

I concur with this review. M. Serabian 11/01/19

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER: STN #125685.000

DATE RECEIVED BY CBER: July 06, 2018 (Pharmacology/Toxicology modules)
 April 05, 2019 (Complete submission)

DATE REVIEW COMPLETED: September 21, 2019

PRODUCT: RETHYMIC (Allogeneic processed postnatal thymus tissue product for surgical implantation; product code name: RVT-802)

APPLICANT: Enzyvant Therapeutics GmbH
 PROPOSED INDICATION: For the immune reconstitution of pediatric patients with congenital athymia

PHARM/TOX REVIEWER: Wei Liang
 PHARM/TOX TEAM LEADER: Sandhya Sanduja
 PHARM/TOX BRANCH CHIEF: Mercedes Serabian
 PRODUCT (CMC) REVIEWERS: Tom Finn, Sukhanya Jayachandra, and Alyssa Kitchel
 CLINICAL REVIEWER: Winson Tang
 PROJECT MANAGERS: Jean Gildner and Adriane Fisher
 DIVISION DIRECTOR: Tejashri Purohit-Sheth
 OFFICE DIRECTOR: Wilson Bryan

EXECUTIVE SUMMARY:

Studies were conducted with thymus tissues to evaluate the effect of a variety of *in vitro* culture conditions on: 1) thymus architecture; 2) thymus epithelial cell (TEC) composition and function; 3) generation of a functional naïve T cell population; and 4) gene expression related to immune tolerance. Resulting data showed that: 1) a normal thymus phenotype was established and survival of TECs able to produce key regulatory genes that support thymopoiesis was observed. Analysis of biopsy samples obtained following transplantation of allogeneic postnatal thymus tissue in patients with Complete DiGeorge anomaly (cDGA) showed development of naïve T cells and the presence of a broad spectrum of T cell receptor beta variable (TCRBV) regions, indicating that the cultured thymus tissue can maintain sufficient numbers of TECs needed to drive development of a functional endogenous T cell population.

Studies were conducted in nude rats to identify potentially optimal culture conditions of allogeneic neonatal thymus that result in engraftment and development of an endogenous T cell population (i.e., thymogenesis) following transplantation. The *in vivo* data showed that: 1) thymopoiesis was observed at the pre-specified time points (1 and 9 months post-transplantation); 2) there was a progressive increase in endogenous generation of total and naïve T cells in the peripheral blood; and 3) there was a time-dependent decrease in donor-derived T cells in the peripheral blood as the endogenous T cell population developed.

Seven clinical studies assessing the safety and effectiveness of RVT-802 in children with cDGA were conducted under IND #9836. Based on the acceptable clinical safety profile of RVT-802 and the lack of relevant animal species/models, no nonclinical toxicology studies with RVT-802 were performed. No *in vitro* or *in vivo* genotoxicity, carcinogenicity, or developmental and reproductive toxicity (DART) studies were conducted with RVT-802.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There were no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of RVT-802. The nonclinical information provided in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:

RVT-802 is an allogeneic, processed postnatal thymus tissue product that is administered via surgical implantation. RVT-802 consists of yellow-to-brown slices of processed thymus tissue with varying thickness and shape. The recommended dose range is (b) (4) to 22,000 mm² of processed thymus tissue/m² recipient body surface area (BSA).

Abbreviations

AIRE	Autoimmune regulator
BSA	Body surface area
cDGA	Complete DiGeorge anomaly
CDR2	Cortical dendritic reticulum antigen 2
CK	Cytokeratin
DART	Developmental and reproductive toxicity
DG	Deoxyguanosine
DGS	DiGeorge syndrome
EpCAM	Epithelial Cell Adhesion Molecule
Eya1	Eyes absent homolog 1
FCS	Fetal calf serum
FOXN1	Forkhead box protein N1
H&E	Hematoxylin and eosin
IHC	Immunohistochemistry
MHC	Major histocompatibility complex
Pax	Paired Box
TCRBV	T cell receptor beta variable
TE	Thymic epithelium

TEC	Thymus epithelial cell
TG	Thyroglobulin
TOM	Thymus organ medium
(b) (4)	

Related File(s)

IND #9836; Enzyvant Therapeutics GmbH; RVT-802 (Allogeneic cultured post-natal thymus tissue); For transplantation as therapy for primary immune deficiency resulting from congenital athymia associated with complete DiGeorge anomaly (cDGA); ACTIVE

Table of Contents

INTRODUCTION	3
NONCLINICAL STUDIES	4
PHARMACOLOGY STUDIES.....	4
Summary List of Pharmacology Studies	4
Overview of Pharmacology Studies	5
SAFETY PHARMACOLOGY STUDIES.....	21
PHARMACOKINETIC STUDIES (Cell Distribution)	21
TOXICOLOGY STUDIES	21
APPLICANT'S PROPOSED LABEL.....	23
CONCLUSION OF NONCLINICAL STUDIES	23
KEY WORDS/TERMS	23

INTRODUCTION

RVT-802 is an allogeneic processed postnatal thymus tissue derived product for the treatment of pediatric patients with congenital athymia associated with cDGA or Forkhead box protein N1 (FOXP1) deficiency. Thymus tissue is obtained as discarded tissue from infants under the age of 9 months that are undergoing cardiac surgery. The tissue is processed by cutting into slices and cultured to partially deplete the tissue of thymocytes, while maintaining a network of TECs. Thymus tissue slices from a single donor constitute one lot, which will treat a single patient. RVT-802 is surgically implanted into the quadriceps muscle.

DiGeorge syndrome (DGS) is a rare congenital disorder characterized by defects in the parathyroids, thymus, and heart. Patients with DGS typically present with clinical features that include congenital cardiac anomalies, a small thymus, reduced numbers of circulating T cells, and hypocalcemia secondary to hypoparathyroidism. Approximately 1% of patients with DGS are athymic; they are classified as having cDGA. There are currently no approved

treatment options for this clinical population. Without therapeutic reconstitution of the immune system, primary immunodeficiency in infants with cDGA is almost 100% fatal by the age of 2 years, most frequently from infections.

FOXN1 deficiency is an exceptionally rare inherited disease, with fewer than 10 cases reported in the literature. It is caused by autosomal recessive loss-of-function mutations in FOXN1. This gene encodes a transcription factor essential for development of the thymus. FOXN1 deficiency results in athymia, absence of hair, and dysplastic nails. The lack of naïve T cell development in these children (as in children with cDGA) makes them susceptible to infection. Without thymus transplantation, these individuals die from infection in the first few years of life.

The proposed mechanism of action involves the migration of recipient bone marrow stem cells to the implanted RVT-802, where they develop into naïve recipient T cells. RVT-802 alters the positive and negative selection process of the developing recipient T cells, enabling the T cells to be tolerant to both donor thymus and recipient tissues, as well as recognize foreign antigens in the context of recipient major histocompatibility complex (MHC) proteins.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of RVT-802 to treat the target clinical population.

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	Effect of Postnatal Thymus Organ Culture on Thymus Tissue for Transplantation in Complete DiGeorge Anomaly Patients	RVT-802-002

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
2	Allogeneic Thymus Transplantation in Nude Rats	RVT-802-001

Overview of Pharmacology Studies

Overview of In Vitro Studies

Study #1: Effect of Postnatal Thymus Organ Culture on Thymus Tissue for Transplantation in Complete DiGeorge Anomaly Patients

Note: The applicant provided Study Report No. RVT-802-002 that summarizes the results of three publications^{1,2,3} reporting the studies conducted by the applicant to support implantation of postnatal thymus tissue in patients with cDGA. The results of the three publications are summarized separately in the study report.

Publication #1: Markert et al. 1997¹

Objective: To evaluate the effect of *in vitro* culture on the growth potential of the thymic epithelium (TE) and the expression of thymic microenvironment antigens in the human postnatal thymus tissue.

Study design:

Discarded thymus tissue (n = 5-9) was obtained from infants at 2 years of age who were undergoing cardiac surgery. Each thymus was cut into 1 mm-thick slices and placed on sterile filters located on sterile sponges in tissue culture plates containing thymus organ medium (TOM). The medium was changed every day for the first 2 weeks and twice a week thereafter. The thymus tissue culture was maintained for up to 12 weeks. Evaluation of the slices occurred at multiple time points during the 12-week culture.

Note: This reviewer presumes that the 'n = 5-9' represents the number of tissue donors.

In addition to assessment of fresh thymus tissue slices, the study was also designed to evaluate the effect of cryopreserved thymus tissue slices on the ability to establish a TE cell monolayer. On days 1 to 7 in culture, thymus slices (on the filters) were placed into cryotubes and frozen. The cryotubes were thawed 2 to 14 days after cryopreservation and the tissue was placed back into the TOM. The medium was changed every day for the first 2 weeks and twice a week thereafter. The thymus tissue culture was maintained for up to 12 weeks. Evaluation of the slices occurred at multiple time points during the 12-week culture.

Thymus tissue slices were examined histologically on day 0 (the day the thymus was obtained) and at weekly intervals during culture using hematoxylin and eosin (H&E) staining. After 6 to 21 days in culture, which is the timeframe when TE monolayers developed, the monolayers were

¹ Markert et al. The human thymic microenvironment during organ culture. Clin Immunol Immunopathol. 1997 Jan;82(1):26-36.

² Li et al. Characterization of cultured thymus tissue used for transplantation with emphasis on promiscuous expression of thyroid tissue-specific genes. Immunol Res. 2009;44(1-3):71-83.

³ Li et al. Thymic microenvironment reconstitution after postnatal human thymus transplantation. Clin Immunol. 2011 Sep;140(3):244-59.

photographed to document their extent of growth, followed by immunostaining with fluorescent monoclonal antibodies to characterize the expression of thymic microenvironment antigens.

Results:

- After 3 weeks of culture, slices were immunostained with antibodies against various thymic microenvironment antigens. The following cell populations were identified: 1) pan cytokeratin (CK)-positive cortical and medullary TECs, 2) thymic cortical epithelial cells, 3) thymic medullary and subcapsular cortical epithelial cells, 4) Hassall's bodies in the thymic medulla, 5) macrophages, and 6) fibroblasts. The tissue slices maintained many features of thymic architecture, including the presence of viable TECs, for up to 6 weeks in culture. However, the number of thymocytes decreased over the initial 3 weeks in culture. Per the publication, this was consistent with the cell losses noted during the daily medium changes; however viable thymocytes were still identified. Beyond 3 weeks in culture no distinct staining patterns of the cortical and medullary areas, with the TECs expressing both cortical and medullary markers, were observed.
- Monolayers of viable TECs were present in the thymus slices for up to 12 weeks of culture.
- When compared to fresh thymus tissue slices, thymus slices that were cryopreserved showed no difference in the growth potential of the TECs or in the ability to express CK-positive cortical and medullary TECs in monolayer cultures.

Publication conclusion: This initial study showed: 1) the long-term growth potential of cultured postnatal thymus tissue *in vitro*; 2) the cultured thymus slices retained expression of antigens characteristic of normal thymic microenvironment; 3) cryopreservation of thymus slices did not affect TEC viability or antigen expression. Thus, maintaining TEC viability and antigen expression of thymus tissues in *ex vivo* cultures would likely support *in vivo* thymopoiesis.

Comments:

- Cryopreserved thymus tissue will not be used to manufacture the final clinical product.
- Based on the data submitted in Report No. RVT-802-002 and the publication by Markert et al. 1997, this reviewer agrees with this conclusion.

Publication #2: Li et al. 2009²

Objectives:

- 1) To evaluate the effect of thymus tissue culture conditions on the composition/differentiation of the TECs.
- 2) To assess the effect of thymus tissue culture conditions on the expression of genes involved in immune tolerance, including thyroid peroxidase (TOP), thyroglobulin (TG), and autoimmune regulator (AIRE).

Background for Objective #2: The incidence of Hashimoto's thyroiditis or Graves disease (thyroid-specific autoimmune disease) is higher in patients with DGA that have undergone thymus transplant compared to healthy individuals, and is characterized by the presence of auto-antibodies against TOP and TG. Within the thymus, tolerance is mediated by initial positive selection, followed by subsequent negative selection. This process is mediated by several key genes, including AIRE. Thus, understanding the thyroid tissue-specific antigen presentation in the thymus may reveal mechanisms underlying the development of autoimmune thyroid disease in the general population and in patients after thymus transplantation.

Study design:

Discarded thymus tissue was obtained from 11 infants under 9 months of age who were undergoing cardiac surgery. Each thymus was cut into 0.5-1 mm thick slices and placed on Millipore filters located on surgical sponges in tissue culture plates containing culture medium. The sliced tissue was cultured using four different media that varied by the presence or absence of fetal calf serum (FCS) and deoxyguanosine (DG): 1) TOM (containing FCS without DG), 2) TOM with DG (containing FCS and DG), 3) X-Vivo™ 10 medium (without FCS or DG), or 4) Cell-Grow Free medium (without FCS or DG). The slices were maintained in culture for up to 21 days. The effect of various culture conditions on the expression of genes (CK5 and CK14) in the TECs was assessed by immunohistochemistry (IHC) and the effect on the expression levels of AIRE, TG, and TOP was assessed by RT-PCR at the time of tissue harvest (day 0) and after 14 and 21 days in culture.

Note: It is not clear whether: 1) thymus tissue from all 11 children was cultured in each of the four different media and for each of the two culture times.

Results #1: Effect of Culture Conditions on Thymus Architecture and TEC Composition

- CK5 and CK14 mRNA was detected in thymus tissue on day 0 and after 14 or 21 days of culture in all four different media. The expression levels were higher after 14 or 21 days of culture than on day 0.

Comments:

- Per the publication, DG was used to deplete thymocytes in the tissue culture. Per CMC Module 2.6.2, "DG was added to the media for the first 43 thymuses that were transplanted to deplete T cells from the thymus tissues. No clinical benefit could be confirmed with the use of DG. After March 2006, DG was no longer added to the media."
- Per the publication, CK proteins are expressed by epithelial cells in the normal thymus; CK5 is expressed in both cortical and medullary epithelium; and CK14 is expressed in the subcapsular cortex and medulla.
- Per the publication, the higher levels of CK5 and CK14 mRNA after culture may be secondary to depletion of thymocytes that do not express these genes and does not necessarily reflect an induction of gene expression.
Note: the CK5 and CK14 mRNA levels were relative to 18s rRNA, which is produced by both thymocytes and TECs.

- Evaluation of the cultures using IHC showed that CK5- and CK14-positive TE were present in the thymus tissue, in a more condensed pattern, that increased with time in culture, compared to the pattern on day 0. The results were similar for thymus tissue cultured in the four different media (Figure 1).

Figure 1 Immunohistological Characteristics of Thymus Tissue Cultured in Different Media

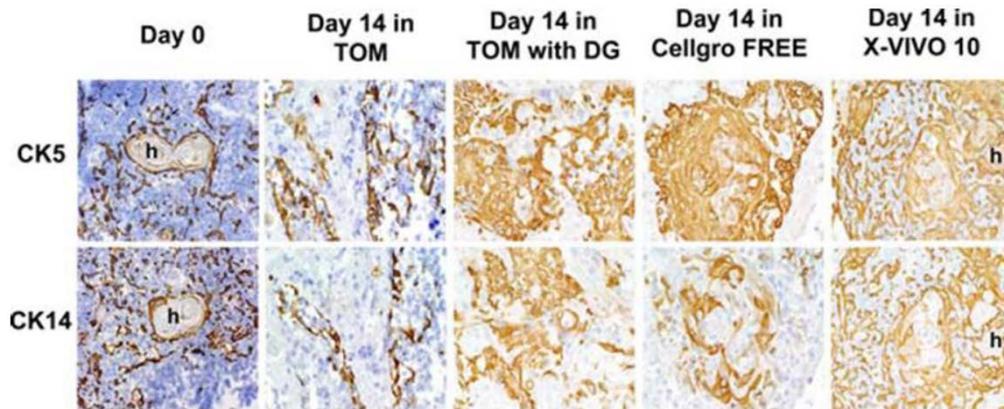


Figure 1. Immunohistological Characteristics of Thymus Tissue Cultured in Different Media. Data adapted from Figure 5 (Li et al., 2009). CK5 and CK14 were consistently expressed in the thymus tissue on the harvest day and in thymus tissue cultured for 14 days in TOM, TOM with DG, Cellgro FREE, and X-VIVO 10 media. In upper and middle panels, viable epithelial cells expressing CK5 and CK14 (brown stain) were detected in the thymus tissue after 14 days of culture although the pattern was more condensed than the lacy pattern seen on day 0. Note Hassall's body (h). (Magnification, x40)

Source: Study Report No. RVT-802-002, submitted in Module 4 of the BLA.

Results #2: Effect of Culture Conditions on Gene Expression Related to Immune Tolerance

Gene expression in cultured thymus tissue:

- mRNA expression of AIRE and TG was detected in the tissue after 14 days of culture at levels similar to day 0 in all four culture media (Figure 6A).
- After 21 days of culture AIRE expression was below the day 0 level, while TG expression was maintained at a level that was similar to day 0 in all four culture media (Figure 6B).
- mRNA expression of TOP was reduced after 14 or 21 days of culture compared to day 0 in all four culture media.

Comment:

- An explanation for the reduction in TOP gene expression following culture was not discussed in the publication or in the study report.

Gene expression in different cellular subsets sorted from thymocytes isolated from the day 0 thymus tissue:

- AIRE expression was not detected in any of the isolated cellular subsets (e.g., thymocytes, CD3+, CD4+, CD8+, etc.) (Figure 6C). TG was expressed by both the thymus tissue and the thymocytes (particularly the CD3^{hi} cells) (Figure 6C). Per the publication, these cells were positive for CD4 or CD8 (i.e., the mature naïve T cell population).

Note: The thymus tissue consisted of both TECs and thymocytes.

Figure 6 mRNA Expression in Thymus Tissue and Isolated Cellular Subsets

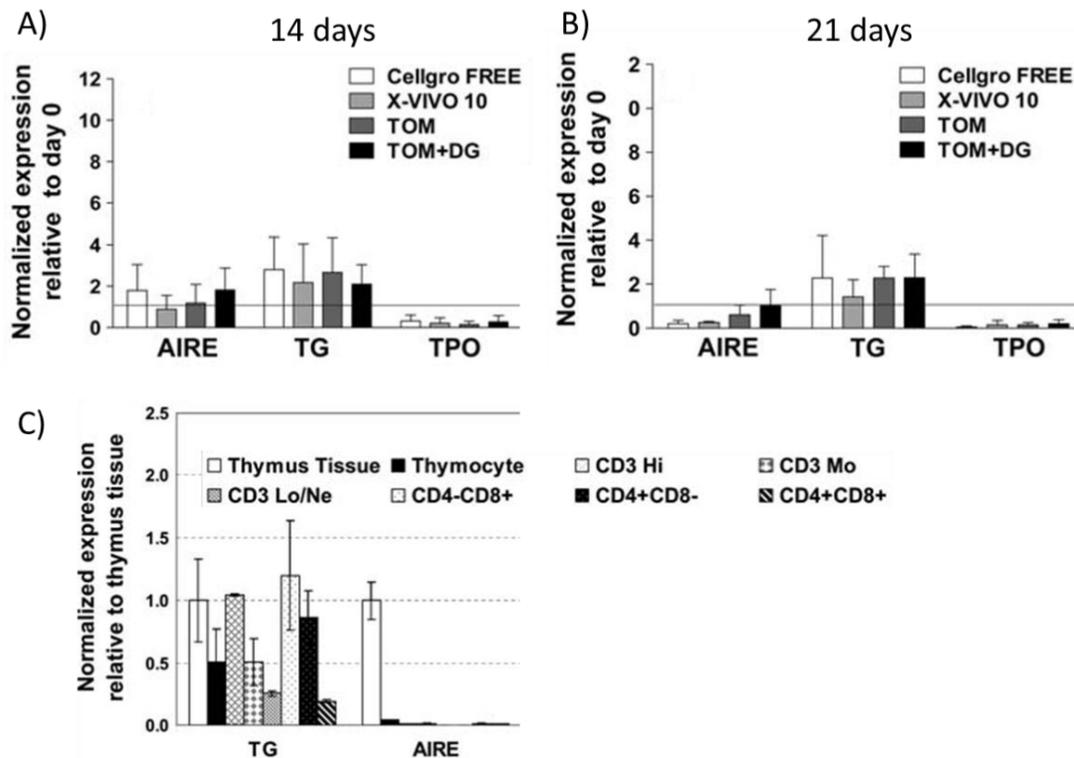


Figure 6. mRNA Expression in Thymus Tissue and Isolated Cellular Subsets. Data adapted from Figure 2 and Figure 4 (Li et al., 2009). (A, B) The relative gene expression of AIRE, TG, and TPO was compared to harvest-day (day 0) thymuses by real-time PCR. The thymus tissue was cultured with media of Cellgro FREE, X-VIVO 10, TOM, and TOM with DG for either (A) 14 days or (B) 21 days. Tissue at day 0 was used as calibrator control (dotted line). (C) Thymocytes were isolated from a fresh thymus. The “thymocyte” population included all thymic cells recovered by density gradient centrifugation. Cell sorting was performed after incubation with mAbs of CD3-PerCP, CD4-PE, and CD8-FITC. The relative gene expression of AIRE and TG was compared across tissue/cellular subsets. The expression in thymus tissue was set to 1. A-C) Error bars represent the average of 3 donors +/- the standard error of the mean.

Source: Study Report No. RVT-802-002, submitted in Module 4 of the BLA.

Publication conclusion: The four culture media used did not affect expression of CK5, CK14, thyroid tissue-specific antigens, or AIRE in the cultured thymus tissue. In the human thymus, the TG gene is expressed in the TECs and the thymocytes. The TG antigen may be directly presented by TECs or cross-presented by thymocytes to antigen-presenting cells. Thus, the data may help further understand the mechanisms underlying the development of autoimmune thyroid disease in patients after thymus transplantation.

Comment:

- Based on the data submitted in Report No. RVT-802-002 and the publication by Li et al. 2009, this reviewer agrees with this conclusion.

Publication #3: Li et al. 2011³

Objective: To understand how a functional thymus develops after implantation of cultured thymus tissue into patients with cDGA.

Study design:

The study compared the phenotype of biopsies of implanted thymus tissue to freshly harvested donor thymus tissue and cultured thymus tissue (prior to transplantation). Biopsy tissues (3-4 samples/biopsy) were obtained from seven infants with cDGA at various time points (2-3 months to 1-4 years) after implantation of unrelated cultured postnatal thymus tissue. The thymus tissue was sliced and maintained in culture for 15-21 days, followed by insertion into the quadriceps muscle of the patients. Samples of biopsied tissue collected at multiple time points post-implant were assessed using flow cytometry to evaluate T cell generation after thymus transplantation. The presence of the following genes was determined using RT-PCR and/or IHC: CK14, cortical dendritic reticulum antigen 2 (CDR2), Foxn1, Epithelial Cell Adhesion Molecule (EpCAM), Paired Box (Pax)1, Pax9, and Eyes absent homolog 1 (Eya1).

Result #1: Effect of *in vitro* Culture on Thymus Architecture and TEC Composition and Function

- The thymic cortex and medulla of the biopsied allograft showed a normal appearance. Most of the TECs expressed both cortical (CDR2) and medullary (CK14) markers.

Comment:

- Per the publication, this TEC phenotype (i.e., CDR2+CK14+) is not found in the normal human postnatal thymus since CDR2 is normally expressed by cortical TECs and CK14 is normally expressed by medullary TECs. Thus, the data indicate that some TECs, possibly expressing CK14 in the cultured thymus, develop into CK14+CDR2+ epithelial cells that act as thymic epithelial progenitors for the reconstitution of the thymus tissue allograft after implantation.
- EpCAM expression was detected in small areas of the biopsies.

Comment:

- Per the publication, EpCAM normally is expressed on medullary epithelium, thus the results suggest early development of a mature medulla.
- Foxn1 mRNA expression was detected in fresh thymus; levels were decreased after time in culture. Foxn1 mRNA expression in the biopsies was increased compared to fresh thymus.

Comment:

- Per the publication, the Foxn1 gene is critical for lineage progression in both medullary and cortical TECs, thus the high levels of Foxn1 in the allografts suggest an important role of Foxn1 in reconstitution of the thymus following implantation.
- Pax1, Pax9 and Eya1 mRNA expression was detected in freshly harvested thymus, in cultured thymus, and in thymus allograft biopsy tissue at varying levels (Figure 3).

Comment:

- Per the publication, the transcription factors Pax1, Pax9, and Eya1 are required for early thymus development, thus the data suggest ongoing thymus development.

Figure 3 Gene Expression in Patient Thymus Allograft Biopsies

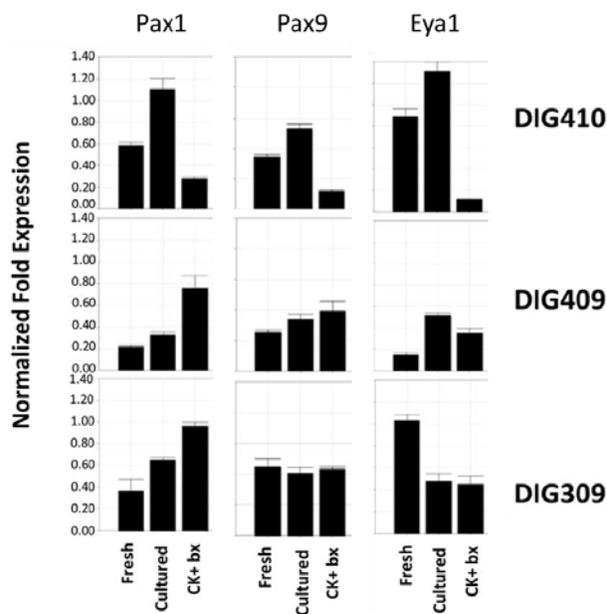


Figure 3. Gene expression in patient thymus allograft biopsies. Data adapted from Figure 10 (Li et al., 2011). For 3 subjects, RNAs from freshly harvested donor thymus (fresh), cultured donor thymus (cultured), and a CK14⁺CK5⁺ positive biopsy (CK⁺ bx) sample, were evaluated by real-time PCR. In each panel the freshly harvested and cultured thymus was the thymus used in transplantation for the subject identified to the right of the panel. In each panel, the control thymus RNA expression is set at 1.0. Abbreviation: bx; biopsy.

Source: Study Report No. RVT-802-002, submitted in Module 4 of the BLA.

Result #2: T Cell Development after Thymus Implantation

- The development of CD3 T cells and naïve CD4 T cells (i.e., CD62L and CD45RA double-positive cells) in peripheral blood mononuclear cells (PBMCs) was observed in all seven infants following thymus implantation (Figures 1A and 1B). These naïve T cells were functional T cells, as evidenced by a broad range of TCR beta variable (TCDBV) regions (Figures 1C and 1D).

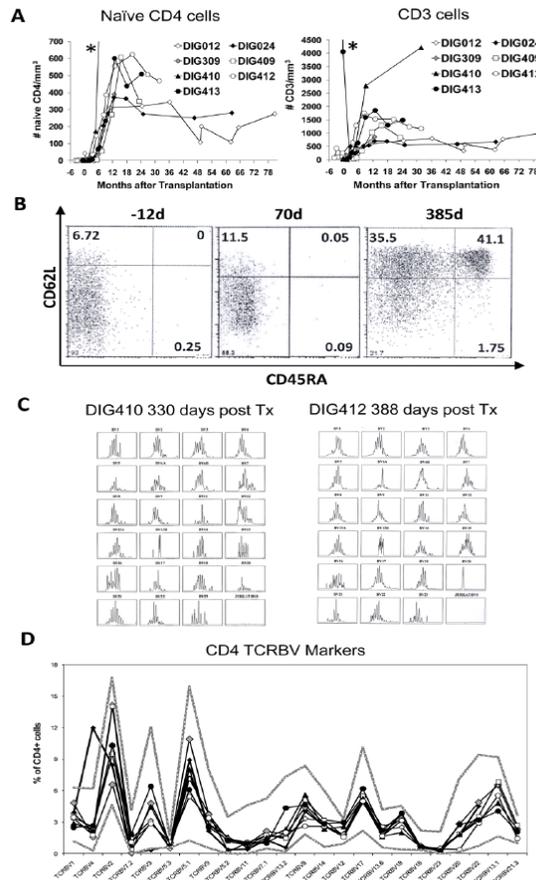


Figure 1. T cell generation after thymus transplantation

(A) The number of naïve CD4 and total CD3 T cells are shown in 7 subjects after transplantation. Subject ID numbers are included. The value of the last naïve CD4 data point of DIG410 (*, not shown) was 1836/mm³ at 9 months after transplantation. The value of the first CD3 data point of DIG413 (*, not shown) was 7264/mm³ at 21 days before transplantation. (B) Representative dot plots of CD4 T cells co-expressing CD62L and CD45RA in one of 7 subjects (DIG413) at 12 days before transplantation, day 70 (the day of biopsy), and 385 days after transplantation. The percentages of CD62L⁺CD45RA⁺ cells in the upper right quadrant are included in each panel. (C) CDR3 spectratyping profiles of CD4 T cells isolated from subjects DIG410 and DIG412 at 330 days and 388 days post thymus transplantation. (D) CD4 TCRBV repertoires were evaluated after transplantation by flow cytometry in 7 subjects. The time points post transplantation for the assays were 1476 days for DIG012, 749 days for DIG024, 379 days for DIG309, 466 days for DIG409, 783 days for DIG410, 388 days for DIG412, and 385 days for DIG413. The symbols for the thymus transplant recipients are the same as in Panel A. The two solid grey lines indicate the normal adult range (3 standard deviations (SD) above and below the mean).

Source: Li et al. Thymic microenvironment reconstitution after postnatal human thymus transplantation. *Clin Immunol.* 2011 Sep;140(3):244-59.

Publication conclusion: The data show that cultured thymus tissue can maintain sufficient numbers of TECs needed to drive development of a functional endogenous T cell population.

Comment:

- Based on the data submitted in Study Report No. RVT-802-002 and in the publication by Li et al. 2011, this reviewer agrees with this conclusion.

Overview of In Vivo Studies

Study #2: Allogeneic Thymus Transplantation in Nude Rats

Date signed: June 8, 2018

Note: Study #2 was conducted as two parts (HRT11 and HRT13). These two experiments are summarized separately in this review memo.

Experiment #1 (HRT11):

Objectives: To explore the feasibility of neonatal thymus transplantation on thymopoiesis in nude rats and to characterize the development of T cell subsets based on the donor thymus pre-transplant condition: 1) cryopreserved, 2) fresh, or 3) 8-day culture.

Test system:

- Donor Animals – Total of 10 (b) (4) rats; male and female
- Recipient Animals – Nude rats (b) (4) on the (b) (4) background]; 6 males(2/group)

Comments:

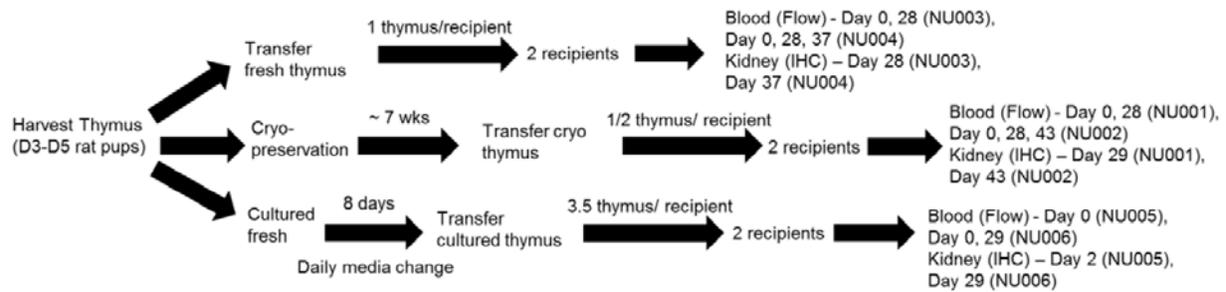
- Since the donors and the recipients had the same (b) (4) background (i.e., the same MHC), the syngeneic transplantation scenario in rats did not reflect the human scenario, which is allogeneic.
- The number of recipients in each group was extremely small (2 males/group).

Study design:

Anesthetized 3-5 day old (b) (4) rats were transplanted with:

- 1) Fresh thymus (one thymus/recipient),
- 2) Cryopreserved/thawed thymus that were not cultured (half of one frozen thymus/recipient), or
- 3) Fresh thymus cultured for 8 days (three thymi/recipient).

The thymus was transplanted under the kidney capsule. The recipients were sacrificed between 2-43 days after transplantation, the kidney was harvested, and thymic architecture and cellular composition was examined using IHC. Flow cytometry was performed on peripheral blood from the recipient rats and two non-transplanted (b) (4) control animals to characterize the development of CD4+ T cells, CD8+ T cells, naïve CD4+ T cells, and naïve CD8+ T cells throughout the course of the study (see the figure below for the overall study design).



Source: Study Report No.RVT-802-001.1, submitted in Module 4 of the BLA.

Comments:

- It is not clear why different the number of transplanted thymi was not consistent between each recipient; no explanation was provided in the study report.
- The transplantation site (kidney capsule) was different from the implant site in humans (quadriceps muscle). The scientific literature reports that the kidney capsule has been the site of transplantation for a variety of tissues in rodents because: 1) the kidney is accessible and 2) the kidney capsule is a highly vascularized site.

Results:

- Cryopreserved thymus did not engraft following transplantation in the nude rats. Fresh thymus (Figure 1) and 8-day cultured thymus (Figure 2) showed engraftment when assessed at 1 month post-transplantation, as supported by expression of CK, Ki-67, and CD3.

Figure 1 Fresh Thymus Transplanted for 28 Days (HRT11)

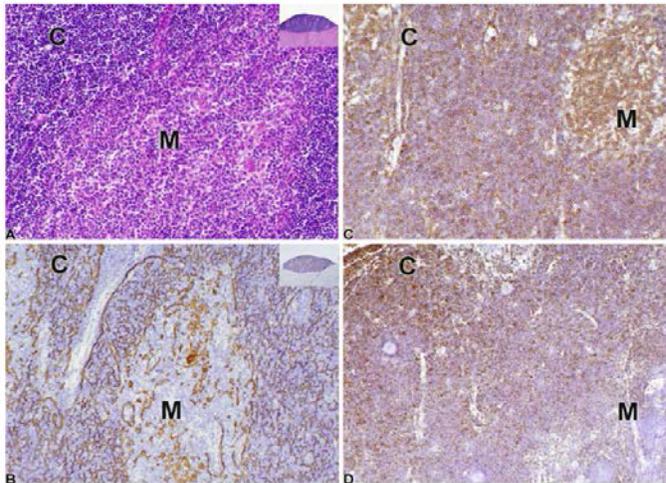


Figure 1. Neonatal thymus tissue allografts 28 days after transplantation of fresh thymus into nude rats. All thymus donors were (b) (4). Recipients were nude rats (b) (4) on the (b) (4) background. A-D) Images taken at 20X magnification except where noted. A) H&E staining of transplanted thymus, inset – 2X view of the transplanted thymus visible under the kidney capsule. B) pan CK (antibody AE1/AE3) expression by IHC, inset – 2X view of the transplanted thymus visible under the kidney capsule. C) CD3 expression in thymic tissue. D) Ki-67 staining supporting cellular proliferation. M – medulla, C – cortex.

Source: Study Report No. RVT-802-001.1, submitted in Module 4 of the BLA.

Figure 2 Cultured Thymus Transplanted for 28 Days (HRT11)

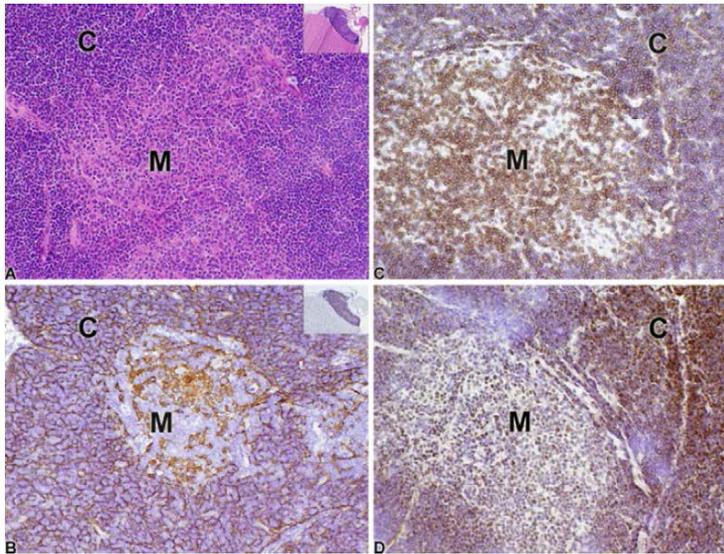


Figure 2. Neonatal thymus tissue allografts 28 days after transplantation of fresh cultured thymus into Nude rats. All thymus donors were (b) (4) Recipients were Nude rats (b) (4) on the (b) (4) background. A-D) Images taken at 20X magnification except where noted. A) H& E staining of transplanted thymus, inset – 2X view of the transplanted thymus visible under the kidney capsule. B) pan CK expression by IHC, inset – 2X view of the transplanted thymus visible under the kidney capsule. C) CD3 expression in thymic tissue. D) Ki-67 staining supporting cellular proliferation especially in the cortex. M – medulla, C – cortex.

Source: Study Report No. RVT-802-001.1, submitted in Module 4 of the BLA.

- The total number of circulating T cells and T cell subsets was analyzed in peripheral blood by flow cytometry prior to transplantation (day 0) and at various time points after transplantation. A time-dependent increase in total CD3+ T cells (Figure 3A) and total CD4+ (Figure 3B) and CD8+ (Figure 3C) T cell subsets was noted in most of the animals. A time-dependent increase in naïve CD4+ (Figure 3D) and CD8+ (Figure 3E) cells was also noted in several animals.

Comments:

- Per the applicant, since both donor and recipient animals had the same genetic (b) (4) background it was not possible to differentiate donor and recipient T cells by flow cytometry. However, the time-dependent increase in both the total and naïve T cell compartments supported some level of *de novo* endogenous generation of these T cells.
- The number of recipients in each group was extremely small (2 males/group), thus the robustness of the data is questionable.

Figure 3 Evaluation of T Cell Populations Following Thymus Transplantation (HRT11)

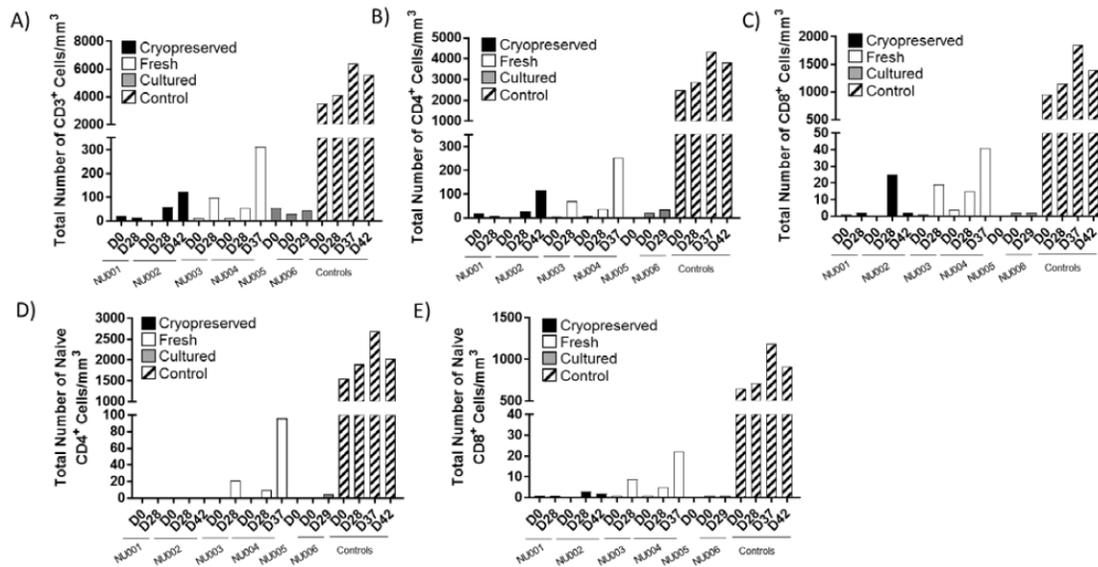


Figure 3. Total number of T cells were quantified in blood obtained pre-transplantation (D0) and at the indicated time point(s) post-transplantation. Data from each animal/treatment group are reported separately. 2 control animals were used for comparison (1 for D0/D28, 1 for D37/42). The frequency and total number of immune cells were quantified by flow cytometry. The total number of A) CD3⁺ (Total T cells – CD4⁺ and CD8⁺), B) CD4⁺ (Total), C) CD8⁺ (Total), D) Naive (CD45RC⁺CD62L⁺) CD4⁺ T cells, E) Naive (CD45RC⁺CD62L⁺) CD8⁺ T cells are shown.

Source: Study Report No. RVT-802-001.1, submitted in Module 4 of the BLA.

Report conclusion: Transplantation of neonatal fresh or cultured fresh thymus in athymic rats resulted in thymopoiesis.

Comment:

- This reviewer agrees with the applicant's conclusion.

Experiment #2 (HRT13):

Test system:

- Donor Animals – Total of 19 (b) (4) rats; male and female; newborn to 3 days old

Note: The number of animals for each age group was not specified.

- Recipient Animals – Nude rats (b) (4) on the (b) (4) background]; 6 males (2/group)

Comment:

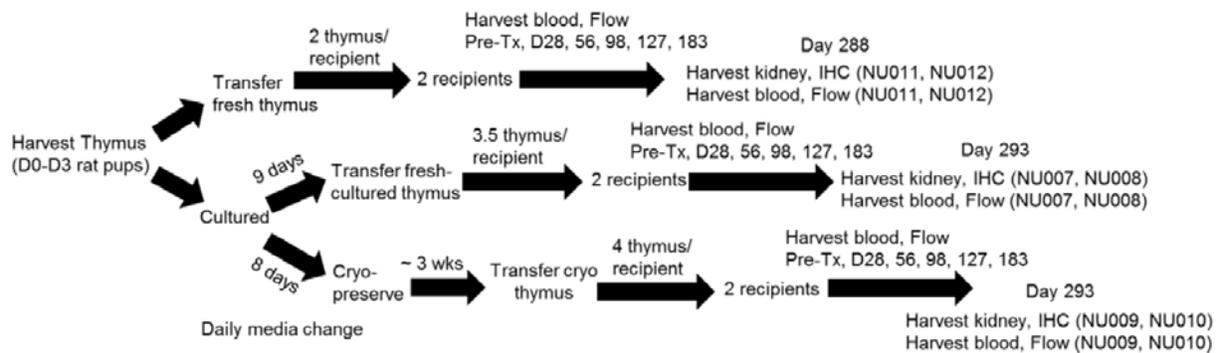
- The donor thymus was half-MHC matched to the recipient, so the donor T cells can be distinguished from the *de novo* generated endogenous T cells.

Study design:

Anesthetized nude rats were transplanted with:

- 1) Fresh thymus,
- 2) Fresh thymus cultured for 9 days, or
- 3) Cryopreserved/thawed thymus cultured for 8 days.

The thymus was transplanted under the kidney capsule. The recipients were sacrificed approximately 9 months (289-294 days) after transplantation, the kidney was harvested, and the architecture and presence of cellular infiltrates in the thymus were evaluated using IHC. Flow cytometry was performed on peripheral blood from recipient and non-transplanted control animals at various time points (pre-transplantation and days 28, 56, 98, 127, 183, and 289/294 post transplantation) to determine the frequency of donor-derived vs. endogenously-generated total T cells and T cell subsets (see the figure below for the overall study design).



Source: Study Report No. RVT-802-001.1, submitted in Module 4 of the BLA.

Results:

- The IHC results show that normal thymopoiesis was observed at 9 months in all thymus allografts transplanted under all three conditions (Figure 5).

Figure 5 Thymic Tissue 9 Months Post Transplantation (HRT13) – High Resolution, 40X

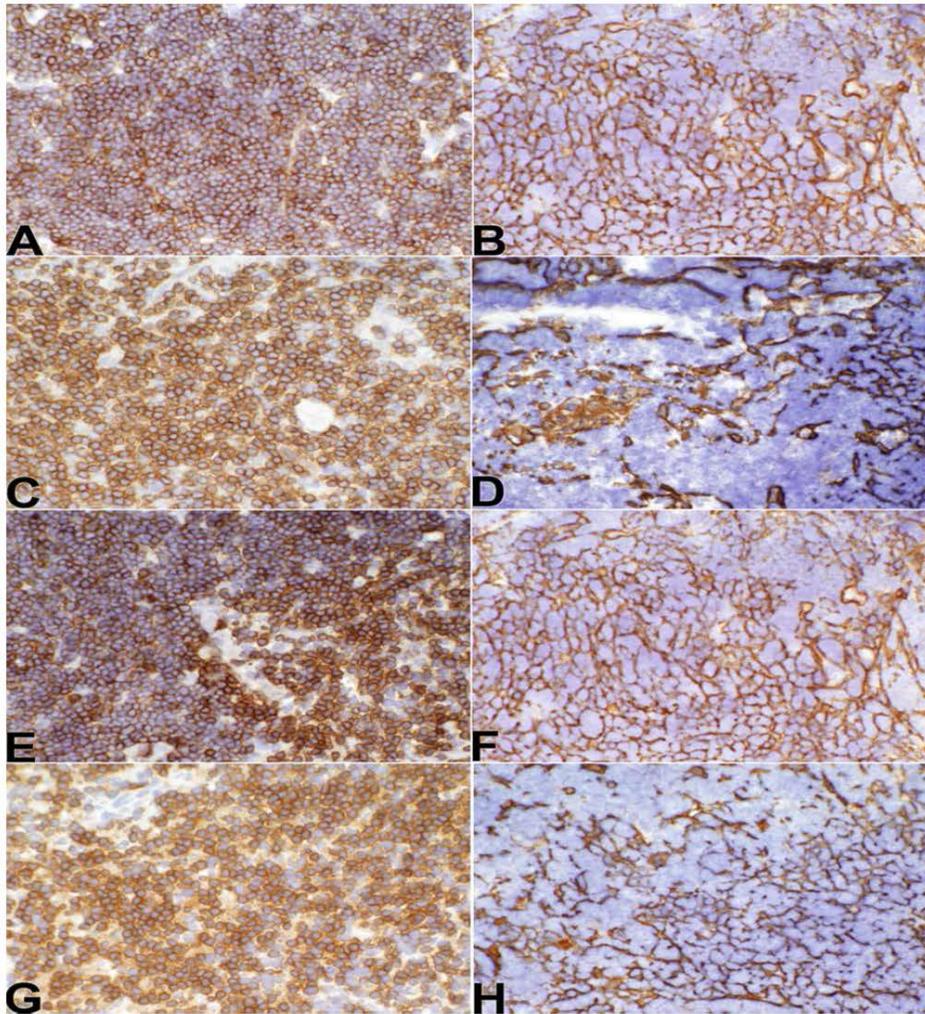


Figure 5. Neonatal thymus tissue allografts 9 months after transplantation into Nude rats. All thymus donors were (b) (4) except in E and F in which (b) (4) donor tissue was used for transplantation. Recipients were nude rats on the (b) (4) background. A-H) Images taken at 40X magnification. A, C, E, G) CD3 expression on A) fresh transplanted thymus, C) cultured fresh transplanted thymus, E) cultured thymus, cryo-preserved prior to transplantation, G) control thymus. B, D, F, H) pan CK expression on B) fresh transplanted thymus, D) cultured fresh transplanted thymus, F) cultured thymus, cryo-preserved prior to transplantation, H) control thymus.

Source: Study Report No. RVT-802-001.1, submitted in Module 4 of the BLA.

- There was a time-dependent increase in circulating total T cells (as measured by TCR $\alpha\beta$ T cells) observed as early as 1 month post-transplantation in recipients administered fresh or cultured thymus. After transplantation of fresh thymus, some donor T cells were present at early time points, but were not detected at later time points as the endogenous T cell population developed (Figure 6).

Figure 6 Evaluation of T Cell Populations Following Thymus Transplantation (HRT13)

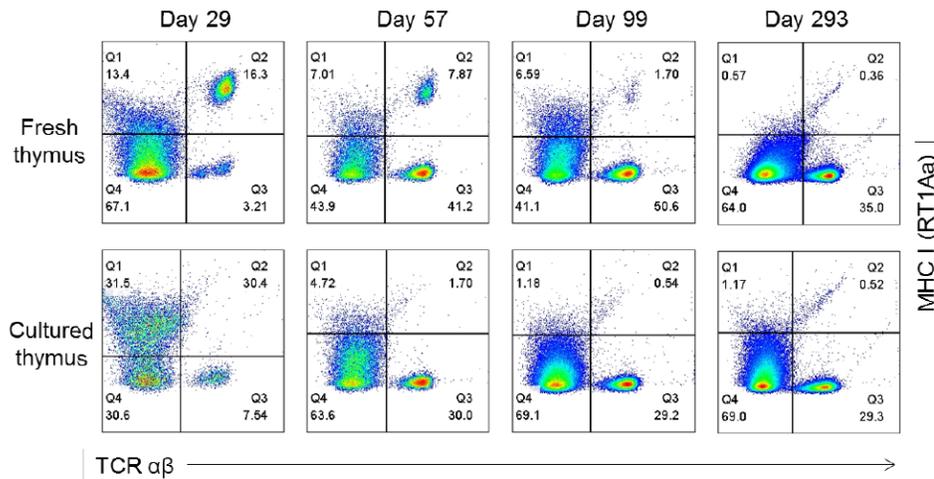


Figure 6. Pseudo-color dot plot analysis of one representative animal from each of the indicated treatment groups (fresh vs cultured) at each of the indicated timepoints. Transferred donor T cells ($\text{TCR}\alpha\beta^+\text{MHC1(RT1Aa)}^+$) Endogenous T cells ($\text{TCR}\alpha\beta^+\text{MHC1(RT1Aa)}^-$).

Source: Study Report No. RVT-802-001.1, submitted in Module 4 of the BLA.

- The total number of circulating T cell subsets increased in transplanted animals compared to non-transplanted controls. A general trend of progressively increasing total T cells and naïve T cells was observed over time. At 9 months, there were minimal differences between the recipient groups (Figure 7).

Figure 7 Evaluation of T Cell Populations Following Thymus Transplantation (HRT13)

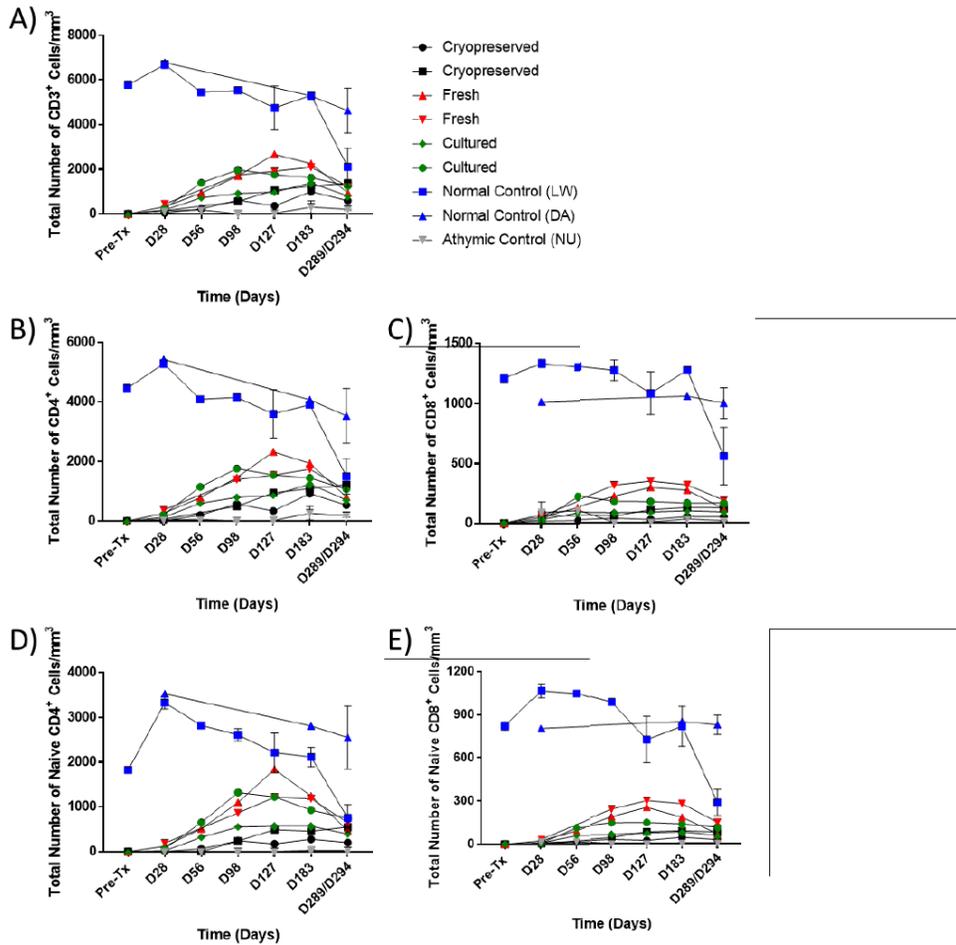


Figure 7. Total number of T cells were quantified in blood obtained pre-transplantation (D0) and at the indicated time point(s) post-transplantation. Data from each animal/treatment group are reported separately. Data from control animals are combined. Error bar indicates average \pm standard error of the mean. The frequency and total number of immune cells were quantified by flow cytometry. The total number of A) CD3⁺ (Total T cells – CD4⁺ and CD8⁺), B) CD4⁺ (Total), C) CD8⁺ (Total), D) Naïve (CD45RC⁺CD62L⁺) CD4⁺ T cells, E) Naïve (CD45RC⁺CD62L⁺) CD8⁺ T cells.

Source: Study Report No. RVT-802-001.1, submitted in Module 4 of the BLA.

Report conclusion: The data support postnatal thymus tissue implantation in infants with cDGA and provide multiple potential pre-transplantation conditions that lead to establishment of endogenous naïve T cells.

Comment:

- This reviewer agrees with the applicant's conclusion.

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies were conducted.

PHARMACOKINETIC STUDIES (Cell Distribution)

No cell distribution studies were conducted.

TOXICOLOGY STUDIES

Per the applicant, based on the acceptable clinical safety profile of RVT-802 and the lack of relevant animal models, no nonclinical toxicology studies with RVT-802 were conducted.

Developmental and Reproductive Toxicology (DART) Studies:

No DART studies were conducted. To assess the DART potential of RVT-802, the applicant examined published assessments of DART outcomes in mice following thymectomy. The findings are summarized in Table 1 below. While the thymus product administered is not identical to RVT-802, these data indicate that the risk of DART after RVT-802 transplantation in humans appears to be minimal.

Table 1: Summary of publications of nonclinical data on the DART risk following thymus transplantation

(Contents modified based on Table 1 submitted in Module 2.4 of the BLA)

Publication	Findings
Nishizuka, et al ⁴	<p>Thymectomy of neonatal mice (at 3 days of age) resulted in developmental arrest of the ovary, but not of the testis. Sterility in the female mice was also observed. The ovaries of the thymectomized mice were small and contained decreased or absent follicles and corpora lutea.</p> <p>The ovarian dysgenesis was prevented in the thymectomized mice given thymus grafting at 7 days of age. All mice (n=8) showed ovaries (n=16 ovaries) of normal size and morphology at 120 days of age by histology examination.</p>
Garcia, et al. ⁵	<p>Thymectomy in 10-day old mice resulted in a delay of puberty, decreased serum 17β-estradiol levels, and a reduced total number of follicles. Injection of thymulin, a zinc-dependent hormone resulted in: an earlier onset of puberty, decreased ovarian and uterine weights, and increased in serum 17β-estradiol levels. The results suggest</p>

⁴ Nishizuka et al. Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. Science. 1969;166(3906):753-5.

⁵ García et al. Effects of infantile thymectomy on ovarian functions and gonadotrophin-induced ovulation in prepubertal mice: role of thymulin. Journal of Endocrinology. 2000; 166: 381–387.

	that the presence of the thymus is necessary for normal ovarian development and function.
--	---

Genotoxicity Studies:

No genotoxicity studies were conducted.

Carcinogenicity/Tumorigenicity Studies:

No carcinogenicity/tumorigenicity studies were conducted. To assess the carcinogenicity/tumorigenicity potential of RVT-802, the applicant examined published assessments of carcinogenicity outcomes in mice following thymus transplantation. The findings are summarized in Table 2 below. Although the thymus products administered were not identical to RVT-802 and the studies were conducted in tumor-bearing mice (which does not reflect the clinical scenario), these data combined with the existing clinical safety data for RVT-802, indicate that the risk of carcinogenicity after RVT-802 transplantation in humans appears to be minimal.

Table 2: Summary of publications of nonclinical data on the carcinogenicity risk following thymus transplantation

(Contents modified based on Table 1 submitted in Module 2.4 of the BLA)

Publication	Findings
Hosaka, et al. ⁶	The study was conducted to evaluate the effects of bone marrow transplantation alone or combined with fetal thymus transplantation (under the kidney capsule) in tumor-bearing mice. Mice receiving bone marrow transplant alone showed a slight improvement in survival compared with nontreated controls. Mice receiving bone marrow and thymus transplantations exhibited longer survival compared to mice with a bone marrow transplant alone. Lung metastasis was also inhibited by the combination. There was a positive correlation between survival days and the number of T cells or T cell function. Results suggest that bone marrow plus fetal thymus transplantation was effective in prolonging survival as a result of the restoration of T cell function in the tumor-bearing mice.
Ikehara ⁷	This review article summarized nonclinical data reporting the effect of bone marrow transplantation in combination

⁶ Hosaka et al. Prolonged survival in mice with advanced tumors treated with syngeneic or allogeneic intra-bone marrow–bone marrow transplantation plus fetal thymus transplantation. *Cancer Immunol Immunother.* 2010; 59:1121-1130.

⁷ Ikehara S. Thymus transplantation for treatment of cancer: lessons from murine models. *Expert Rev Clin Immunol.* 2011; 7(2): 205-211.

	with thymus transplantation on tumor growth and survival in tumor-bearing mice. Based on the data, the author concluded that bone marrow and thymus transplantation could be a ‘valuable strategy’ for suppressing tumor growth, thereby prolonging survival.
--	---

Comment:

- This reviewer appraised each publication cited in Tables 1 and 2 and confirmed that the contents of these tables reflect the respective publications.

Other Safety/Toxicology Studies

No other safety/toxicology studies were conducted.

APPLICANT’S PROPOSED LABEL

Subsections 8.1-8.3 of Section 8 (‘Use in Specific Populations’) should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14), as applicable.⁸

Section 13.2 (‘Animal Toxicology and/or Pharmacology’) is not included in the label. However, this is appropriate because the data generated were primarily proof-of-concept data and the studies conducted were not robust in nature.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

RETHYMIC, RVT-802, cDGA, DGS, allogeneic, postnatal thymus tissue, immune reconstitution, congenital athymia, rats, kidney capsule

⁸ *Pregnancy, Lactation, and Reproductive Potential: Labeling for Human Prescription Drug and Biological Products – Content and Format at:*
<https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm450636.pdf>