999; Yagi et al 2003). Regarding the prevalence of these conditions in complete DiGeorge anomaly, the sponsor has found (based on 72 subjects with complete DiGeorge anomaly) 46% with 22q11 hemizigosity, 28% with CHARGE association, 14% with diabetic embryopathy, 1% with ectodermal dysplasia, and 11% with no syndromic associations (Markert et al 2003; Markert et al 2004a; Markert et al 2004b; Markert et al 2007; and unpublished data).

2.6.1.3. All children with athymia suffer from recurrent infections and usually die in the first two years of life.

2.6.1.4. **Patients with Typical complete DiGeorge Anomaly** usually have a) less than 50 naive T cells/mm^3 or naive T cells that are less than 5% of T cells and b) a low proliferative responses to mitogens (e.g. < a 20 fold response to the mitogen phytohemagglutinin or a response of fewer than 5,000 cpm). These patients do not have diffuse rashes or lymphadenopathy. Occasional patients with typical complete DiGeorge anomaly have proliferative responses to mitogens. Patients with the typical phenotype can develop an atypical phenotype with time.

2.6.1.5. **Patients with Atypical complete DiGeorge Anomaly** meet the criteria for athymia (less than 50/mm^3 naive T cells or less than 5% of the T cells having the naive phenotype). Patients with the atypical phenotype have developed rash, lymphadenopathy, and oligoclonal T cells. The T cells may infiltrate the liver resulting in elevated liver transaminases. The oligoclonal T cells developing in patients with DiGeorge anomaly may or may not respond to the mitogen phytohemagglutinin (PHA) *in vitro*.

2.6.1.6. The study will include
2.6.1.6.1. Subjects with *typical* phenotype of complete DiGeorge anomaly.

2.6.1.6.2. Subjects with *atypical* phenotype of complete DiGeorge anomaly.

2.6.2. The incidence and survival of patients with complete DiGeorge anomaly who are

not given immune reconstitution therapy have not been well studied. Driscoll and Sullivan (1999) estimate that approximately 1 in 4,000 live births have 22q11 hemizygosity. Only 1 to 2 in 500 of those will have the profound immunodeficiency of complete DiGeorge anomaly (Ryan et al 1997; and Dr. K. Sullivan, Children's Hospital of Pennsylvania, Philadelphia PA, personal communication).

2.6.2.1. The original investigator-sponsor, Dr. Markert, reviewed survival data on 34 consecutive pediatric patients with complete DiGeorge anomaly who did not undergo treatment for restoration of the immune system, but were only provided supportive care. At the time of the review, ten of these pediatric patients were not yet enrolled but were awaiting insurance approval for thymus transplantation. The physicians of all 34 pediatric patients had contacted Dr. Markert about possible enrollment in the thymus transplantation protocol. Nine of these 34 pediatric patients had preexisting CMV infection or ventilator dependence and are thus excluded from this historical control group (because these conditions are exclusions for groups 2, 3, and 4 in this protocol). Outcomes were determined by Dr. Markert by email or by discussion with the pediatric patient's physician. Of the 25 subjects, who did not have CMV or ventilator dependence, 17 died. Eight of the 25 were living and one was over 1 year. Of the 18 who either died (n=17) or were surviving past 1 year (n=1), 5 of 18 (28%) survived to one year, thus, the one year death rate was 72%. The median survival of these 18 subjects was 7.1 months. No subject had survived past 18.8 months.

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3. Trial Objectives.
   Survival, naïve CD4 T cell counts, and the effect of thymus graft dose on naïve CD4T cell counts will be assessed at one year after transplantation. Additional descriptive studies will be performed.

   Rationale: The immune monitoring will be limited to one year after thymus transplantation. Cumulative data on the previous thymus transplant recipients (60 subjects from 5 protocols 'Thymus Transplantation in Complete DiGeorge, Protocol #668'; 'Thymus Transplantation and Immunosuppression, #884'; 'Parathyroid and Thymus Transplants in DiGeorge Syndrome, #931'; 'Dose Study of Thymus Transplantation in DiGeorge Anomaly, IND #9836, #932'; and, 'Phase I/II Trial of Thymus Transplantation with Immunosuppression, #950')

support the change from long term monitoring to one year after transplantation. Although subjects do not establish immunological stability (i.e. stable T cell counts and B cell function) until almost 2 years after transplantation, the one year parameters of T cell counts, proliferative responses to antigens, T cell receptor excision circles (TRECs), and T cell receptor diversity are positive indicators of successful immune reconstitution.

3.1. Assess survival at 1 year.
  3.1.1. The primary hypothesis is that greater than 50% of subjects will survive thymus transplantation.

3.2. Assess naïve CD4 T cell counts at 12 months post-thymus transplantation.
  3.2.1. A secondary hypothesis is that greater than 50% of subjects will have >100 naïve CD4 T cells at one year post-transplantation.

  3.2.2. Another secondary hypothesis is that the dose of thymus tissue transplanted will affect the naïve CD4 count at one year.

3.3. Perform descriptive studies.
  3.3.1. Tabulate immune outcomes at 12 months.

  3.3.2. Tabulate the presence of donor T cells in the blood at 3 months post transplantation.

  3.3.3. Tabulate graft versus host disease in first 12 months post transplantation.

  3.3.4. Tabulate infection rates in first 12 months post transplantation.

  3.3.5. Tabulate autoimmune disease in first 12 months post transplantation.

  3.3.6. Tabulate persistent rashes present in first 12 months post transplantation.

  3.3.7. Tabulate other adverse events that are possibly, probably or definitely related to the thymus transplant detected in first 12 months post transplantation.

4. Trial Design.
  4.1. Primary and secondary endpoints.
    4.1.1. Primary endpoint.
      4.1.1.1. Survival at 1 year after transplantation. Survival at 2 years after transplantation will be analyzed as supportive data.

4.1.2. Secondary endpoints include the following, as data permit.

Secondary Endpoints (at year 1 unless otherwise specified)	
CD3 T cells	6 and 12 months
CD4 T cells	6 and 12 months
CD8 T cells	6 and 12 months
naïve CD4 T cells	6 and 12 months
naïve CD8 T cells	6 and 12 months
Total TCRαβ T cells	6 and 12 months
Total TCRγδ T cells	6 and 12 months
Total B cells	6 and 12 months
Total NK cells	6 and 12 months
Proliferative T cell responses to stimulants including: phytohemagglutinin (PHA), Concavalin A (ConA), immobilized CD3, soluble CD3, tetanus toxoid, and Candida	X
TCR repertoire variability	X
TREC/TREG	X
Biopsy of Transplanted Thymus[1]	X
[1] For biopsy results, the following data will be recorded for each subject: presence of thymopoiesis, Hassall bodies, and graft rejection.	

4.1.3. Safety Endpoints include the following, as data permit:

Safety Endpoints	**25966**
Vital Signs	X
Adverse Events / Serious Adverse Events	X
Infection-Related Adverse Events	X
Adverse Events of Special Interest (Protocol Defined)[1]	X
Medical History	X
Clinical Chemistry	X
Liver Function Studies	X
Thyroid Studies	X
Hematology	X
[1] Adverse events of special interest include: GVHD, infections, rashes, cancers, and granulomas.	

4.2. Study design.

4.2.1. The study is considered an expanded access protocol to allow continued treatment of subjects with thymus tissue; however, the study is still intended to generate data on the safety and efficacy of thymus tissue for transplantation. This is a Phase I/II, single site, open, nonrandomized clinical protocol done at Duke University Medical Center. All subjects will have been diagnosed as having complete DiGeorge anomaly, characterized by very low T cell numbers (<50/mm^3) or less than 50/mm^3 naive T cells ($CD3^+CD45RA^+CD62L^+$) or naïve T cells being less than 5% of total T cells. Subjects will be enrolled in one of four Groups. Subjects will receive a postnatal cultured allogeneic thymus transplant with or without immunosuppression prior to transplantation.

4.2.2. Four Groups will be utilized for the following populations.

4.2.2.1. Group 1.

4.2.2.1.1. Subjects.

4.2.2.1.1.1. Subjects with ***typical*** complete DiGeorge anomaly whose T cells have a response of less than 5,000 counts per minute (cpm) to PHA and less than a 20 fold response to PHA.

4.2.2.1.1.1.1. If the response increases prior to transplantation to over 5,000 cpm, the subject will be put in Group 2.

4.2.2.1.2. Group 1 will receive

4.2.2.1.2.1. No pre or post-transplantation immunosuppression.

4.2.2.2. Group 2.

4.2.2.2.1. Subjects.

4.2.2.2.1.1. *Subjects with* ***typical*** complete DiGeorge anomaly whose T cells have a response of greater than 5,000 cpm and less than 50,000 cpm to PHA and greater than a 20 fold response to PHA

4.2.2.2.1.1.1. If the response increases prior to transplantation to over 50,000 cpm, the subject will be moved to Group 3.

4.2.2.2.2. Group 2 will receive

4.2.2.2.2.1. Three doses of 2 mg/kg of rabbit anti-thymocyte globulin IV pre-transplantation. Medications (diphenhydramine, steroids, and acetaminophen) are given with rabbit anti-thymocyte globulin.

4.2.2.2.2.2. No additional pre or post-transplantation immunosuppression.

4.2.2.3. Group 3.

4.2.2.3.1. Subjects.

4.2.2.3.1.1. *Subjects with **typical*** complete DiGeorge anomaly whose T cells have a response of more than 50,000 cpm to PHA.

4.2.2.3.1.1.1. Subjects may first appear to be in Group 2 but will be changed to Group 3 if the PHA response increases to over 50,000 cpm.

4.2.2.3.1.2. *Subjects with **atypical*** complete DiGeorge anomaly whose T cells have a response of less than 40,000 cpm in response to PHA when on immunosuppression or less than 75,000 cpm to PHA when not on immunosuppression.

4.2.2.3.1.3. *Subjects with **typical*** complete DiGeorge anomaly who have maternal engraftment.

4.2.2.3.1.4. *Subjects with **atypical*** complete DiGeorge anomaly with maternal engraftment who have a PHA response that meets criteria for Group 3.

4.2.2.3.1.5. If the atypical subject has an initial PHA of less than 75,000 cpm prior to immunosuppression but is greater than 40,000 cpm to PHA while on cyclosporine with trough levels of approximately 180 to 220 ng/ml and on 2 mg/kg/day of steroids, the subject will be moved into Group 4 for additional immunosuppression in the peri-transplant period.

4.2.2.3.2. Group 3 will receive

4.2.2.3.2.1. Pre-transplant cyclosporine is started when medically indicated and is based on symptoms and flow cytometry results performed in a CLIA or CAP Certified Laboratory.

4.2.2.3.2.2. The cyclosporine is continued with target trough levels of 180 to 220 ng/ml. When trough levels are outside of this target range, dosing will be modified appropriately.

4.2.2.3.2.2.1. If the subject cannot tolerate cyclosporine secondary to adverse events, then the immunosuppression may be changed to tacrolimus (FK506). The tacrolimus target trough level is 7 to 10 ng/ml. When trough levels are outside of this target range, dosing will be modified appropriately.

4.2.2.3.2.3. Pre-transplant steroids (methylprednisolone or prednisolone) are used for atypical subjects if pre-transplantation T cell numbers are greater than 4,000/mm^3.

4.2.2.3.2.4. For all subjects, three doses of 2 mg/kg of rabbit anti-thymocyte globulin IV are given pre-transplantation. Medications (diphenhydramine, steroids, and acetaminophen) are given with rabbit anti-thymocyte globulin.

4.2.2.4. Group 4.

4.2.2.4.1. Subjects.

4.2.2.4.1.1. *Subjects with **atypical*** complete DiGeorge anomaly whose T cells have a response of greater than 40,000 cpm in response to PHA when on immunosuppression with cyclosporine and steroids or a response of greater than 75,000 cpm in response to PHA with no immunosuppression.

4.2.2.4.1.2. *Subjects with **atypical*** complete DiGeorge anomaly and maternal engraftment who have a PHA response that meets criteria for Group 4

4.2.2.4.2. Group 4 will receive

4.2.2.4.2.1. Immunosuppression with cyclosporine and steroids (methylprednisolone or prednisolone) is started when medically indicated and is based on symptoms and flow cytometry results performed in a CLIA or CAP Certified Laboratory.

4.2.2.4.2.1.1. After PHA is documented at over 40,000 cpm on suppression, pre-transplant cyclosporine is maintained with target trough levels of 250 to 300 ng/ml. (When trough levels are outside of this range, the dose will be modified appropriately.)

4.2.2.4.2.1.2. If the subject cannot tolerate cyclosporine due to adverse events, the immunosuppression may be changed to tacrolimus (FK506). The target trough level for FK506 is 10 to15 ng/ml. When trough levels are outside of this target range, dosing will be modified appropriately.

4.2.2.4.2.2. Three doses of 2 mg/kg of rabbit anti-thymocyte globulin are given IV pre-transplantation. Medications (diphenhydramine, steroids, and acetaminophen) are given with rabbit anti-thymocyte globulin.

4.2.2.4.2.3. Additional immunosuppression.

4.2.2.4.2.3.1. Mycophenolate Mofetil (MMF), 15 mg/kg/dose every 8 hours IV or enteral.

4.2.2.4.2.3.1.1. May be given if T cells remain elevated 5 days after rabbit anti-thymocyte globulin is given.

4.2.2.4.3. Proliferative Response (PHA).
4.2.2.4.3.1. Groups 1, 2, 3 and 4.
4.2.2.4.3.1.1. PHA assays must be done prior to transplantation.

4.2.2.4.3.2. For Groups 3 and 4.
4.2.2.4.3.2.1. PHA assays are not required before starting cyclosporine or steroids.

4.2.2.4.4. Flow cytometry is conducted in a CLIA or CAP Certified Laboratory before or as soon as possible after initiation of cyclosporine or steroids.
4.2.2.4.4.1. The flow cytometry is expected to show naive T cells less than 5% of T cells or < 50/mm^3.

4.2.2.5. Because of the morbidity associated with oligoclonal T cells in subjects with atypical complete DiGeorge anomaly, subjects in Group 3 and 4 may start cyclosporine and steroids as part of clinical management and before enrollment in this study.

4.2.3. Subjects will receive discarded thymus tissue from infants undergoing heart surgery. The tissue is sliced and held in culture for up to 21 days. After testing for infectious agents, the thymus slices are surgically implanted into the DiGeorge anomaly subject's quadriceps muscles in one or both legs. A thymus allograft biopsy may be performed at approximately 2 months post-transplantation. If a biopsy is performed, the biopsied tissue is examined for evidence of thymopoiesis and graft rejection by immunohistochemical staining for the presence of cytokeratin, a marker of thymic epithelium, and for T cells populating the thymus.

4.2.4. Immune monitoring: The immune status of the recipient is monitored by the number and phenotype of T cells as measured at 6 and 12 months and the proliferative response of the T cells to mitogens.

4.2.5. Clinical monitoring: During the first 2 to 3 months of the post-transplantation period, the recipient will be monitored for evidence of GVHD, lung or breathing problems that may result from inflammation of the lungs by T cells, rashes and lymphadenopathy infections by routine methods when symptoms develop, and autoimmune disease by monitoring complete blood counts, thyroid function, liver function, and renal function.

4.2.6. There is no enrollment goal nor requirement for replacement in any Group.

4.3. Measures to minimize bias.
4.3.1. This is an open label study. Subjects meeting eligibility criteria will be enrolled. The primary safety endpoint is survival at one year.

4.4. Description of trial treatment, dosage and dosage regimen, description of the dosage form, packaging, and labeling.
4.4.1. Thymus for transplantation.
4.4.1.1. The trial treatment is thymus transplantation using allogeneic cultured

postnatal tissue from unrelated donors ≤9 months of age. The thymus tissue from the donor is processed in the GMP Laboratory as per standard operating procedures.

The surface area of tissue used for transplantation is provided by the manufacturer. The thymus tissue dose used in transplantation is 4,500 to 22,000 mm^2 of thymus tissue per recipient BSA in m^2.

4.4.1.2. This is a single site trial.

4.5. Expected duration of subject participation.

4.5.1. Participation in this study will last for 2 years after thymus transplantation.

4.5.2. Follow-up in this study will be completed after the 1 year flow cytometry and PHA testing is done and the 2 year telephone contact is done.

4.5.3. Subjects may be asked to enroll in a Congenital Athymia Patient Registry.

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

4.6.1. All serious adverse events (SAE) that are unanticipated but related to the investigational interventions are reviewed by Enzyvant, the IRB, and FDA. These do not stop enrollment unless the Sponsor, Principal Investigator, FDA, or IRB requests this. Examples of SAE are GVHD and cancer.

4.6.2. Survival will be assessed after transplantation of 8, 16 and 25 subjects who do not have CMV infection. The protocol will be placed on hold for enrollment and transplantation if 7 of the first 8 transplanted subjects, 11 of the first 16 transplanted subjects, or 15 of the first 25 transplanted subjects die within the first 12 months after transplantation. If this occurs, consultation with the FDA will be undertaken and a plan developed. The plan and any changes to the protocol documents will be submitted to the FDA, and IRB prior to removal of the hold on new enrollment and transplantation.

4.6.3. The year one naïve CD4 T cell counts will be assessed after 8, 16, and 25 subjects reach 1 year after transplantation. The protocol will be put on hold for enrollment and transplantation if 7 of the first 8 survivors at one year, or 11 of the first 16 survivors at one year or 15 of the first 25 survivors at one year have ≤100/mm^3 naïve CD4 T cells at 12 months after transplantation. The naïve CD4 count (≤100/mm^3) applies whether or not all immunosuppression has been weaned. If a hold occurs, consultation with the FDA will be undertaken and a plan developed. The plan and any changes to the protocol documents will be submitted to the FDA, and IRB, prior to removal of the hold on new enrollment and transplantation.

Naïve CD4 T cells are those $CD4^+$ T cells coexpressing CD45RA and CD62L.

- The treatment groups are
  - ⇒ Group 1. No immunosuppression
  - ⇒ Group 2: Rabbit anti thymocyte globulin alone

⇒Group 3: Rabbit anti thymocyte globulin plus cyclosporine
⇒Group 4: Rabbit anti thymocyte globulin plus cyclosporine plus mycophenolate

4.6.4. Although not a stopping rule for the entire protocol, there will be a stopping rule for enrollment of CMV infected subjects with typical complete DiGeorge anomaly. Please see section 9.

4.7. Data on Case Report Forms (CRF).
4.7.1. All data on the CRF are derived from source data in the medical chart. Source data may be obtained from the subject's DUMC medical record and/or data obtained from the local physician(s)/hospital. Data from outside sources are placed in the subject's research record.

5. Selection and Withdrawal of Subjects. Note that at the time of this amendment (November 2021) the clinical study is closed to enrollment.
5.1. Selection. Potential subjects referred with the diagnosis of DiGeorge anomaly who meet the criteria below will be considered for enrollment into this study.
5.1.1. Primary Diagnosis.
5.1.1.1. The subject must have DiGeorge anomaly.

5.1.1.2. The subject may have DiGeorge anomaly as part of 22q11.2 or 10p13 hemizygosity or CHARGE association (with or without CHD7 mutations) or diabetic embryopathy.

5.1.2. Number of Subjects.
5.1.2.1. There is no enrollment goal nor requirement for replacement in any Group.

5.1.2.2. Subjects with typical and atypical complete DiGeorge anomaly will be enrolled into one of the four Groups.

5.1.3. Inclusion and Exclusion Criteria.
5.1.3.1. Inclusion Criteria for Thymus Graft Recipients.
5.1.3.1.1. A parent or guardian of the potential subject with DiGeorge anomaly must sign the consent form.

5.1.3.1.2. To fit the diagnosis of DiGeorge anomaly, the subject must have one symptom from the following list
- Congenital heart disease
- Hypocalcemia requiring replacement
- 22q11.2 hemizygosity or 10p13 hemizygosity
- CHARGE association (Pagon et al 1981; Lin et al 1987; DeLonlay-Debeney et al 1997; Blake et al 1998) or CHD7 mutation (Vissers et al 2004)
- Although not sufficient to make the diagnosis of complete DiGeorge anomaly, a subject with abnormal ears plus mother with diabetes (type I, type II, or gestational) (Gosseye et al 1982; Wang et al 2002) will have this risk factor recorded.

5.1.3.1.3. To Fit the Category of ***Typical*** complete DiGeorge anomaly
5.1.3.1.3.1. Circulating $CD3^+$ $CD45RA^+$ $CD62L^+$ T cells will be <50/mm^3 or <5% of the total T cell count.
5.1.3.1.3.1.1. The phenotypic evaluation of T cells is done by flow cytometry.
5.1.3.1.3.1.2. Flow cytometry must be performed twice.

5.1.3.1.3.1.3. Two studies must show similar immunological findings.

5.1.3.1.3.1.4. One assay must be done within 3 months of transplantation.

5.1.3.1.3.1.5. One assay must be within 1 month of transplantation.

5.1.3.1.3.1.6. These assays will be done in a Clinical Laboratory Improvement Amendments (CLIA) Certified Laboratory, or a College of American Pathologist (CAP) Certified Laboratory.

5.1.3.1.3.2. PHA proliferative response.
5.1.3.1.3.2.1. For Group 1.
5.1.3.1.3.2.1.1. T cell proliferative response to PHA of <5,000 cpm or less than 20 fold above background.

5.1.3.1.3.2.2. For Group 2.
5.1.3.1.3.2.2.1. T cell proliferative response to PHA of > 5,000 cpm and <50,000 cpm or >20 fold over background.

5.1.3.1.3.2.3. For Group 3.
5.1.3.1.3.2.3.1. T cell proliferative response to PHA of >50,000 cpm.

5.1.3.1.3.2.4. For all three Groups, two assays of T cell numbers and PHA responses must show similar immunological findings (eg both must meet criteria).
5.1.3.1.3.2.4.1. One assay must be done within 3 months of transplantation.

5.1.3.1.3.2.4.2. One assay must be done within 1 month of transplantation.

5.1.3.1.3.2.4.3. Assays will be done in a CLIA or CAP Certified Laboratory.

5.1.3.1.3.3. Optional tests in subjects with typical complete DiGeorge anomaly.
5.1.3.1.3.3.1. TRECS (T cell receptor rearrangement excision circles)
5.1.3.1.3.3.1.1. TRECs are not assayed prior to transplantation.

5.1.3.1.3.3.1.2. Newborn screening or other TREC data, if available,

may be recorded.

5.1.3.1.4. To Fit the Category of ***Atypical*** complete DiGeorge anomaly.

5.1.3.1.4.1. Rash.

5.1.3.1.4.1.1. The subject must have, or have had, a rash. If the rash is present, a biopsy of the rash must show T cells in the skin. If the rash and adenopathy have resolved, the subject must still have >50/mm^3 T cells and the naive T cell (CD45RA$^+$ CD62L$^+$ CD3$^+$ T cells) count must be <50/mm^3 or <5% of the T cells.

5.1.3.1.4.1.2. Usually there is lymphadenopathy. If the rash has just developed, the subject may not yet have lymphadenopathy.

5.1.3.1.4.2. PHA proliferative response.

5.1.3.1.4.2.1. For Group 3, the PHA proliferative response must be less than 40,000 cpm on immunosuppression or less than 75,000 cpm off immunosuppression.

5.1.3.1.4.2.2. For Group 4, the PHA proliferative response must be greater than 40,000 cpm on immunosuppression or greater than 75,000 cpm off immunosuppression.

5.1.3.1.4.2.3. This assay must be done *twice*.

5.1.3.1.4.2.3.1. One assay must be done within 3 months of transplantation

5.1.3.1.4.2.3.2. One assay must be done within 1 month of transplantation.

5.1.3.1.4.2.3.3. The last assay is used to assign the Group.

5.1.3.1.4.2.3.4. These assays will be done in a CLIA or CAP Certified Laboratory.

5.1.3.1.4.3. Circulating CD3$^+$ T cells.

5.1.3.1.4.3.1. Circulating CD3 T cells usually will be > 50/mm^3 *but* CD45RA$^+$ CD62L$^+$ CD3$^+$ T cells must be < 50/mm^3 or < 5% of the total CD3 count.

5.1.3.1.4.3.2. The phenotypic evaluation of T cells is done twice by flow cytometry.

5.1.3.1.4.3.2.1. One assay must be done within 3 months of transplantation.

5.1.3.1.4.3.2.2. One assay must be done within 1 month of transplantation.

5.1.3.1.4.3.2.3. These assays will be done in a CLIA or CAP Certified Laboratory.

5.1.3.1.4.4. TCRBV Flow cytometry.

5.1.3.1.4.4.1. Examines T cell receptor beta variable (TCRBV) repertoire.

5.1.3.1.4.4.2. This assay may be done once but there is no requirement regarding the results. It is anticipated that the TCRBV flow will show oligoclonality.

5.1.3.1.4.4.3. A second assay may be done per Principal Investigator's discretion.

5.1.3.1.4.4.4. This is done prior to transplantation if there are sufficient T cells.

5.1.3.1.4.4.5. If there is an increase in T cell numbers or activation status, this assay is repeated at the discretion of the Principal Investigator.

5.1.3.1.4.5. TRECS (T cell receptor rearrangement excision circles)

5.1.3.1.4.5.1. There is no requirement for a TREC assay.

5.1.3.1.4.5.2. Newborn screening or other TREC data, if available, may be recorded.

5.1.3.1.5. Medical screening as defined in 6.2.1.2 (Pre-transplantation medical screening) is complete.

5.1.3.1.6. The subject can be male or female.

5.1.3.1.7. All ages.

5.1.3.2. Exclusion Criteria for Thymus Graft Recipient.

5.1.3.2.1. Heart surgery conducted less than 4 weeks prior to projected transplantation date.

5.1.3.2.2. Heart surgery anticipated within 3 months after the proposed time of transplantation.

5.1.3.2.3. Rejection by the surgeon or anesthesiologist as surgical candidate.

5.1.3.2.4. Lack of sufficient muscle tissue to accept a transplant.

5.1.3.2.5. HIV infection.

5.1.3.2.6. Prior attempts at immune reconstitution, such as bone marrow transplant

or previous thymus transplantation.

5.1.3.2.7. CMV Infection.
5.1.3.2.7.1. For Group 2, 3, and 4, CMV infection is documented by >500 copies/ml in blood by PCR on two consecutive assays.

5.1.3.2.8. Ventilator Dependence or Positive Pressure Support.
5.1.3.2.8.1. Ventilator support or positive pressure support, such as Continuous Positive Airway Pressure (CPAP) or Bi-level Positive Airway Pressure (BiPAP) support for a condition that is deemed to be severe or irreversible or which renders the subject too clinically unstable to undergo the procedures.

5.2. Withdrawal/Replacement of Subjects from Trial.
5.2.1. Removal of Subjects.
5.2.1.1. A subject may withdraw or be withdrawn from the study for any of the following reasons:
- if the parent(s) will not comply with the protocol, or
- if there are medical reasons not to proceed, or
- at the sponsor's discretion, or
- withdrawal of consent for any reason.

5.2.1.1.1. In all cases of study withdrawal, permission would be requested to receive updates on the subject's condition. This information would allow the Principal Investigator to better counsel parent(s) in the future.

5.2.1.1.2. With respect to the data analysis, any subject withdrawn because of lack of function of the thymus will be included in the data analysis. Their final data point at the time of withdrawal or at one year (if the parent(s) give permission for this sample and if no other treatment has been given) will be used in the calculations.

5.2.2. Replacement of Subjects.
5.2.2.1. There is no requirement for replacement of subjects.

6. Treatment of Subjects. Note that at the time of this amendment (November 2021) the clinical study is closed to enrollment. Patients currently remaining in the trial are all in the Post-Transplantation Period and have been returned to Care of Referring/Local Physician.

6.1. Assessment Periods-Thymus Graft Recipient.
6.1.1. Pre Transplantation Period.
6.1.1.1. This phase usually lasts from 2 to 8 weeks. The recipient is enrolled and screened. The recipient screening can be done as an inpatient or an outpatient.

6.1.2. Day of Operative Procedure(s).
6.1.2.1. Immediately after thymus transplantation, the subject will be an inpatient until medically stable.

6.1.3. Post Transplantation Period (From thymus transplantation to 24 months post-thymus transplantation).

6.1.3.1. Duke University Medical Center (DUMC).

6.1.3.1.1. The subject may be followed as an inpatient or outpatient.

6.1.3.2. Care of Referring/Local Physician.

6.1.3.2.1. The subject is usually discharged to home/local hospital (medical care provided by local immunologist or other specialist such as hematologist or bone marrow transplant physician) after the subject is medically stable.

6.1.3.2.2. The referring immunologist (or other specialist) should see the subject at least monthly for the first year and then annually.

6.1.3.2.3. After transfer back to the care of the immunologist (or other specialist), the subject is monitored for adverse events by requesting information from the local physicians.

6.1.3.2.4. After transfer back to the care of the referring immunologist (or other specialist), local testing results and/or blood samples for flow cytometry are requested. Blood draws and testing are dependent on the parent, the subject, and the local physicians.

6.2. Plan of treatment. See timelines for thymus recipient (Appendix A).

6.2.1. Thymus Graft Recipient.

6.2.1.1. Pre-transplantation enrollment and screening of thymus graft recipient.

6.2.1.1.1. The consent form must be signed before screening testing and thymus transplantation can be done. If immunosuppression is started for medical reasons, the consent form does not need to be signed prior to initiation of immunosuppression.

6.2.1.1.2. After consent is obtained, the unique ID# and the subject ID# are assigned per Standard Operating Procedure and entered in the password protected enrollment log.

6.2.1.1.3. The PI and/or designee completes the Determination of Eligibility Form.

6.2.1.2. Pre-transplantation medical screening.

6.2.1.2.1. The PI and/or the study staff reviews laboratory values.

6.2.1.2.2. For subjects on calcium replacement.

6.2.1.2.2.1. Urine for calcium/creatinine ratio. The urine for calcium/creatinine ratio is obtained initially and then every 1 to 2 weeks until transplantation. The ratio is kept less than 0.4 for older children and <0.6 for infants under the age of 6 months if possible, to prevent nephrocalcinosis. Additional testing is done if the ratio is too high.

6.2.1.2.3. Physical Examination.

6.2.1.2.3.1. The Principal Investigator or the study staff will perform a history and physical examination at enrollment.

6.2.1.2.3.2. Physical examinations will be done monthly prior to transplantation when at Duke.

6.2.1.2.4. The following studies are completed within ***one month*** prior to thymus transplantation.

6.2.1.2.4.1. Complete Blood Count (CBC) with Manual Differential (MD).

6.2.1.2.4.1.1. Weekly prior to transplantation or as medically indicated.

6.2.1.2.4.2. Serum Electrolytes. Serum sodium, potassium, chloride, bicarbonate, glucose, creatinine, and BUN.

6.2.1.2.4.2.1. This testing is repeated at least monthly prior to transplantation or as medically indicated.

6.2.1.2.4.3. Serum albumin, protein, calcium, magnesium, and phosphorus, and ionized calcium (if on calcium supplementation).

6.2.1.2.4.3.1. This testing is repeated at least monthly prior to transplantation or as medically indicated.

6.2.1.2.4.4. Liver Function studies: ALT, AST, Alkaline Phosphatase, and total bilirubin.

6.2.1.2.4.4.1. This testing is repeated at least monthly prior to transplantation or as medically indicated.

6.2.1.2.4.5. Type and Screen. This is done prior to transplantation and any surgery so that blood will be available in the event of a bleeding problem. Blood for transfusion is irradiated, CMV seronegative and leukocyte reduced. These conditions for transfusions of blood products are requested for all profoundly immunodeficient pediatric patients. Note: The time period that the type and screen needs to be done prior to transplantation and biopsy (if performed) depends on the subject's age and the blood bank guidelines.

6.2.1.2.4.6. Direct Coombs.

6.2.1.2.4.6.1. This is done once prior to transplantation to test for immune-mediated hemolytic anemia.

6.2.1.2.4.7. Reticulocyte Count.

6.2.1.2.4.7.1. This is done once prior to transplantation to test for decreased red cell production.

6.2.1.2.4.8. PT and PTT.

6.2.1.2.4.8.1. These coagulation tests are performed prior to surgeries to ensure safety for procedures.

6.2.1.2.4.8.2. If these values are prolonged, such that surgery would entail more risk, the subjects are treated appropriately.

6.2.1.2.4.9. Chest X-ray.

6.2.1.2.4.10. Serum Lipase
6.2.1.2.4.10.1. This testing is done at least once prior to transplantation if on calcineurin inhibitors or as medically indicated.

6.2.1.2.5. The following studies are completed within ***two months*** prior to thymus transplantation.
6.2.1.2.5.1. Urinalysis.
6.2.1.2.5.1.1. This is done at least one time prior to transplantation or as medically indicated.

6.2.1.2.5.2. Stool for electron microscopy (EM).
6.2.1.2.5.2.1. This is done to screen for infection. The presence of enterovirus or adenovirus in stool is not exclusion criterion.

6.2.1.2.5.2.2. Stool samples for electron microscopy must be in the testing laboratory prior to thymus transplantation; however, results may not be available at the time of transplantation.

6.2.1.2.5.2.3. Testing of stool is repeated if the subject develops diarrhea or as medically indicated.

6.2.1.2.5.3. Nasopharyngeal (NP) aspirate for viral culture.
6.2.1.2.5.3.1. This is done to screen for infection. The presence of RSV or parainfluenza virus on nasopharyngeal screen is not exclusion criterion.

6.2.1.2.5.3.2. This testing is repeated if the subject develops respiratory symptoms or as medically indicated.

6.2.1.2.5.4. Thyroid Function Studies.
6.2.1.2.5.4.1. This is done at least one time prior to transplantation.

6.2.1.2.5.4.2. If thyroid function studies indicate hypothyroidism, anti-thyroglobulin and anti-thyroperoxidase antibodies will be measured.

6.2.1.2.6. The following studies are done within ***three months*** prior to thymus transplantation.
6.2.1.2.6.1. Evidence of the following infections are not exclusion criteria.
6.2.1.2.6.1.1. Evidence of Epstein Barr Virus (EBV) by PCR of blood

6.2.1.2.6.1.2. HHV6 (Herpes Human Virus 6) by PCR of blood

6.2.1.2.6.1.3. West Nile Virus by PCR of blood

6.2.1.2.6.1.4. Hepatitis C by PCR of blood

6.2.1.2.6.1.5. Hepatitis B by PCR of blood

6.2.1.2.7. Evidence of the following are exclusions.

6.2.1.2.7.1. HIV-1 is exclusion criterion for Groups 1, 2, 3, and 4.

6.2.1.2.7.1.1. This test is done any time after birth and before the thymus transplant.

6.2.1.2.7.1.2. If the HIV antibody test is positive, a nucleic acid test (NAT) is done. A positive nucleic acid test is an exclusion criterion.

6.2.1.2.7.2. CMV infection is an exclusion criterion for Groups 2, 3 and 4.

6.2.1.2.7.2.1. This initial testing (blood by PCR) can be done within 3 months prior to thymus transplantation.

6.2.1.2.7.2.2. A second test (blood by PCR) must be done within 1 month prior to thymus transplantation.

6.2.1.2.7.2.3. For subjects receiving immunosuppression, evidence of CMV by PCR in the blood is an exclusion criterion.

6.2.1.2.8. The following study is done within ***six months*** prior to thymus transplantation.

6.2.1.2.8.1. T Cell Chimerism.

6.2.1.2.8.1.1. T cell chimerism is done if there are sufficient T cells.

6.2.1.2.8.1.2. This testing examines the origin of the recipient's T cells. It is important to be able to exclude transplacental transfer of maternal T cells as the source of developing T cells.

6.2.1.2.8.1.3. Chimerism is a clinical test for standard of care.

6.2.1.3. Pre-transplantation medical testing on the recipient to characterize the DiGeorge subject's phenotype.

6.2.1.3.1. These tests are done once but can be repeated as necessary for the medical care of the subject.

6.2.1.3.1.1. A renal ultrasound is used to look for renal anomalies. Renal anomalies are very common in DiGeorge anomaly and each subject will be characterized. The renal ultrasound can be delayed until after transplantation if there are logistic issues. An ultrasound done prior to admission at DUMC can be used.

6.2.1.3.1.2. A cardiac evaluation will be done in each subject with a history of heart disease or in whom heart disease has not been ruled out to assess heart defects and function. In addition, the evaluation will

determine if the subject is at a high risk for anesthesia, whether bacterial endocarditis prophylaxis is needed for surgery/procedures, if a particular hemoglobin value must be maintained, and what oxygen saturation is needed to be maintained and, if applicable, whether central line can safely be inserted into the vessels near the heart (e.g. is the anatomy normal or will any vessels be needed for future heart operations). Many subjects receive central lines as part of medical care.

6.2.1.3.1.3. A parathyroid hormone assay is done to assess for hypoparathyroidism with simultaneous ionized calcium in subjects requiring calcium supplementation. This can be delayed until after transplantation.

6.2.1.4. Pre-transplantation testing with research objectives.

6.2.1.4.1. HLA typing – HLA matching is not a requirement in this protocol; however, HLA typing is done on the recipient. The data are kept for reference. PCR-based HLA typing is done. The typing of the recipient can be done in the Duke Hospital HLA Laboratory or by an outside CLIA or CAP Certified Laboratory.

6.2.1.4.2. HLA antibodies. The subject's serum is screened for anti-HLA antibodies.

6.2.1.4.2.1. If present, the alleles against which antibodies react will be exclusion criteria for the thymus donor.

6.2.1.4.3. 22q11.2 hemizygosity by FISH or microarray. This is usually done prior to transfer to DUMC.

6.2.1.4.4. DNA is stored for future molecular analysis.

6.2.1.4.4.1. The thymus recipient blood sample for DNA must be obtained prior to transplantation.

6.2.1.4.4.2. The thymus recipient buccal sample for DNA must be obtained prior to transplantation. The buccal sample is compared to the DNA isolated from purified recipient T cells and maternal DNA prior to the transplant to confirm that the recipient circulating T cells are genetically recipient. This also rules out the presence of T cells from graft versus host disease from an unirradiated blood transfusion.

6.2.1.4.4.3. If maternal sample is not available.

6.2.1.4.4.3.1. High resolution HLA typing will be done on isolated T cells to test for the presence of a 3rd haplotype.

6.2.1.4.4.3.2. Alternatively in male recipients and in male atypical subjects, cytogenetic studies on isolated T cells may be done for XX versus XY chromosomes.

6.2.1.4.5. T Cell Chimerism.
6.2.1.4.5.1. T cell chimerism is done prior to thymus transplantation if there are sufficient T cells.

6.2.1.4.6. Serum is stored prior to transplantation in the event that a serum factor may be found to correlate with athymia in the future.

6.2.1.4.7. Serum Immunoglobulins.
6.2.1.4.7.1. Serum immunoglobulins should be measured monthly prior to transplantation.

6.2.1.4.8. Flow cytometry is done to assess T, B, and NK numbers and phenotypes.
6.2.1.4.8.1. This is done as per inclusion criteria.

6.2.1.4.9. PHA proliferation response. The ability of peripheral blood mononuclear cells (PBMC) to proliferate in response to *in vitro* exposure to PHA.
6.2.1.4.9.1. This is done as per inclusion criteria.

6.2.1.4.10. TRECs.
6.2.1.4.10.1. No requirement for testing.

6.2.1.4.10.2. Newborn screening or other TREC data, if available, will be recorded.

6.2.1.4.11. Flow cytometry for TCRBV repertoire.
6.2.1.4.11.1. This is done prior to transplantation if there are sufficient T cells.

6.2.1.4.11.2. If there is an increase in T cell numbers or activation status, this assay may be repeated at the discretion of the PI.

6.2.1.5. Immunosuppression.
6.2.1.5.1. Rabbit Anti-Thymocyte Globulin (RATGAM).
6.2.1.5.1.1. Dose.
6.2.1.5.1.1.1. Three doses of 2 mg/kg/dose IV. Each dose given over approximately 12 hours.

6.2.1.5.1.1.2. This dose may be modified as medically indicated.

6.2.1.5.1.2. Administration.
6.2.1.5.1.2.1. RATGAM is usually given on days -5, -4, and -3 (relative to thymus transplantation). Days -2 and -1 are usually rest days.

6.2.1.5.1.2.2. The rabbit anti-thymocyte globulin is started at 0.125 ml/kg/hr, after 60 minutes the rate is increased to 0.25 ml/kg/hr, at 120 minutes it is increased to 0.35 ml/kg/hr and the rate is left at this rate for the rest of the infusion. Note rabbit anti-thymocyte

globulin is reconstituted at 0.5 mg/ml in normal saline.

- If there are adverse reactions with an infusion, such as fever, rash, and/or hypoxia, the rate of rabbit anti-thymocyte globulin administration is decreased per Intensive Care Unit (ICU)/Bone Marrow Transplant Unit (BMTU) guidelines. An example would be to halve the rate.
- Hypersensitivity/anaphylactic reactions will be addressed in accordance with hospital policy ICU/BMTU. The infusion will be stopped temporarily and necessary clinical interventions will be performed per hospital policy to stabilize the subject. Once the reaction has been controlled, the infusion will be resumed at a tolerable rate as directed by the subject's attending physician.

6.2.1.5.1.2.3. The timing of the rabbit anti-thymocyte globulin and days of rest can be modified per the Principal Investigator. Other arrangements of treatment and rest days are permissible as long as the subject receives 3 doses of rabbit anti-thymocyte globulin. The changes are usually due to the subject's medical condition.

6.2.1.5.1.2.4. The thymus transplant must be done within 7 days of completion of the last dose of RATGAM.

- If the transplant is to occur after 7 days from the last dose of RATGAM, a T cell count will be repeated:
  - ⇒ If the T cell count is <50/mm^3, no more RATGAM will be administered
  - ⇒ If the T cell count is >50/mm^3, RATGAM will be repeated at the same schedule and dose as the initial infusion.

6.2.1.5.1.3. Rabbit Anti-Thymocyte Globulin Associated Medications.

6.2.1.5.1.3.1. Methylprednisolone.

6.2.1.5.1.3.1.1. Dose.

- A dose of 2 mg/kg IV is given approximately 4 hours prior to the first dose of rabbit anti-thymocyte globulin.
- A dose of 2 mg/kg/day in divided doses every 6 hours (0.5 mg/kg every 6 hours IV) is given during the administration of rabbit anti-thymocyte globulin prior to transplantation.
  - This dose of steroids continues with every 6 hour dosing for 24 hours after the last dose of rabbit anti-thymocyte globulin is finished.

6.2.1.5.1.3.1.2. For Group 2, methylprednisolone is stopped 24 hours after the last dose of RATGAM ends.

6.2.1.5.1.3.1.3. For Group 3 and Group 4, pre RATGAM steroid therapy, if any, is resumed 24 hours after the last dose of RATGAM ends.

6.2.1.5.1.3.2. Acetaminophen.

6.2.1.5.1.3.2.1. Three doses of 10 mg/kg orally, rectally, or by tube every 4 hours are given on the days of rabbit anti-thymocyte globulin treatment beginning immediately prior to the start of each dose of rabbit anti-thymocyte globulin. This dose may be modified as medically indicated.

6.2.1.5.1.3.2.2. The first dose is given 15 minutes prior to the start of the infusion.

6.2.1.5.1.3.2.3. Additional doses may be given every four hours as needed.

6.2.1.5.1.3.3. Diphenhydramine.

6.2.1.5.1.3.3.1. Three doses of 0.5 mg/kg IV every 4 hours are given on the days of the rabbit anti-thymocyte globulin treatment beginning immediately prior to the start of each dose of rabbit anti-thymocyte globulin. This dose may be modified as medically indicated.

6.2.1.5.1.3.3.2. The first dose is given 15 minutes prior to the start of the infusion.

6.2.1.5.1.3.3.3. Additional doses may be given as needed every 4 hours.

6.2.1.5.2. Steroids.

6.2.1.5.2.1. Steroids, if given, depend on the T cell count or the Absolute Lymphocyte Count (ALC).

6.2.1.5.2.2. Methylprednisolone (intravenously) or Prednisolone (enterally).

6.2.1.5.2.2.1. Prednisolone can be used at the same dose as the methylprednisolone if the subject does not have diarrhea.

6.2.1.5.2.2.2. Methylprednisolone/prednisolone may be started prior to arrival at DUMC.

6.2.1.5.2.2.3. For Group 3 and Group 4 – Pre Transplantation.

6.2.1.5.2.2.3.1. The most recent T cell count or ALC is used.

- If the T cell count or ALC is less than 4,000/mm^3 and the subject does not have clinical problems related to these T cells

⇒ Steroids are not used

- If the T cell count or ALC is less than 4,000/mm^3 and the subject has clinical problems from the atypical T cells, such as T cell infiltration of the liver, or severe rash
  ⇒ Methylprednisolone or prednisolone will be started at 1 to 2 mg/kg/day and weaned based on the medical condition of the subject and T cell count.
- If the T cell count or ALC is 4000 to 10,000/mm^3 (on or off cyclosporine
  ⇒ Methylprednisolone or prednisolone will be started at 1 mg/kg/day.
- If the T cell count is over 10,000/mm^3 (on or off cyclosporine)
  ⇒ Methylprednisolone or prednisolone will be started at 2 mg/kg/day.
- An extra dose of 2 mg/kg of methylprednisolone is given approximately 4 hours prior to the first dose of rabbit anti-thymocyte globulin. This is the peri-rabbit anti-thymocyte globulin steroid and must be methylprednisolone.

6.2.1.5.2.2.4. Weaning steroids before and after transplantation.

- Steroid use is minimized. An absolute lymphocyte count [ALC] (the maximum possible T cell count) or the number of T cells should be checked prior to each wean.
- The steroids are weaned 10 to 30% per week if the T cell count or the ALC drops less than 4,000/mm^3 and there are no organ toxicities for host oligoclonal T cells such as elevated liver enzymes, diarrhea associated with GVHD appearance, or rash.
- When the steroids are weaned to every other day dosing for one week, an 8 am cortisol level (on the day the steroid dose is given and just prior to the dose) is obtained. If the cortisol level is normal, the steroid is stopped.
- The subject may need to be placed on maintenance hydrocortisone to continue a slow wean after the methylprednisolone/prednisolone is weaned.

6.2.1.5.3. Cyclosporine.

6.2.1.5.3.1. Group 1 and Group 2.

6.2.1.5.3.1.1. No cyclosporine is used.

6.2.1.5.3.2. Group 3 and Group 4.

6.2.1.5.3.2.1. Cyclosporine will be started as soon as the diagnosis of atypical complete DiGeorge anomaly is made if the subject is symptomatic or as medically indicated.

6.2.1.5.3.2.2. Cyclosporine is started at least 7 days prior to the administration of rabbit anti-thymocyte globulin.

6.2.1.5.3.2.3. Dosing of Cyclosporine.

- Enteral: Begin at 4-8 mg/kg every 12 hours.
  - May increase enteral dosing to every 8 hours.
  - Oral dosing is preferred.
- Intravenous: Begin at 4 mg/kg every 12 hours.
- The starting dose depends on the other medications that the subject is taking. For example, if the subject is on steroids or fluconazole, lower doses are used because of the potential drug interaction with cyclosporine leading to high cyclosporine levels.
  - Renal function is monitored and renal toxicities are evaluated. Alternative immunosuppression may be needed.
  - Other toxicities are handled on an individual basis.

6.2.1.5.3.2.4. Trough Cyclosporine Levels.

6.2.1.5.3.2.4.1. Every 1 to 2 weeks, or as medically indicated, until the cyclosporine wean begins.

6.2.1.5.3.2.4.2. At least twice between the end of the first dose of rabbit anti-thymocyte globulin and thymus transplantation.

6.2.1.5.3.2.4.3. Cyclosporine dose should be halved prior to the administration of the first dose of rabbit anti-thymocyte globulin to maintain target CSA trough levels in anticipation of the effects of the 2mg/kg methylprednisolone on cyclosporine levels.

6.2.1.5.3.2.4.4. Group 3.

- The cyclosporine trough target range is 180 to 220 ng/ml.
- If the PHA response on suppression is >40,000 cpm, the subject will move to Group 3.

6.2.1.5.3.2.4.5. Group 4.

- The cyclosporine target range is 250 to 300 ng/ml while the PHA response remains greater than 40,000 cpm pre-transplantation or prior to the development of 10% naïve T cells.
- Once the PHA response is less than 40,000 cpm, the cyclosporine trough level is decreased to 180 to 220 ng/ml.

6.2.1.5.3.2.5. Weaning of Cyclosporine.

6.2.1.5.3.2.5.1. Once naïve T cells are >10% of CD3 T cells
- Cyclosporine is weaned over 2 months after steroids have been weaned off.
- The cyclosporine dose is decreased weekly.
- If the ALC increases by over 2000/mm^3 over the pre wean ALC, then a T cell count is done.
  ⇒ If the T cells show <10% naïve T cells, the cyclosporine is increased with or without addition of steroids for a cyclosporine trough level of 180 to 220 ng/ml.

6.2.1.5.3.2.5.2. Additional testing of ALC or T cell counts may be done at the discretion of the Principal Investigator. If the subject cannot tolerate cyclosporine, the cyclosporine is stopped and Tacrolimus (FK506) is started.

6.2.1.5.4. Tacrolimus (FK506).

6.2.1.5.4.1. If the subject is unable to tolerate cyclosporine, then tacrolimus (FK506) may be used.

6.2.1.5.4.2. Dosing of Tacrolimus.

6.2.1.5.4.2.1. The initial starting dose is 0.1 mg/kg/day divided into every 12 hours enterally.

6.2.1.5.4.2.2. The enteral dosing can be given every 8 hours, if necessary.

6.2.1.5.4.2.3. The intravenous dose used is 1/4 of the enteral dose and this is given over 2 to 4 hours every 12 hours.

6.2.1.5.4.2.4. If the trough levels are outside of the desired range, the dose will be modified appropriately.

6.2.1.5.4.3. Tacrolimus Trough Levels.

6.2.1.5.4.3.1. Every 1 to 2 weeks, or as medically indicated, until the tacrolimus wean begins.

6.2.1.5.4.3.2. At least twice between the first dose of rabbit anti-thymocyte globulin and transplantation.

6.2.1.5.4.3.3. Tacrolimus dose should be halved prior to the administration of the first dose of rabbit anti-thymocyte globulin to maintain target Tacrolimus trough levels in anticipation of the effects of the 2mg/kg methylprednisolone on Tacrolimus levels.

6.2.1.5.4.3.4. For Group 3, the desired tacrolimus target trough level is 7 to 10 ng/ml.

6.2.1.5.4.3.4.1. If the PHA response on suppression is >40,000 cpm, the subject will move to Group 4.

6.2.1.5.4.3.5. For Group 4, the desired tacrolimus target desired trough level is 10 to 15 ng/ml.

6.2.1.5.4.4. Weaning of Tacrolimus.

6.2.1.5.4.4.1. Once naïve T cells are >10% of CD3 T cells

- Tacrolimus is weaned over 2 months after steroids have been weaned off.
- The tacrolimus dose is decreased weekly.
- If the ALC increases by over 2000/mm^3 over the pre wean ALC, then a T cell count is done.
  - ⇒ If the T cells show <10% naïve T cells, the tacrolimus is increased with or without addition of steroids for a tacrolimus trough level of 7 to 10 ng/ml.

6.2.1.5.4.4.2. Additional testing of ALC or T cell counts may be done at the discretion of the Principal Investigator.

6.2.1.5.5. Additional Immunosuppression for Group 4.

6.2.1.5.5.1. Between days +3 to +5 (inclusive) after transplantation, one flow cytometry assay is repeated

- ➢ If the T cell count is greater than 5000/mm^3 after day +5, mycophenolate mofetil (MMF), 15 mg/kg IV every 8 hours is given.

6.2.1.5.5.2. Mycophenolate Mofetil (MMF) Wean.

6.2.1.5.5.2.1. MMF may be stopped at 35 days if there is no extensive rash, if the AST and ALT are less than 3 times the upper limit of normal, and if T cells are <5000/mm^3. MMF can be continued up to 6 months if these parameters are not met earlier.

6.2.1.6. Pre-transplantation safety testing.

6.2.1.6.1. For subjects receiving immunosuppression (Groups 2, 3, and 4), CMV testing of blood by PCR **must be repeated** after the end of the last dose of rabbit anti- thymocyte globulin which is prior to the thymus transplantation.

6.2.1.6.2. For subjects not receiving immunosuppression (Group 1), CMV testing of blood by PCR must be repeated in the week prior to thymus transplantation.

6.2.1.6.3. Samples for pre-transplantation CMV safety testing need to be in the testing laboratory; however, the CMV results do not need to be available to proceed with the thymus transplant. If the test results are available prior to transplantation, a positive test does not exclude the subject at this time.

6.2.1.7. Operative Procedures.

6.2.1.7.1. Procedures on Day of Thymus Transplantation.
6.2.1.7.1.1. Thymus Transplantation.
6.2.1.7.1.1.1. Thymus transplantation is an open procedure.

6.2.1.7.1.1.2. The subject is taken to the Operating Room and given general anesthesia.

6.2.1.7.1.1.3. The cultured thymus tissue slices are placed into one or both quadriceps(s) muscle in the recipient by a pediatric surgeon.

6.2.1.7.2. Procedures on the Day of Thymus Allograft Biopsy, if performed.
6.2.1.7.2.1. Thymus Allograft Biopsy.
6.2.1.7.2.1.1. Thymus allograft biopsies after thymus transplantation are not required.

6.2.1.7.2.1.2. If there is some concern regarding the thymus transplant, a thymus biopsy may be done at the discretion of the PI. If the biopsy is performed, the subject is taken to the Operating Room and given general anesthesia. A biopsy of the thymus allograft is obtained. The site of the allograft is exposed and several samples of tissue, approximately 4 x 4 mm each, are removed.

6.2.1.7.2.1.3. The biopsied tissue is stained with antibodies to keratin, CD3, CD1a (cortical thymocytes), and Ki-67 (proliferation marker of cortical thymocytes) to evaluate the biopsy for thymopoiesis.

6.2.1.8. Monitoring from thymus transplantation to 24 months post thymus transplantation.
6.2.1.8.1. When the subject is discharged to the care of the referring/local immunologist, requests are submitted to the referring/local immunologist for the testing/blood samples. Recommendations for the frequency for post thymus transplantation follow up is listed below; however, obtaining the testing/blood samples is dependent upon the parent(s), the referring/local immunologist, and the subject's medical condition.

6.2.1.8.2. Immune evaluations.
6.2.1.8.2.1. Complete Blood Count (CBC).
6.2.1.8.2.1.1. A CBC with differential will be done weekly for the first two months after thymus transplantation to look for sudden elevations in the absolute lymphocyte count (ALC). This could indicate graft versus host disease, sudden growth of pre-existing host clones, or B cell lymphoproliferative disease. The CBC with differential may be repeated as medically indicated.

6.2.1.8.2.1.2. A CBC with differential will be done as follows
- Month 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.
- As medically indicated

6.2.1.8.2.1.3. For subjects receiving a calcineurin inhibitor

6.2.1.8.2.1.3.1. A CBC with differential will be done weekly until the calcineurin inhibitor is weaned off. Once the calcineurin inhibitor is weaned off, the CBC will be done monthly as above.

6.2.1.8.2.2. Flow cytometry.

6.2.1.8.2.2.1. Flow cytometry, along with a CBC with (manual differential) (MD), is done to assess T, B, and NK cell numbers. Evaluation of naïve T cells is included.

6.2.1.8.2.2.2. Flow cytometry will be done as follows

- Month 3 ± 1 month
- Month 6 ± 1 month
- Month 9 ± 2 months
- Month 12 ± 2 months
- As medically indicated

6.2.1.8.2.2.3. Flow cytometry is done if there is an adverse event thought to be related to an increase in T or B cells. In particular, if the ALC remains greater than 4,000/mm^3, and the subject has symptoms suggestive of autoreactive clones, the flow cytometry may be repeated sooner to help guide immunosuppressive therapy.

6.2.1.8.2.3. *In vitro* T cell proliferative responses to PHA.

6.2.1.8.2.3.1. PHA is done at Month 12 ± 2 months.

6.2.1.8.2.3.2. **Additional testing may be done at the PI's discretion.** If additional PHA testing is done, it is done in a CLIA or CAP Certified Laboratory.

6.2.1.8.2.4. TREC (T cell receptor rearrangement excision circles)

6.2.1.8.2.4.1. TREC data, if done, may be recorded.

6.2.1.8.2.5. Serum immunoglobulins including IgG, IgA IgM, and IgE.

6.2.1.8.2.5.1. Immunoglobulins are checked for evidence of development of IgA, IgM, and IgE.

6.2.1.8.2.5.2. Immunoglobulins levels are tested as follows

- Month 1 and 2
- Month 3 and 6 ± 1 month
- Month 9 and 12 ± 2 months

6.2.1.8.3. Testing for immune-based adverse events.

6.2.1.8.3.1. Complete Blood Counts with Differential.

6.2.1.8.3.1.1. Individuals with DiGeorge anomaly can have autoimmune

cytopenias.

6.2.1.8.3.1.2. The hemoglobin, platelet count and neutrophil counts are most important for surveillance for immune based adverse events.

6.2.1.8.3.2. Flow Cytometry.

6.2.1.8.3.2.1. The CD19 and/or CD20 antibodies will identify clonal or polyclonal amplifications of B cells. Such amplifications may be related to EBV and CMV infection.

6.2.1.8.3.3. Extended T cell receptor beta variable chain (TCRBV) flow cytometry.

6.2.1.8.3.3.1. This assay may be done to assess diversity of T cell receptor expression and to assess if new clones are developing, or is a single clonal population of T cells is developing.

6.2.1.8.3.4. Isolated $CD3^+$ T cells may be assessed for sex chromosome or 22q11.2 status or DNA analysis to distinguish host cells from maternal cells or thymus donor cells.

6.2.1.8.3.4.1. This testing will be done Month 3 ± 1 month after transplantation.

6.2.1.8.3.4.2. This testing will be repeated in 3 to 12 months, if maternal or thymic donor cells are found in the 3 month sample.

6.2.1.8.3.4.3. This testing may be done after transplantation if there are adverse events, such as rashes, that could be explained by GVHD from donor T cells.

6.2.1.8.3.5. Liver enzymes (AST and ALT)

6.2.1.8.3.5.1. This testing will be done to look for signs of GVHD, self reactive host T cell clones, or autoimmune disease.

6.2.1.8.3.5.2. AST and ALT will be done as follows

- Month 1 and 2
- Month 3 and 6 ± 1 month
- Month 9 and 12 ± 2 months

6.2.1.8.3.6. Serum albumin, protein, calcium, magnesium, and phosphorus.

6.2.1.8.3.6.1. This testing will be done as follows

- Month 1 and 2
- Month 3 and 6 ± 1 month
- Month 9 and 12 ± 2 months.

6.2.1.8.3.6.2. This testing may be repeated as medically indicated.

6.2.1.8.3.7. Serum sodium, potassium, chloride, bicarbonate, BUN, creatinine,

and glucose.

6.2.1.8.3.7.1. This testing will be done as follows

- Month 1 and 2
- Month 3 and 6 ± 1 month
- Month 9 and 12 ± 2 months.

6.2.1.8.3.7.2. This testing may be repeated as medically indicated.

6.2.1.8.3.7.3. Serum creatinine is tested to look for immune based renal disease.

6.2.1.8.3.8. Serum magnesium, serum creatinine, serum potassium, and serum lipase.

6.2.1.8.3.8.1. These tests are obtained to look for calcineurin inhibitor toxicity.

6.2.1.8.3.8.2. This testing will be done monthly until the calcineurin inhibitor is weaned off.

6.2.1.8.3.9. Urinalysis.

6.2.1.8.3.9.1. This testing will be done to look for signs of nephrotic syndrome or infection.

6.2.1.8.3.9.2. This testing will be done as follows

- Month 1 and 2
- Month 3 and 6 ± 1 month
- Month 9 and 12 ± 2 months.

6.2.1.8.3.10. Serum immunoglobulins.

6.2.1.8.3.10.1. If the IgE increases to over >500 IU/ml, the EBV and CMV status will be checked (after the initial high IgE level). The EBV PCR and CMV PCR after subsequent IgE levels do not need to be repeated if the IgE remains elevated. Spikes in other isotypes will be evaluated. B cell clonal proliferations are possible and may need treatment if present.

6.2.1.8.3.11. Rashes.

6.2.1.8.3.11.1. If new rashes develop, the rash will be monitored daily (while the subject is at DUMC).

6.2.1.8.3.11.2. Biopsies will be taken on rashes that are severe and persist for more than 2 weeks to assess the etiology of the rash. If granuloma are found, evidence for sarcoidosis including an angiotension converting enzyme (ACE) level and eye examination will be sought.

6.2.1.8.3.12. Diarrhea.

6.2.1.8.3.12.1. If severe diarrhea develops, endoscopy and colonoscopy will be done as indicated with biopsies to assess for the etiology of the diarrhea.

6.2.1.8.3.13. Endocrine studies.
6.2.1.8.3.13.1. Thyroid Studies.
6.2.1.8.3.13.1.1. Thyroid function studies will be done to screen for thyroid disease.

6.2.1.8.3.13.1.2. Anti-thyroglobulin and anti-thyroid peroxidase antibodies will be measured if the subject develops thyroid disease.

6.2.1.8.3.13.1.3. Thyroid function studies will be done as follows
- Month 6 ± 1 month
- Month 12 ± 2 months

6.2.1.8.3.13.1.4. If thyroid disease develops, the endocrine evaluation is directed by the subject's endocrinologist or primary care physician.

6.2.1.8.4. Testing for medical conditions.
6.2.1.8.4.1. Medically indicated studies are done as necessary and may include the following
6.2.1.8.4.1.1. Laboratory studies, for example, ionized calcium, magnesium, blood counts, and/or cultures.

6.2.1.8.4.1.2. Calcium.
6.2.1.8.4.1.2.1. For subjects on calcium supplementation

6.2.1.8.4.1.2.2. Serum calcium levels will be done monthly for 1 year.

6.2.1.8.4.1.2.3. Urine calcium/creatinine ratio will be done as follows
- Months 1, 2, 3, 4, 5, 6
- Months 9 and 12
- As medically indicated

6.2.1.8.4.1.3. Periodic cardiac evaluation(s) as recommended by the subject's cardiologist.

6.2.1.8.4.1.4. Endocrinology may recommend additional test(s).

6.2.1.8.4.1.5. Follow up renal ultrasound(s) may be done.

6.2.1.8.5. Safety Testing.
6.2.1.8.5.1. CMV testing of blood by PCR and EBV by PCR
- Month 3 ± 1 month

6.2.1.8.6. Physical Examination.
6.2.1.8.6.1. Physical examinations will be done monthly.
6.2.1.8.6.1.1. The length of the subject is recorded, as autoimmune thyroid

disease can first be detected when the length stops increasing.

6.2.1.8.6.1.2. The subject will be observed for the development of new rashes that are severe and persist for more than 2 weeks.

6.2.1.8.6.1.3. After returning to the care of the local immunologist and/or referring physician, physical examinations will be requested to be done monthly for the first year after transplantation.

6.2.1.8.6.1.4. If the subject is on cyclosporine, the blood pressure should be monitored every 2 weeks, or as medically indicated, until the cyclosporine is weaned.

6.2.1.9. Infections.

6.2.1.9.1. Monitoring for Infections.

6.2.1.9.1.1. While at Duke, this is done from the time of enrollment until discharge.

6.2.1.9.1.2. After discharge, information is requested from the local immunologist/referring physician and/or parent(s) by telephone or email at the following intervals after transplantation.

- Month 3 ± 1 month
- Month 6 ± 1 month
- Month 12 ± 2 months
- Month 24 ± 2 months
- As medically indicated.

6.2.1.9.2. Recording of Infections.

6.2.1.9.2.1. The definitions are taken directly from the Blood and Marrow Transplant Clinical Trials Network https://web.emmes.com/study/bmt2/public/Definition/Definitions_of_Inf_Severity.pdf All adverse events are to be reported as described in Section 8.3.

6.2.1.10. Possible additional biopsies. Note: These will not be done if the subject is not medically stable.

6.3. Concomitant Therapy - all of the below therapies are permitted.

6.3.1. Other procedures for medical care of research subject.

6.3.1.1. Protective Isolation: DUHS and Clinical Care Unit policies and procedures are followed while at DUMC.

6.3.1.1.1. Discontinuation of protective isolation is recommended when the subject meets criteria for permanent discontinuation of IVIG (see section 6.3.1.2.4) and/or discontinuation of PJP prophylaxis (see section 6.3.1.3.3).

6.3.1.2. Immunoglobulin Replacement Therapy.

6.3.1.2.1. Dosing.

- Immunoglobulin replacement is given to maintain IgG at ≥ 800 mg/dL.
- Intravenous immunoglobulin (IVIG) replacement monthly or
- Subcutaneous immunoglobulin replacement weekly to biweekly

6.3.1.2.2. Replacement therapy frequency depends on the subjects' IgG levels. It may be given more frequently if indicated clinically.
- If the IgG levels drops below 800 mg/dl, give replacement therapy

6.3.1.2.3. Guidelines for *initial* discontinuation of Immunoglobulin Replacement.
- Subject is not on immunosuppression
- Subject is at least 9 months post transplantation
- PHA response is > 100,000 cpm
- Normal serum IgA is desirable but not required

6.3.1.2.4. Guidelines for *permanent* discontinuation of Immunoglobulin Replacement.

6.3.1.2.4.1. Two months after stopping immunoglobulin, the IgG trough level will be checked by the local immunologist and/or referring physician.
- If the IgG trough level is in the normal range for age, the subject may remain off immunoglobulin.
- If the IgG level is lower than the normal range for age, the subject will restart immunoglobulin.
  - The subject will continue on immunoglobulin for a year and then be retested by the local immunologist/referring physician using the above guidelines. Recommendations will be sent to the local/referring physician.

6.3.1.3. *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis.

6.3.1.3.1. Subjects are maintained on PJP prophylaxis before and after transplantation.

6.3.1.3.2. PJP Prophylaxis.
- Septra/Bactrim – 2.5 mg trimethoprim (TMP)/kg twice a day, three days a week, usually Monday, Wednesday, Friday.
- Alternatively, Pentamidine 4 mg/kg IV every 4 weeks.

6.3.1.3.3. Guidelines for discontinuation of PJP prophylaxis.
- Subject is not on immunosuppression
- Subject is at least 9 months post transplantation
- PHA response is >100,000 cpm
- CD4+ count is >200/mm^3

6.3.1.4. Other Immunizations.

6.3.1.4.1. No live vaccinations are administered until the recipient has received killed vaccinations.

6.3.1.5. Steroid Treatment.

6.3.1.5.1. Methylprednisolone or prednisolone 1 to 2 mg/kg/d (followed by a slow taper) may be required for extreme elevations of eosinophil counts (>30,000/mm^3) or IgE levels >10,000, or pulmonary deterioration. Evidence of EBV or CMV infection should be sought for IgE levels >500/mm^3.

6.3.1.5.2. Methylprednisolone or prednisolone 1 to 2 mg/kg/d (followed by a slow taper) may be required for treatment of aggressive host T cell clones that appear after the initial steroids have been weaned. The clones may cause elevations in serum bilirubin, in pulmonary compromise, or other clinical problems.

6.3.1.6. Other Immunosuppressive Medications

6.3.1.6.1. Alemtuzumab may be used prior to and/or after transplantation to prevent long term use of steroids and calcineurin inhibitors.

6.3.1.6.2. Other immunosuppression medications may be used as needed for the medical care of the recipient.

6.4. Procedures for Monitoring.

6.4.1. Case Report Forms.

6.4.1.1. Case report forms are completed.

6.4.1.2. When the subject is discharged to home, protocol required testing is provided to the local immunologist and parent(s). Results of this testing are requested to be sent to the Principal Investigator. Information may be requested from the local immunologist/referring physician and obtained as possible. (see 6.1.3.2.4)

7. Assessment of Efficacy.

7.1. Specification of the efficacy parameters

7.1.1. Refer to 4.1

7.2. Methods and timing for assessing, recording, and analyzing of efficacy parameters.

7.2.1. The studies will be obtained on the time frame as detailed above. The data are recorded by the PI or the study staff as they accrue.

7.2.1.1. Efficacy data will be analyzed as outlined in the statistical analysis plan (SAP). See Appendix B.

8. Evaluation of Safety.

8.1. Specification of safety parameters.

8.1.1. Procedures to enhance research subject safety.

8.1.1.1. *Inpatient subjects* are monitored daily by physicians and staff familiar with management of immunodeficient patients. Subjects who are medically stable may be followed as *outpatients* at DUMC pre and post thymus transplantation.

8.1.1.1.1. Subjects are monitored for signs of rashes and/or infection, such as

fever, etc. If a fever develops, the subject is assessed by blood and other cultures and treated with antibiotics as medically indicated.

8.1.1.1.2. DUHS and Clinical Care Unit Infection Control policies are followed while at Duke.

8.1.1.2. GVHD.

8.1.1.2.1. Chimerism studies on circulating T cells are performed at 3 ± 1 month after transplantation to detect thymus donor T cells.

8.1.1.2.2. If clinical symptoms resembling those of GVHD develop, the relevant tissue is biopsied and examined by DUMC pathology. Blood will be tested to determine if the T cells are recipient, maternal, or thymus donor. GVHD will be treated, if medically indicated, by standard approaches.

8.1.1.3. Infection.

8.1.1.3.1. Infection information during the first 24 months post transplantation is requested from the primary and or referring physician.

8.1.1.4. Autoimmune disease.

8.1.1.4.1. Autoimmune disease is recorded for the first 24 months post transplantation.

8.1.1.5. Malignancy.

8.1.1.5.1. Development of a malignancy is always a possibility in patients with poor T cell function. EBV and CMV-related lymphomas have been reported in the past (Dictor et al 1984; Borzy et al 1979).

8.1.1.5.2. The recipient is tested prior to transplantation for presence of EBV or CMV to help assess the risk of lymphoproliferative disease after transplantation.

8.1.1.6. CMV Testing.

8.1.1.6.1.1. Thymus transplant recipients are tested before thymus transplantation and at Month 3 ± 1 month after thymus transplantation to screen for the presence of CMV.

8.1.1.7. EBV Testing.

8.1.1.7.1.1. Thymus transplant recipients are tested before thymus transplantation and at Month 3 ± 1 month after thymus transplantation to screen for the presence of EBV.

8.1.2. Previously observed adverse events.

8.1.2.1. Individuals with complete DiGeorge anomaly are at risk (even without therapy) from serious infections, aspiration, graft-versus-host disease from maternal cells or from unirradiated blood transfusions, death from cardiac complications, or complications from laryngomalacia. Individuals with

complete DiGeorge anomaly often develop skin rashes and cytopenias (even without therapy). For the purposes of this protocol, all pre-existing conditions are not considered adverse events and are not recorded as such. Pre-existing conditions are reported in the medical history.

8.1.2.2. Autoimmune disease is a risk in the thymus allograft group because T cells develop in a donor thymus and may react against the host. If autoimmune disease develops, it will be fully characterized and treated. Examples of this are thyroid disease, cytopenias, hepatitis, enteritis/colitis, nephrotic syndrome, arthritis, partial albinism, and alopecia.

8.1.2.3. Subjects with typical complete DiGeorge anomaly may develop rashes after transplantation.

8.1.2.4. Subjects with typical complete DiGeorge anomaly can develop oligoclonal amplifications of T cells associated with rashes after transplantation. These can result in marked lymphadenopathy.

8.1.2.5. Subjects with typical complete DiGeorge anomaly may develop a pulmonary inflammatory condition early after transplantation if oligoclonal T cells develop.

8.1.2.6. Death has occurred in a number of patients prior to and after transplantation because of respiratory, cardiac, infectious, and reflux problems. These problems (tracheomalacia, cardiac defects, aspiration, and gastroesophageal reflux [GER]) are related to the underlying DiGeorge anomaly.

- Tracheomalacia is so severe in some patients that tracheostomy is required.
- Many patients/subjects have underlying cardiac defects. Heart surgery is frequently needed to correct defects associated with DiGeorge anomaly. The cardiac status of the patients will be stable at screening for thymus transplantation.
- Aspiration is a significant problem because of the GER.
- Many patients/subjects require Nissen fundoplication. Gastric tubes may be needed because of abnormal esophageal motility or nasopharyngeal reflux.
- Because of the immune deficiency in these patients/subjects, many develop fevers, diarrhea, and have multiple infections.

8.1.2.7. Hypocalcemia is a frequent severe problem associated with DiGeorge anomaly.

8.1.2.8. Fevers and infections are expected adverse events in immunodeficient individuals. Infections are recorded. The decrease in infectious complications is a measure of efficacy.

8.1.2.9. Wound dehiscence, wound infection, and inflammation around stitches can

occur with the transplantation or the biopsy surgical procedures.

8.1.2.10. There was an occurrence of ovarian failure in one adolescent female. It is unknown at this time if it is genetic or related to thymus tissue transplantation.

8.1.2.11. Risk of immunosuppressive medications.

8.1.2.11.1. Rabbit anti-thymocyte globulin.

8.1.2.11.1.1. Rabbit anti-thymocyte globulin can be associated with fever, chills, low white blood cell counts, low platelets, pain, headache, abdominal pain, diarrhea, nausea, alterations in blood pressure, abnormal clotting, swelling, difficulty breathing, high potassium levels, and high heart rate. Many of the side effects are a result of killing the T cells. Rabbit anti-thymocyte globulin is made from animal serum. Individuals treated with animal serum are at risk of serum sickness approximately 1 week later. Serum sickness symptoms include fever, rash, and elevated liver enzymes. There may be other unknown risks of rabbit anti-thymocyte globulin. To try to prevent side effects from the rabbit anti-thymocyte globulin, the subject is treated with 3 doses of acetaminophen, 3 doses of diphenhydramine, and methylprednisolone. These medicines are started 15 minutes to 4 hours (as per protocol) prior to the rabbit anti-thymocyte globulin. The acetaminophen and diphenhydramine will be repeated with each dose of rabbit anti-thymocyte globulin; the steroid will be continued until 24 hours after the last rabbit anti-thymocyte globulin dose.

8.1.2.11.2. Cyclosporine.

8.1.2.11.2.1. There are a number of likely side effects from cyclosporine.

- Cyclosporine can affect the pancreas, liver, kidneys and decrease kidney function. This will be monitored by checking cyclosporine levels, blood studies of pancreatic function, liver function, kidney function, and urine examinations. Abnormalities in the pancreas, liver or kidney will result in reducing cyclosporine doses.
- Cyclosporine can cause low magnesium levels and high potassium.
- Cyclosporine can cause increases in blood pressure. This usually is mild or moderate but may persist. Blood pressure will be monitored daily during the inpatient hospitalization and after discharge at least every 2 weeks. Medication may be given to lower the blood pressure.
- Cyclosporine can result in excessive body hair.
- Cyclosporine can increase risk of infection in people born with thymuses, but this risk does not apply to complete DiGeorge anomaly because these pediatric patients are already at risk for infection. Cyclosporine does not change this risk.
- Infrequently cyclosporine can result in seizures (less than 1 in

20). More frequently, a tremor (trembling or quivering) can develop.

- Cyclosporine can increase the risk of cancer, although the risk remains less than 1%. As mentioned above, patients with poor T cell function are already at increased risk of cancer; this risk continues whether or not cyclosporine is used. Cyclosporine should not affect this risk.

8.1.2.11.3. Tacrolimus (FK506).

8.1.2.11.3.1. The possible side effects of tacrolimus are the same as those listed above for cyclosporine.

- Tacrolimus can also cause anemia and hyperglycemia.

8.1.2.11.4. Mycophenolate Mofetil (MMF).

8.1.2.11.4.1. The main risks of MMF are abdominal pain, diarrhea, vomiting, fever, and increase in blood pressure. Use of MMF has been associated with low neutrophil counts, low platelet counts, and low red blood cell counts. Bleeding in the gut can be seen with the use of MMF.

8.1.2.11.5. Steroids.

8.1.2.11.5.1. Prolonged use of daily steroids is associated with slowed growth, fluid retention and weight gain, muscle weakness, poor wound healing, peptic ulcer with possible complications, increased blood pressure, osteoporosis, increase in infection risk, poor wound healing, and possible development of diabetes-like syndrome.

8.2. The methods and timing for assessing, recording, and analyzing safety parameters.

8.2.1. The methods are described above. Please see the timeline for the times of the assessments. Data are recorded on case report forms. Safety parameters are reviewed by the Principal Investigator, and sponsor.

8.3. Procedures for eliciting reports of and for recording and reporting adverse event and intercurrent illnesses.

8.3.1. Adverse Event Monitoring.

8.3.1.1. Thymus Recipient.

8.3.1.1.1. The Principal Investigator (or an attending physician) examines the recipient daily during DUMC hospitalizations.

8.3.1.1.2. A recipient, who is an outpatient, is examined in the outpatient clinic while at DUMC (as per the assessment periods-section 6.1).

8.3.1.1.3. It is recommended that the subjects are followed by their local immunologist/referring physicians at least monthly through one year.

8.3.1.1.4. While the subject is at DUMC or in the Durham area, the investigator will aggressively work up any fevers, difficulty in breathing etc.

8.3.2. Adverse Event Recording.

8.3.2.1. All adverse events are tabulated.

8.3.2.1.1. The classification of the severity and attribution of the adverse event is determined using guidelines derived from the Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 at http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf

8.3.2.1.2. The classification of the severity of infections is determined using the definitions taken directly from the Blood and Marrow Transplant Clinical Trials Network.

8.3.2.2. All adverse events that meet the criteria for serious (see Section 8.3.3.2) and deaths are to be entered into the clinical database and to be forwarded to the safety vendor (Appendix D). Review of SAEs and any that require expedited reporting will be conducted according to the procedures at Enzyvant and safety vendor. Expedited safety reports will be submitted to the DUHS Institutional Review Board. These reports are sent to the FDA.

8.3.2.3. The IRB annual continuing renewals and the FDA annual report will provide the following information:

8.3.2.3.1. Summary of New Adverse Events (AES) reported since the previous report.

8.3.2.3.2. Summary of New Serious Adverse Events (SAEs) reported since the previous annual report.

8.3.2.3.3. Adverse Events that occur after discharge from Duke. The following information will be obtained:

- Survival
- Infections
- Autoimmune Disease (examples may include cytopenias, thyroid disease)
- Rashes persisting over 2 weeks
- Other problems likely related to the transplanted thymus tissue (examples may include granuloma, lymphoma or other cancers related to thymus transplantation)

8.3.2.3.3.1. The above are requested and obtained from the local physician and /or parent(s) by telephone or email at the following intervals after transplantation

- Month 3 ± 1 month
- Month 6 ± 1 month
- Month 12 ± 2 months
- Month 24 ± 2 months

8.3.2.3.4. All adverse events are reviewed at least yearly with the Thymus Transplantation Data and Safety Monitoring Board at DUMC.

8.3.2.4. If there is transmission of an infectious agent because of the thymus transplant this is to be reported to [redacted] and Enzyvant for management of AE and product complaint.

8.3.2.5. After the subject is discharged, the subject will be followed by the local immunologist and/or referring physician. However, blood for flow cytometry is requested to be sent to the Duke University Hospital Clinical Immunology Laboratory. If the subject lives near to DUMC, the subject will come to DUMC for follow up. Adverse event reports based on information obtained from the local physician will continue to be submitted.

8.3.3. Adverse Event Definitions.

8.3.3.1. An adverse event is defined as any undesirable experience occurring to a subject during the clinical trial, whether or not the event is considered related to the treatment.

8.3.3.2. Serious adverse events are fatal, life-threatening, disabling, or result in in-patient hospitalization or prolongation of hospitalization. Occurrence of malignancy and adverse events resulting from overdose are considered serious adverse events.

8.3.3.3. An unexpected adverse event is one not described in this document or in the consent form, see Appendix C for US Prescribing Information.

8.3.3.4. Relationship to Transplantation: The assessment of the relationship of an adverse event to transplantation (possible, probable, or definitely related) is a clinical decision based on all available information.

8.3.4. Reporting of Serious Adverse Events.

8.3.4.1. Serious Adverse Events which are unexpected and possibly, probably or definitely related to treatment will be reported to the DUHS IRB, and the FDA within 7 calendar days (5 business days).

8.3.4.2. Other SAEs will be reported in the annual report. See 8.1.2 for expected adverse events.

8.3.4.3. Any deaths will be reported in expedited fashion.

8.3.4.3.1. Unexpected study-related deaths will be reported to the DUHS IRB within 24 hours and to the FDA within 7 days.

8.3.4.3.2. Expected and/or non-study related deaths will also be reported to the agencies.

8.3.5. Grade 4 and 5 Adverse Event Reporting.

8.3.5.1. Adverse events that are severe (grade 4 or higher in CTCAE tables at http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf) and related and unexpected will be reported within 7 days to the IRB, and FDA.

8.3.5.2. Other severe (grade 4 or 5 in CTCAE Tables) adverse events (e.g. expected or not related to the transplant) will be reported in the annual report to the FDA, the DUHS IRB, and, if applicable, to the appropriate funding agencies.

8.4. The type and duration of the follow-up of subjects after adverse events.

8.4.1. Thymus Recipients.

8.4.1.1. Subjects will be followed for at least 2 years.

9. Statistics

9.1. A description of the statistical methods to be employed, including timing of any planned interim analysis(es), is included in the statistical analysis plan. Refer to the statistical analysis plan (SAP) for a full description of the planned analysis. Appendix B.

For the primary efficacy endpoint, survival at Year 1 > 50% will be tested using the binomial exact test. Survival at Year 2 will also be reported as supportive data. Summary of Kaplan-Meier survival will be calculated. Kaplan-Meier estimates of survival at Years 1, 2, 3, 4, and 5 post-transplantation, will be presented with number at risk, number with events, and estimated survival probability.

Summary statistics of the secondary efficacy endpoints will be calculated at baseline, Month 6, Year 1 and Year 2 post-transplantation, as data permit. Summary statistics including the change from baseline to Month 6, Year 1 and Year 2 will also be calculated as data permit.

Summary statistics of the laboratory evaluations for safety and vital signs will be calculated at baseline and post-baseline. Summary statistics including the change from baseline to the timepoints post baseline will be calculated.

Safety assessments (AEs, laboratory evaluations, vital signs measurements, and physical examinations) will be assessed according to the protocol schedule of events for up to 2 years following transplantation. Summaries of safety parameters including adverse events (AEs) reported within 2 years of transplantation will be summarized. The number of AEs, number of subjects in whom AEs occurred, and the percentage of occurrence (%) will be tabulated by system organ class (SOC), and by preferred term (PT). All reported events, regardless of time of onset, will also be listed.

9.1.1. Basis of stopping rule regarding deaths of subjects.

9.1.1.1. The sponsor reviewed survival data on 34 consecutive pediatric patients with complete DiGeorge anomaly who did not undergo treatment for restoration of the immune system, but were only provided supportive care. At the time of the review, ten of these pediatric patients were not yet enrolled but were awaiting insurance approval for thymus transplantation. The physicians of all 34 pediatric patients had contacted Dr. Markert about possible enrollment in the thymus transplantation protocol. Nine of these 34 pediatric patients had preexisting CMV infection or ventilator dependence and are thus excluded from this historical control group (because these conditions are exclusions for groups 2, 3, and 4 in this protocol). Outcomes were determined by Dr. Markert by email or by discussion with the pediatric patient's physician. Of the 25 subjects, who did not have CMV or ventilator dependence, 17 died. Eight of the 25 were living and one was over 1 year. Of the 18 who either died (n=17) or were surviving past 1 year (n=1), 5 of 18 (28%) survived to one year, thus, the one year death rate was 72%. The median survival of these 18 subjects was 7.1 months. No subject had survived past 18.8 months.

9.1.1.2. Taking the historical data into account, we decided that a death rate of ≥ 0.67 would indicate that the procedure had unacceptable safety and that a death rate of ≤0.5 would be acceptable. Only subjects without CMV infection will be evaluated under this rule.

9.1.1.3. Dr. Barry Moser, biostatistician, designed the following overall survival stopping rules: Survival will be evaluated one year after transplantation of $n_1 = 8, n_2 = 16, n_3 = 25$ subjects. The measure of safety is the number of deaths at each of the three evaluation stages, twelve months after transplantation of the first 8, 16, and 25 subjects. An overall safe prognosis is that 1/2 or less of the subjects die within the first 12 months; an unsafe prognosis is that 2/3 or more of the subjects die within the first 12 months. Formally, the null and alternative hypotheses are $H_0 : p \le 0.5$ versus $H_1 : p \ge 0.67$ where p is the proportion of deaths. (H1 = Ha = alternative hypothesis.) Stopping rules were designed to stop enrollment to the trial at the three evaluation stages and conclude that the procedure is unsafe as soon as 7 of the first 8 transplanted subjects, 11 of the first 16 transplanted subjects, or 15 of the first 25 transplanted subjects die within the first 12 months after transplantation. This interim analysis procedure provides a Type I error rate equal to 0.24 (the probability of incorrectly stopping the trial early or continuing the trial through all 25 subjects and concluding the procedure is unsafe when the procedure is safe) and a Type II error rate equal to 0.16 (the probability of incorrectly continuing the trial through all 25 subjects and concluding the procedure is safe when the procedure is unsafe). The stopping rules in Section 4.6.2 and 4.6.3 were chosen under the following criteria: spend a reasonable proportion (near 1/3) of the total Type I error rate at each evaluation stage (to protect against inflating the Type II error rate at any evaluation stage) and minimize both the Type I and Type II error rates while maintaining a Type II error rate below the Type I error rate to more stringently protect against concluding an unsafe procedure is safe. An explanation of terms follows.

9.1.1.3.1. The null hypothesis, H_0, is a good outcome and denotes that the one year death rate is ≤ 0.5.

9.1.1.3.2. The alternative hypothesis, H_1, is a bad outcome and denotes that the one year death rate is ≥ 0.67.

9.1.1.3.3. n_i is the number of transplanted subjects to be examined in the i^{th} evaluation stage for $i = 1, 2, 3$, that is, $n_1 = 8, n_2 = 16, n_3 = 25$.

9.1.1.3.4. The stopping rule is $X_i \ge x_i$ where X_i is the observed number of deaths occurring before one year at the i^{th} evaluation stage for $i = 1, 2, 3$

and $x_i \le n_i$ is a number of deaths such that if $X_i \ge x_i$ at any stage for $i = 1, 2, 3$, $H_0 : p \le 0.5$ is rejected, and the trial is considered unsafe and stopped at that stage. That is, in the 9.1.7.3 discussion $x_1 = 7, x_2 = 11, x_3 = 15$.

9.1.1.3.5. The one year death rate is evaluated with respect to the stopping rules as soon as 7 of the first 8 subjects have died within one year of transplantation or as soon as 2 of the first 8 subjects are alive one year after transplantation, and as soon as 11 of the first 16 subjects have died within one year of transplantation or as soon as 6 of the first 16 subjects are alive one year after transplantation, and as soon as 15 of the first 25 subjects have died within one year of transplantation or as soon as 11 of the first 25 subjects are alive one year after transplantation.

9.1.1.3.6. α_1 , α_2,and α_3 are the Type 1 errors after each evaluation stage (after the number of deaths occurring before one year have been examined in the first $n_1 = 8, n_2 = 16, n_3 = 25$ transplanted subjects).

9.1.1.3.7. α is the cumulative Type 1 error rate (the sum of the three error rates, α_1 , α_2,and α_3, from each evaluation stage). This error rate reflects the overall probability of stopping the trial early or continuing the trial through all 25 subjects and concluding that the procedure is unsafe (high death rate) when in fact it is safe.

9.1.1.3.8. β is the Type 2 error rate. This error rate reflects the overall probability of concluding that the procedure is safe (acceptable death rate) when in fact it is not safe.

9.1.1.3.9. All possible x_1, x_2, x_3 combinations were evaluated. The stopping rule shown in the table below was chosen to have $\beta < \alpha$ (to more stringently protect against continuing the study if it is unsafe) and to have the α_1, α_2 and α_3 be as uniform as possible so that each evaluation contributes some Type I error rate to the analysis while not inflating the Type II error rate at any one stage.

9.1.1.3.10. Using the data in Table 3 below, as soon as 7 of the first 8 transplanted subjects die prior to one year, or 11 of the first 16 transplanted subjects die by 1 year, or 15 of the first 25 transplanted subjects die by 1 year, the protocol will be stopped and the treatment declared unsafe. If none of these three criteria are met, the treatment will be declared safe.

Table 3: Analysis of survival outcomes for activation of stopping rule.

Stopping rule to be used				
x_1 x_2 x_3	n_1 n_2 n_3	α_1 α_2 α_3	α	B
7 11 15	8 16 25	0.035 0.082 0.122	0.23	0.156
Examples of stopping rules rejected				
6 10 16	8 16 25	0.145 0.127 0.017*	0.289	0.17
6 11 17	8 16 25	0.145 0.042 0.011#	0.197	0.289#

*Rejected because α_3 is relatively small that is contributes little to the overall α
Rejected because β is greater than α, and α_3 is relatively small

9.1.2. Basis of stopping rule regarding number of naïve CD4 T cells at one year

9.1.2.1. We have set 67% of subjects surviving to one year having ≤100 naïve CD4 cells/mm^3 as unacceptable. We have set that ≤ 50% having < 100 naïve CD4 cells/mm^3 as acceptable.

9.1.2.2. The data analysis is based on a review of 50 consecutive subjects followed prior to transplantation. None of 50 developed > 100 naïve CD4 T cells even in subjects who were not transplanted until after one year. Data is shown in Figure 4 for 27 subjects who had two naïve CD4 counts tested. Data are similar for the 23 subjects (17 typical, 6 atypical) who had total T cells or naïve CD3 cells tested instead of naïve CD4 counts. None developed cell counts consistent with >100/mm^3 naïve CD4 cells by year 1.

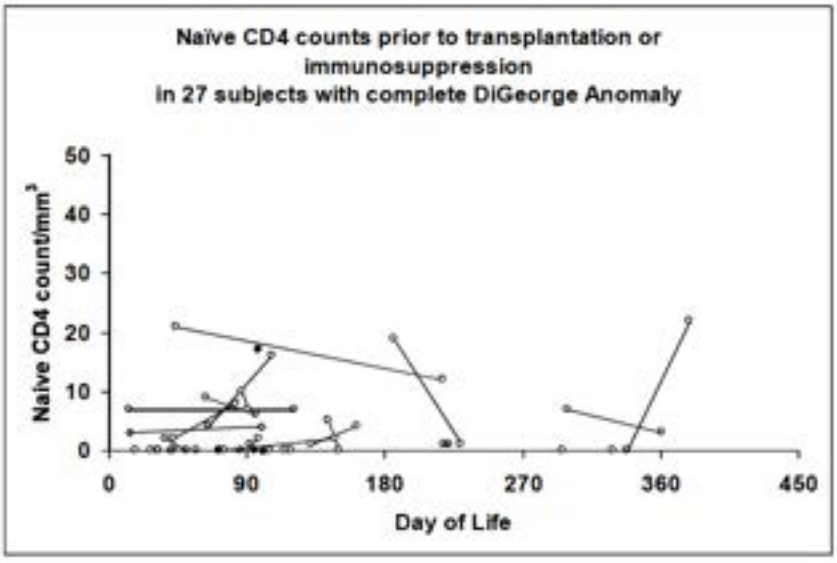


Figure 4. Naïve CD4 T cell counts prior to transplantation and prior to immunosuppression in 27 subjects with complete DiGeorge Anomaly. These data include 14 typical subjects and 13 atypical subjects. The two time points for each subject (open circles) are connected by lines. Four atypical subjects only had this count performed once prior to immunosuppression. Their single data points are indicated by filled circles.

9.1.2.3. Of note there have been 32 subjects with complete DiGeorge anomaly who would have met entrance criteria for this protocol who have been treated under protocols 668, 884, 931,932, or 950 and had naïve T cell counts done at 1 year ± 2 months. Twenty five of the 32 (78%) reached the target of 100 naïve CD4 cells/mm^3 at one year ± 2 months. Thus 22% did not reach the target of 100 naïve CD4 cells/mm^3. Data were similar for subjects treated with immune suppression and those not treated with immune suppression.

9.1.2.4. The statistical plan is analogous to that in 9.1.7. The results are evaluated after 8, 16 and 25 subjects reach 1 year after transplantation.

9.1.2.5. Dr. Barry Moser, biostatistician, designed the following one year naïve CD4 T cell count stopping rules. The year one naïve CD4 T cell counts will be assessed after 8, 16, and 25 subjects reach 1 year after transplantation. For any immunosuppression group, the endpoint in this analysis is the number of naïve CD4 T cells at 12 months ± 2 months. A count of 100/mm^3 naïve CD4 T cells or less at any assessment stage is considered unacceptable. An overall acceptable prognosis is that 1/2 or less of the subjects have naïve CD4 T cells counts of 100/mm^3 or less at 12 months; an unacceptable prognosis is that 2/3 or more of the subjects have naïve CD4 T cells counts of 100/mm^3 or less at 12 months. Formally, the hypotheses under consideration are $H_0 : q \le 0.5$ versus $H_1 : q \ge 0.67$ where q is the proportion of subjects who have naïve CD4 T cells counts of

100/mm^3 or less. Since the hypothesis and the number of subjects at each evaluation stage is the same as the survival safety analysis in Section 4.6.2 the same stopping rules apply with the same Type I and II error rates. That is, stop enrollment to the trial at the three evaluation stages and conclude that the procedure is unacceptable as soon as 7 of the first 8 transplanted subjects, 11 of the first 16 transplanted subjects, or 15 of the first 25 transplanted subjects have naïve CD4 T cells counts of 100/mm^3 or less at 12 months ± 2 months. The naïve CD4 count (≤100/mm^3) applies whether or not all immunosuppression has been weaned.

9.1.2.6. The null hypothesis, H_0, is a good outcome and denotes that the proportion of subjects with a naïve T cell count <100/mm^3 at one year after transplantation is ≤ 0.5.

9.1.2.7. The alternative hypothesis, H_1, is a bad outcome and denotes that the proportion of subjects with a naïve T cell count <100/mm^3 at one year after transplantation is ≥ 0.67.

9.1.2.8. n_i is the number of subjects to be examined in the i^{th} evaluation stage for $i = 1, 2, 3$ one year after transplantation, that is, $n_1 = 8, n_2 = 16, n_3 = 25$.

9.1.2.9. The stopping rule is $X_i \geq x_i$ where X_i is the observed number of subjects with a one year naïve CD4 count of <100/mm^3 at the i^{th} evaluation stage for $i = 1, 2, 3$ and $x_i \leq n_i$ is a number of subjects with a naïve T cell count <100/mm3 at one year after transplantation such that if $X_i \geq x_i$ at any stage for $i = 1, 2, 3$, $H_0 : q \leq 0.5$ is rejected, and the trial is considered not efficacious and stopped. That is, in the 9.1.8.5 discussion, $x_1 = 7$, $x_2 = 11$, $x_3 = 15$.

9.1.2.10. The subjects with naïve CD4 counts of <100/mm^3 are evaluated with respect to the stopping rule after $n_1 = 8, n_2 = 16, n_3 = 25$ subjects have reached one year after transplantation.

9.1.2.11. α_1, α_2, and α_3 are the Type 1 errors after each evaluation stage (after $n_1 = 8, n_2 = 16, n_3 = 25$ subjects have been transplanted and reached one year).

9.1.2.12. α is the cumulative Type 1 error rate (the sum of the three error rates, α_1, α_2,and α_3, from each evaluation stage). This error rate reflects the overall probability of stopping the trial early and concluding that the procedure not efficacious (naïve CD4 counts <100/mm^3) when in fact it is efficacious.

9.1.2.13. β is the Type 2 error rate. This error rate reflects the overall probability of concluding that the procedure is efficacious (acceptable naïve CD4 count) when in fact it is not efficacious.

9.1.2.14. All possible x_1, x_2, x_3 combinations were evaluated. The stopping rule shown in the table below was chosen to have β < α (to more stringently protect against continuing the study if it is not efficacious) and to have the α_1, α_2 and α_3 be as uniform as possible so that each evaluation would contribute some Type I error rate to the analysis while not inflating the Type II error rate at any one stage.

9.1.2.15. Using the data in the Table 4 below, after 8 subjects have been transplanted, if 7 have <100 naïve CD4 cells one year ± 2 months after transplantation, or after 16 have been transplanted and reached one year, if 11 have < 100 naïve CD4 T cells/m^3, or after 25 have been transplanted and reach one year, if 15 have < 100 naïve CD4 cells/mm^3, the protocol will be stopped and the treatment declared to be not efficacious.

Table 4: Analysis of naïve CD4 T cell outcomes for activation of stopping rule.

Stopping rule to be used				
x_1 x_2 x_3	n_1 n_2 n_3	α_1 α_2 α_3	α	B
7 11 15	8 16 25	0.035 0.082 0.122	0.23	0.156
Examples of stopping rules rejected				
6 10 16	8 16 25	0.145 0.127 0.017*	0.289	0.17
6 11 17	8 16 25	0.145 0.042 0.011#	0.197	0.289#

*Rejected because α_3 is so small can contributes little to the overall α
Rejected because β is greater than α, and α_3 is so small

9.1.3. Stopping Rule regarding survival of CMV infected subjects with typical complete DiGeorge anomaly.

9.1.3.1. The stopping rule for inclusion of CMV infected subjects will be based on the sequential probability ratio test (SPRT) designed to evaluate whether the estimated probability of survival within 12 months favors *p1* = 0.1 or *p2* = 0.5, clinically important values chosen to facilitate decisions about early stopping. The decision of stopping enrollment of this subgroup of subjects will be evaluated sequentially using the accumulating data, and enrollment of CMV infected subjects will be stopped if:

Four of 4 subjects die, otherwise

- (if 1 of the first 4 subjects survives) 7 of 8 subjects die, otherwise
- (if 2 of the first 8 subjects survive) 10 of 12 subjects die, otherwise
- (if 3 of the first 12 subjects survive) 12 of 15 subjects die, otherwise
- (if 4 of the first 15 subjects survive) 15 of 19 subjects die, otherwise (if 5 of the first 19 subjects survive) 18 of 23 subjects die, otherwise
- (if 6 of the first 23 subjects survive) 21 of 27 subjects die, otherwise
- (if 7 of the first 27 subjects survive) 23 of 30 subjects die.

9.1.3.2. It is assumed here that no more than 30 CMV infected subjects with typical complete DiGeorge anomaly will be considered for enrollment. Under these stopping rules, there is less than 8.4 percent chance that enrollment will be stopped if the true survival rate is 50% or greater. The probability of stopping enrollment is more than 92 percent if the true survival rate is 10% and more than 55 percent if the true survival rate is 25%. These stopping rules are designed to serve as guidelines for the sponsor, and the decision to stop or continue enrollment of CMV infected subjects may depend on a variety of clinically important factors.

9.2. The number of subjects planned to be enrolled. Reason for choice of sample size, including reflections on the power of the trial and clinical justification.

9.2.1. It is anticipated that approximately 25% will die in the early post transplant course (based on past experience) from infections or other medical complications. We want sufficient numbers to assure us that we can determine whether the protocol as written will result in the development of naïve T cells without excessive adverse events.

9.3. The level of significance to be used.

9.3.1. See Statistical Analysis Plan (SAP) in Appendix B

9.4. Criteria for the termination of the trial.

9.4.1. Termination can be by the IND sponsor at any time. If a stopping rule is hit, the sponsor can terminate.

9.5. Procedure for accounting for missing, unused, and spurious data.

9.5.1. Data that appear spurious are reviewed and an assessment is made regarding the result.

9.5.2. Documentation as to the reason that data are missing will be recorded in the research record (for an example, an intercurrent illness or anemia).

9.6. Procedure for reporting any deviations from the original statistical plan

9.6.1. Not applicable since this is a descriptive study. DUHS IRB amendments will be submitted if deviations from the original statistical plan are desirable.

9.7. The selection of subjects to be included in the analyses.
9.7.1. All subjects who receive thymus transplants will be included in the analyses.

10. Direct access to source data/documents.
10.1. The sponsor will permit trial-related monitoring, audits, IRB review, and regulatory inspections providing direct access to source data/documents. This is a single site study and all participants sign the consent form which allows inspection of the medical record and research records which have the primary data.

10.2. Audits of Good Clinical Practice (GCP) procedures.
10.2.1. These may be done by an external auditor.

11. Quality Control and Quality Assurance.
11.1. Audits of Good Clinical Practices may be performed.

12. Ethics.
12.1. Informed Consent Process and Subject Information.
12.1.1. Potential Subject with DiGeorge Anomaly.
12.1.1.1. The Principal Investigator discusses the research protocol with the parent(s) of the potential subject with complete DiGeorge anomaly who is/are considering thymus transplantation. Usually information is sent to the parent(s) and referring physician for their review prior to transfer of the patient to DUMC. A 1.5-2.5 hour conference call usually is conducted with one or both parent(s) present on the call for the Principal Investigator to be able to discuss thymus transplantation procedures, medications, and risk and benefit and to review the consent document. The entire consent form is reviewed during the conference call and all questions are answered. After the parent(s) and potential subject arrive at DUMC, the entire consent document is again reviewed with the parent(s) and questions answered. If the parent(s) is agreeable, the consent form is signed at this time.

12.2. Documentation.
12.2.1. Subject with complete DiGeorge Anomaly-Transplant Recipient.
12.2.1.1. The original signed consent form is kept in the research study record.

12.2.1.2. A copy of the signed consent form is placed in the recipient's research record and medical chart.

12.2.1.3. A copy of the signed consent form is given to the parent(s) of the subject.

13. Data Handling and Record Keeping.
13.1. All clinical data are reviewed by the Principal Investigator. The Principal Investigator is responsible for record keeping of the clinical data.

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Prior to thymus transplantation					Following transplantation											
	Wk -4	Wk -3	Wk -2	Wk -1	0	Wk +1	Wk +2	Wk +3	Wk +4	Wk +5	Wk +6	Wk +7	Wk +8	Wk +9	Wk +10	Wk +11	Month 3
					TX												
Inpatient/Outpatient DUMC	Depends on clinical assessment.				Inpatient												
Informed Consent	Consent is signed when patient arrives at DUHS																
CBC (Hemoglobin, Hematocrit, WBC, Platelets, Differential	At least once within 1 Month of TX usually done every 1-2 weeks				x	x	x	x	x	x	x	x	x				x
CBC (Hemoglobin, Hematocrit, WBC, Platelets, Differential (on calcineurin inhibitor)	At least once within 1 Month of TX usually done every 1-2 weeks				Weekly until the calcineurin inhibitor is weaned off												
Serum sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose	At least once within 1 Month of TX and monthly prior to TX or as medically indicated								x				x				x
Serum albumin, protein, calcium, magnesium, phosphorus	At least once within 1 Month of TX and monthly prior to TX or as medically indicated								x				x				x
Ionized calcium if on Calcium supplementation	At least once within 1 Month of TX and monthly prior to TX or as medically indicated																
Serum magnesium, lipase, potassium, creatinine	Monthly until off calcineurin inhibitor				Monthly until off calcineurin inhibitor												
Liver function studies (AST, ALT, Alkaline phosphatase, total bilirubin)	At least once within 1 Month of TX and monthly prior to TX or as medically indicated								x AST ALT				x AST ALT				x AST ALT
Type and screen	At least once within 1 Month of TX				X								once before biopsy (if biopsy performed)				
Direct Coombs	At least once within 1 Month of TX																
Reticulocyte Count	At least once within 1 Month of TX																
Coagulation Tests PT/PTT	At least once within 1 Month of TX				X								once before biopsy (if biopsy performed)				
Chest x ray	At least once within 1 Month of TX																

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Prior to thymus transplantation					Following transplantation										
	Wk -4	Wk -3	Wk –2	Wk -1	0	Wk +1	Wk +2	Wk +3	Wk +4	Wk +5	Wk +6	Wk +7	Wk +8	Wk +9	Wk +10	Wk +11	Month 3
					TX												
Urinalysis	At least once within 2 months of TX								x				x				x
Stool for EM	At least once within 2 months of TX and repeat stool testing if develops diarrhea or as medically indicated					as medically indicated											
NP for viral culture	At least once within 2 months of TX and repeated if respiratory symptoms develop or as medically indicated					as medically indicated											
Thyroid Studies	Once within 2 months of TX																
Anti-thyroglobulin and anti-thyroperoxidase antibodies	if thyroid studies indicate hypothyroidism																
Urine for calcium/creatinine ratio (every 1-2 weeks if on calcium replacement)	x		x		x				X				x				x
EBV PCR of Blood As needed if IgE is >500IU/ml	Once within 3 month of TX																x
HHV6 PCR Blood	Once within 3 month of TX																
West Nile Virus PCR blood	Once within 3 month of TX																
Hepatitis B PCR	Once within 3 month of TX																
Hepatitis C PCR	Once within 3 month of TX																
HIV antibody	Birth to before tx																

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Prior to thymus transplantation					Following transplantation										
	Wk -4	Wk -3	Wk –2	Wk -1	0	Wk +1	Wk +2	Wk +3	Wk +4	Wk +5	Wk +6	Wk +7	Wk +8	Wk +9	Wk +10	Wk +11	Month 3
					TX												
HIV NAT (done if antibody +)	If HIV ab + before tx																
CMV PCR blood As needed if IgE is >500 IU/ml	2 tests (1 test within 3 months and 1 test within 1 month of TX				Groups 2, 3, 4 once after RATGAM and before TX Group 1 within 1 week of Tx												x
HLA antibodies	Once																
HLA typing	Once																
22q11.2 hemizigosity	Once																
T cell chimerism (if T cells present)	Once within 6 months of TX																x
DNA storage for molecular analysis-blood sample	Once																
Buccal swab	Once																
Serum Storage (depends on blood volume)	Once																
Serum immunoglobulins	Once and monthly prior to TX								x				x				x
Flow Cytometry	2 assays (1 assay within 3 months and 1 assay within 1 month of TX)																x
PHA proliferative response	2 assays (1 assay within 3 months and 1 assay within 1 month of TX)																
TCRBV flow	Once if sufficient T cells – maybe repeated at the discretion of the PI																

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Prior to thymus transplantation					Following transplantation											
	Wk -4	Wk -3	Wk –2	Wk -1	0	Wk +1	Wk +2	Wk +3	Wk +4	Wk +5	Wk +6	Wk +7	Wk +8	Wk +9	Wk +10	Wk +11	Month 3
					TX												
TRECs	Newborn screening or other TREC data, if available, may be recorded.																
CSA levels	Every 1-2 weeks, if applicable				End of 1st dose of RATGAM and TX (2 levels done)	Every 1-2 weeks until wean begins											
Trough Tacrolimus levels	Every 1-2 weeks, if applicable				End of 1st dose of RATGAM and TX (2 levels done)	Every 1-2 weeks until wean begins											
Cortisol level								when steroids are weaned to every other day for 1 week – get 8 am cortisol level pre steroid dose									
Blood Pressure if on CSA	Every 2 weeks or as medically indicated					Every 2 weeks (or as medically indicated) until wean begins											

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Prior to thymus transplantation					Following transplantation										
	Wk -4	Wk -3	Wk -2	Wk -1	0	Wk +1	Wk +2	Wk +3	Wk +4	Wk +5	Wk +6	Wk +7	Wk +8	Wk +9	Wk +10	Wk +11	Month 3
					TX												
PTH with simultaneous Ionized calcium (in subjects requiring Ca supplementation),	Once but can be delayed until after transplantation																
Renal ultrasound	Once, but can be delayed until after transplantation				as needed												
Cardiac Evaluation	Once				as needed												
Thymus transplantation					X												
Biopsy of thymus tissue, if performed													X, if performed				
Cardiac evaluation	If patient has cardiac history				as needed												
Colonoscopy/Endoscopy - as needed					as needed												
Examine for rashes	Daily, if an inpatient, or at outpatient visits.																
Immunoglobulin (IG) (schedule depends on whether IV or SQ)	At least monthly for IV and weekly to biweekly for SQ																
PCP prophylaxis	Septra three times a week or Pentamidine every 4 weeks																
Protective isolation	Throughout this time																
Interval history and Physical Examination	x			x					x				x				x

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Prior to thymus transplantation					Following transplantation											
	Wk -4	Wk -3	Wk -2	Wk -1	0	Wk +1	Wk +2	Wk +3	Wk +4	Wk +5	Wk +6	Wk +7	Wk +8	Wk +9	Wk +10	Wk +11	Month 3
					TX												
Recording of infections	X	X	X	X	X	X	X	X	X	X	X	X	X				X
Recording of Adverse Events	X	x	x	x	x	x	x	x									x
Medications																	
RATGAM Groups 2, 3, 4				days -5, -4, -3													
Methylprednisolone (RATGAM associated medications)				days -5, -4, -3													
Acetaminophen (RATGAM associated medications)				days -5, -4, -3													
Diphenhydramine RATGAM associated medications)				days -5, -4, -3													
Steroids – Groups 3, 4 Methylprednisolone or prednisolone as needed	as needed																
Cyclosporine as needed Group 3, 4	as needed																
Mycophenolate Mofetil (MMF) Group 4	After Day +5 if T cells over 5000/mm^3 (can be given for 35 days or up to 6 months)																
Tacrolimus Group 3, 4 (if unable to tolerate cyclosporine)	as needed																

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Follow Up After Thymus Transplantation									
	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12	Month 24
CBC (Hemoglobin, Hematocrit, WBC, Platelets, Differential)	X	X	X	X	X	X	X	X	x	
CBC (Hemoglobin, Hematocrit, WBC, Platelets, Differential (on immunosuppression)	Weekly until off calcineurin inhibitor, then monthly as above									
Serum sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose			x			x			x	
Serum albumin, protein, calcium, magnesium, phosphorus			x			x			x	
Serum magnesium, lipase, potassium, creatinine	Monthly until off calcineurin inhibitor									
Liver enzymes (AST/ALT)			x			x			x	
Urinalysis			x			x			x	
Immunoglobulin (IG) (schedule depends on whether IV or SQ	At least monthly for IV and weekly to biweekly for SQ									
PCP prophylaxis	Septra three times a week or Pentamidine every 4 weeks									
Protective isolation	Recommended but done as per local/referring MD									
Calcium (if on Calcium supplementation)	X	x	x	x	x	x	x	x	x	
Urine for calcium/creatinine ratio (if on Calcium supplementation)	X	x	x			x			x	
Thyroid studies			x						x	
Thyroid antibodies, if thyroid function studies show hypothyroidism			x						x	

Appendix A. Timeline for Thymus Transplant Recipient

Procedures/Tests	Follow Up After Thymus Transplantation									
	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12	Month 24
Interval history and Physical Examination	X	x	x	x	x	x	x	x	x	
Examine for Rashes – biopsies as needed	X	x	x	x	x	x	x	x	x	
Serum immunoglobulins			x			x			x	
Flow cytometry			x			X			x	
PHA Proliferation Response									x	
T cell chimerism	Repeated if maternal or donor T cells present in the 3 month sample post transplantation sample									
Recording of infections			x						x	X
Recording of AEs			x			X			x	X
Telephone Contact										X
Cyclosporine as needed	As needed									
Trough Cyclosporine levels	Every 1-2 weeks until wean begins									
Tacrolimus (if unable to tolerate cyclosporine	As needed									
Trough Tacrolimus levels	Every 1-2 weeks until wean begins									
Cortisol Level	(when steroids are weaned to every other day for 1 week- get 8 am cortisol level pre-steroid dose									

Appendix B. Statistical Analysis Plan

Statistical Analysis Plan is a separate document titled *"Appendix B. Enzyvant_RVT-802 Program SAP-Final v2_19Sep2018"*

Enzyvant on file

Appendix C. RETHYMIC US Prescribing Information

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 833-369-3868 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

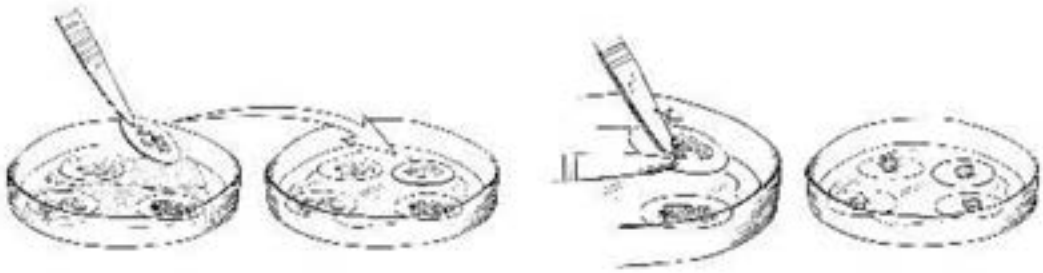
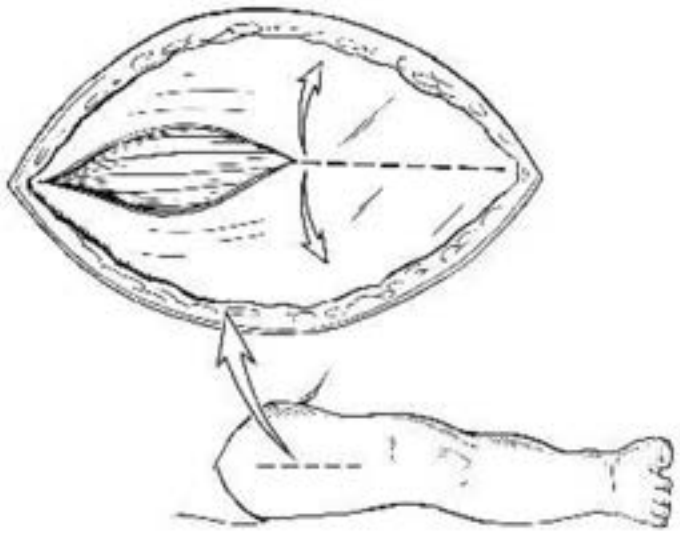


Figure 1: Within the sterile field, forceps are used to move individual RETHYMIC slices with their filter membranes from the drug product dish to the operating room culture dish (left images). A pair of forceps is used to gently scrape and lift the RETHYMIC slice off the filter membrane in the operating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

Implantation of RETHYMIC:

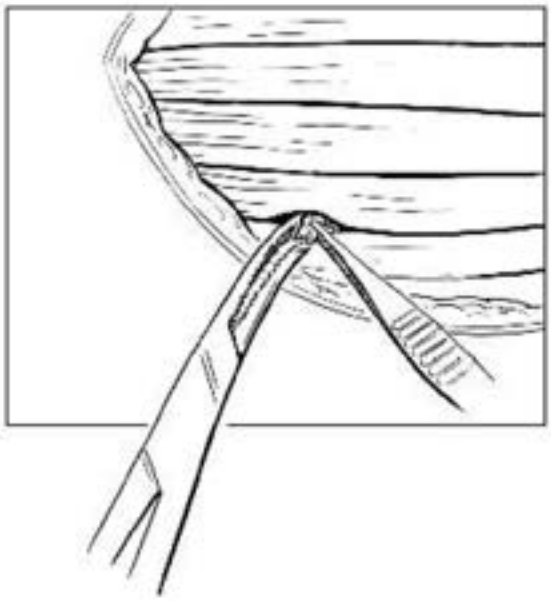
1. After induction of general anesthesia, a cranial-caudal skin incision (typically ~5 cm in length; Figure 2) should be made over the anterior thigh compartment. The size of the incision and the use of one or both legs for the implantation procedure is determined by the size of the patient, his/her 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



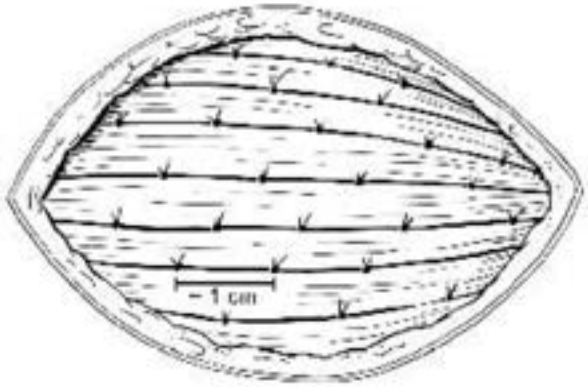
2. Open fascia to expose the anterior compartment muscles (Figure 2).
3. Create a pocket in between the muscle fibers using a tonsil clamp or similar instrument. Each pocket should be made along the natural furrows throughout the quadriceps muscle group.
4. Individual RETHYMIC slices should be implanted 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 Preferred Term	RETHYMIC (N=105) 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 or < 20-fold response to PHA over background	None
Typical	≥ 5,000 cpm and < 50,000 cpm or Evidence of maternal engraftment	• ATG-R • Methylprednisolone
Typical	≥ 50,000 cpm	• ATG-R • Methylprednisolone • Cyclosporine[2]
Atypical	< 40,000 cpm on immunosuppression or < 75,000 cpm when not on immunosuppression	• ATG-R • Methylprednisolone • Cyclosporine[2]
Atypical	≥ 40,000 cpm on immunosuppression or ≥ 75,000 cpm when not on immunosuppression or Evidence of maternal engraftment	• ATG-R • Methylprednisolone • Cyclosporine[2] • Basiliximab[3] • 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) • 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 • RETHYMIC implantation occurred within 7 days of last dose of ATG-R o If the implant occurred more than 7 days after the last dose of ATG-R, a T cell count was repeated: – If the T cell count was <50/mm³, no more ATG-R was administered – If the T cell count was >50/mm³, ATG-R was repeated at the same schedule and dose as the initial infusion. • 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

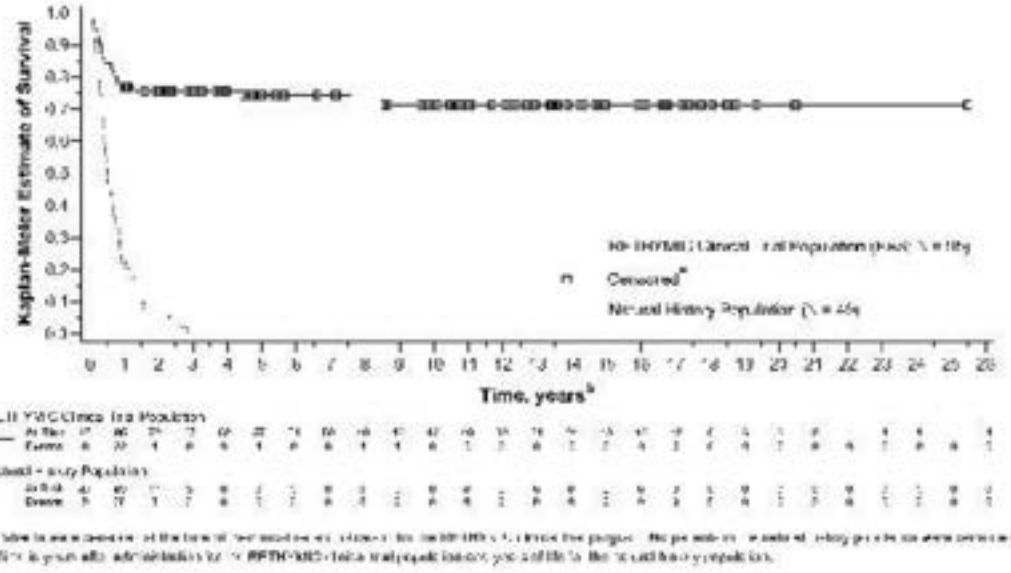
1. Additional pre-implantation corticosteroids (methylprednisolone) were used for atypical patients if pre-implantation CD3⁺ T cell numbers or the absolute lymphocyte count (ALC) was greater than 4,000 cells/mm³. A starting dose of 1 mg/kg/day was used if the T cell count or ALC was between 4,000 and 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 D. 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 E. Investigator Agreement

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.

[redacted] [redacted]

Principal Investigator Name (Printed) Principal Investigator Signature

[redacted]

[redacted] [redacted]

Date Site