

CBER CMC BLA Review Memorandum

BLA STN 125685

Allogeneic processed thymus tissue-agdc

(RVT-802, RETHYMIC)

Enzyvant Therapeutics GmbH

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BLA#: STN 125685

APPLICANT NAME AND LICENSE NUMBER

Enzyvant Therapeutics GmbH, License # 2100

PRODUCT NAME/PRODUCT TYPE

Non-Proprietary/Proper/USAN: Allogeneic processed thymus tissue-agdc

Proprietary Name: RETHYMIC

Name used under product development: RVT-802

NDC: 72359-001-01

UNII code for cellular product: XD66YK3YY3

UNII code for fetal bovine serum: K5DD3J879P

UNII code for (b) (4) sponge: (b) (4)

UNII code for (b) (4)

GENERAL DESCRIPTION OF THE RVT-802 MANUFACTURING PROCESS AND FINAL PRODUCT

- a. **Pharmacological category (product class):** Currently under review and a product class has not yet been assigned.
- b. **Dosage form:** Yellow to brown slices of processed and cultured tissue with varying thickness and shape supplied adhered to filter membranes. The slices are teased away from the filters prior to surgical implantation into thigh muscle. Dose is based on total surface area of cultured thymus slices, with a dose range of 5,000 to 22,000 mm²/m² recipient body surface area
- c. **Strength/Potency:** (b) (4)
- d. **Route of administration:** Ectopic surgical implantation into thigh muscle of recipient
- e. **Indication(s):** Congenital athymia

The product is slices of allogeneic unrelated thymus tissue cultured in (b) (4) medium supplemented with (b) (4) fetal bovine serum (FBS) for up to 21 days. Cultures are set up as suspension cultures with slices adhered to individual (b) (4) filter membranes that rest on top of surgical (b) (4) sponges soaked with culture medium. (b) (4) medium is applied (b) (4) to cultures by (b) (4) onto the thymus slices (b) (4). A "slice" is defined as the total amount of material covering a (b) (4) filter. A slice can be the sum of multiple slices placed on the same filter at the time of culture. Slice thickness varies from approximately (b) (4) thick. A product lot is intended for a single patient and can be composed of up to (b) (4) slices, with up to 4 slices per (b) (4) culture dish (final container closure).

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Histology data provided on a subset of lots used for transplantation was essential for review of this resubmission. This was especially true in terms of assessing product quality, and for interpreting results from new process validation results (CRL item #6). It was also critical for determining the adequacy and reproducibility of the histological assays used for lot release (CRL item #3), the adequacy of in-process and final product sampling points (CRL item #2), for addressing previous concerns raised about in-process hold times (CRL item #4), product stability (CRL item #5), and product transported in the final container closure assessed as part of process validation (CRL item # 6).

The manufacturing process has significant flexibility built in: 1) the process allows for different numbers of slices to be established given similar amounts of starting material; 2) the thickness of slices is not well controlled; 3) some tissue is held (b) (4) before processing; 4) the amount of tissue that must cover a filter to be considered a slice is (b) (4) and can be composed of multiple pieces; 5) the length of time in culture before the final product is harvested can vary from 12 to 21 days and the product continues to change over time in culture; 6) the number of different batches of culture medium used has varied substantially; and 7) the time between culture medium replenishment/flushing the slices is (b) (4) the time point that in-process and final product is sampled by histology is variable.

MAJOR MILESTONES

7/6/2018	Module 1 and 4 submitted
12/20/2018	Module 5 submitted
4/5/2019	Module 3 submitted, start of PDFUA clock
6/4/2019	Filing date
9/8/2019	Midcycle meeting
9/27/2019	Late cycle meeting
12/4/2019	CRL letter issued
03/19/2020	Type A meeting held
4/9/2021	BLA resubmitted
10/8/21	Resubmission PDUFA action date

CMC/QUALITY REVIEW TEAM

Reviewer/Affiliation	Section/Subject Matter
Alyssa Kitchel, CBER/ OTAT/DCGT/CTB	3.2.S.6 – Container Closure System 3.2.S.7 – Stability 3.2.P.2.4 – Container Closure System 3.2.P.7 – Container Closure System 3.2.P.8 – Stability
Sukhanya Jayachandra, CBER/ OTAT/DCGT/CTB	3.2.S.2.3 – Control of Materials
Irina Tiper CBER/OTAT/DCGT/CTB	1.14 – Labeling 3.2.S.4 – Control of Drug Substance 3.2.P.4 – Control of Drug Product

Reviewer/Affiliation	Section/Subject Matter
Thomas Finn, CBER/ OTAT/DCGT/CTB	1.4.4 – Cross-reference 1.12.5 – Request for a waiver 1.12.14 – Environmental analysis 1.14 – Labeling 1.18 – Proprietary names 3.2.S.1 – General Information 3.2.S.2 – Manufacture 3.2.S.2.3 – Control of Materials 3.2.S.2.4 – Controls of Critical Steps & Intermediates 3.2.S.2.5 – Process Validation 3.2.S.2.6 – Manufacturing Process Development 3.2.S.3 – Characterization 3.2.S.4 – Control of Drug Substance 3.2.S.6 – Container Closure System 3.2.S.7 – Stability 3.2.P.1 – Description and Composition of the DP 3.2.P.2 – Pharmaceutical Development 3.2.P.2.4 – Container Closure System 3.2.P.3 – Manufacture 3.2.P.3.4 – Controls of Critical Steps & Intermediates 3.2.P.3.5 – Process Validation 3.2.P.4 – Control of Drug Product 3.2.P.5 – Control of Drug Product 3.2.P.6 – Reference Standards or Materials 3.2.P.7 – Container Closure System 3.2.P.8 – Stability 3.2.A.1 – Facilities and Equipment 3.2.R – Regional Information

INTER-CENTER CONSULTS REQUESTED

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations (Yes/No)
Samanthi Wickramasekara CDRH/OSEL/DBCMS	3.2.P.2 – Final container leachables and extractables	Yes

SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
BLA Original Submission		
7/6/2018	125685.0	Original submission with Module 1 and Module 4 information
10/30/2018	125685.1	Proprietary name review
12/20/2018	125685.2	Module 5 rolling submission
4/5/2019	125685.3	Module 3 of rolling submission

Date Received	Submission	Comments/ Status
4/29/2019	125685.5	Proposed non-proprietary name suffix
5/15/2019	125685.7	(b) (4) sterility and mycoplasma validation
6/14/2019	125685.8	Calculation of tissue slice dose by (b) (4)
6/17/2019	125685.9	Information on 3rd party testing laboratories; (b) (4) facility information; copy of (b) (4) batch record
6/21/2019	125685.10	Qualitative histology assay method and reference images
6/26/2019	125685.11	Mycoplasma limit of detection validation
6/28/2019	125685.12	Source material hold times; in-process container closures; (b) (4) final container closure, acceptable endotoxin level in materials and final product; drug product batch listing with histology testing times; calculation of residual excipient administered to patient; additional examples of (b) (4) histology from PV lots; donor eligibility; in-house testing of critical materials
7/17/2019	125685.14	Source material (b) (4) storage; culture medium; histology testing time points; justification for culture time window; final product shipping and handling; (b) (4) filters; histology protocol and histological analysis
7/17/2019	125685.15	Facility environmental monitoring; Thymus Organ Media (TOM) medium preparation; (b) (4) validation studies; (b) (4) sampling; facility equipment and procedures; final product visual inspection; aseptic process validation; (b) (4) final container closure; drug product packaging and shipping procedures; container; DP endotoxin levels; DP patient label; DP shipping temperature.
7/18/2019	125685.16	Facility environmental monitoring; (b) (4) sampling;
7/26/2019	125685.17	Manufacturing consistency; batch analysis; clinical experience with (b) (4) container; comparison of (b) (4) final container packaging process; (b) (4) validation; histology assay methods; histology assay validation (b) (4) variability (b) (4)
8/15/2019	125685.18	Process validation; chemokine assays
8/20/2019	125685.19	Donor qualification; donor eligibility
8/23/2019	125685.20	15-day response to 483 observations
8/23/2019	125685.21	Relationship between length of culturing and clinical outcome
8/30/2019	125685.22	(b) (4) sterility validation; endotoxin validation
8/30/2019	125685.24	Donor eligibility
9/6/2019	125685.25	Endotoxin validation; batch records for PV lots (b) (4)
9/13/2019	125685.27	Revised product labeling

Date Received	Submission	Comments/ Status
9/13/2019	125685.28	Justification for specifications; histology acceptance criteria; histology sample selection; reference histology images; final product visual inspection; Preliminary T cell counts on subjects transplanted with product lots using (b) (4) final container; source material container closures; 6-month T cell counts on subjects (b) (6) who received products lots formulated and stored in (b) (4) final container
9/17/2019	125685.29	Sample pooling for sterility, endotoxin and mycoplasma
9/18/2019	125685.30	Biopsy data on subjects (b) (6) (days 98 and 139), (b) (6)
9/25/2019	125685.32	Dose calculation on PV lots (b) (4)
9/26/2019	125685.33	Cross-reference to IND 9836
10/1/2019	125685.34	Clinical outcome of 7 recently treated subjects
10/1/2019	125685.36	Slicer Performance Qualification Protocol
10/4/2019	125685.37	(b) (4) sterility validation; mycoplasma validation
10/10/2019	125685.39	Donor eligibility and donor qualification
10/15/2019	125685.40	Thymus source material pre-processing and processing hold times
10/15/2019	125685.41	Sterility testing of (b) (4)
10/15/2019	125685.42	Material qualification sterility and endotoxin testing; (b) (4) sterility assurance method; ancillary material shelf life; facility environmental monitoring; telecon summary of 483 discussions
10/23/2019	125685.43	Results of tissue slicer qualification
10/25/2019	125685.44	Justification for lot size; relationship between surface area of tissue slices and intended dose; proposed labeling information about dose; procedures for ensuring minimum dose and exceeding maximum dose; comparison of dose calculation with (b) (4) methods and historical method
10/28/2019	125685.45	Revised proposal for histology test window; request for extension to update BLA eCTD with (b) (4) final container information
10/28/2019	125685.46	Details of 20 subjects with low or delayed naïve T cell counts
11/1/2019	125685.47	Updated Module 3 with (b) (4) culture dish final container closure
11/1/2019	125685.48	List of treatment related adverse events
11/1/2019	125685.49	2nd response to 483 observations
11/5/2019	125685.50	Additional details on 20 subjects with low or delayed naïve T cell counts
11/7/2019	125685.51	(b) (4) final container closure transport study report

Date Received	Submission	Comments/ Status
11/12/2019	125685.52	Additional data on subjects with T cell counts <50/mm ³ and statistical analysis of responders and non-responders
11/15/19	125685.53	Revised package insert in response to first round of FDA feedback, and notice of address change
11/26/19	125685.54	Updated statistical analysis for Infection-Related Adverse Events
	125685.55	
11/26/19	125685.56	Revised draft package insert
BLA Resubmission		
2/13/20	125685.57	Type A meeting request and briefing package
11/6/20	125685.58	BLA resubmission date extension request
2/10/21	125685.59	BLA resubmission date extension request and change of address for sponsor contact
4/9/21	125685.60	BLA resubmission and complete response to CRL items
4/21/21	125685.61	DSCSA Exemption Request
4/23/21	125685.62	Proprietary Name Review Request for RETHYMIC
5/7/21	125685.63	Response to clinical information request #35 and Pharmacovigilance plan for patients administered RETHYMIC
5/19/21	125685.64	Partial response to clinical information request #36 for updated clinical datasets and analyses
6/4/21	125685.65	Complete response to clinical information request #36 for clinical datasets and analyses from patients treated with product lots from the different RVT-802 manufacturing facilities
6/25/21	125685.66	Response to clinical information request #37. Provides a safety management plan and a draft post approval registry protocol.
7/30/21	125685.67	Partial response to clinical information request #38 for updated autologous GVHD, SAE, and safety /efficacy analysis related to product culture time
7/30/21	125685.68	Response to clinical information request #39 and Pharmacovigilance plan for patients administered RETHYMIC
8/5/21	125685.69	Response to clinical information request #40 for subjects experiencing acute kidney injury (AKI), a shift table for serum creatinine and a list of subjects with >20% shift in baseline serum creatinine.
8/5/21	125685.70	Partial response to clinical IR #38 on aGVHD, SAEs following the resubmission, and updated analyses of efficacy and safety with regards to culture time
8/13/21	125685.71	Response to clinical IR #38 on aGVHD, SAEs following the resubmission, and updated analyses of efficacy and safety with regards to culture time
9/13/21	125685.72	Response to DMPQ IR #42 on 483 items

Date Received	Submission	Comments/ Status
9/20/21	125685.73	Response to CMC IR #43; Resubmission of missing PV histology data; electronic submission of PV digital histology files, part 1 of 3.
9/20/21	125685.74	Electronic submission of PV digital histology files, part 2 of 3
9/20/21	125685.75	Electronic submission of PV digital histology files, part 3 of 3
9/20/21	125685.76	Submission of primary and secondary container labels for DCSA review
9/22/21	125685.77	Response to DMPQ information request #44
9/22/21	125685.78	Applicant revisions to PMC potency draft language and agreement on container closure PMC draft language
10/1/21	125685.79	Applicant agreement to revised potency PMC draft language
10/4/21	125685.80	Applicant response to CMC IR on container labels and revised minimum final in-process product dose

Referenced REGULATORY SUBMISSIONS (e.g., IND BLA, 510K, Master File, etc.)

Submission #	Sponsor	Referenced Item	Letter of Cross-Reference	Comments/Status
IND 9836	Dr. Mary Louise Markert	The Applicant referenced but did not directly refer to the IND for any specific information. The review team used information from the IND in the preparation of information requests.	Submitted 9/26/19 in 125685.33	CMC information in IND is at the level of Phase 2 manufacturing. No formal Phase 3 conducted. Patients still being treated under 2 expanded access trials.

REVIEWER SUMMARY AND RECOMMENDATION

i) EXECUTIVE SUMMARY

To accommodate the very limited supply of source material, and complex logistics with patient scheduling and pre-conditioning, certain flexibilities needed to be built into the RVT-802 (RETHYMIC) manufacturing process. It was unclear whether the flexible manufacturing and testing strategies proposed in the original submission provided confidence in product quality. A pre-license facility inspection resulted in numerous observations, and two attempts by the Applicant, with FDA input, did not adequately resolve these issues during the original

submission review. A type A meeting was held on March 19, 2020 at which Enzyvant outlined their plan for responding to all complete response letter (CRL) items, including 483 observations. FDA offered advice for all CRL and 483 items and provided additional suggestions and considerations. The BLA resubmission reflects the large degree to which the Applicant adhered to FDA recommendations and suggestions. Substantial changes were made to Module 3 to accommodate all the changes made as a result of responding to the CRL. New CMC studies were conducted, and many new standard operating procedures (SOP) were developed or updated.

Manufacturing was conducted in a dedicated manufacturing space in the laboratory of (b) (4) from 1993 through 2015, after which it was transferred to the (b) (4) campus as a contract manufacturer. The (b) (4) multiproduct facility has a dedicated room for RVT-802 manufacturing used under IND and will be the sole manufacturing space for RETHYMIC. Facility 483 observations that remained unresolved by the original submission action date covered a wide range of deficiencies. Resolution of these observations occurred through: physical modification to areas; improved qualification of critical materials and material control strategies; improvements to risk assessment and change control procedures; enhanced environmental monitoring and cleaning procedures; and improved quality control (QC) data backup procedures. The corrections were found adequate.

To address CMC CRL items, the Applicant enhanced the existing set of histology assays that were a critical part of product specifications and developed and implemented an SOP to document these procedures. Product testing was revised to repeat histology testing further (b) (4) in manufacturing to within (b) (4) days of release. An additional in-process (b) (4) test and an (b) (4) were implemented to provide greater assurance prior to patient conditioning with immunosuppressive agents the intended recipient will be able to receive the product. Additional information and supportive data were included to justify source material hold times and conditions, and final product expiry. Additional supportive data was also provided to demonstrate the ability to transport the product in the intended container closure system while maintaining product sterility. These changes addressed our concerns.

The evaluation of the adequacy of RVT-802 manufacturing and testing control strategies relied heavily on the long manufacturing experience and positive clinical outcome data on individual patients treated with single RVT-802 lots. Determination of product potency and overall quality is made largely through histology evaluation on sections prepared from a single slice. In the original submission, very few examples were provided on histology from product lots used to treat patients. In the resubmission, substantially more histology examples were provided on product lots in patients with positive clinical outcomes. These examples were essential in understanding the acceptable range for lot release and how the (b) (4) histology scoring system is applied, and are now part of the histology SOP. It was expected that the thymus tissue slices would change over time in culture, but the examples demonstrated a larger shift in phenotype the slices can undergo than we anticipated. Even within the same section there is a wide range of different cellular phenotypes and slice architecture. The approach used in reviewing the BLA was to use the range of phenotypes presented from those patients with positive clinical outcome as an acceptable range, which were then used as a benchmark for interpreting process validation and stability data for product quality. To address

Agency concerns that 29 patients had slower recovery of naïve peripheral T cells in the first two years post-transplant than the average patient that might be due to lower product quality in those lots, the Applicant performed a retrospective study. Their retrospective analysis concluded there was no difference in the histological features, and therefore product quality, in these patients. Our independent evaluation of the histology images is in agreement with that assessment.

No separate phase 3 study was conducted in the development of this product. The Applicant based safety on all 105 subjects treated since 1993, and efficacy on all 95 subjects treated since the IND was submitted in 2001. However, limited manufacturing information exists prior to 2001, and the manufacturing process, testing, and specifications have changed over the 20-year history of the IND, especially with the change in manufacturing facility and methods in 2015. Although clinical data of positive outcomes were used to help support a particular lot was of adequate quality, in many cases only a subset of product lots could be used for analysis because of differences in manufacturing or test procedures. Only (b) (4) product lots have been manufactured in the (b) (4) facility, (b) (4) of which were formulated differently and used a different container closure system. Of the (b) (4) lots, only (b) (4) lots were evaluated by histology using the (b) (4) sectioning process as intended for the commercial process. Only a small number of lots were exposed to the maximum hold conditions proposed for the commercial product. The (b) (4) histology scoring system was only recently introduced. Clinical efficacy data is compelling and consistent across all clinical protocols conducted over the long history of this product. Statistical analysis conducted by the Applicant shows the same level of 2-year survival for subjects treated with product lots manufactured in the (b) (4) facility as those treated with lots manufactured in the previous facility.

Updated and revised manufacturing and testing procedures implemented since the issuance of the CRL provide confidence that manufacturing is under an adequate state of control. Importantly, the basic approach to manufacturing and testing intended for the commercial product is largely as was conducted under IND. Product test methods are limited in their ability to demonstrate comparability should a significant manufacturing change occur in the future. Changes to date are not a concern because clinical data exists to verify quality of individual lots. A substantial change could be a concern, as was the case for the proposed change in primary container in the original submission, which also involved a large change in formulation and shelf life, but for which clinical data did not exist. For this reason, we have proposed a post-marketing commitment (PMC) for the development of a (b) (4) assay. Though a histology-based evaluation of a tissue product is scientifically justifiable, it is not a (b) (4) measure of a biological activity, and interpretation as a product quality metric is complicated by substantial product variation. Though procedures have been modified to make the assay a more (b) (4) determination of surrogate measures of biological function, the thresholds for release are low. (b) (4) studies do not shed much light on the sensitivity of the assay because the tissue is (b) (4), and even with (b) (4) many of the same histological thymus hallmarks are still present. We reached agreement with the Applicant on a PMC for the development of a (b) (4) assay that can be used in cases where a (b) (4) assessment of a potential change in product quality may be needed, such as part of a comparability study or stability.

We also reached agreement with the Applicant on a second PMC for the development of a new container closure. The current container closure system is polystyrene culture dishes placed inside a single-use acrylic secondary container with a tight-fitting lid and gasket. The product tissue slices remain in the same configuration as during culture, with the slices adhered to (b) (4) filters that are suspended on surgical (b) (4) sponges above a small volume of culture medium. Up to 11 primary container closure dishes, each containing up to 4 tissue slices, are used to hold one product lot. All dishes are stacked in rows inside the secondary container. Both the primary and secondary containers are single use. The culture dishes have been in use as the container closure system since 1993 and the secondary container was introduced more recently. While suitable for licensure for the short transportation from the manufacturing facility to the treating hospital on the same campus, the primary container does not offer a strong barrier and the container closure system relies heavily on the secondary container for integrity

RECOMMENDATION

We recommend that the BLA be approved. Two CMC postmarketing commitments have been agreed upon for developing and establishing a (b) (4) assay and a new container closure system.

(1) SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Thomas Finn, PhD CBER/OTAT/ DCGT/CTB	Concur	Thomas P. Finn - S DN: c=US, o=U.S. Government, ou=HHS, Digitally signed by Thomas P. Finn -S, ou=FDA, ou=People, cn=Thomas P. Finn -S, 0.9.2342.19200300.100.1.1 1300386089 Date: 2021.10.06 14 27 52 -04'00'
Alyssa Kitchel, PhD CBER/OTAT/ DCGT/CTB	Concur	Alyssa Kitchel - S DN: c=US, o=U.S. Government, ou=HHS, Digitally signed by Alyssa Kitchel -S, ou=FDA, ou=People, cn=Alyssa Kitchel -S, 0.9.2342.19200300.100.1.1=2001736484 Date: 2021.10.06 14:48:04 -04'00'
Sukhanya Jayachandra, PhD CBER/OTAT/DCGT/CTB	Concur	Melanie Eacho - S Digitally signed by Melanie Eacho -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Melanie Eacho -S, 0.9.2342.19200300.100.1.1 1300391929 Date: 2021.10.06 17 31 09 -04'00'
Irina Tiper, PhD CBER/OTAT/DCGT/CTB	Concur	Melanie Eacho -S Digitally signed by Melanie Eacho -S DN: c=US, o=U.S. Government, ou=HHS, ou=People, cn=Melanie Eacho -S, 0.9.2342.19200300.100.1.1=1300391929 Date: 2021.10.06 17:31:47 -04'00'
Melanie Eacho, PhD, Branch Chief CBER/OTAT/DCGT/CTB	Concur	Melanie Eacho -S Digitally signed by Melanie Eacho -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Melanie Eacho -S, 0.9.2342.19200300.100.1.1 1300391929 Date: 2021.10.06 17 32 31 -04'00'
Steven Oh, PhD, Dept. Division Director CBER/OTAT/DCGT	Concur	Steven Oh -S Digitally signed by Steven Oh -S DN: c=US, o=U.S. Government, ou=HHS, ou=People, cn=Steven Oh -S, 0.9.2342.19200300.100.1.1 1300409381 Date: 2021.10.06 17 40 22 -04'00'
Raj Puri, PhD, Division Director CBER/OTAT/DCGT	Concur	Raj K. Puri -S Digitally signed by Raj K. Puri -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Raj K. Puri -S, 0.9.2342.19200300.100.1.1=1300048757 Date: 2021.10.06 18:14:54 -04'00'

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COMPLETE RESPONSE ITEMS

Chemistry Manufacturing, and Controls

CRL item #1 (Outstanding PLI Issues): Outstanding issues identified during the pre-license inspection (PLI) at your contract manufacturing facility conducted July 29 to August 2, 2019, as detailed in Form FDA 483, have yet to be resolved. Please submit documentation that demonstrates that all outstanding inspectional issues identified during the PLI have been resolved.

Observation #1 (CAPA Documentation). Corrective actions and preventive actions (CAPA) implemented are not always being documented per the CAPA SOP COMM-QA-076. For example,

- a. Deviation IR-0111 is regarding failures in the sterile packaging of ethylene oxide sterilized critical supplies, which resulted in modified packaging configurations as captured in Change Controls (b) (4)-CCR-434. However, no CAPA was initiated.
- b. Deviation DEV-0602 is regarding personnel monitoring samples being discovered by the contract lab in the sample receipt area (i.e., “garage of disposed materials” per contract lab deviation DEV2018_0042) one week after receipt. As a corrective action, the contract lab tested the samples and found negative growth on any of the (b) (4), including the control (b) (4). No growth promotion testing results, if performed on these (b) (4), was reported. No CAPA was initiated to capture the corrective action taken and no additional corrective and/or preventive action was deemed necessary.
- c. Deviation DEV-0556 is regarding incorrect lot number of fetal bovine serum (FBS) being recorded on Thymus Organ Medium (TOM) Batch Record due to failure in secondary verification by the operators. The error was not detected during QA review but was discovered 25 days later by the operator who initially reported the incorrect number. As a corrective action, the operator determined the correct lot of FBS and the batch record was corrected and re-reviewed. All staff were reminded of the importance of GDP entries. No CAPA was initiated to capture the corrective action taken and no additional corrective and/or preventive action was deemed necessary.

Applicant’s response to CRL item #1, observation #1: This CRL item was jointly reviewed by DMPQ and DCGT.

Enzyvant states that extensive procedural updates that ensure Quality oversight and drive improvement to the QMS have been implemented. Revisions to SOP COMM-QA-077 Risk Assessment Procedure and associated SOPs have been made to ensure that the

procedures for investigation of deviations and requirements for risk assessment and corrective action adequately protect product quality and the patient. Concerning the 3 cited deviations they have re-reviewed these deviations to establish the root cause of the procedural or systemic problems that led to the lack of CAPA initiations. Reviews were captured in QA 2019-010-P (b) (4) Deviation, Investigation, Risk Assessment, and Corrective and Preventive Action Assessment Report and the QA 2019-011-P (b) (4) Change Control System Assessment Report. The investigation determined that the root cause of failing to initiate CAPAs for these deviations was inadequate and unclear procedures governing the deviation, risk assessment, and CAPA processes. To prevent recurrence of similar situations, improved procedures and training have been implemented to ensure CAPAs are opened for all corrective or preventive actions, and Change Control is now better linked to the CAPA process (e.g., relevant Change Control numbers and details are captured within CAPA Reports). Previously, risk assessment performed was narrowly focused on the specific event and the overall risk was deemed below the risk threshold to require initiation of a formal CAPA. Improvements to the risk assessment and related processes have been implemented. If similar deviations to those cited in Observation 1 were to occur under the current SOPs, CAPAs would be initiated.

Major changes to key SOPs include:

- Quality Risk Management requirements are now implemented through COMM-QA-077 Risk Assessment (effective 3/17/21). Change Control, Deviations and Investigations, and CAPA are now coordinated through use of a common risk process and expands upon the use of Risk Assessment Tools. Risk assessment matrix now includes detectability in addition to severity and probability (frequency). The matrix will assign a score from 1-125, with 125 being the most severe. Scores <50 a CAPA is not required, but recommended and no effectiveness check is needed.
- COMM-QA-076 (effective 10/30/20) covers CAPA. In addition to risk driven CAPA initiation, a CAPA may be triggered from other quality systems, including, but not limited to, in response to internal/external audit findings, management review, COMM-QA-080 Quality Risk Management risk assessments, and/or identified trends.
- COMM-QA-080 (effective 10/30/20) covers quality risk management and outlines a process for Risk Assessments (RA) and identifies Risk Assessment Tools to enable effective risk assessments. It follows the principles described in (b) (4).
- COMM-QA-042 (effective 10/30/20) covers deviations and investigations. Clearer instruction for Root Cause Analysis includes the use of problem-solving tools. The Quality Unit is now involved earlier in the deviation process. A defined timeline now includes a target timeframe for deviation/investigation resolution of ::30 days.
- COMM-QA-019 (effective 10/30/20) covers change control. It has been revised to provide more detailed instructions on when effectiveness checks are required, and greater detail for process changes.

Review of response: This CRL item was primarily reviewed by DMPQ. The actions taken also impact the response to observation #2. A review of SOP COMM-QA-076, COMM-QA-

077, and COMM-QA-078 showed improvements to these procedures over previous versions, and no serious new concerns were identified.

To assess the current state of their quality system, we reviewed recent deviations reported for the four process validation lots produced in response to the original submission CR.

Note: PV lots (b) (4) were initiated on 10/22/20 and lots (b) (4) on 12/11/20, and therefore the latest versions of quality risk management, deviation investigation, CAPA, and change control were not yet in effect, and the latest version of the risk assessment SOP was not in effect until 3 months after the process validation lots were completed. A risk assessment report (b) (4) 2020-009.1-P) was initially approved prior to the 2020 Process Validation study.

A total of 29 deviations were associated with manufacturing and testing the (b) (4) PV lots.

Note: This is a sizable number of deviations considering the (b) (4) process lots were generated using a (b) (4) manufacturing approach where the source material was (b) (4) lots, so it does not reflect (b) (4) individual full manufacturing runs. Second, in the 5 years that the thymus product has been manufactured in the (b) (4) facility, 80 previous deviations occurred. The 29 PV deviations occurred over a span of a total of two months of processing and culturing.

None of the PV lot deviations were deemed to negatively affect the validation study or the ability of the (b) (4) facility to provide evidence of controlled processing, testing, and release of RVT-802 using current standard operating procedures. Most of the deviations were not substantial and appropriate corrective actions were taken. Three of the deviations were associated with an unplanned facility HVAC shutdown due to either a fire alarm or a coil freeze sensor. In one of these cases that meant a PV lot had to be terminated and a new lot generated. PV lot (b) (4) was released without full results of an interim mycoplasma time point being available due to a test issue. There was also a failure to complete (b) (4) pass-through cleaning for (b) (4) PV lots. (b) (4) slice surface area images for one of the days on one lot could not be uploaded to the server, and therefore analysis couldn't be conducted on that time point. It was not clear in these cases that adequate risk management had been employed. In response to an information request the Applicant provided copies of the CAPA reports and responded to concerns raised. In the case of the (b) (4) software the root cause was determined to be a communication error with the camera and the software that could not be overcome on the day of sampling. The issue was not found on subsequent days of culture and had not occurred in the past. Since it hadn't occurred in the past no corrective action was taken. However, in discussions with the vendor this is a known issue and no software updates are available. The Applicant considers this to be related to the daily acquisition of the cultures, which is not used for commercial manufacturing. While this may be a low frequency event, we do not agree that this shouldn't have been elevated to a higher risk category. Commercial production may involve a higher number of lots being cultured at the same time and the camera may be in greater use in the future. The images are needed to conduct required in-process and final product release for dose calculation, and these are time sensitive

events. If the data cannot be collected a final product lot might not be released. The older, manual method with a ruler could possibly be reverted back to, but that was not described. We suggest that potential problems with this equipment/software be followed up on the next inspection to determine if there were any repeat occurrences and whether this had any significant consequences. DMPQ separately reviewed this deviation and came to the same conclusion. Please refer to the DMPQ review memo. A review of the other deviations appears reasonable.

Overall Reviewer's Assessment of CRL Item 1, observation #1:

The response is acceptable

Observation #2 (Deviation and Root Cause Analysis). Report # DEV-0723 019 documented a deviation for (b) (4) lots (b) (4) where final product mycoplasma test results provided by contract testing lab were determined by the contract lab to be invalid. (b) (4) lots (b) (4) were transplanted into the intended patients on April 12, April 15 and April 9, respectively. Repeat testing on (b) (4) samples of all three lots were negative for the presence of Mycoplasma. Root cause was determined to be an error on the part of the contract testing lab.

- a. No investigation was performed as to whether appropriate corrective actions were taken by the contract lab to prevent the problem from reoccurring. No CAPA was initiated to capture the corrective action taken and no additional corrective and/or preventive action was deemed necessary.*
- b. The likelihood of repeat occurrence was deemed improbable due to the fact that this event has not occurred in the past, though three individual lots were affected.*

Applicant's response to CRL item #1, observation #2: The applicant refers to changes in written procedures described in response to observation #1. They also re-reviewed deviation DEV-0723 and conducted a gap analysis. A CAPA for DEV-0723 was not previously initiated because the risk assessment was narrowly focused on the specific event, and thus the overall risk was deemed below the risk threshold to require initiation of a formal CAPA. The improved procedures for Risk Management, Risk Assessment, Deviations and Investigations, CAPA, and Change Control have tightened the requirements for assessing risk and implementing corrective actions, and thus a deviation similar to DEV-0723 would now result in a CAPA being initiated.

Upon review of this deviations and their current procedures, the following SOPs were updated:

- (b) (4)-QA-003 Non-conforming Products and Out of Specification Results was updated to clarify the procedure for investigation and notification of positive results to the clinical team. Actions that are needed when results are delayed in being reported or initially flagged as invalid/indeterminate have been detailed.
- (b) (4)-GEN-008 Shipping Mycoplasma Samples was updated to request expedited testing and (b) (4) mycoplasma samples for RVT-802 are shipped with the primary samples.
- (b) (4)-TRM-001 Communication Regarding Suitability of Donor Thymus Tissue for Recipient was created to formalize communication between the (b) (4) facility and the recipient's clinical team, such as any failed release testing or product issues to the recipient's attending physician.
- (b) (4)-QA-016 Out of Specification Investigations for RVT-802 was updated to reference (b) (4)-TRM-001 for communication with the clinical team in the cases of any final result failing to meet specifications and/or when results are found to be invalid or delayed.

The Applicant also responded to Type A discussions and the recommendation for a retrospective review of deviations that have occurred in the (b) (4) facility since 2016 when thymus product manufacturing was transferred to (b) (4). FDA also had recommended revisions to their quality risk management needs to consider the multiproduct nature of the facility, for which little information was provided, and there was not an opportunity to view this on inspection.

Review of response: During inspection, the inspection team noted how this and other events were deemed not to require a corrective action because mitigating actions taken at the time addressed the issue at hand, but did not necessarily solve a larger, underlying problem, or prevent a similar situation from occurring again. The updates to the main risk management and corrective action written procedures is an improvement. The more specific revisions to SOPs related to communicating delays in product test results also helps address concerns raised.

The main corrective action taken in this re-review of the deviation by Enzyvant and (b) (4) staff is to help expedite repeat testing of mycoplasma samples by the contract testing lab by supplying the mycoplasma (b) (4) testing at the (b) (4) as the original sample. Should a repeat test be necessary the contract lab can initiate repeat testing immediately. We agree this will speed up getting mycoplasma results as quickly as possible. However, this approach should not really be necessary. It is also a little risky to send (b) (4) along with the original because if the sample were to be lost or damaged in shipment, there would be no additional sample available for repeat testing. It is not clear if this risk has been considered. An information request was sent on Sept. 8, 2021. In response to this concern, the Applicant refers to (b) (4)-2020-046-P, RETHYMIC Testing and Sampling Strategy Risk Assessment. That report documents their risk management approach. The assessment took into consideration the medical status of the recipient including additional risks associated with

RATGAM, unexpected events and deviations such as those related to product transport which could potentially impact product quality. The option of sending (b) (4) samples independent of the primary sample shipment was considered and evaluated relative to the option for shipping (b) (4) the primary (b) (4) sample within one shipment. The risk associated with multiple shipments outweighed the potential benefits of that approach, and thus the procedures were updated to include the (b) (4) sample with the original sample shipment. The response is adequate.

The problem wasn't so much on the part of the (b) (4) facility, but on the contract testing lab being very slow to notify (b) (4) of the failed test due to an incorrect assay, and the time it took repeat testing to be performed. Once (b) (4) had been notified of the failed test they immediately send culture medium needed for dilution (b) (4) for repeat testing by overnight deliver. The contract lab, however, did not expedite repeat testing and did not immediately provide the results. This deviation has more to do with the adequacy of the contract testing lab than with actions taken by (b) (4) staff. To address concerns raised about vendor and contract testing lab qualification, (b) (4) executed QA 2019-015-P (b) (4) RVT-802 "External Service Provider Assessment Report," which assessed critical vendors and outlined changes to vendor management. (b) (4) vendor quality agreements have been updated to clarify timelines for notification of deviations, out of specification (OOS), invalid results and other investigations. Specific to the cited deviation (DEV-0723), the quality agreement with (b) (4) Labs for mycoplasma testing has been updated to establish a (b) (4) notification for deviations, out-of-specification, and invalid test results. The changes to external test lab and vendor qualification address our concerns.

During the Type A meeting discussion FDA suggested that since they are making significant changes to their risk management and corrective action procedures, they should consider reviewing all deviations associated with RVT-802 lots since the process was transferred to the (b) (4) facility in 2016. If such a review is conducted, we asked they provide a list of any corrective actions they have taken as a consequence, along with a summary of their risk assessment for each. A review of all deviations associated with RVT-802 lots since the process was transferred to the (b) (4) facility in 2016 has been completed and documented in QA 2020-001-P, "Rethymic deviation, investigation, and CAPA retrospective review- final report." A total of 80 deviations were listed, but the category and severity were not indicated. The majority were associated with either equipment, materials, or testing (Fig 1). The Applicant will use data trending to monitor future deviations for improvements to each category and a reduction of repeat problems.

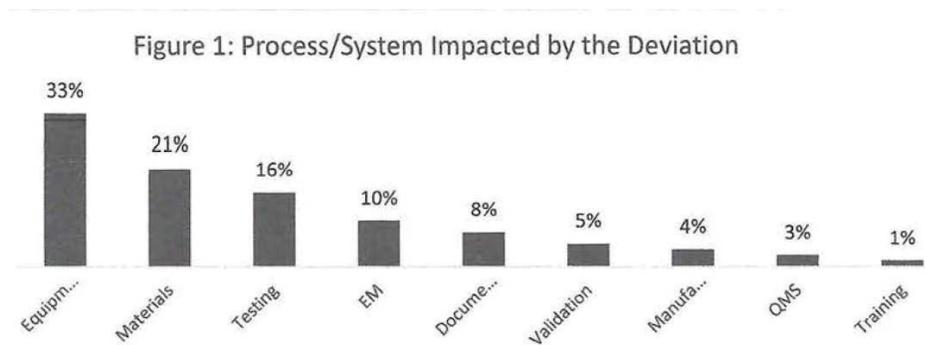


Figure 1. Graph of (b) (4) facility deviations by category (supplied by Applicant)

All deviations reviewed were closed, but 41% still had open CAPAs. Their review revealed that root cause analysis needed improvement. Based on the risk assessment score provided at the time they evaluated whether a CAPA was deemed necessary, and if so were corrective actions taken. They found 100% of the time when a CAPA was required it was initiated, and 38% of the time when it was optional.

Note: A review of risk assessment for some of these and other deviations examined on inspection suggested that no corrective actions were taken because risk assessment scores were assigned a low value. It was questionable if these scores were accurate in some cases (please refer to the Establishment Inspection Report). If they are only looking to see if corrective actions were taken as dictated by the original score, then the review would be fully informative. For example, in response to CRL item #1, observation #1, they indicated that if these cited deviations would occur now with the new quality risk management system in place, that corrective actions that were not previously taken would be taken now.

The retrospective review was helpful in identifying situations where a corrective action was taken to address a problem associated with a deviation, but a formal CAPA process was not initiated. 30% of the deviations had corrective actions that were documented in the deviation report instead of launching a CAPA Report. The Applicant states that although CAPAs were not opened in some of these events, in the context of this review, there was no detected adverse impact to the product or patient in these scenarios. Therefore, no additional corrective actions, investigation, or documentation is deemed necessary at this time because appropriate changes were made. The important point is that corrective actions were taken at the time, even if they did not follow their CAPA procedures. Based on revisions made to main SOPs listed in observation #1, it appears this may have largely been due to unclear written CAPA procedures. These procedures have now been modified. The response is adequate.

FDA also noted that the original submission did not provide much information on the multiproduct nature of the facility, and the multiproduct manufacturing and shared resources. (b) (4)-2020-065-P describes “A Cross-Contamination Risk assessment” that was performed. To mitigate risk of cross-contamination a risk control plan was identified.

Mitigation includes: 1) multiple procedural and facility changes made to further minimize impact and the potential for contamination to RETHYMIC, such as facility upgrades, improved cleaning, updated personnel flows, and environmental monitoring procedures; 2) the inventory control system has been updated to ensure segregation of commercial product from research and development materials is maintained; 3) QMS improvements detailed ensure events related to issues associated with a multi-product facility are detected and investigated with appropriate corrective or preventive actions; and 4) trend analysis will be performed to ensure the facility is maintained in a state of control. The enhanced procedures and updated documents address concerns about multiproduct manufacturing.

Note: Modules 3.2.S, 3.2.P, and 3.2.A are built on the knowledge that thymus tissue processing and RETHYMIC manufacturing only occurs within clean room (b) (4) (Room (b) (4)). This is no longer true due to a recent change that now allows clinical manufacturing under a different IND for essentially the same product for a different clinical indication. That product is handled in clean room (b) (4). The potential impact to the commercial product and multiproduct manufacturing was not updated in the BLA. In response to an information request the Applicant indicated there are no other immediate plans to conduct manufacturing of cultured thymus tissue outside of (b) (4). Before a thymus would be brought into the facility again and manufacturing activities would commence in a suite different from (b) (4) (b) (4) commits to initiating a change control, which would include formal review of related risk assessments and implementation of any required documentation updates. Section 3.2.A.1 has been updated to reflect that cultured thymus for treatment of patients may be processed in other areas of the facility. The response is acceptable.

Note: Additional responses were provided in response to this observation as a follow-up to comments communicated at the Type A meeting. However, these comments relate to histology testing and not the mycoplasma deviation, or risk quality risk management procedures. We are including a review of these responses as part of the response to CRL item #3 that covers histology testing, instead of as CRL item #1, observation #3.

Overall Reviewer’s Assessment of CRL Item 1, observation #2:

The changes to written procedures, enhanced vendor and contract test lab agreements, and the corrective action to provide a (b) (4) sample along with the original sample adequately addresses the observation. The retrospective analysis of previous deviations provided useful insight to the nature of the deviations, and led the Applicant to identify root cause analysis needed improvement, and that was corrected in the updated quality risk management procedures. The retrospective study is limited because they relied on the old risk assessment scoring matrix to determine whether a corrective action was needed. No additional corrective actions were identified as a result, even though they say in response to Observation #1 that additional corrective actions would be taken now that were not previously. Since the risk assessment and mitigation strategies have been recently strengthened, there are few examples to assess the level of improvement they provide. This will be easier to assess in the

future should new deviations occur, and the revised process can be evaluate at that time. Enhancements to the facility and written procedures help address concerns about multiproduct manufacturing. Since multiproduct manufacturing could not be observed during the pre-license inspection, it should be evaluated at a future inspection when other products are being made at the same time. **The response is acceptable.**

Observation #3 (Environmental Monitoring Program). The environmental monitoring program is deficient in ensuring that the cleanrooms are operating in a state of environmental control. For example,

- a. *The 2017 EMPQ performed following the modifications to the HVAC system is inadequate as it was limited in the number of samples collected and the type of sampling performed: (b) (4) sampling was limited to (b) (4) per location for (b) (4)*
- b. *The routine environmental monitoring program does not include (b) (4) monitoring for (b) (4) in the ISC^{(b) (4)} and ISC^{(b) (4)} areas.*
- c. *A single (b) (4) sample (b) (4) is collected during the aseptic processing in the (b) (4) (ISC^{(b) (4)}) during the manufacturing process, which can take up to (b) (4)*
- d. *Routine (b) (4) sampling for (b) (4) is not performed for the ISC^{(b) (4)} manufacturing area and the associated passthroughs under (b) (4) conditions.*
- e. *There is no (b) (4) sampling (post operations) in the (b) (4) (ISO^{(b) (4)}) used for the aseptic manufacturing of the product.*
- f. *There is no (b) (4) sampling, or sampling of the (b) (4) in the incubator after the manufacturing of a lot. The incubator is located in manufacturing room (b) (4) (ISO^{(b) (4)}) and used for the in- process incubation of the product up to 21 days.*
- g. *The differential pressure between the ISO^{(b) (4)} and ISO^{(b) (4)} cleanrooms and between the ISO^{(b) (4)} and the CNC areas is not alarmed or recorded to ensure compliance with the established settings.*
- h. *The settings for humidity (b) (4) and temperature (b) (4) are too wide.*

Applicant's response to CRL item #1, observation #3: To address these concerns the following studies and changes were made:

- Multiple modifications were made to the facility, procedural controls, and engineering controls to ensure (b) (4) personnel flows.
- Updated procedures for cleaning, gowning, and materials transfer/personnel flow were implemented upon recertification of the facility and prior to conducting the June 2020 aseptic process validation
- Completion of (b) (4) environmental monitoring performance qualification (EMPQ) studies (executed from 29 June – 01 August 2020)
- Significant revisions and advancements in the environmental monitoring (EM) program
- Inconsistencies in the original EM risk assessment summary report (b) (4)-2019-045-P) has been superseded by a new risk assessment conducted in first half of 2020 (b) (4)-2019-045.1-P)
- The Applicant concluded the 2020 EMPQ showed that the cleaning program and operational procedures in place during the 2020 EMPQ provided a high level of microbial control, with low excursion rates and an expected distribution of different types of organisms commonly found in cleanrooms
- SOPs have been updated for (b) (4) samples collected in the (b) (4)
- At the end of RETHYMIC manufacturing incubator (b) (4) is sent for bioburden testing
- Tighter controls for targets and alarm ranges of room temperature and room humidity
- Differential pressures throughout the facility were rebalanced

Review of response: This CRL item was reviewed by DMPQ and found acceptable. There are no additional comments from DCGT. Please refer to DMPQ review memo for details.

Overall Reviewer's Assessment of CRL Item 1, observation #3:

The response is acceptable.

Observation #4 (Cleaning Procedures Not Qualified). The current cleaning procedures used in the cleanroom have not been qualified.

- a. Disinfectant effectiveness studies have not been performed for the sanitizing agents routinely utilized in the manufacturing facility.*
- b. The procedures established and followed for cleaning the facility are inadequate; for example,*

- i. Procedure (b) (4) -SOP-060 is deficient in that it does not describe in detail the process for cleaning the (b) (4) specifically, during observation of the simulated manufacturing operations, we noted the following:
 - a. the operators cleaned the whole (b) (4) with one surface of a disposable sterile (b) (4) pad using an (b) (4).
 - b. the (b) (4) cleaning solution was dry before the (b) (4) set time required in the procedure.
 - c. one (b) (4) wipe was used multiple times for wiping several items before placing them in the (b) (4).
- ii. Procedure (b) (4) -SOP-066, includes two different procedures for cleaning the (b) (4) incubator even though only one procedure is used at the facility.
- iii. Procedure (b) (4) -SOP-006 states that (b) (4) cleaning is required for the cleanrooms (floors and surfaces) even though the production room and supportive areas are used (b) (4) for manufacturing product lots.
- iv. The cleaning of the passthroughs is not performed (b) (4) use. It is cleaned once a (b) (4)

Applicant's response to CRL item 1, observation #4: The cleaning program has been qualified by completing disinfectant efficacy (DE) studies and by completing the EMPQ. (b) (4) -2019-044.1-P was conducted to assess the cleaning and disinfection program and determined additional DE testing and environmental monitoring need to be conducted to qualify cleaning procedures.

In May/June 2020 multiple modifications were made to the facility, procedural controls, and engineering controls in order to address FDA concerns about personnel flow (covered in CRL item #12).

Updated procedures for cleaning, gowning, and materials transfer/personnel flow were implemented in June 2020 upon recertification of the facility and prior to conducting the 2020 aseptic process validation, 2020 EMPQ, and 2020 process validation studies.

The 2020 EMPQ was executed in July 2020 and the DE study, which included microbial and viral arms, was executed February-November 2020, with additional DE studies completed in January-February 2021. The 2020 EMPQ was conducted in parallel with the DE studies, rather than completing validation of the cleaning program and then performing the post-change EMPQ. Through successful completion of these two studies, the cleaning program has now been qualified.

Key changes to cleaning, gowning, and materials transfer/personnel flow that came out of the 2020 risk assessments are as follows:

- Added detailed description for (b) (4) cleaning of (b) (4)
- Added details regarding all disinfectants used in the facility, including composition, modes of usage, and storage requirements.
- Added details regarding all cleaning supplies used in the facility, including specifics of the cleaning supplies used, where used, how used, and where stored.
- Provide a daily cleaning of the ISO (b) (4) manufacturing rooms.
- Line clearance occurs (b) (4) with each working session and may occur up to (b) (4) times per day in the RETHYMIC manufacturing suite.
- Replaced (b) (4) of the ISO (b) (4) workspace and required recording a (b) (4) contact time for equipment that will be transferred into the (b) (4) (e.g., pipet aid and EM equipment).
- Added procedures for cleaning all passthroughs (b) (4). Passthroughs on the (b) (4) rooms are cleaned at the (b) (4) when it was used
- Fully described the cleaning procedures after spills
- Added a (b) (4) cleaning (b) (4) of the Changing Room performed by (b) (4) personnel, performed with (b) (4).
- Added details to the section related to cleaning procedures performed by contracted cleaners, including specific details for each room/area.
- Gowning SOP changes
- Upon completion of the study, data review indicated that broader use of (b) (4) would be beneficial, and supplemental DE studies were executed in January 2021 to support use of (b) (4) on more surface types and under soiled conditions.
- The DE study included evaluation of (b) (4) different microbial organisms (b) (4) organisms plus (b) (4) facility isolates) plus (b) (4) viruses (b) (4). A total of (b) (4) different materials were selected to represent the surface types present throughout the facility (based on being a worst-case example of a particular surface type or being the most commonly occurring example of a particular surface type). These data demonstrated that within the set of disinfectants in use within (b) (4) there are disinfectants that are effective against all the types of organisms and viruses that are expected to occur in the facility. Thus, the set of disinfectants included in the cleaning program is adequate and appropriate for ensuring the cleanroom meets FDA guidelines and EU Annex 1.

The DE data suggest that (b) (4) bacteria (except (b) (4) a facility isolate) and (b) (4) viruses should be effectively inactivated during (b) (4) facility cleaning with (b) (4) and through the routine cleaning of work surfaces,

passthroughs, and materials with (b) (4). The (b) (4) combination used before each use in the (b) (4) is effective against the (b) (4) bacteria plus (b) (4) and (b) (4) viruses. All organisms (mold, fungi, bacterial spores [like (b) (4), a facility isolate] and (b) (4) bacteria [including (b) (4), a facility isolate]) and all virus types ((b) (4)) will be effectively inactivated during (b) (4) facility cleaning with (b) (4).

The EM data from the 2020 EMPQ show that there were few spore-formers found throughout the facility (b) (4)-2020-034-P Final Report). A total of (b) (4) contaminants were identified during the EMPQ (among the (b) (4) samples collected), and of those (b) (4) contaminants, (b) (4) were mold/fungus and (b) (4) were bacterial spores. The majority of those spore-formers (b) (4) were obtained on samples collected in the CNC areas, with (b) (4) obtained in the ISO (b) (4) areas. Spore-formers were rare in ISO (b) (4) of all identified spore-formers occurred in ISO (b) (4) and ISO (b) (4) of all spore-formers occurred in ISO (b) (4).

Review of response: This CRL item was reviewed by DMPQ. There are no additional comments from DCGT. Please refer to DMPQ review memo.

Overall Reviewer’s Assessment of CRL Item 1, observation #4:

The response is acceptable.

Observation #5 (Alarm System Deficient). The existing alarm system and its implementation are deficient. Specifically,

- a. *Temperature probe in (b) (4) used to store released critical reagent, (b) (4), is not placed in the worst-case location as determined during equipment qualification.*
- b. *The firm did not perform IQ/OQ of the (b) (4) alarms and probes installed after 2014, including those installed in (b) (4) incubators (b) (4) instrument. The equipment is used for manufacture and release sterility testing of RVT-802 and storage of critical reagents and source material.*
- c. *The firm failed to provide records of preventive maintenance for any of (b) (4) alarms used for monitoring of differential pressure, temperature, and humidity within the facility, (b) (4) system, as well as the following critical equipment: (b) (4) incubators (b) (4) levels and temperature), (b) (4) used for storage of critical reagents, source material, and (b) (4) samples, and (b) (4) instrument used for release sterility testing of the product.*

- d. *The alarm notification and response are not adequate. Per deviation IR-0114 dated December 5, 2017 and a corresponding (b) (4) log for events #7285 and 7286: On December 3, 2019 temperature within the incubator was out of range between 12:35 and 15:12 and (b) (4) was out of range between 12:03 and 14:46. No alarm notification was received until 12:30, and notified employee failed to immediately respond to the alarm. The incubator contained (b) (4) lot of thymus tissue, which was implanted on December 19, 2017.*

Applicant's response to CRL item #1, observation #5: There are (b) (4) systems that monitor the operation of the facilities and the equipment a (b) (4) and provide alarms when out-of-specification conditions occur: (b) (4). The (b) (4) system monitors the equipment and rooms within the manufacturing space (b) (4). The (b) (4) system monitors the equipment and rooms within the receiving and storage area used for RETHYMIC and the (b) (4) Processing Laboratory space. To address the deficiency the Applicant conducted extensive analyses of the alarm systems and procedural updates

Additional temperature mapping was performed in multiple (b) (4) throughout the (b) (4) facility to thoroughly assess hot spots and cold spots. This additional temperature mapping was used to identify the worst-case locations for sensor placement. The sensors were then re-located as recommended.

The (b) (4) were evaluated to identify gaps and determine appropriate corrective actions to improve the alarm systems. For the (b) (4) system, the identified gaps were related to inadequate detail regarding system access, data review, data backup, and lack of assessment of potential impact to product when excursions occur.

All (b) (4) sensors were re-calibrated in August-September 2020 for the (b) (4) calibration.

During the (b) (4) calibration, the sensor and sensor connections will be cleaned and inspected, as well as all components of the installed sensor. (b) (4), the backup batteries in the Collection Points and Access Points will be replaced.

(b) (4)-SOP-094 (formerly known as CT2-SOP-094), was updated:

- To require Installation/Operation Qualification (IQ/OQ) of sensors at time of installation.
- Now includes details regarding procedures for data review, including frequency of data review and what is included in the data review. On a (b) (4), a review of (b) (4) data is performed, which includes retrieving the (b) (4) Day event report, and assessing whether all events have been properly responded to within appropriate timeframes.
- Details regarding procedures for handling excursions/events, how to acknowledge alarms, and expected response times were added to (b) (4)-SOP-094.

Review of response: This CRL item was reviewed by DMPQ. There are no additional comments from DCGT. Please refer to DMPQ review memo.

Overall Reviewer’s Assessment of CRL Item 1, observation #5:

The response is acceptable.

Observation #6 (Quality Unit Oversight of Batch Record Review). The Quality Unit oversight of batch record review is deficient. Specifically,

- a. *A review of the (b) (4) batch record, Preparation of Final Product (b) (4)-SOP-031, FRM14, dated April 19, 2019) and the Room (b) (4) incubator use log form does not include: 1) verification or periodic recording of (b) (4) and temperature during this time period, and/or 2) inclusion of the (b) (4) graph printout, which shows continuous monitoring of (b) (4) and temperature over the (b) (4) day time period, (b) (4). The manufacturing process requires (b) (4) to be maintained between (b) (4) and the temperature (b) (4) during the incubation period.*
- b. *Not all time limits for the completion of each process step follow limits defined by process validation, and batch record review does not confirm adherence to step times.*

Applicant’s response to CRL item #1, observation #6: A risk assessment of validation and System Unit (QSU) oversight was completed in November 2019 and documented in “The Validation and Quality System Unit Oversight Assessment Report”, QA-2019-016-P. (b) (4) personnel determined that SOP COMM-QA-044 “Approaches to Validation” warranted updates to provide clarity regarding when to use the deviation/investigation procedure versus protocol deviations and to require improved connection to change control to ensure updates to procedures and batch records as a result of validation activities are implemented correctly. SOP COMM-QA-044 has been revised to clarify responsibility, PQ requirements, change control, and the use of protocol deviations.

Revisions have been made to the manufacturing SOPs related to batch record review, including (b) (4)-QA-006, (b) (4)-SOP-029, (b) (4)-SOP-030, (b) (4)-SOP-031, (b) (4)-THY-009, and (b) (4)-QA-007.

Further steps have been taken to ensure that time limits for the completion of each process step follow limits defined by process validation, and batch record review confirms adherence to step times. Specifically, a careful review of how process step times and hold times are defined and justified was conducted. Justification of all step and

hold times, and other critical and non-critical process parameters was documented in a process risk assessment report (b) (4) 2020-009.1-P), which was initially approved prior to the 2020 Process Validation and updated in March 2021 (to incorporate data from the 2020 clinical batches and PPQ batches, add acceptable ranges for two new parameters [slice yield and in-process dose], and update the Risk Reduction and Control Plan). The acceptable ranges for hold times and all other parameters were established based on the clinical manufacturing process history, which encompassed (b) (4) manufacturing areas (ie, the (b) (4) facility), multiple operators, and multiple lots of raw materials. Batch records have been revised to clarify these hold times and calculation steps for each process time/hold time were added to allow QA to easily confirm adherence to the process and hold times and all other critical and non-critical process parameters during batch record review.

Step (b) (4) of (b) (4)--SOP-029 FRM1 was revised to emphasize that tissue slicing must begin within (b) (4) of when notification is received from the operating room (OR) that thymus tissue is available. The time that the notification call is received is recorded on the batch record, and the date and time that the thymus tissue expires is calculated based on this notification time and recorded on the form. In (b) (4)-SOP-029 FRM2, the time that tissue slicing begins is now recorded alongside the notification time for the batch from (b) (4)-SOP-029 FRM1.

The time between media changes during culturing is now calculated immediately (b) (4) to each feed to confirm that it is in the acceptable range. The acceptable times for performing the media change on the (b) (4) are also calculated after the media change is complete and dishes have been returned to the incubator to guide manufacturing planning and improve control.

At the end of each batch record, relevant processing and hold times for the lot are now recorded in a table alongside the acceptance criteria specification.

Note: This was a recommendation made during prelicense inspection

All other critical and non-critical manufacturing process parameters identified in (b) (4) 2020-009.1-P were incorporated into manufacturing SOPs and batch records where appropriate, and into (b) (4)-QA-007. Batch records include steps for verification of parameters during the review process, and (b) (4)-QA-007 outlines the process for review of these required parameters.

Review of response: The response to observation 6a was reviewed by DMPQ. The response to observation #6b was reviewed by DCGT.

The changes described by the Applicant address concerns about monitoring, recording, and ensuring compliance with defined process step and hold times.

For Lot (b) (4) the tissue was stored (b) (4). The expiration of the source material is set as (b) (4) from the time of notification. Step (b) (4) on form (b) (4)-SOP-029-FRM1 reviewed for inspection states that the tissue must be processed within (b) (4) of notification. The time of notification was (b) (4) and the expiration

was (b) (4). Tissue processing (b) (4) storage began at (b) (4). Step (b) (4) on (b) (4)-SOP-029-FRM2 involves (b) (4), which was completed at (b) (4). Steps (b) (4) involve (b) (4) for use. Tissue processing does not begin until Step (b) (4) and the time is not recorded. It is unclear how long steps (b) (4) took to complete. Step (b) (4) on form (b) (4)-SOP-029-FRM1 is unclear as to whether tissue processing must be initiated by (b) (4) or must be completed by (b) (4). As previously stated, it implies that all processing must be completed. Tissue processing was completed at (b) (4). Since the time for Step (b) (4) was not documented it is unclear whether the tissue had expired prior to the initiating of processing, but it had expired prior to completion of processing. The language in the revised SOP is clearer and states that *tissue slicing* must begin within (b) (4)-SOP-029-FRM1 and (b) (4)-SOP-029-FRM2 both include places where the start times of tissue processing are recorded.

During Type A discussions FDA asked for clarification for why processing tissues sometimes in initiated until near the (b) (4) expiry the next day, rather than being expedited. Lot (b) (4) is an example. The Applicant responded in the resubmission by stating the decision to process the same day or next day "...is informed by, not strictly driven by, the time of receipt". Section 3.2.S.2.2 and (b) (4)-SOP-029 have been updated which procedures to follow if (b) (4) storage is used. The rationale and the decision-making process is not described in more detail in either documents, other than to say the manufacturing team makes the decision based on scheduling and resource considerations. The facility director makes decisions about suite availability. Considering the small staff size of the facility, the (b) (4) they operate under, the shortage of available tissue donations, and the intention to manufacture up to (b) (4) lots at any one time and to treat a larger patient population that under IND, it is highly likely that (b) (4) storage will be used to a greater extent for the commercial process than was used under IND. The use of (b) (4) storage has been used more frequently than when processed in the (b) (4). This places a greater emphasis on evaluating product stability and safety and efficacy of product lots held for up to (b) (4) under (b) (4) conditions before slicing and initiation of thymus cultures. This was reviewed in CRL item #4.

Overall Reviewer’s Assessment of CRL Item 1, observation #6:

The changes described by the Applicant address concerns about monitoring, recording, and ensuring compliance with defined process step and hold times. **The response is acceptable.**

Observation #7 (Procedures & Process Control for Microbiological Contamination). Procedures and process control designed to prevent microbiological contamination of drug product are not established with appropriate acceptance criteria. Specifically,

- a. (b) (4)-SOP-060, "Operation and Maintenance of the (b) (4) [redacted] dated July 29, 2019, states certification is performed every (b) (4) [redacted] for every (b) (4) [redacted] Section 8.11.3 of (b) (4)-SOP-060, states the (b) (4) [redacted] are certified using standards traceable to the (b) (4), but does not include the acceptance criteria. Additionally, the acceptance criteria for (b) (4) [redacted] was not specified but was calculated in the most recent (b) (4) [redacted] certification, dated April 16, 2019.
- b. The (b) (4) System Qualification Summary Report (b) (4)-2019-025-E, dated March 25, 2019, Ongoing Monitoring, did not include acceptance criteria for (b) (4) [redacted], which were calculated in the supporting (b) (4) [redacted] Testing Results from October 2018.

Applicant's response to CRL item 1, observation #7: (b) (4)-SOP-060 and (b) (4)-2020-025.1-E were revised to include acceptance criteria for (b) (4) certification and operational/performance qualification of the (b) (4). These revisions were already provided in a BLA amendments 125685.49 and 125685.50. Since then, the documents have been further updated ensure repair of HEPA filters is restricted to a (b) (4) area before complete filter replacement is required. In addition, the appropriateness of the acceptance criteria has been established by comparison to ISO guidelines (ISO (b) (4) [redacted]) and manufacturer's specifications. Acceptance criteria for (b) (4) [redacted] have been verified through aseptic process simulation, (b) (4) EM, (b) (4) studies related to the thymus tissue processing, and other process validation activities. (b) (4)-EQUIP-003 was updated to describe the acceptance criteria for calibration of all equipment.

Review of response: This CRL item was reviewed by DMPQ. There are no additional comments from DCGT. Please refer to DMPQ review memo.

Overall Reviewer's Assessment of CRL Item 1, observation #7:

The response is acceptable.

Observation #8 (Inadequate Quality Unit Control over Critical Materials). The Quality Unit does not have adequate control over critical materials. For example:

- a. A new container closure was implemented before approval by (b) (4) Quality Assurance for use in the (b) (4) facility on July 27, 2019. It was used for the manufacturing of (b) (4) lots (b) (4) [redacted] initiated beginning on April 27, 2019.

- b. *Sterility of critical product-contact equipment sterilized by external vendors (i.e., sterile (b) (4) specimen final container closure system, tissue slicer, blades, blade handles, forceps, filter papers) is not being verified through periodic sampling of incoming lots.*
- c. *The firm does not have controls in place to ensure that critical product contact supplies, such as support filters included in final product formulation, dissection instrument, and tissue culture implements are sterile and (b) (4) .*
- d. *Identity tests are not in place for critical raw materials used in the manufacture of TOM media. These include (b) (4) filter, and surgical sponge.*
- e. *No expiration dates exist for critical materials for the final drug product container closure system or secondary sterile overlap for the final product. Materials that do not have an expiration date assigned by the vendor are to be assigned by (b) (4) according to SOP GEN-009. Expiration for these materials is currently designated "Not Applicable". For other critical supplies that are sterile with direct product contact, such as tissue slicers, scissors, and forceps, expiration dates were not provided.*

Applicant's response to CRL item 1, observation #8: To address item 8a the Applicant assessed their change control procedures. They determined the root cause was the insufficient change control procedure that was in place at the time of the event. Changes had been made to the COMM-QA-019 Change Control and associated procedures in May 2019:

- Addition of an Implementation Step in the Change Control process to help detect and confirm that necessary pre-requisites were completed as required before a change is implemented.
- Addition of an Effectiveness Check
- Revision of the forms to prompt the change owner for information and documentation to support each phase of the change and to ensure all approvals are obtained prior to implementation
- Providing more detailed instruction to help the user know when risk assessments are required.
- Linkage to COMM-QA-077, Risk Assessment Procedure, ensures the risk assessment within change controls is conducted consistently with other major QMS systems, like Deviations and CAPA.
- Improved management oversight by adding Medical Director review and approval on FRM2
- Addition of a projected implementation date to be completed prior to implementation approval helps with planning for changes as well as ensuring any pre-requisites are complete.

For item 8b and c the Applicant clarifies that the (b) (4) container that is now used only to transport and hold incoming thymus tissue prior to processing, and it is no longer the final container closure system. Only (b) (4) container is used for transporting the thymus tissue.

(b) (4)



For item 8d identity assays for critical materials have been established, and the identity methods validated. We have summarized these methods in the following table.



(b) (4)

(b) (4)

Review of response: The response to observation 8a and 8d were reviewed by DCGT. The response to observation #8b, 8c, and 8e were reviewed by DMPQ.

A review of COMM-QA-019 Change Control procedures appears to address concerns that changes should not take place before proper oversight is conducted and signed off. The comprehensive changes to other risk management procedures reviewed in response to Observation #1 demonstrate a serious effort by the Applicant to make improvements to their quality system.

In the case of the new process validation study being executed before the latest versions of quality risk management, deviation investigation, CAPA, and change control documents were in effect, a risk assessment report (b) (4) 2020-009.1-P) was initially approved prior to the 2020 Process Validation study.

Note: The date of (b) (4) 2020-009.1-P Rev 1 “Risk Assessment of the RVT-802 Manufacturing Process, including Parameter Criticality and Justification of Acceptable Ranges/Acceptance Criteria” is March 25, 2021. This version of the document must have been revised after the initial preliminary report was generated to conduct the PV study. The risk assessment document is not specific to just the PV study.

Information gathered from the PV and other studies were used to further revise these risk management documents. The PV study involves several elements not normally part of commercial manufacturing, such as a (b) (4) day time point analysis for evidence of (b) (4) with extended culture time. On Day (b) (4) there was not a sufficient number of single use bottle of medium to perform the feeding and so a bottle was used (b) (4). Although this is a small deviation, it is an example of a consequence of a manufacturing change, in this case to conduct the PV study, that should have been considered and planned for. It is not clear that the final version of risk assessment procedures would have identified and mitigated this risk versus the earlier version. (b) (4) 2020-009.1-P is a comprehensive risk assessment report. In the report, materials were evaluated and the TOM culture medium was listed as a critical material. The PV report includes a list of all materials used for the study and their criticality. The amount of TOM medium needed was not described. There

does not appear to have been a separate risk consideration for changes to the manufacturing and test procedures used for PV that are not part of commercial production, such as (b) (4) image acquisition for product slices to evaluate tissue (b) (4) during culture. As noted in the review of Observation #1, there was a deviation in the ability to perform the analysis on all days because a set of Day (b) (4) images could not be copied to the server for data analysis. Both of these incidents are examples of risk assessment that should have been more comprehensive, rather than an issue that the study was executed before proper oversight was in place.

EMPQ studies were repeated while other changes to written procedures and the facility were still being made. More typically, the written procedures and facility modifications would have been revised first. EMPQ, media fills, and the PV studies were successfully executed without any sterility, mycoplasma, or endotoxin failures.

We reviewed the identity assays that have been established for the following critical reagents: (b) (4), fetal bovine serum (FBS), (b) (4) filters, and (b) (4) sponges. We agree the assays methods are appropriate. Each assay method validation report was reviewed and the validation methods and results found acceptable.

Overall Reviewer's Assessment of CRL Item 1, observation #8:

The revised written procedures address concerns about changes being implemented before proper oversight had been conducted. Identity assay methods for critical reagents have been established using methods that are appropriate and have been validated. **The response is acceptable.**

Observation #9 (Deficient Inventory Control of Raw Materials). The inventory control of raw materials is deficient. Specifically,

- a. *Per deviation DEV-0455 dated August 22, 2016: thymus organ media lot TOM-(b) (4) was conditionally released without sterility testing results due to insufficient volume of released TOM available to complete manufacture of lot (b) (4)*
- b. *Expired supplies were used in manufacture of thymus lots (b) (4) (per deviation DEV-0667 dated February 4, 2019).*
- c. *The control system to prevent mix-ups for materials, components, samples, and containers, intended for use in the RVT-802 manufacturing process does not include inventory records that show the current real-time inventory for (b) (4) used for storage of critical reagents, source material, (b) (4) and QC samples.*

- d. *Materials are not being properly segregated. (b) (4) used to store RVT-802 (b) (4) samples is also used to store (b) (4) samples for other products manufactured in the facility, along with research materials. Aside from the RVT-802 mycoplasma (b) (4) sample log on the front of the (b) (4) there is no log of the contents of the (b) (4)*

Applicant's response to CRL item 1, observation #9: Updated procedures for Inventory logs, tracking, restocking, reconciliation, and storage as well as segregation of materials, have been implemented. (b) (4) modified the supply management process to help ensure enhanced control and management of material inventory. Key updates to this system include creation of routine, defined inventory checks, new inventory log documentation and reconciliation, as well as regular restocking. The new processes focus on implementation of (b) (4) new forms/inventory logs to ensure complete and accurate inventory records are kept at (b) (4) from initial receipt to final use during manufacturing: (b) (4)

[Redacted]

(b) (4)

[Redacted]

[Redacted]

(b) (4)



Review of response: The response to observation 9a -9c were jointly reviewed by DCGT and DMPQ. The response to observation 9d was reviewed by DCGT. Much of the response covered how inventory was managed and tracked. There was not much information provided on avoidance of the use of expired materials. During PLI it was noted supplies were located visually and that there was a (b) (4) inventory review. Supplies did not seem organized in any particular way and bins of material do not have any assigned place. No real-time inventory control was maintained for any of the storage rooms or (b) (4) storage. (b) (4) staff stated that expired materials can be used if no other supply is available. For example, toolkits for product manufacturing can be assembled for use in the cleanroom using expired materials if no other materials are available. Several deviations were noted on inspection. A contributing factor was the lack of a robust inventory control system to keep track of how much material is available for use at any given time. The inspection team recommended that a better system to monitor inventory would help reduce the need for using expired materials. The updated SOPs and the use of an excel spreadsheet would help improve keeping track of which materials they have on hand, review of material qualification, and with expiration dating. The updated procedures are still not real-time because they are only updated (b) (4). For this small facility with low production capacity that may be sufficient. The (b) (4) logs have been improved, especially with regards to (b) (4) sample storage.

SOP (b) (4)-GEN-009 states that expiration dates are required for all supplies used in the manufacture of licensed products. The expiration dates are determined as described in project specific SOPs. TOM expiration is assigned based on the first expiration date among all the components used for generating the medium. TOM medium, like all

manufacturing materials, is to be used on a (b) (4) basis, and medium lots are to be used according to expiration date. The updated SOPs also indicate this same policy. However, a review of the batch record for lot # (b) (4) on PLI indicated manufacturing operators (b) (4)

(b) (4)

Prioritized use of materials is not specifically covered in this CRL item. Revised procedures state they will target no more than (b) (4) different lots of medium for any RVT-802 product lot. Adherence to (b) (4) can be evaluated as part of standard future inspection procedures.

SOP (b) (4)-QA-002 revision 4 (no implementation date was specified) states that if expired supplies or supplies that will expire in the upcoming (b) (4) are noted during (b) (4) inventory checks, they are removed from the supply room and properly disposed. It does not say how the material will be replaced, or how much material of any one type needs to be on hand at any one time to meet current manufacturing needs. Inventory updates occur each (b) (4) in order to update items removed and items added to each (b) (4). Rhythmic manufacturing requires (b) (4) feeding and therefore a use of a lot of single use materials. The updated procedures would help address concerns with deviations resulting from using expired materials if no other material is available because now materials that have expired or are about to expire will be discarded. Since the materials are discarded (b) (4) and the inventory is checked (b) (4) there should not be any expired materials kept in the supply room. It does not explain how a proper level of inventory is maintained to avoid disruptions in manufacturing or product testing. The amount of material that must be available at any one time is unclear, as is how the projected need for commercial manufacturing and testing supplies is determined. In response to an information request the Applicant indicated that a (b) (4) lot supply of all materials is to be maintained, and if less is available additional supplies are ordered or prepared. The response is acceptable.

Note: For process validation lot # On Day (b) (4) there was not a sufficient number of single use bottles of medium to perform the feeding of lot (b) (4) and so a bottle was used (b) (4)

Regarding segregation of commercial (b) (4) samples, IND, and research samples, (b) (4) samples for commercial production will now be segregated from other materials by shelf. The placement, sample description, and date/time in and out time of the (b) (4) is now documented on log form (b) (4)-SOP-027 FRM1 (b) (4) Sample Storage and Disposition Log". This addresses concerns about (b) (4) sample storage, but not (b) (4) materials in the supply room or the Material Prep Lab Room. Materials in (b) (4) located in Material Prep Lab Room were not previously not segregated, and (b) (4) samples were stored together with research materials. The Applicant responded to an

information request and have clarified the (b) (4) in the material supply room is not utilized for storage of raw materials for the manufacturing of RETHYMIC. (b) (4) is used to (b) (4) for the manufacture of (b) (4) products. These (b) (4) are separated by (b) (4) for RETHYMIC that are stored in (b) (4) are not used in any other products manufactured at (b) (4) -QA-002 FRM6 has been revised to include (b) (4) location. The response is adequate.

(b) (4) of the (b) (4) supply room (Room (b) (4)) was included. Supplies for (b) (4) product manufacturing are kept on (b) (4). Thymus product specific materials only occupy (b) (4) of the (b) (4) total carts in the room. IND and commercial lots of thymus reagents are stored on (b) (4), but the materials are identical.

Overall Reviewer’s Assessment of CRL Item 1, observation #9:

The assignment of dedicated areas for ambient material storage in the supply room and weekly review and confirmation of inventory addresses concerns about material management. Segregation of (b) (4) samples and the establishment of a (b) (4) log addresses concerns about (b) (4) sample storage. Previous issues with the use of expired materials has been addressed by assigning an expiration date to all materials used for commercial production, and (b) (4) inventory of all materials, with disposal of any material that has expired or will expire within (b) (4). Updated procedures since the last inspection include keeping at least a (b) (4) lot supply of all materials on hand. **The response adequately addresses the inspection observation.**

Observation #10 (Data Protection of Computer System). A means of assuring data protection has not been established for the following computerized system. There is failure to maintain a backup file that is assured as secure from alteration, erasure or loss through keeping hard copy or alternate systems. Specifically, the current (b) (4) -EQUIP-021, Operation, Maintenance and Sterility Culture using the (b) (4) dated September 27, 2018, does not include criteria for back up of data from (b) (4), a (b) (4) based data management software application used for the (b) (4), to removable (b) (4) and verification of back up to a networked path.

Applicant’s response to CRL item 1, observation #10: Enzyvant has updated computer equipment, upgraded the operating system and application software to ensure Part 11 compliance where technically possible, and took measures intended to improve data protection for the (b) (4) system as well as other computerized systems supporting RETHYMIC manufacture. Procedures have been established to maintain a backup file that is secure from alteration, erasure, or loss. (b) (4)

(b) (4). This process has completed verification testing including evaluation of the associated audit trails. The backup schedule has been increased from (b) (4) backups during (b) (4) working periods and minimally every (b) (4). The backup tests (b) (4)-2020-067-E) confirmed that the audit trail is indelible and SOP (b) (4)-EQUIP-021 was updated to reflect the revised audit trail review process. A (b) (4) is used because the equipment the equipment is not currently networked. A PDF report is generated and also copied with the associated metadata. They consider this a true copy of the original data and is now in a format compatible with the original format to allow data recovery. Additional validation was completed of the backup process for (b) (4) under (b) (4)-2019-055-E. SOP (b) (4)-QA-022 Computerized System Access and Administration was developed to ensure only pre-approved qualified staff members are allowed access to the computerized systems that generate and store (b) (4) data.

The (b) (4) computer operating system was upgraded to a (b) (4) version. The (b) (4) Management Software was upgraded to Version (b) (4) (latest available from manufacturer). The (b) (4) software is already at the most current software version (b) (4) IQ/OQ/PQ was updated to the latest configuration.

The (b) (4)-2020-006-P Computerized System Data Integrity Risk Assessment evaluated the computerized laboratory and manufacturing systems used in the (b) (4) facility and in the manufacture of RVT-802 and identified opportunities for improvement of several systems that process and store (b) (4) data. These include:

- (b) (4) monitoring system was upgraded to (b) (4), a Part 11 compliant software.
- (b) (4) Imaging software installed on upgraded (b) (4) computer
- Updated (b) (4)-SOP-047 to indicate that the printed record is the official record as the current (b) (4) software platform is not Part 11 compliant. The (b) (4) upgrade plan detailed in CAPA Report- 0152 details plans for upgrades of this testing system to a Part 11 compliant version.
- MasterControl system upgraded (b) (4) to ensure continued Part 11 compliance and implementation of current software version

Review of response: This CRL item was reviewed by DMPQ. There are no addition comments from DCGT. Please refer to DMPQ review memo.

Overall Reviewer's Assessment of CRL Item 1, observation #10:

The response is acceptable.

Observation #11 (PQ of Critical Equipment not Completed Prior to Process PQ). Performance qualification (PQ) of numerous critical equipment was not completed prior to conducting process performance qualification runs (November 16, 2018 – January 17, 2019) and aseptic processing runs (August 2018). Specifically,

- a. *PQ of the (b) (4) incubators, (b) (4), used to incubate the thymus tissue slices, was approved in March 2019.*
- b. *PQ of the (b) (4) system, used to maintain (b) (4) level inside the incubators, was approved in March 2019.*
- b. *PQ of (b) (4), used during the processing of RVT-802, was approved in March 2019.*

Applicant's response to CRL item 1, observation #11: In responding to this CRL the Applicant took two approaches:

- Ensured that the repeat process validation being conducted in response to CRL item #6 was performed after equipment qualification was completed on all critical equipment.
- DEV- 0829 was initiated to investigate this issue, identify root cause, and link to any identified CAPAs.

The updated Validation Master Plan (b) (4)-2018-012.1), and a PV Readiness Report (b) (4)-2020-068-P) documented the status of each prerequisite prior to the initiation of PPQ. Individual reports were prepared to assess the qualification status and calibration status of each piece of critical equipment in the RETHYMIC process. For qualification deficiencies identified, appropriate qualification tests were promptly executed. The more extensive qualification confirmed that the (b) (4) system was appropriately qualified prior to the PPQ. CAPA Report-0184 captures identified remediations, including defining expectations and qualification requirements for new equipment, establishing equipment decommissioning procedures, updating the list of critical equipment for RETHYMIC, clarifying procedures for (b) (4) qualification assessments, and better defining the calibration and preventative maintenance program. Preventative maintenance and calibration criteria, specifically the tasks to be performed and frequency of those activities, have been specified in individual equipment SOPs. (b) (4)-EQUIP-003 was updated to outline a general approach to calibration and preventative maintenance.

In review of DEV-0829 they found the equipment qualification performed in February/March 2019 (just after PPQ) was qualified to operate within the same acceptable ranges that were used during the prior PPQ (November 2018 – January 2019). Review of performance data indicated that these pieces of equipment operated within their acceptable ranges during the PPQ. The incubators and (b) (4) were found to be up-to-date

on calibration and maintenance at the time of the prior PPQ. Thus, it was determined that the delayed equipment qualification had no impact on the PPQ or aseptic process validation. COMM-QA-044 “Approaches to Validation” states that perform validations and qualifications are to occur in a specific order, and the SOP was not followed. Contributing factors as to why it was not followed included validation procedure and template gaps, such as the need for clear guidance to review pre-requisites and order of processes when performing the validation or qualification process. SOP COMM-QA-044 and associated template/forms have been updated to ensure that critical equipment PQ is always completed prior to PPQ. For the new process validation study a new risk assessment was first conducted (QA 2019-016-P) on the validation system and quality oversight.

Review of response: This CRL item was reviewed by DMPQ. There are no addition comments from DCGT. Please refer to DMPQ review memo.

Overall Reviewer’s Assessment of CRL Item 1, observation #11:

The response is acceptable.

CRL item #2 (Testing and Sampling Strategy): *The proposed sampling and testing strategy for your histology-based potency assay is not acceptable because it neither fully supports the need for making an informed decision on initiating treatment of the patient with rabbit anti-thymocyte globulin (RATGAM) nor provides an adequate assessment of the product the patient will receive. Please address the following concerns:*

- a. (b) (4) testing: *We agree that the risk RATGAM treatment presents to the intended RETHYMIC patient makes it important to have confidence that the intended product lot is consistent with lot release at the time of interim testing. Your proposed strategy appears to be inconsistent with this goal. Please revise your strategy to take into account the following:*
 - i. *Even if histology results from early sampling, as proposed, meet your acceptance criterion, the product lot could fail for other reasons. Results from donor qualification should also be known but are not available until Day 12. Further, in-process testing for (b) (4) should be implemented.*
 - ii. *If there are delays in the scheduling of the transplant that extend beyond the (b) (4) window, you do not have a plan in place once this window is exceeded, such as discarding the product lot or retesting.*
 - iii. *You indicate that if histology testing spans a (b) (4), then more than (b) (4) may be needed to obtain results. In such a case, if RATGAM treatment involves 3 daily doses followed by 2 days prior to transplant, it does not appear the product lot could be released within (b) (4) days.*

- b. *Testing of the final product: You failed to demonstrate that testing (b) (4) by (b) (4) days is reasonably representative of the drug product (DP). Please implement testing by histology on another slice taken from the drug substance as close as is feasible for product release. Please propose a window of histology sampling for DP release.*

Applicant's response to CRL item 2: To address FDA concerns about the timing of product testing the Applicant has made several significant changes:

- A revised histology testing plan has been developed for both patients pre-conditioned with RATGAM and those who are not
- Addition of in-process testing for (b) (4), in addition to existing histology assay, with results available before RATGAM treatment
- Addition of final product sampling point for histology testing, with results available for release. All lots will be sampled for histology within (b) (4) days of product harvest. Target dates within the (b) (4) day sampling window were identified to lessen the difference to (b) (4) days when possible. If there is a change in the surgical schedule and the initial histology testing that was completed is outside of the testing window before product release, then another tissue slice from the product will be tested.
- The creation of a new SOP to ensure clear communication between the manufacturing team and the clinical team
- Modifications to existing SOPs
- A risk assessment was conducted for endotoxin, and a control plan was created to ensure total endotoxin levels in all combined materials remain (b) (4) of RETHYMIC
- Should a serious product deviation occur after histology testing was completed the deviation will be investigated and the potential impact to product quality assessed, including addition histology testing, if needed

A cross- functional Testing and Sampling Strategy Risk Assessment (b) (4)-2020-046-1.P) was conducted to ensure that the proposed sampling and testing strategy for the RETHYMIC manufacturing process minimizes risk to the recipient patient and that the results of the testing conducted on the manufactured product are representative of the quality of the final drug product that is administered to the recipient. The assessment included analyses for recipients receiving RETHYMIC with or without pre-treatment with RATGAM. Modifications to the histology testing and sampling strategy have been designed to allow for an informed decision on initiating treatment of the patient with RATGAM.

The revised histology testing windows and target times, which incorporate multiple scheduling considerations, have been added to the histology assay SOP (b) (4)-SOP-030). SOP (b) (4)-TRM-001 was created facilitate and document communication between the manufacturing team and the clinical team about product specifications and the clinical decision to administer RATGAM. This SOP includes processes to ensure

RATGAM is not administered prior to obtaining acceptable donor qualification results, (b) (4)
 Day (b) (4) and day (b) (4) sterility results must also be negative to date.

In-process testing for (b) (4) is complicated by tissue (b) (4) during culture. To compensate, a study was performed (b) (4)-2020-008-P) on the process validation lots measuring the total surface area (b) (4) of culture. The majority of the (b) (4) occurred prior to day (b) (4) (primarily between day (b) (4) and day (b) (4)). Based on results of this study the final product dose acceptance criteria (i.e., (b) (4) mm²/m²) was raised to (b) (4) mm²/m² for in-process surface area. If the in-process (b) (4) criteria are not met for a specific recipient, a smaller recipient may be considered for treatment with the lot. If there is not a smaller patient available that could use this lot, then it will not be further processed and RATGAM treatment will not be initiated.

Note: The minimum product dose has been increased from (b) (4) to 5,000 mm²/m² based on an assessment by the clinical review team. An interim dose of (b) (4) mm²/m² may not be sufficient to account for (b) (4) in surface area with culturing. Based on data from PV studies, tissue (b) (4) from Day (b) (4) to Day (b) (4) was on average about (b) (4) and from day (b) (4) to Day (b) (4) about (b) (4). Therefore, an in-process dose of (b) (4) mm²/m² may be more appropriate. Based on manufacturing data since 2001, the change in dose would have not resulted in any additional lots being unable to be released, therefore the impact would expected to be minimal to commercial manufacturing. In response to an information request the Applicant has revised the in-process minimum dose to (b) (4) mm²/m². The change is acceptable.

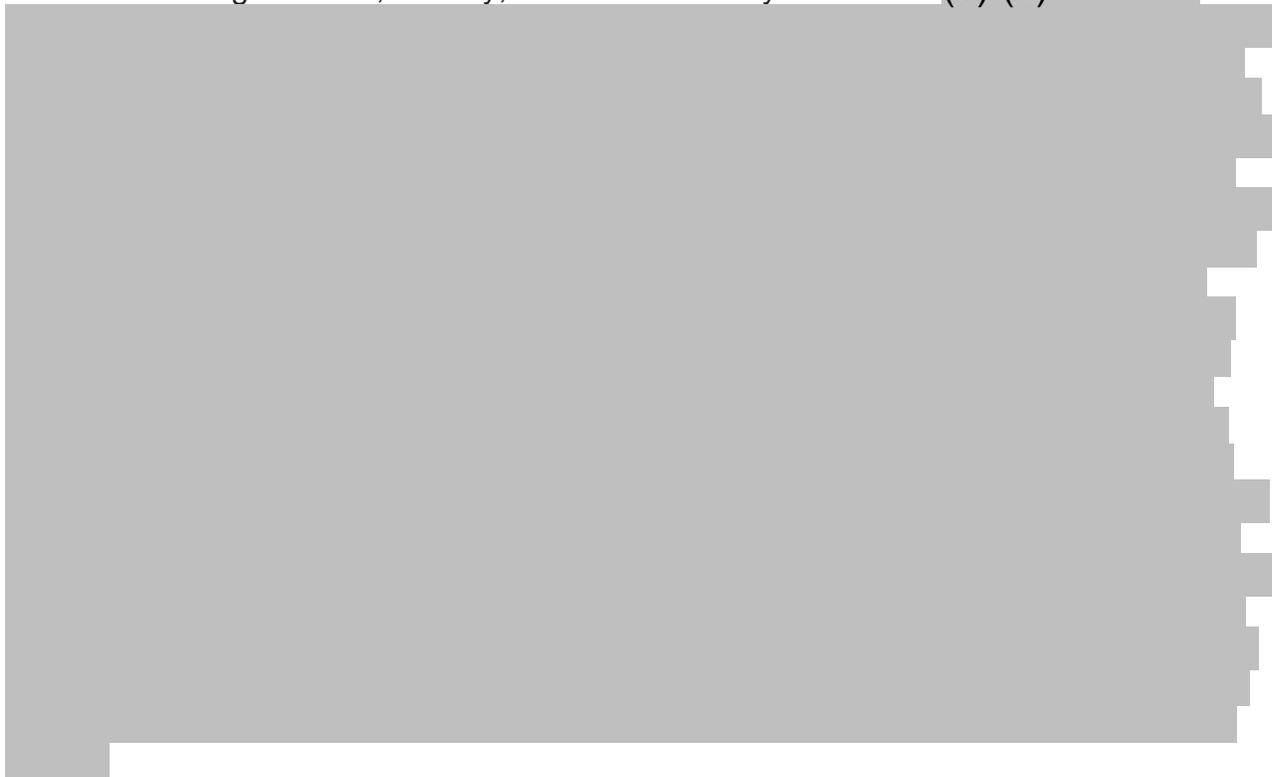
No product lot has failed endotoxin testing, but revised procedures implemented after prelicense inspection intended to increase the sensitivity of the assay could potentially lead to a failure in the future that might have passed previously. To mitigate the risk of a failure from endotoxin, a new (b) (4) endotoxin assessment has been added at day (b) (4). In the event of a failed (b) (4) endotoxin test result, SOP (b) (4)-TRM-001 includes processes to facilitate and document communication with the clinical team and ensure RATGAM is not administered to potential recipients. All incoming lots of each product contact component are tested for endotoxin and must be below the new acceptance criteria to be released for use in manufacture of RETHYMIC.

Review of response: FDA regulations state that the final product must be tested and must meet quality standards set by the manufacture for release. Testing of the final using the histology-based assays is not feasible because the shelf life of the final product is (b) (4) and preparing product samples for histology requires (b) (4)

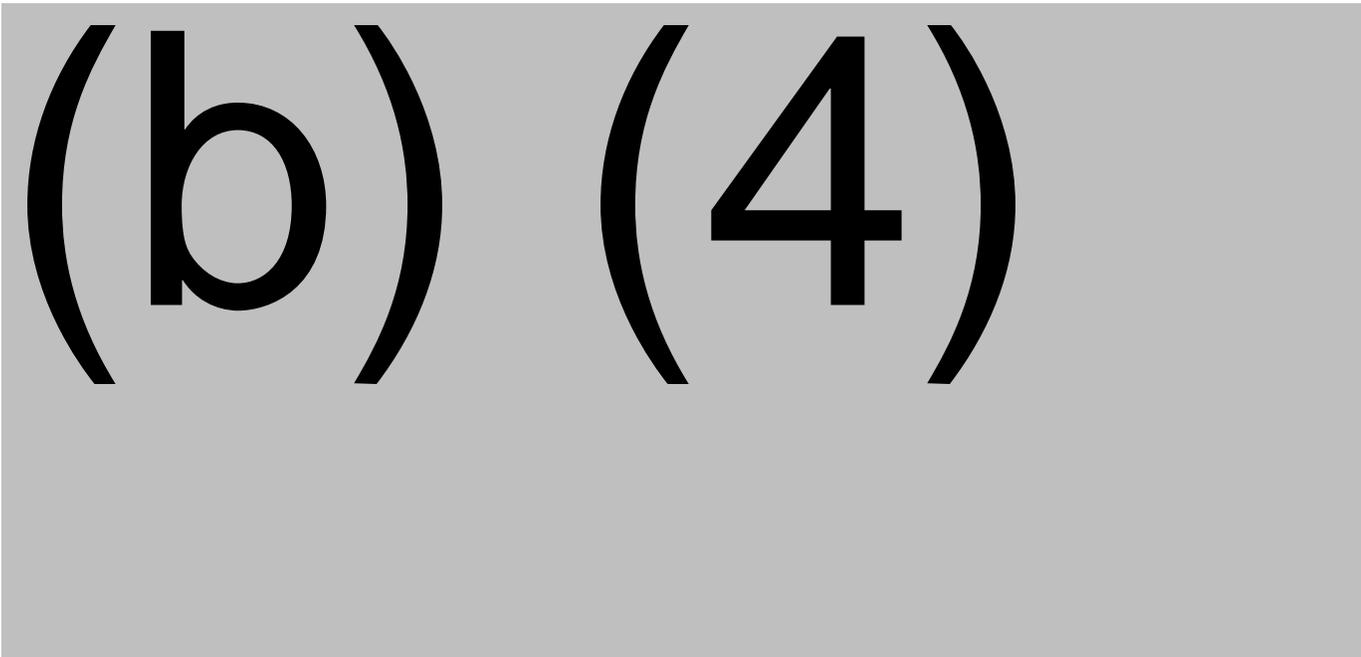
(b) (4) evaluation of prepared sliced by a pathologist for grading and determination of release. Therefore, testing must be conducted on a sample collected at an earlier point in manufacturing.

In the original submission the Applicant proposed to conduct testing on a single slice per lot on a sample collected on (b) (4) between Day (b) (4) and Day (b) (4) of culture. Transplantation of allogeneic thymus tissue or slices of Day (b) (4) product would not lead to the same clinical

outcome because the donated tissue has a very large number of allogeneic thymocytes which would cause problems for the recipient. The primary purpose of the manufacturing process is to greatly reduce allogeneic thymocyte levels, while trying to maintain similar overall tissue organization, viability, and function. They stated that (b) (4)

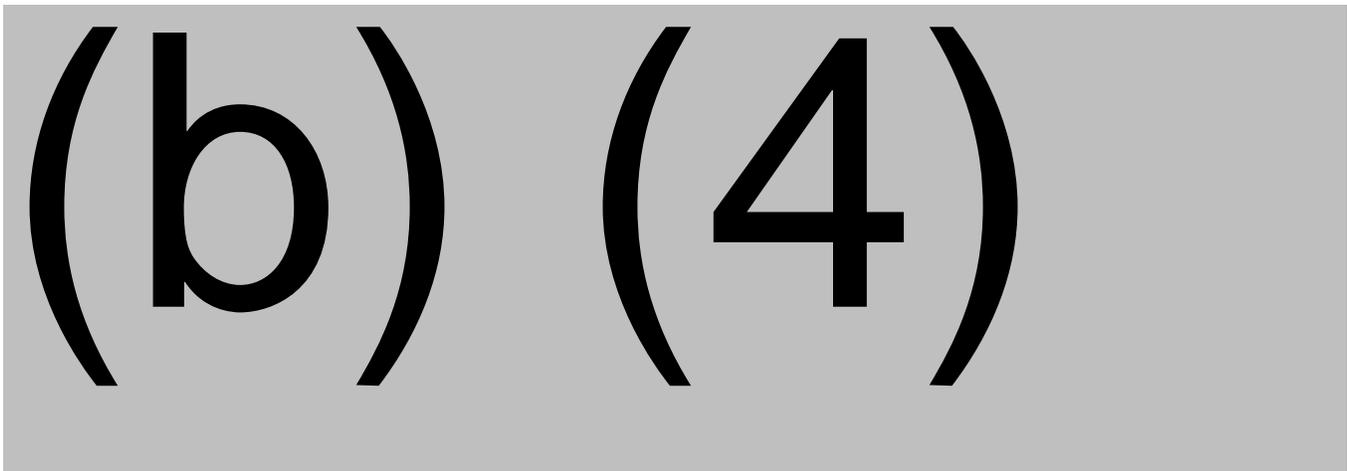


During BLA original submission review the Applicant revised the test window to mitigate FDA concerns. A Day (b) (4) test window was proposed with product still harvested between Day 12-21. The review team was concerned this would still allow for product to be tested as far upstream as Day (b) (4) and released on Day 21. To alleviate this concern an (b) (4) day span was proposed in which the product would have to be tested and released. Please refer to Fig. 2, which we generated to describe the proposal.



This did not address all concerns because a product lot could still be tested on Day ^{(b) (4)} and released on Day ^{(b) (4)} so the testing for final product release would occur halfway through the manufacturing process, and the fact remained that Day ^{(b) (4)} product was not always representative of Day ^{(b) (4)} product. This strategy would also appear to limit product release in cases where testing might have been done (b) (4) to this schedule but the surgery had to be delayed. This also did not appear to fit with information provided on coordinating histology testing and analysis with RATGAM treatment, which requires 3 days doses followed by one to two days off. Histology was said to take ^{(b) (4)} days to have results once a slice is collected for testing. In some cases, histology results might not be known before RATGRAM treatment would need to be initiated in accordance with the time frame for the product harvesting window.

In the table below we have compiled information provided in the submission on product lots used for patient treatment that were produced in the (b) (4) manufacturing facility. A total of ^{(b) (4)} lots were produced and reported where survival data exists out to at least one year.



(b) (4)

(b) (4)

In Mid-cycle and late-cycle discussions during the original submission review, the Applicant expressed the desire for greater flexibility and to have testing information earlier to coordinate patient treatment. Some patients have other serious other medical conditions and timing treatment is not always easy, and they have carefully evaluated the time needed. We appreciated that point, but little information on the actual logistics was not provided in the submission. Understanding the logistics of product sampling, the time until

results are available, and the time the surgery is scheduled is important for review of this issue. It was also not clear why for the majority of lots transplanted since 1993 manufactured in the (b) (4) that had fewer resources, why product lots could typically be tested much closer to release than was being conducted in the (b) (4) facility. On inspection they stated that processing and evaluating the samples for release is on a priority schedule at the (b) (4) services. Results can be achieved in (b) (4). However, in late-cycle meeting discussions additional information was provided and up to (b) (4) days is sometimes needed. The Applicant still felt Day (b) (4) samples were representative of the final product. We were not confident of that assessment because the histology images provided at that time suggested significant additional changes in the product by overall appearance, residual thymocyte levels, and (b) (4). It was difficult to evaluate these differences because most of the histology images provided to date were from product development lots and process validation lots, not actual product that had been used for patient treatment. Therefore, there was little data to reference as what would be acceptable.

Note: For a more in-depth discussion of changes in the tissue by histology and validation of the histology assay, please refer to review of CRL item #3.

During review of the original submission the Applicant also related that patient treatment with immunosuppressive agents has varied, but they now believe treatment with RATGAM is important for the survival of the transplant and nearly all patients now receive RATGAM to condition the recipient to receive the transplant. But treatment can be life threatening in this patient population and so it is critical to receive the transplant once conditioned. Therefore (b) (4) testing is critical in order to have knowledge prior to patient conditioning that the product will be released for transplant. The FDA stance was that if RATGAM treatment will be standard for this population and receiving the final product is critical, then in-process testing should be in place so that there is a high level of confidence the product will be released. Such (b) (4) testing should not be a substitute for testing the final product. Confidence in product quality should exist in the product lot at the time of patient conditioning and in the final product. FDA advice was to keep the Day (b) (4) testing as an added (b) (4) test, but to move final product testing closer to the time of harvest. Since confidence in product quality at the time of conditioning would also need to include adequate dose and (b) (4) results, the Applicant was also asked to determine total tissue slice area (b) (4) and (b) (4) as part of in-process testing. The Applicant has now implemented both (b) (4) histology testing and surface area calculations, as requested. The same histology measures, scoring system, and acceptance criteria are used for (b) (4) and final product testing.

At the Type A meeting the Applicant proposed a revised testing strategy. (b) (4) staff had carefully evaluated the time required to obtain results and have set a range of up to (b) (4) days prior to final harvest as the time window for slice collection for testing. Part of the need for (b) (4) days is to allow additional time for histology results to be available if a (b) (4) was involved. (b) (4) days is consistent with the approach used by the (b) (4) and where the bulk of clinical data supporting this approach exists. It was recommended that, when possible, a shorter period within that (b) (4) days is targeted. For example, if a weekend is not involved and results can be obtained in as little as (b) (4) they target (b) (4) days

instead of the full (b) (4) days. That would be more consistent with regulatory expectations for testing a final product for release. In the resubmission they provided examples of all possible timing scenarios and how the (b) (4) day test window would be used. They also indicated what the target day would be in each case (for examples please refer to Fig 3).



A total 20 possible scheduling scenarios were presented for patient who would be treated with RATGAM and 45 for those who would not. The strategy is summarized in the following diagram (Fig. 4) we generated to overlay the histology testing with all other testing done on the product during manufacturing.

(b) (4)

(b) (4)

In their presentation of histology data, they did not directly compare Day 21 product with product slices taken ^{(b) (4)} days prior (Day ^{(b) (4)} or perform a time course on Day (b) (4) slices to compare day by day differences in slice appearance or marker expression. However, our review of all slice data present in pre-BLA discussions, the original submission, and new data in the resubmission suggests that differences between Day ^{(b) (4)} and ^{(b) (4)} slices would likely be less than between different lots. Though differences in Day 12 versus Day 21 slices are sometimes evident, there is less difference in the later time points the closer you get to Day 21, at least based on the limited examples provided.

Overall Reviewer’s Assessment of CRL Item 2:

The addition of final product histology testing to the existing in-process testing, along with changes in the time window of testing to within (b) (4) days of release addresses concerns about product sampling for final product release being tested too far (b) (4). The use of a target date for testing and release should further reduce the difference between testing and release to just (b) (4) days in most cases. The inclusion of product (b) (4) to in-process testing addresses concerns about adequate assurance of quality before conditioning the patient with RATGAM. More careful evaluation of endotoxin levels in (b) (4) should reduce the chance of a lot failure due to endotoxin, even with the more sensitive endotoxin testing being performed.

The revisions to product testing are adequate to address the concerns raised. The CR item has been resolved.

CRL item #3 (Histology Assay): Please address the following deficiencies related to the histology assay used to assess product safety and quality including identity, potency, and purity:

- a. You provided the Histology Training Guide that serves as a training manual for pathologists performing evaluation of RETHYMIC. However, there are no Standard Operating Procedures (SOPs) for the procedures performed by the pathologists. Written procedures are required for both manufacture and process controls designed to ensure that the DPs have appropriate levels of identity, strength, quality, and purity (21 CFR 211.100). Please provide an SOP for the histological evaluation.
- b. The histology assay performed during method validation implemented (b) (4) criteria. The use of (b) (4) criteria is a more rigorous reflection of the depth of analysis performed by the pathologists than the (b) (4) criteria you proposed for release testing and provides further assurance of product quality. Furthermore, your batch records reported (b) (4) histology results, including reporting a percentage of residual thymocytes. Thus, please revise your histology acceptance criteria to include a (b) (4) measure for assessment of (b) (4).
- c. Data from twenty-nine subjects treated under IND used to support the safety and efficacy of RETHYMIC documented low naïve T cell counts during the first 2 years post-transplant. You have not adequately excluded the possibility that these patients received lower quality lots. You indicated that histology testing met the release criteria for these product lots but did not provide examples of histology images from patients who had positive or negative clinical outcomes. In order to

establish a basis by which (b) (4) histology results can be evaluated, please perform a retrospective (b) (4) histological analysis of product lots used to support clinical safety and efficacy, including new product lots produced in the (b) (4) facility. Please discuss how the retrospective analysis supports the setting of (b) (4) histological criteria. Please also include examples of the evaluated histology images in your BLA resubmission.

Applicant's response to CRL item 3a: The Applicant has developed SOP (b) (4)-THY-018 for evaluating histology for potency and overall product quality. The SOP and supporting information were provided in the submission. This builds on the original "Histology Training Guide" by providing more detailed procedures, a (b) (4) scoring system for all histological features being evaluated, and provides many reference images for all stains and immunocytochemistry used, at low, medium, and high magnification. Examples are provided from multiple lots at multiple time points. Pathologists who conduct the evaluation of RETHYMIC sections are trained on this SOP. The SOP includes over 1000 examples of histology images, most of which were from actual product lot samples used to treat patients and who had successful clinical outcomes. The scoring for these images were included in most cases, and served as a reference. The inclusion of many reference images and fields of view, along with annotations, and justifications for why a score should be assigned in a particular way was in direct response to advice provided at the Type A meeting as to what should be included in the SOP. Acceptance criteria are in place, and the scores are included in a report that is part of the batch record. A copy of SOP (b) (4) THY-018 was included in the submission.

In 2012, (b) (4) facility started work to transition to (b) (4) sections to obtain (b) (4) of the images. (b) (4) sections generally have (b) (4), and this change was intended to provide an improvement to the histology assay, although the lab continued to (b) (4) tissue in (b) (4) for (b) (4) samples. Starting with lot (b) (4) for subject (b) (4) in October 2015, histology samples were prepared as (b) (4) sections by the (b) (4).

Histology measures continue to include assessing for the presence of (b) (4)

A (b) (4) scoring system has been developed for (b) (4)

Scoring ranges

from (b) (4) depending on the analyte. Intra and inter-pathologist assessment was performed as an estimate of precision for assay validation. This new SOP was applied to process validation lots. In-process and final process specifications have been updated.

In addition to the (b) (4) assessment, they also evaluate the histology sections for (b) (4) attributes, as has been previously done since the IND was first submitted. The (b) (4) assay is used for (b) (4) final product testing, and for verification that the source material represents normal, healthy thymus tissue. A “global overall histology assessment” looks for the presence of cortical and medullary areas within cultured thymus slices. Day (b) (4) sections are evaluated for (b) (4)

. Also evaluated is whether (b) (4) is acceptable. The SOP includes these procedures, describes what to look for at the cellular and gross level, and includes low and high magnification images representative of acceptable quality.

Pathologists responsible for performing the histological assay will be trained using the SOP. Also, in response to FDA advice, the Applicant has included training for Quality Assurance representatives who are responsible for making batch release determinations so that they may interpret the pathologist results relative to the requirements per the specifications. Training also ensures familiarity with the scoring process. Quality personnel from the (b) (4) facility responsible for batch release train on (b) (4)-THY-018 prior to releasing any batches.

Review of response: SOP (b) (4)-THY-018 now conforms to a formal SOP format. The SOP covers purpose, materials, step by step procedures, references to related documents, references, and revision history. The latest version is version 6 release March 12, 2021. It is being handled under change control according to their quality system. Procedures cover the “Guide to Pathologic Evaluation of Cultured Thymus Slices”. The evaluation includes:

- Determination if prepared and stained tissue section is appropriate for evaluation (e.g. (b) (4)).

Note: Previously there was no lower limit on the size of the slice area that must be present. As the pathologists have experience regularly examining needle biopsies as part of their hospital work, they had placed no restriction on the minimum slice area needed for histological analysis of RVT-802. It appears they now do have a lower limit. Also, orientation is now more critical because part of the qualitative assessment is to evaluate for the presence of both medullary and cortical tissue regions – both are needed according to the mechanism of action.

- Identification of tissue as thymus
- Expected histological changes
- Assessment (b) (4) portions)

Not covered in this SOP are the tissue sample processing, (b) (4) [REDACTED]. Those procedures are conducted as contract services by (b) (4) [REDACTED] according to their procedures for clinical samples.

The SOP includes 315 figures (over 1000 examples) of histology images, many annotated, to aid in describing how the assessment should be performed. The batch record includes a QC histology report form with tabled results of (b) (4) [REDACTED] scoring.

SOP (b) (4)-THY-018 adequately documents the procedures to be performed and satisfies the requirements of the CRL item.

Applicant's response to CRL item 3b: The Applicant has revised the histology acceptance criteria to include a (b) (4) [REDACTED] measure for assessment of (b) (4) [REDACTED].

Scoring previously assigned in the training guide has also been revised at FDA request. A new table of histology specifications has been established, which are consistent with the scoring system outlined in SOP (b) (4)-THY-018. The scoring system is based on the long history of evaluating histology sections for RVT-802 during development and the knowledge that these lots resulted in positive clinical outcomes for all patients other than those who did not benefit due to pre-existing conditions or other medical reasons. A large number of representative images of tissue samples sectioned and stained for the (b) (4) [REDACTED] were selected to help support the scoring system.

Histology specifications have been updated to incorporate the new (b) (4) [REDACTED] scoring system for some attributes:

(b) (4)

(b) (4)

The overall global histology assessment (Table 5) provides an opportunity for the pathologist to provide additional input regarding lot quality that may not be recognized using the (b) (4) criteria alone. The results reported here support the continued use of the currently established acceptance criteria for the (b) (4) histology assay.

Table 5. Quality overall assessment by histology

Qualitative assessment

Assay	Rating Criteria	Acceptance criteria
Global overall histology assessment	(b) (4)	Met
Global overall histology assessment	(b) (4)	Acceptable

For thymus slices to be considered to have normal appearance they must generally look acceptable based on prior pathologist experience, including (b) (4). Classification of the tissue as normal thymus on Day (b) (4) also requires the absence of other histologic features that would classify it as (b) (4). Such features might include, but are not limited to: (b) (4).

Review of response to CRL item 3b: The Applicant has set (b) (4) limits for release based on a scoring system designed to assess key phenotypic features of thymus tissue. The previous assessments of product (b) (4) by histology was based on a similar evaluation of the same histological markers; however, the threshold is now more clear and the same level of evaluation is performed and recorded on each lot.

Examples of changes by histology were documented in the original submission review. The Applicant also provided example images of the typical appearance of tissue slices at various points in manufacturing. In Fig. 5 below using images from what the Applicant considers “typical” we have compiled the following time course conducted over 21 days in culture.

(b) (4)

(b) (4)

Examples provided by the Applicant shows how the product changes over time in culture relative to D^{(b) (4)}. Some features are still present at D21, but the architecture has changed and the staining pattern. The provided images do supply an example of what the product can look like over time. However, aside from Day^{(b) (4)} histology of normal, healthy thymus tissue prior to culturing, it is difficult to identify a typical appearing tissue slice at the in-process or final product evaluation time point. Though some slices can retain a remarkable degree of the normal hallmarks of thymus tissue even out to 21 days, others can change in appearance and expression considerably within the (b) (4). At (b) (4), the phenotype can differ significantly area to area, and different slices can have different levels of (b) (4) overall morphology. Illustrative examples are supplied as part of the review of the response to CRL items, especially CRL #3 and 6. We have summarized some of the most important changes we have noted during our review in the Table 6 below.

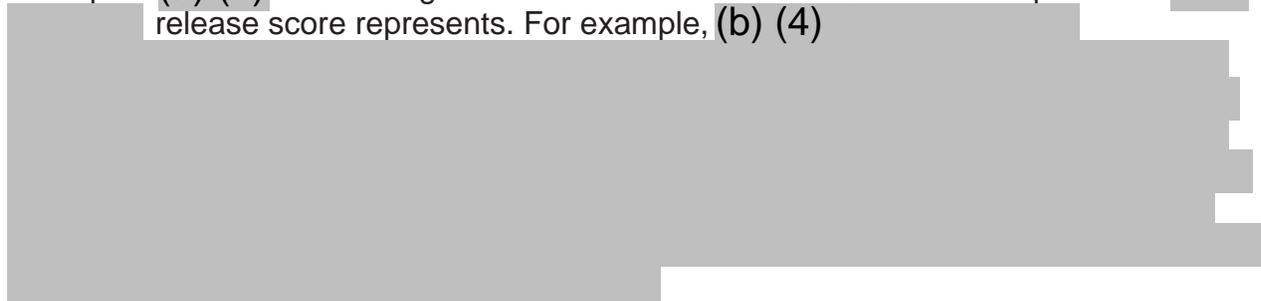
Table 6. Typical changes in RETHYMIC tissue slices during culture

Change	Typical observation	Cause
		Intentional
		Intentional
		Consequence of culturing and loss of thymocytes
		Consequence of culturing and loss of thymocytes
		Consequence of culturing and loss of thymocytes

In the original submission the Applicant indicated they had no scientific or medical justification to set limits or ranges by histology for lot release. Considering the purpose of establishing release criteria is to decide whether a product lot is acceptable for patient treatment, this statement was problematic. The (b) (4) nature of the assay in the original submission also was a cause of concern for reasons of reproducibility and sensitivity. None of the individual high magnification fields of view on a slice that were presented in response to information requests in the original submission were designated by the Applicant as being an example of tissue of unacceptable quality. The Applicant provided data on a subset of lots. The provided examples were absolutely critical for the evaluation of RETHYMIC product lot quality, interpreting process validation data, and assessing QC testing by histology.

Note: These examples may not represent the extremes to which the phenotype of product slices may have changed during culture in lots used to treat patients under IND. However, these were the examples provided and therefore we are treating the phenotype of these examples as the acceptable range to define what a score of (b) (4) represents. Should future regulatory submissions provide histology data on new product lots exceeding what was presented in this BLA resubmission additional justification would have to be provided.

The slices clearly change over time in culture. Sometimes they appear to change a lot, sometimes very little. It is difficult to say whether those changes have a big impact on product function in the patient, or if they might have no impact. In our review we focused on how much the slices changed in culture for those lots that that were transplanted and the patients had a positive clinical outcome. Histology data and other measures from these lots help define what a quality lot is, and how much a slice might be able to deviate from the appearance of freshly cut tissue at Day^{(b) (4)} and still represent quality. We did not attempt to (b) (4) these images. We tried instead to understand what a particular (b) (4) release score represents. For example, (b) (4)



(b) (4)

4 pages determined to be not releasable: (b)(4)

(b) (4)

The revised testing procedure including the (b) (4) measures was implemented for release testing in 2020.

Note: Only (b) (4) lots were produced in 2020, therefore lots that met the full, current proposed commercial product release criteria represent only (b) (4) lots used to support safety and efficacy.

We do not believe there is a substantial difference in the pathologist review of histological features prior to adoption of the (b) (4) criteria and after. However, the more in-depth analysis that was always being performed was not reflected in the previous Histology Training Guide, and typically not reflected in the batch record. Upon interviewing the main pathologist during pre-license inspection performing the histology review, it was obvious that a more careful and (b) (4) evaluation was being conducted, but was not being captured by the written procedures or QC reports. The fact that in some cases (b) (4) results were reported in the batch record was further evidence that more than a purely qualitative approach was being used and could be captured. The development of the histology SOP, (b) (4) scoring system, and batch record form would lead to a more uniform approach to evaluating and documenting histology testing.

Variabilities observed in reviewing the histological data include:

- The size of the slices and the section of the slice vary by product lot. If this were a (b) (4) assay the (b) (4) value derived from sampling a smaller slice versus a larger slice might differ. Since this is a (b) (4) assessment based on whether a feature is present or not, that is less relevant. At their discretion, a pathologist can request a section taken from a deeper part of a tissue block if necessary.

(b) (4)

- A “slice” is defined by the minimum percent area covered by a filter, and multiple tissue pieces can make up one slice.
- The Applicant states that any one slice is representative of the whole product lot, but that is not clear. No histology data was provided on different slices from the same lot at the same time point.
- Not all areas of a slice are either cortical or medullary tissue, and the percent that is not depends on the individual slice/section and on the time in culture.
- It is not always easy to distinguish cortical from medullary regions, especially at high magnification and with slices cultured for longer periods.
- Expression of (b) (4) is associated with medullary thymic regions, as are (b) (4) but not every section has the same percent medullary area.
- It is not always easy to distinguish individual cells, even at high magnification. However, in the case of evaluating (b) (4) this is not usually an issue.
- The intensity of staining can vary between different histology series, as is typical for immunohistochemical assays (for an example see review of process validation).
- Even with freshly isolated tissue stained at D^{(b) (4)} differences exist in the (b) (4). Therefore, no one field of view may necessarily be representative of the whole. In general, (b) (4) expression is much more (b) (4) than in cultured slices (for examples of variability among cultured slices please refer to Fig. 9).

Cell and therapy products often have wide lot-to-lot variation, and the acceptance criteria for product release, therefore, have correspondingly wide ranges for manufacturing to be feasible. Clinical outcome data can be highly useful in helping to justify the proposed commercial range for a release criterion. In the case of potency by histology this is harder to define because histology data was only provided on (b) (4) patients (b) (4) and the assay is much less (b) (4). The phenotypic appearance and marker expression of the slices provided at low and high magnification provide valuable examples of actual patient lots. Since all (b) (4) the patients had a positive clinical outcome, these lots would represent a range of acceptable product quality, especially the (b) (4) product lots where the rate of naïve T cell development was proceeded according to typical rates seen across the different clinical studies (see review of CRL items #3c below).

The acceptance criteria for the (b) (4) histology assay were tested as part of the March 2019 method validation. The (b) (4) criteria were refined in 2020 and the method validation report was amended, though no revalidation was performed. We reviewed the revisions to the acceptance criteria and agree no revalidation would be necessary. The scoring criteria were tightened for viability by (b) (4) -stained samples. A more detailed description of tissue morphology for normal thymus tissue and additional reference images were added.

Evaluation of precision for assay validation included repeat evaluation of the same slides by the same pathologist on different days, and between (b) (4) different pathologists. Two protocol deviations occurred during assay validation where there was a discrepancy between the (b) (4) pathologist's assigned score, otherwise all scoring on all samples and all days was the same.

With the change in the histology assay and changes in the timing of product testing (See CRL item #2), as well as changes to the minimum dose (determined by the clinical review team), the specifications have been revised. The revised specification table covering source material testing, in-process and final product testing are shown in Table 7 below. Revisions to the specifications proposed in the resubmission are in blue text.

Table 7. Revised specification table



1 page determined to be not releasable: (b)(4)

(b) (4)

Note: The acceptance criterion for histology for D^{(b) (4)} tissue is a comparison to normal thymus tissue, for in-process and final product testing it is compared to cultured thymus.

A footnote in the specification table states that the product is released “at risk” because results of final product mycoplasma and sterility are not known at the time of product release for transplantation. It would be more accurate to state that release is based on the sum of all testing to date, including upstream sterility and mycoplasma testing. It is common practice for non-cryopreserved cell therapy products to be released under these conditions.

Not shown in the table is an additional visual inspection that occurs by (b) (4) personnel in the operating room after product transport.

Applicant’s response to CRL item 3c: The Applicant referred to BLA original submission Amendment 54 where they summarized underlying clinical reasons that may have contributed to lower naïve T cell counts during the first 2 years post-implant for the 29 patients that had low naïve T cell counts during the first 2 years post-implant. The slower T-cell responders have demonstrated durable and robust overall survival: 26 of the 29 recipients (89.6%) were alive at the time of the analysis. Long-term survival curves were similar regardless of naïve T cell status (i.e., for both “responders” and “non- responders,” defined as subjects with naïve T cells <100 cells/mm³) at Year 1 and Year 2 post-implantation. The delayed development of naïve T cells could frequently be attributed to their underlying medical issues or concurrent treatments. Additional retrospective analysis does not suggest that RETHYMIC treatment would have been able to prevent the 3 deaths observed in this slow responder group.

A retrospective analysis of product lots from patients used to support clinical safety and efficacy has been completed. The analysis included the new (b) (4) histologic

scoring system. This study was conducted to establish the basis by which (b) (4) histology results can be evaluated. The study compared product lots received by subjects who had a positive outcome (e.g., >100 naïve T cells/mm³ at year one post-implant) with those who had potentially reduced or delayed naïve T cell development (i.e., <100 naïve T cells/mm³ at year one).

Note: The athymic population in this clinical study was defined as individuals having < 50 naïve T cells/mm³. The normal range is from 396 to 3111 cells/mm³ at birth for newborns without immune disorder.

The analysis included an evaluation of the data for potential differences in the established product quality characteristics with the clinical outcomes. As described in “Report of Retrospective Analysis of Clinical Samples by (b) (4) Histology”, no differences in product quality were observed between lots administered to subjects who had a positive outcome with those who had potentially reduced or delayed naïve T cell development. All lots examined on day (b) (4) scored identically, and all lots examined after at least (b) (4) days of culture scored identically. The scoring rubric distinguished between day (b) (4) and cultured thymus, with differences scored in viability of (b) (4).

In addition to meeting the (b) (4) acceptance criteria, the overall appearance of the tissue at each time point examined was judged to be acceptable from a histologic standpoint. The images illustrating the histologic features of the lots evaluated in the retrospective study were included in the submission. Images from (b) (4) of the lots were annotated for clarity in how the slices were assessed.

Overall, the study data did not show differences in the product quality characteristics that may be associated with the clinical outcomes (i.e., rate of development of naïve T cells or 1-year survival). Specifically, the retrospective histologic scoring study detected no differences between the histologic features of thymus lots whose recipients had >100 naïve CD4 T cells/mm³ at 1 year post-implantation compared with lots whose recipients had delayed reconstitution. The results of the retrospective analyses support that the current (b) (4) histologic criteria, combined with the global overall histology assessment, are capable of identifying thymus lots of acceptable quality and support the continued use of the established acceptance criteria for the (b) (4) histology assay.

Review of response to CRL item 3c: In performing the retrospective analysis the Applicant was limited to a small set of product lots. Of the (b) (4) lots used in the clinical and efficacy data set (b) (4) lots were chosen for analysis- (b) (4) lots from patients with delayed naïve T cell recovery and 6 patients whose recovery was on schedule. Most of the historical lots produced in the (b) (4) were based on (b) (4). While the basic principle of the assay is the same and the same histological features are evaluated, the Applicant does not feel that the (b) (4) scale they have recently developed is directly applicable to (b) (4). In addition, there can be variability between different laboratories conducting the same immunostains, and the Applicant wanted to restrict the analysis to sections cut and stained by the same laboratory (b) (4).

Note: This is a reasonable justification, but also highlights the difficulty in evaluating the full manufacturing (b) (4) product lot history. The commercial process proposed builds on the manufacturing, testing, and specifications put in place beginning in 2016, after product manufacturing was transferred to the (b) (4) facility. The process and testing were further modified in preparation for submission of the BLA and in response to CRL items. Though the data on these (b) (4) lots is highly valuable, only (b) (4) of the (b) (4) lots where delay was observed clinically is presented. Further, only (b) (4) of the (b) (4) clinical lots were included where no delay was seen. Any conclusion made would have to be based on the small subset included.

Of the patients who had delayed naïve T cell development (b) (4) product lots were produced in the (b) (4) facility and (b) (4) in the (b) (4) facility. For those with normal development (b) (4) lots were produced in the (b) (4) facility and (b) (4) in the (b) (4) facility. Therefore, there are (b) (4) variables involved: 1) the rate of naïve T cell development in the patients, and (b) (4) the facility they were produced in. This is in addition to the typical lot-to-lot and slice-to-slice variability of the product.

Note: Of the (b) (4) product lots that have been manufactured since 2016 in the (b) (4) facility, >2 year clinical outcome data does not exist on all of these patients. Of the (b) (4) lots, only (b) (4) were produced in 2017 or earlier, (b) (4) of which were included.

The Applicant provided a table of the (b) (4) subjects, the product lots, and the assessment result for each. We have amended that table with our own impressions of the images supplied on each lot. In Table 8 we have color coded what we perceived as the degree of similarity with Day (b) (4) slice properties. We did not attempt to score them according to the rank scale developed by the Applicant, as our goal was not to question the score assigned by the Applicant. Instead, we compared the same histological features and looked at how similar the intermediate and final time points were to Day (b) (4) slices, and then compared the overall rankings between the two groups. Our results are presented as gray shading for each time point with the darkness of the shading corresponding to degree of difference from Day (b) (4) with the darkest shade being the most different.

(b) (4)

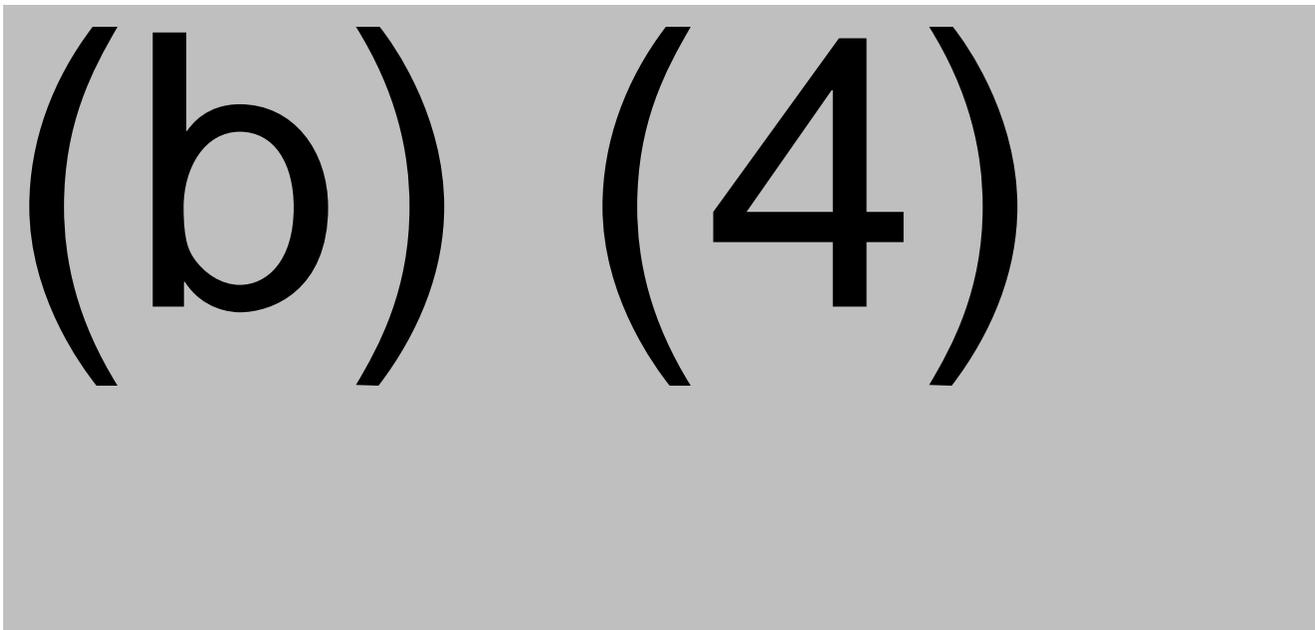
(b) (4)

Several observations were made in our assessment: 1) all slices changed over time in culture, but to varying degrees; 2) the degree change is not simply a function of length of time in culture, though typically the greatest alteration from the Day^{(b) (4)} phenotype is seen in the final time point; 3) in any one slice there can be regions that have a high degree of similarity with Day^{(b) (4)} histological features, but also significant differences; 4) although any one slice from a product lot is considered to be representative for assessment of quality and slices are selected at random by manufacturing personnel for submission for testing, we noted at least two cases where the final product slices resembled more closely Day^{(b) (4)} slices by at least one attribute, compared to the intermediate time point - it seems unlikely that product attributes would reverse during extended time in culture.

Note: For the purpose of making a determination of product quality for lot release, we agree that selection of product slice is not critical because all lots and all time points met the release criteria, and images provided on other lots support that a determination can be made using the (b) (4) rating scale on a wide range of slice sizes and time points. However, in cases where one is trying to make a side-by-side comparison, relying on a single slice may not provide the most accurate representation. Future comparability studies would be best conducted using several slices for each analysis.

At high magnification all slices at all time points had areas of TEC that looked healthy, with clear immunoreactivity to (b) (4). The (b) (4) network that is indicative of the normal distribution of TEC was present in all slices to varying degrees. At

high magnification some slices resembled Day (b) (4) network staining to a significant degree, while other areas of the same or different slices did not. We compiled some examples of high magnification views in the figure below.



(b) (4)

Although the slices in general appear to continue to change over time in culture, in one case Day (b) (4) and Day (b) (4) slices had the same general appearance, which appeared altered compared to Day (b) (4). So it appeared that whatever shift in phenotype that occurred, did so early on in culture.

We could find no clear difference between these (b) (4) delayed naïve T cell lots and the (b) (4) product lots that resulted in the normal time course of naïve T cell development. We also did not observe any significant difference among the (b) (4) delayed naïve T cells lots as a whole in terms of number of slices transplanted, dose, or days in culture. We therefore agree that based on the level of information provided there is no indication these lots represented lower product quality.

Overall Reviewer's Assessment of CRL Item 3:

As requested, the Applicant has established an SOP for the histology measures used for assessment of Day (b) (4) in-process, and final product release, and the SOP is adequate.

Also as requested, they have developed and implemented a (b) (4) scale to better reflect the degree of analysis that has been part of the analysis, but was not formally captured in their procedures, or consistently reflected in the batch record. The multi-point scales for (b) (4), presence of (b) (4), presence and pattern of (b) (4) immunoreactivity, along with (b) (4) assessment appears adequate. It has been successfully used in several recent studies, including process validation. It has been assessed by the Applicant for intermediate precision with a high degree of reproducibility. The threshold for lot release is a low by several criteria (e.g. viability, (b) (4), normal phenotype), but consistent with what has been used for the clinical lots being used to support safety and efficacy of RVT-802. There are still elements of subjectivity for this kind of assay despite the efforts made to better define the procedures, the inclusion of a large number of reference images, and the establishment of the (b) (4) scales. The sensitivity of the histology method as a whole for assurance product quality is unclear, as it was not directly examined except by tissue slices exposed to extreme (b) (4) conditions. Even under these situations, the (b) (4) samples scored better than might be expected. Given the 28 year history of using histology for release and the consistently high efficacy profile in this patient population, and that the manufacturing method is largely the same process, we find the proposed histology method suitable for the intended purpose and the release criteria adequate. However, should there be a significant manufacturing change in the future, it may be difficult to establish comparability using histology. Therefore, we recommend that a (b) (4) assay be developed as a PMC (see PMC section at the end of the review). The Applicant has agreed to develop a (b) (4) assay as a PMC (please refer to BLA amendment 125685.79).

Based on our review of the Applicant's assessment of product lots by histology, and our own assessment of representative images provided in the submission for each, we could find no clear difference between these (b) (4) delayed naïve T cell lots and the (b) (4) product lots that resulted in the normal time course of naïve T cell development. We therefore agree that based on the level of information provided, there is no indication product lots used in (b) (4) patients who had delayed naïve T cell development were of low product quality. Full determination was limited by the small histology sample size (n=(b) (4)). **Our concerns have been addressed and the CRL item has been resolved.**

CRL item #4 (Thymus Source Material Hold Time): Support for a thymus source material hold time of (b) (4) is insufficient:

- a. Manufacturing instructions for clinical lots produced under IND in the (b) (4) state the tissue was to be immediately processed or stored (b) (4) at (b) (4). Thus, the data supporting clinical safety and efficacy appear to be based on source material handled differently than what you propose in the BLA. You did not provide any additional clinical data based on source material held for (b) (4)
- b. Your process validation (PV) study intended to support the (b) (4) hold time was based on (b) (4) histology results from (b) (4) tissue slice from (b) (4) lot (b) (4) held at room temperature in a (b) (4) sterile specimen cup. The study is insufficient to determine whether the (b) (4) staining profile for overall quality on the Day 21 slice represents adequate product quality, and whether the assay is sufficiently sensitive to support stability of the tissue under these storage conditions. No (b) (4) stability-indicating assay was included in your analysis.
- c. You propose a maximum hold time of (b) (4) in the operating room (OR) from notification of thymus harvest up to time of pick-up; however, PV lot (b) (4) was only exposed to a hold time of (b) (4). Other (b) (4) lots have been held for as long as (b) (4), but those were not exposed to the same conditions as lot (b) (4). You propose a total hold time of (b) (4) from the time of notification of tissue availability, but this does not factor in the full (b) (4) of OR hold time.

To support your proposed full hold time, you will need to either 1) provide historical clinical data from patients treated with source material held for (b) (4) that covers all intended maximum step times, 2) conduct a stability study using a (b) (4) stability-indicating assay on multiple lots tested using multiple slices, or 3) establish a shorter expiry based on historical clinical data of safety and efficacy.

Applicant's response to CRL item: The maximum allowable room temperature hold time covers the period from when the manufacturing facility receives the notification fresh thymus tissue is available to the time when either tissue slicing begins or when TOM is added prior to placing the tissue in the (b) (4) for an (b) (4) hold. In accordance with the 2020 (b) (4)-2020-009.1-P Risk Assessment report, source thymus hold time is now considered a critical process parameter. The room temperature hold time has been (b) (4) for incoming thymus source material from (b) (4). A hold time of (b) (4) is supported by historical manufacturing experience associated with clinical safety and efficacy data and by the most recent process validation data where source material was held for (b) (4) at room temperature. No reduction was observed in product quality for the process validation study.

The historical manufacturing data is based on product lots where at least one year clinical safety and efficacy data was demonstrated. A total of (b) (4) lots had corresponding 1-year survival data. However, only (b) (4) of these lots were actually held using the using the (b) (4) as are currently being used and as intended for the commercial process. These conditions involve either (b) (4)

(b) (4). For this reason the hold time data is based on (b) (4) lots, not (b) (4). For these (b) (4) batches:

- (b) (4) lots were manufactured in (b) (4) where hold times ranged from (b) (4).
- (b) (4) were manufactured at (b) (4) where hold times ranged from (b) (4)
- The average hold time for the (b) (4) batches was (b) (4)
- (b) (4) batches had hold times (b) (4)
- (b) (4) batches showed hold times longer than (b) (4), supporting an acceptable range of (b) (4)

Of the (b) (4) clinical lots that constitute the clinical safety and efficacy data set, (b) (4) recent clinical lots do not yet have 1-year survival data, but the hold times at room temperature before start of slicing ranged from (b) (4)

Note: The Applicant indicated these values are slightly different than what was presented in the Type A meeting request due to an earlier error in the interpretation of the batch record data for the processing start time, but the data continue to support an acceptable range of (b) (4)

The (b) (4) batch records have been updated to clearly state that the source thymus hold time (from notification until start of slicing or media addition for (b) (4) hold) cannot be greater than (b) (4) -SOP-029).

Note: The Applicant often refers to (b) (4) storage at (b) (4) temperatures. The actual allowable range according to SOP is (b) (4), and nearly the full range has been used for some lots manufactured since manufacturing was moved to (b) (4).

The new process validation study conducted in 2020 was designed to include the proposed maximum hold time. For process validation (b) (4) source thymus tissues collected on (b) (4)

(b) (4) after notification of availability. The specific hold times for the (b) (4) lots that were (b) (4)

Review of response to CRL item 4: The approach of using existing hold time data from product lots used to treat patients that ultimately had a good clinical outcome is a reasonable approach, and one of three options suggested to the Applicant.

The 2020 process validation was not designed to test worst case. Partially this is due to the fact that the design originally proposed to respond to CRL items included the PV lots

could be used to treat patients, and it would be inadvisable to purposefully manufacture lots under worst case and then give to patients before FDA had agreed the data supported adequate product quality. However, the PV study design did include (b) (4) time prior to processing.

The Applicant supplied a table summarizing the lots used to support the proposed (b) (4) source material holding time based on lots where at least 1 year positive clinical outcome data exists (see Table 8).

Note: 2 year survival data is more definitive for this clinical indication. Most patients die within the first year and nearly all by 2 years. Clinical outcome partially depends on when a patient is challenged with a serious infection, and that doesn't always occur in the first year. How old a patient was at the time of treatment is also a factor because those already nearly 2 years old at the time of treatment might statistically have less time to live post-transplant than very young recipients. These are more recently manufactured lots and 2 year clinical outcome data exist for even fewer lots.

(b) (4)

1 page determined to be not releasable: (b)(4)

(b) (4)

A review of the (b) (4) histology files provided shows that the change in histological features over time is within the range provided across all lots where histology images were provided. The sensitivity of the histology assay is unclear, however, and we would recommend should a (b) (4) assay be established that the hold times be re-evaluated at that time (see PMC section at end of document).

Overall Reviewer’s Assessment of CRL Item 4:

The data on product lots held either for (b) (4) at room temperature, (b) (4) at (b) (4) temperatures, or both, are supported by > 1 year survival data on patients treated with these lots. Some of these patients had slower recovery of naïve T cells compared to the whole (b) (4) patient efficacy data set, but this is believed to be due to clinical reasons and not product quality. It is further supported by process validation data. A review of histology images on sections from lots held (b) (4) showed no greater change in histological features compared to data on other lots provided. The room temperature hold and (b) (4) hold conditions were established before moving into the (b) (4) facility, though the (b) (4) hold option is currently used more often. The (b) (4) and (b) (4) hold times are acceptable. **The results addressed our concerns and the CRL item has been resolved.**

CRL item #5 (Drug product Expiry): You propose an expiry of (b) (4) for the Drug Product (total time outside incubator until end of surgery) based on one clinical lot manufactured in 2018 that experienced a hold time of (b) (4). However, these data do not support the proposed hold time because that lot was formulated and transported in the (b) (4) container, not the (b) (4) final product container, and therefore does not represent the commercial process. Clinical data provided on product lots packaged in the (b) (4) tissue culture dish that were manufactured and transported from the (b) (4) facility were held for less than (b) (4) between product formulation and administration. Please establish an expiry based on relevant clinical data using the proposed formulation and (b) (4) final product container or provide additional stability data using a (b) (4) stability-indicating assay.

Applicant’s response to CRL item: A new risk assessment was performed in 2020 and product expiry was categorized as a critical process parameter. Based on the risk assessment, manufacturing experience, and clinical experience, an expiry of (b) (4) is proposed. The Applicant refer to data provided in the Type A meeting briefing document (Amendment 125685.57).

The new drug product expiry of (b) (4) is based on historical manufacturing data associated with product lots packaged in the (b) (4) tissue culture dish from both the (b) (4). Data on (b) (4) lots and (b) (4) lots were included in the analysis.

During the process validation, a tissue slice was sampled for histology testing on the day of release (prior to transport to the OR) as part of the standard product release process. The remaining slice were then (b) (4)

Manufacturing SOP (b) (4)-THY-009 has been revised to include the (b) (4) expiry. The package insert has been revised to state "Use RETHYMIC prior to the time and date of expiration printed on the package"

Review of response: In the original submission the proposed shelf life was (b) (4). Final product hold times prior to surgical transplantation was not recorded for lots manufactured in the (b) (4). For (b) (4) lots the hold time on (b) (4) lots was (b) (4). Process validation data was intended to support (b) (4), but it was concluded that (b) (4) was not justified because: 1) the PV lots were not used to treat patients and there was no available clinical data beyond a (b) (4) hold time; 2) the PV study involved a complicated design among the (b) (4) PPQ lots, with different lots exposed to different conditions, and a very different final container, formulation, and storage temperature.

Data submitted in amendment 125685.57 indicated that the average time out of the incubator, including surgical time was (b) (4) with a maximum time of (b) (4) (based on (b) (4) lots). Data provided on (b) (4) lots (b) (4) lots had an average time out of incubator of (b) (4), with the maximum time of (b) (4). The minimum was only (b) (4), which is impressive considering the product has to be packaged and transported to the clinical site and surgery has completed.

Note: The Applicant clarified in this submission that the product is formulated in the final primary container (b) (4) culture dish) (b) (4)

Technically, this (b) (4) hold could be considered part of the final product holding conditions as final product sampling has already occurred and the product is now "formulated". However, since the same the final product is formulated in the same culture medium, (b) (4), and the only difference is that the culture medium is (b) (4) 5 mls. This is similar enough conditions that the (b) (4) hold time could be considered as part of final product preparation and not part of the shelf life.

(b) (4) of the (b) (4) had hold times of (b) (4), all of which had positive clinical outcomes. The only product lot with a hold time of (b) (4) or greater was (b) (4). This patient died from unrelated causes. The product lot was not associated with delayed naïve T cell recovery.

A (b) (4) study where the culture plates were (b) (4) did not show a huge difference in culture slice histology.

The 2020 repeat PV study did include a hold time of (b) (4) for all (b) (4) lots that included transport to and from the clinical site (double the normal transport time). The histology data is within the range of histological phenotypic characteristics as other lots where positive clinical outcome data exists. However, the sensitivity of the histology assay to detect changes in quality over time is questionable. Another limitation to this study is only one slice was used for analysis after transport and hold.

Given the limited clinical data on product held for the full (b) (4), a shorter expiry might be justified. However, it is important to consider that the shelf life is taking into account sufficient time for surgery. Although the surgical procedure is not complex, some of these patients have other congenital defects, such as cleft palate and heart problems. According to a publication by Dr. Markert, management of anesthesia for these patients can be difficult. Therefore, it would be best for patient care for expiry to include an adequate amount of time to perform surgery without being rushed. In the case of lot (b) (4) the surgical time took up nearly (b) (4) of the total time post removal from the incubator. Also relevant is the fact that during much of the 28 year history of patient treatment with this product the elapsed time post-harvest was not controlled. It is highly likely that some lots were held for as much as (b) (4).

Overall Reviewer's Assessment of CRL Item 5:

The clinical data is limited by the fact that hold time data post-harvest is only available on about a third of the safety and efficacy lots. Of the lots that were included in the analysis, some lots spent far less time post-harvest than (b) (4), and the average was only held at the room temperature shelf life conditions for about (b) (4) of the proposed expiry. Only a single lot was actually held for the full (b) (4) and that was for a patient who died of other causes. Based purely on this information, a shorter expiry might be justified. However, it is important to consider that the shelf life is taking into account sufficient time for surgery. The new 2020 process validation study supports the (b) (4) hold time, though is limited by the sensitivity of the histological assay. The totality of the data, along with considerations for safe product transplantation time support a (b) (4) expiration as acceptable. **The results addressed our concerns and the CRL item has been resolved.**

CRL item #6 (Process Validation Studies): *The PV study does not adequately demonstrate manufacturing and product consistency for all elements. A successful PV*

study should demonstrate that each unit operation is performing as intended, and manufacturing is consistent lot-to-lot. However, this was not fully demonstrated in Process Validation CT2-2017-013-P. Please perform an additional study to address the following concerns:

a. Unlike (b) (4) staining performed for the purposes of identity, which had successfully demonstrated substantial reduction in donor thymocyte levels by Day (b) (4) and the presence of key hallmarks of thymus tissue at all stages, the same methods applied to potency and overall tissue quality are not conclusive for the following reasons:

- i. Results of this study and other data provided in the submission show wide variation in the phenotype of tissue slices and the expression pattern of (b) (4) within different regions of the same slice, different slices, different lots, and different culture times.
- ii. Though all lots and time points met release criteria, the criteria are broad, raising concerns about the sensitivity of the assay.
- iii. The impact on tissue slice quality is difficult to assess because data on the tissue received by subjects treated under IND was not provided (no retrospective comparison was made of product lots received by subjects who had either a positive outcome, negative outcome, or a reduced/delayed naïve T cell development).
- iv. (b) (4) staining profile in the PV lots appears to change to a greater degree over the course of 21 days compared to other time course examples provided to date. It is unclear if this is related to differences between lots or to longer step and holding times included in the PV study intended to represent worstcase.

b. The design of the study is complicated by the range of variables included in the study. No (b) (4) lots were treated the (b) (4), and no (b) (4) was exposed to the (b) (4). While we appreciate your efforts to cover the range of conditions the lots would be exposed to for commercial manufacturing, PV is typically performed after critical process parameters for all manufacturing steps have been established. In general, for a PV study, a minimum of three lots should be manufactured under the same conditions. The information provided in this study is typically reported under Sections 3.2.S.2.6 (Manufacturing Process Development) or 3.2.S.2.4 (Control of Drug Substance). You also considered a process step to be validated based on the outcome from a single lot. Further, the study was designed to use only (b) (4) of culture medium per lot, yet most clinical lots used multiple lots, and you report that up to (b) (4) lots of culture medium were used for some clinical lots. Testing of your manufacturing process should represent conditions typically used.

c. Unit operations:

- i. The extension of the culture medium exchange time to (b) (4) is not adequately supported, since (b) (4) intervals were not tested on (b) (4) medium

exchanges for cultures beyond Day (b) (4). The study is also limited by the fact that only (b) (4) product lot was exposed to these conditions, and there was no comparison made to elucidate the effects of these conditions on thick versus thin or small versus large slices.

- ii. At the initiation of culture, the (b) (4) filters must be covered with (b) (4) of tissue, but no (b) (4) method was used to verify filter coverage.
- iii. The clinical data set indicates that about (b) (4) of slices produced for clinical lots with the tissue slicer are thick and (b) (4) are thin, though the proportion varies by product lot. Slice surface area varies greatly within a lot and between lots. No evaluation of the consistency of slice thickness or size was included in the study. Since it is unclear whether slice thickness or size has a meaningful impact on clinical outcome, the commercial process should be better controlled to maintain consistency in the properties of clinical lots.
- iv. No calculation of yield was performed, and no comparison was made with clinical lot production under IND.

Applicant's response to CRL item: A new process validation (PV) study was conducted to demonstrate manufacturing and product consistency, that all process steps are performed as intended, and that manufacturing is consistent lot-to-lot. The PV study design incorporated the Agency comments provided during the Type A Meeting.

A new risk assessment was conducted to re-evaluate all critical process parameters (b) (4) 2020- 009.1-P), including identification of critical process parameters and justification of acceptance criteria.

The study included the recently implemented (b) (4) histology rating scale and the individual scores were provided for all elements at all time points across all product lots.

Product lots were handled under worst case conditions for hold times (b) (4) maximum hold at room temperature and up to (b) (4) hold at (b) (4) prior to processing, and a (b) (4) maximum hold time at (b) (4) for the shelf life).

(b) (4) pathologists evaluated the histology sections from samples collected at specified time points through the Day 12 or Day 21 commercial process. In the case of the Day 21 process, additional samples were collected (Day (b) (4) beyond the normal culture window to help support manufacturing out to the full 21 days, and to further assess for the potential for (b) (4) to occur within the slices over time. The (b) (4) pathologists who did not distinguish any unusual changes in (b) (4) staining profiles with time, concluding that differences were within the expected range of variations that are intrinsic between thymic cortex and medulla.

To address Agency concerns about the previous PV study several additional elements were included in the 2020 PV study:

- The typical number of lots of media (b) (4) lots of TOM per batch) were used for the PV lots. Moving forward, the intention is to use no more than (b) (4) lots of TOM to manufacture each lot of RETHYMIC in the (b) (4) facility. This aligns with the manufacturing history data from the (b) (4) facility.
- Yield was determined by calculating the number of slices (b) (4) of source thymus tissue. Yield is not normally calculated. The number of slices created (b) (4) of source material was (b) (4) slices for (b) (4). Historical data from (b) (4) lots was (b) (4) slices (range (b) (4)). The number of slices created (b) (4) of tissue actually slices was (b) (4) for (b) (4).
- (b) (4) images were captured daily throughout the culture period to determine changes in the tissue appearance and surface area over time. The degree of tissue (b) (4) per slice was estimated. The data on Day (b) (4) slices was used to demonstrate that the filters are initially set up with slices in the range of (b) (4) coverage of the (b) (4) filter, as required by manufacturing protocol. The data show that while some slices fell below the minimum (b) (4), the average of all slices in the first lot was (b) (4) for the second lot.
- (b) (4) technique to estimate slice thickness on the day before release was used (typically there is no estimate or measurement of slice thickness). For subplot (b) (4) all slices but one were categorized as thick at the time of sampling (b) (4) for harvest, and at Day (b) (4) slices were considered thick. For (b) (4) were considered thick, and for (b) (4) were rated as thick. Historically, on average (b) (4) of slices were considered thick (range (b) (4) thick).

- (b) (4)



As an addition study, the ability to remove tissue slices from the filters for surgery without damage was assessed by visual examination and by histology. The smallest and thinnest slices were removed from the filters and the ease of removal was compared to larger, thicker slices from the same lot and timepoints. For (b) (4) Both thick and thin slices were rated as easy to remove. The smallest slices were associated with a medium level of difficulty to remove, and the largest slice with the hardest. For (b) (4) different results were obtained with the largest and thickest slices as being the easiest to remove, the smallest moderate, and the hardest was the thinnest. Histology performed after removal confirmed that that all slices met histology acceptance criteria.

The 2020 PV study was completed successfully and all study acceptance criteria were met. Product consistency was demonstrated, as well as manufacturing consistency, and the in-process and release testing results from the PV lots were similar to the historical range and the range from previous larger clinical lots (i.e., lots produced at maximal scale). The process risk assessment was updated with data generated upon completion of the PV batches (b) (4) 2020-009.1-P). The results of the study provide evidence that the RETHYMIC manufacturing process generates drug product that consistently meets all in-process and release acceptance criteria.

Review of response: The Applicant states that the PV study replaces the previous PV study in the original submission. Images from the previous PV study raised concern because some slices look substantially different compared to D^{(b) (4)} tissue- more so that what some product development lots looked like, or what was presented in pre-BLA discussions and scientific publications. The new PV study does supplement the previous study, but it should be pointed out that (b) (4) staff and Enzyvant believed that the previous study data meet release criteria and would be suitable for transplant into patients. For the most part that would still be true, even though the histology assay has adopted a (b) (4) scoring system. The main purpose of repeating the process validation study was because not all important thymus slice attributes had been evaluated as part of the original validation design, and the inconsistent nature of how the lots were processed and handled made assessment across the three previous PPQ lots difficult. The 2020 validation study incorporates the missing elements, and a split lot approach was used to better evaluate manufacturing consistency.

The process validation design involves a (b) (4) manufacturing approach. A total of (b) (4) lots was generated from (b) (4) donor lots of thymus tissue. This strategy was recommended by FDA at the time of the Type A meeting in order to minimize the number of lots of donor source tissue that would be needed to satisfy regulatory requirements and thereby not be available for patient treatment. It would also allow for a more direct comparison between lots manufactured according to a 12 day culture period with a 21 day culture period, since the same donor tissue would be used for each.

Note: As a consequence of the Applicant wanting to adhere as closely as possible to the 12 day and 21 day commercial manufacturing processes, there is no histology sample other than Day^{(b) (4)} and Day^{(b) (4)} that was performed on both.

(b) (4) attempts were made to manufacture lots for this study, but (b) (4) lots (b) (4) did not meet acceptance criteria and manufacturing was not continued. Therefore, the (b) (4) donor lot was actually conducted on the (b) (4) attempt. This approach was acceptable because FDA removed the expectation for (b) (4) consecutive lots in order to minimize disruption of patient treatment under IND.

The process validation design is complicated. Beyond the difference of using (b) (4) manufacturing, the study included the newly implemented (b) (4) histology test time point to satisfy final product testing requirements (see CRL item #2). Lots cultured for 21 days had included testing at (b) (4) a final time point within (b) (4) days of harvest. Some product testing was conducted for the sole purpose of the validation study and would not normally be conducted for commercial lot manufacturing, but was included at the request of FDA. We have depicted the overall strategy in the following diagram (see Fig. 10).

5 pages determined to be not releasable: (b)(4)

(b) (4)



Overall Reviewer's Assessment of CRL Item 6:

The design of the repeated PV study conducted in 2020 follows FDA advice provided at the Type A meeting and adequately addresses concerns raised about insufficient attributes being evaluated. It was conducted using the (b) (4) manufacturing approach recommended, and (b) (4) lot follows commercial manufacturing procedures except those done in addition to provide information not normally collected. The study was conducted under worst case conditions for hold times (b) (4) maximum hold at room temperature and up to (b) (4) hold at (b) (4) prior to processing, and a (b) (4) maximum hold time at (b) (4) for the shelf life). All in-process and final product acceptance criteria were met. The histological evaluation for quality included the newly implemented (b) (4) analysis scoring system and all lots met the acceptance criteria. We have reviewed digital files of the actual histology slides. Although the cultured slices clearly change over time in culture, and the normal architecture of unprocessed thymus is altered over time in culture, we agree that the phenotypic features present are consistent with other product lots where positive clinical outcome has been demonstrated. **The results addressed our concerns and the CRL item has been resolved.**

CRL item #7 (Transport Study): Transport study (b) (4)-2019-050-A failed to demonstrate microbial protection of DP during packaging, transportation to the OR, and hold in the OR in the (b) (4) culture dish and (b) (4) secondary container. If you intend to proceed with

commercialization of the (b) (4) final DP container, please investigate the media growth promotion failures and take appropriate corrective actions prior to conducting a new study demonstrating that the final DP container adequately maintains a sterile environment. Please submit the summary reports.

Applicant's response to CRL item: The Applicant intends to proceed with commercialization using the (b) (4) culture dish as the final drug product (DP) container.

Distribution of the product involves transporting the product from the (b) (4) facility in the (b) (4) building to the surgical suite in the (b) (4) on the (b) (4) medical campus. Prior to transport the final product slices still adhered to the filters are transferred to a new set of dishes containing the surgical (b) (4) sponge strips and culture medium. The culture medium is (b) (4) 5 mls to help avoid the liquid from leaking outside the loose-fitting culture lid. The same number of dishes are used as were used during culture, with up to 4 slices/dish. The dishes are placed inside the single use (b) (4) secondary container that has been pre-cleaned. The secondary container is placed inside a (b) (4) loaded with (b) (4) to maintain temperature within (b) (4).

Note: Transport temperature according to Section 3.2.P.3.4 and the PV study is considered non-critical. For the PV study the acceptance criterion for transport was (b) (4). This temperature range includes the maximum incubator temperature of (b) (4). The product is (b) (4) prior to shipping and placed back into the incubator until (b) (4) staff receive a call from the surgical staff they are ready for the product. This is expected to be a short period as the (b) (4) staff coordinate with surgical staff. The hold temperature inside of the cooler was (b) (4) and the full (b) (4) was not tested. In response to an information request the Applicant revised the acceptable transport temperature to (b) (4).

The (b) (4) is hand carried by (b) (4) manufacturing staff to the hospital on foot. The transport time is typically (b) (4). At the surgical suite manufacturing personnel gown up and handle the product inside the surgery room. They take the (b) (4) secondary container out of the cooler and perform a visual inspection, including inspecting the dishes for signs of leaking or contamination. If any signs of leakage or contamination are detected, (b) (4) staff will determine the lot should be rejected and will notify the surgical staff (see CRL item #13 for CMC comments about proposed product labeling information).

Note: Given the short transport time it is unlikely that any microbial contamination, if present in the final product and not detected before transport would be detected after. It is also unlikely that any foreign material, such as dust, that could be detected by a visual inspection would occur because the (b) (4) container has an airtight seal and is precleaned and disinfected before use. The main value of the visual inspection would be to make sure that leakage did not occur and the slices didn't shift during transport.

Transport studies are conducted using surrogate product and 5 mls of (b) (4) instead of culture medium to increase the chance of detecting contamination. The surrogate product is package and handled the same way as would be for the commercial

product. Worst case conditions of transport and hold times were used for the new study. At the end of the transport and hold times the final product culture dishes and secondary container are taken back to the (b) (4) facility and the transport medium is collected for sterility testing. The transport study, therefore, represents more than worst case because additional handling is involved, and the final product is transported twice. At the (b) (4) facility the medium is collected, (b) (4), packaged and sent to (b) (4) for sterility testing using the (b) (4) method. The transport study conducted for the original submission failed because the positive control growth promotion test failed for some samples.

(b) (4) transport studies were conducted. The sterility testing showed no signs of contamination, and all growth promotion tests were successful. The Applicant considers these results support the use of the single use (b) (4) culture dish primary container and single use (b) (4) secondary container as an appropriate container closure system for RETHYMIC.

An investigation of the growth promotion failure with the original transport study was conducted. No definitive root cause could be assigned.

In addition, the aseptic process validation (APV) study protocol was designed to fully incorporate DP transport, enabling a robust assessment of the microbial protection of DP during packaging, transportation to the OR, and hold in the OR in the (b) (4) culture dish and (b) (4) secondary container. All sterility and growth promotion results from this APV, which included (b) (4) lots, met specifications, demonstrating successful aseptic processing of RETHYMIC by (b) (4) technicians and that the final DP container adequately maintains a sterile environment.

Review of response: Additional root cause analysis was performed, new samples were generated for testing, and additional testing was performed. Due to the sporadic nature of the initial failures, larger sample sizes were prepared for the second round of investigational testing, samples were collected on different days of simulated culture, and samples were generated that directly replicated the initial study. Similar to the initial investigation the tests showed no growth promotion failures (including the (b) (4) samples replicating (b) (4) timepoints from the initial study), and thus, again, no definitive root cause was identified. These investigations are documented in the transport study report, (b) (4) 2019-050.1-A. Three aspects of the testing design that may have contributed to the initial growth promotion failures were identified: shorter time in dishes prior to testing than during routine processing/aseptic process validation; (b) (4) media change prior to testing instead of (b) (4) media changes over 12-21 days as during routine processing/aseptic process validation; and use of (b) (4) for sample holds prior to analysis instead of the (b) (4) used routinely. The level of investigation conducted appears reasonable.

The transport study design was previously reviewed in the original submission and found acceptable. No changes were recommended to the study (see however CRL item #8

regarding the secondary container). The transport study was (b) (4) studies showed no signs of contamination and the results of the transport studies met the Applicant's acceptance criteria. We agree that the (b) (4) studies with no evidence of contamination is acceptable.

Container closure testing as recommended in FDA guidance could not be followed for the (b) (4) culture dish primary container. The loose-fitting lids would not provide the level of integrity needed for some of the testing, such as (b) (4) testing. Nevertheless, the primary container has been in use for 28 years for patient treatment in this same location. No adverse events were associated with the product that might have suggested a product lot was contaminated for any of the 105 patients who have received RVT-802 to date. The secondary container was introduced in 2016 to further reduce the risk of contamination during transport and has been in use since then. The culture medium volume was (b) (4) to 5 mls for transport as a further mitigation step. There have been no reports of dishes leaking in clinical use under IND. Commercial distribution of the product will be the same as has been used under IND. (b) (4) will remain the only manufacturing facility and the Duke University Hospital is the only clinical center where the surgical procedure is performed. Given the totality of evidence and the high level of experience with the current container closure system, we consider the culture dish and (b) (4) secondary container as adequate.

Although it is a remote possibility the dishes could leak during transport, or foreign material could enter through the loose-fitting culture dish lid inside the air-tight secondary container, this is an immunocompromised patient population due to being athymic. In most cases patients scheduled to receive Rethymic are preconditioned prior to surgery with immunosuppressive agents, putting them at greater risk for infection. It is therefore important to assure sterility of the product. Further, it would represent a difficult situation for the patient and medical team if the product lot could not be used because according to (b) (4) procedures the (b) (4) staff determine a product lot cannot be used. Although the manufacturing facility has the capacity to produce up to (b) (4) product lots at any one time, there is no guarantee another lot could be substituted on short notice. The source material is in short supply and not all planned donor thymus collections actually occur, or meet the minimum tissue weight requirements for manufacturing to proceed. Not all lots initiated meet donor screening requirements and manufacturing is terminated in those cases. Product dose is based on the total surface area of all slices within a lot divided by the body surface area of the recipient. Not all lots produced would have adequate surface area for all target recipients, and even though there is no MHC matching requirement for RETHYMIC, not all lots could be considered interchangeable. For these reasons it would be important to make sure that a product lot that meets release requirements could be packaged in a container closure system with greater integrity. We recommend that a new container closure system be developed and validated and eventually replace the current container closure system (please see PMC section at the end of this document).

Overall Reviewer’s Assessment of CRL Item 7:

The successful completion of (b) (4) transport studies **support use of the single use (b) (4) culture dish primary container and single use (b) (4) secondary container as an appropriate container closure system for RETHYMIC**. Due to the limited availability of RETHYMIC lots, the immunocompromised intended patient population, and great medical need, we recommend as a PMC a new container closure system be developed with greater integrity to avoid a situation where a product lot could not be used due to a container leak, even if a leak is unlikely. The Applicant has agreed to develop a new container closure system as a PMC (please refer to BLA amendment 125685.78).

CRL item #8 (Sterility of (b) (4) Container): *You failed to assure sterility of direct product contact materials. Specifically, (b) (4) validation of the (b) (4) container used for source material transport and (b) (4) storage was deficient. The study was performed on a different container, and (b) (4) was not performed. Please provide the summary report for sterilization validation of the (b) (4) container.*

Applicant’s response to CRL item: Proper sterilization of (b) (4) containers (b) (4) used to transport the source thymus tissue has been demonstrated by the successful validation and continued validity of the (b) (4) sterilization processing of (b) (4) alternate master product containers per the requirements outlined in ISO (b) (4). To support the (b) (4) container is representative of (b) (4) with respect to bioburden and sterilization challenges additional information is included.

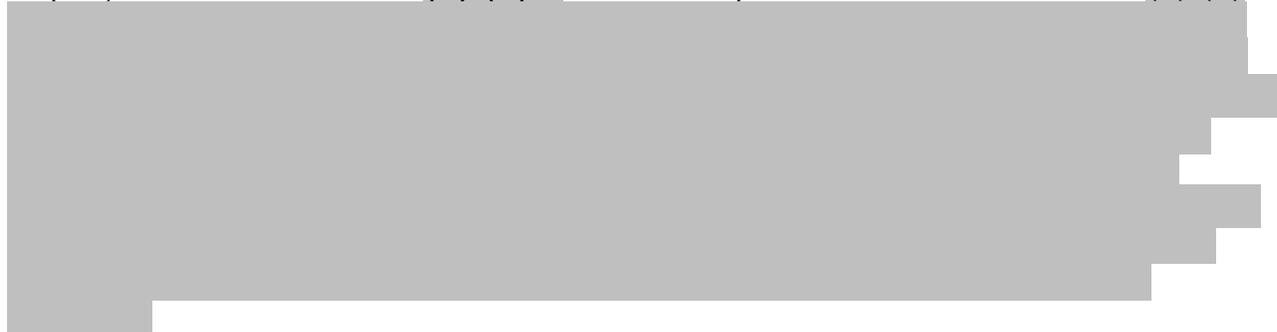
A master product approach was used to validate the sterilization process for the (b) (4) by employing worst-case within a given manufacturer’s production line to represent a challenge for sterilization that is greater than that of other family members. The (b) (4) container (part (b) (4)) is a member of (b) (4) for sterilization. Product code (b) (4) was selected by (b) (4) as the master product for (b) (4) since it is the (b) (4) item in the (b) (4) when packaged for shipment; it also contains a towelette with each container and is therefore considered the most challenging to sterilize. Worst case includes source of raw materials and components; product design and size, including container opening diameter and product dimensions and packaging; manufacturing processes and equipment, as well as the manufacturing location and environment. A summary table comparing the (b) (4) container to the (b) (4) was included in the submission. All (b) (4) containers are made of (b) (4) Polypropylene, with the same (b) (4) polyethylene cap and (b) (4)

openings, packaged in units of (b) (4) , with a bioburden level of (b) (4) , and validated with the same validation cycle and processing instructions.

(b) (4)



During the 2017 sterilization validation study (b) (4) Sterilization Validation Report), in conformance with (b) (4) verification procedures described in ISO (b) (4)



(b) (4)



(b) (4)



Review of response: This CRL item was reviewed by DMPQ and found acceptable. No new concerns were raised. Please refer to the DMPQ review memo for details.

Overall Reviewer's Assessment of CRL Item 8:

The response is acceptable.

CRL item #9 (Drug Product Shipper Validation): Adopting the (b) (4) culture dish as your primary DP container changed your DP packaging and configuration of the shipping container used for DP transport to the OR. Therefore, the validation of this shipping container to maintain the appropriate temperature is no longer valid. Please revalidate and provide the summary report.

Applicant's response to CRL item: This deficiency has been addressed through successful validation of the Drug Product (DP) shipping container using the packaging configuration that will be used for RETHYMIC. Each RETHYMIC lot includes 3 to 11 primary container culture dishes, with each dish containing up to 4 processed tissue slices. The culture dishes are packaged in racks, and placed in the sealable polycarbonate (b) (4) Container System secondary container for transport to the OR. The (b) (4) is placed

(b) (4) box to maintain an acceptable temperature for transport to the OR. Enzyvant validated the new DP packaging in the shipping container used for transport to the OR to ensure that the shipping container provides adequate protection of the DP during typical handling and maintains the appropriate temperature throughout transport.

The results of the shipping validation study (b) (4)-2019-063-E, Drug Product Shipper Validation Report) demonstrated controlled transport of outgoing drug product from the (b) (4) facility to the surgical suite at Duke Hospital under current approved SOPs, both in terms of physical protection and temperature control. During the validation, transport was simulated to ensure that the configuration of the shipping container can withstand the impact of the typical distribution environment (ie, typical handling) during worst case (longest) delivery times of (b) (4). After the (b) (4) walking period, the cultures dishes were visually inspected for evidence of leakage/spillage and photographs were taken. The study included a total of (b) (4) tests to evaluate temperature stability (b) (4) test (b) (4)

The total and outdoor transport times are now described in (b) (4)-THY-009 Packing and Transport of Thymus Drug Product to the OR. The total transport time begins at the time (b) (4) operators leave the manufacturing suite and ends upon entrance to the OR, while the outdoor transport time begins when the (b) (4) operators exit the (b) (4) building and ends upon arrival at the Duke Hospital. Given that the typical transport time is (b) (4), with a portion of this time indoors, the demonstrated temperature control under extreme cold conditions for (b) (4) indicates negligible risk to temperature deviations during the timeframe for product shipping when the product is manufactured at and administered to a patient at (b) (4). The addition of temperature monitoring during transport of each RETHYMIC batch using a temperature logger at the shipper wall (worst case) location, as is now required per procedure, provides evidence of the temperature during shipment in order to demonstrate that the drug product remained within acceptable temperature range throughout transport.

In addition, (b) (4)-EQUIP-043 Operation and Maintenance of Shipping Boxes for RVT-802 Manufacturing was updated to ensure that the appropriate (b) (4) requalification is performed using the parameters and methods that were used for the most recent validation.

SOP (b) (4)-THY-009, Packing and Transport of Thymus Drug Product to the OR, was updated to include temperature monitoring and recording of both total shipping time and the outdoor shipping time during every shipment. The procedure in case of spills, damage, or leakage being detected in the OR was updated to include immediate notification of Management to support a product disposition decision. SOP (b) (4)-THY-010 was modified

such that if evidence of contamination, damage, spills, or leakage is noted following transport of product to the OR, the lot will be rejected and the surgical team will be informed.

Review of response: This CRL item was reviewed by DMPQ and found acceptable. No new concerns were raised. Please refer to the DMPQ review memo for details.

Overall Reviewer’s Assessment of CRL Item 9:

This CRL item was reviewed by DMPQ and found acceptable. No new concerns were raised. **The item is considered resolved.**

CRL item #10 (Secondary Container): *Due to the nature of your primary DP container, the environment inside your secondary (b) (4) container becomes more critical to ensure microbial protection of the product. We recommend cleaning and/or sterilization validation of the secondary container and packing of the (b) (4) container in the ISO (b) (4) environment. Additionally, please implement and provide procedures and lot disposition for spill incidents in transport.*

Applicant’s response to CRL item: *Cleaning validation of the (b) (4) secondary container has been completed according to the planned manufacturing cleaning procedures, including cleaning the (b) (4) in the ISO (b) (4) environment, where it may be held until receiving the notification from the OR, at which point the (b) (4) will be transferred into the ISO (b) (4) environment and packed with the drug product.*

An initial cleaning validation study (b) (4)-2020-005-E Validation of (b) (4) Cleaning Interim Report) was executed and the updated cleaning procedures were implemented prior to conducting process validation. All results from samples taken after cleaning for all time points of the (b) (4) container and racks were (b) (4). Swab results indicated no contaminants in either the (b) (4) samples. An investigation concluded the technique used during swab sampling likely led to the lack of contaminants detected on the swabs. As part of the investigation, swabbing technique for the (b) (4) rack and chamber was evaluated per (b) (4)- 2020-064-E (Investigational Protocol for (b) (4) Cleaning), but inadequate recoveries were obtained despite trained personnel using best practice techniques. Thus, it was concluded that swabs were not an appropriate sampling method to collect representative samples from these surfaces. Based on contact plate results the cleaning method was deemed acceptable and was implemented for the 2020 Aseptic Process Validation and PPQ batches until the investigation of the swab sampling was completed. While the investigation into swab sampling methods was ongoing, additional cleaning verification was performed. Because swab sampling was determined to be inappropriate for sampling the (b) (4) posts and corners, and contact plates could not be used for sampling these locations, a rinse method was developed to

ensure the (b) (4) cleaning procedures are effective. The (b) (4) method involved (b) (4)

The cleaning validation was re-executed with the rinse method. Results demonstrated the cleaning method described in the protocol (b) (4)-2020-005.1-E Validation of (b) (4) Cleaning Final Report was highly effective at reducing bioburden, and all acceptance criteria were met. On average, bioburden levels as detected by the (b) (4) method were (b) (4) as a result of the cleaning. Hold times of (b) (4) had bioburden levels of around (b) (4) while average bioburden levels after a (b) (4) hold were (b) (4). It is believed the lower CFU levels with longer hold times was due to the death of microorganisms over time. They concluded the cleaning validation studies demonstrated the cleaning procedures effectively cleaned all components of the (b) (4), and supported a hold time of (b) (4) for the cleaned single use secondary containers.

During process validation batches, the cleaning verification included collecting (b) (4)

(b) (4)

Review of response: This CRL item was reviewed by DMPQ and found acceptable. No new concerns were raised. Please refer to the DMPQ review memo for details.

Overall Reviewer's Assessment of CRL Item 10:

The item is considered resolved.

CRL item #11 (b) (4) System): Regarding your (b) (4) system:

- a. Qualification of your (b) (4) system is deficient in scope and duration. Specifically, it did not include monitoring of (b) (4) over a period of time, and only a limited number of locations were sampled. (b) (4) sampling did not demonstrate that (b) (4) is within ISC^{(b) (4)} acceptance limits.
- b. Your strategy and schedule for routine (b) (4) sampling is unclear, as not all testing is performed (b) (4), and locations vary for different dates and types of tests. The sampling procedure description is inconsistent (e.g., use of (b) (4)), and vague about (b) (4) use during sampling, which could interfere with bioburden testing.

Please provide information and/or data to address these issues.

Applicant's response to CRL item: A qualification of expanded scope and duration that demonstrated that all use points meet ISO^{(b) (4)} limits and (b) (4) purity requirements was performed in May/June 2020 (SOP REP-020, (b) (4) System Qualification Summary Report). SOP (b) (4)-2020-031.1-E, (b) (4) System Qualification Final Report provides a description of the (b) (4) system and a summary of the studies that have been conducted for IQ, OQ and PQ. Every active point of use throughout the system was sampled on (b) (4) different days. Sampling duration was defined in the protocol to provide adequate sample volume and a representative sample for each test, and each site (b) (4) to the start of sampling to ensure that a representative sample was obtained. Test results on (b) (4) passed all acceptance criteria at all tested points of use. (b) (4) requirements. The acceptance criteria for (b) (4) were established to match ISO^{(b) (4)} requirements (ISO (b) (4)). The acceptance criterion for (b) (4) matches (b) (4) requirements for (b) (4). The testing results from the qualification study demonstrated that the (b) (4) system provides (b) (4) to all (b) (4) active points of use throughout the system, with (b) (4) levels (b) (4) that meet ISO^{(b) (4)} criteria. The qualified state of the (b) (4) system is confirmed (b) (4) through the routine monitoring program (b) (4)-EQUIP-042). For each (b) (4) system check, (b) (4) point of use

on (b) (4) is tested, for a total of (b) (4) points of use tested during each (b) (4) test. The selected use point on each (b) (4) to ensure that all use points are sampled over time. Testing of (b) (4) provides a representative sampling of the entire system each (b) (4), as the (b) (4) design includes a primary (b) (4) are installed per room (the number of manifolds per room is dependent upon the number of incubators in the room). Results from the Q1 2021 routine monitoring testing program confirmed the qualified state of the (b) (4) system, as all acceptance criteria were met.

The sampling procedures for the (b) (4) system are now clearly defined in the revised (b) (4) equipment SOP, (b) (4)-EQUIP-042. Details for collecting each sample type are provided in the SOP, including sampling duration. For most sample types, the sample volume is the same for routine sampling as was collected during qualification. For (b) (4), the sample size is about (b) (4) the volume collected during qualification. That (b) (4) sample size is expected to be adequate for assessing (b) (4) levels given the low variability in (b) (4) at different points of use that were measured during qualification. To improve the consistency of the sampling, (b) (4)-EQUIP-042 was updated to provide details regarding sampling for the (b) (4) testing, including specific instructions for the use of the (b) (4). In addition, procedures were added to the SOP to require training of staff and/or contractors prior to performing sampling of the (b) (4) system.

Review of response: This CRL item was reviewed by DMPQ and found acceptable. No new concerns were raised.. Please refer to the DMPQ review memo for details

Overall Reviewer's Assessment of CRL Item 11:

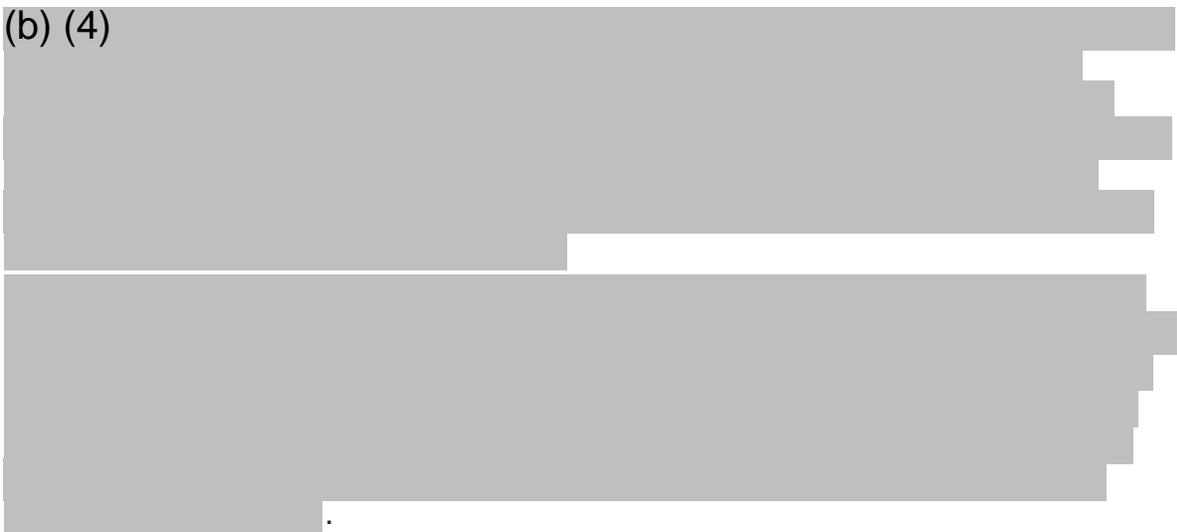
The item is considered resolved.

CRL item #12 (Personnel Flows): *The personnel flows at your multi-product facility create an increased risk of product contamination and cross-contamination. Specifically,*

- a. *You allow (b) (4) of your facility. This allows simultaneous presence of personnel working on different products in (b) (4).*
- b. *Additionally, personnel enter Gown-In Room (b) (4) and exit Gown-Out Room (b) (4) of the facility through the same Receiving/Supply Room (b) (4). This allows simultaneous presence of personnel entering and exiting the manufacturing areas in Room (b) (4)*

2 pages determined to be not releasable: (b)(4)

(b) (4)



Review of response: This CRL item was reviewed by DMPQ and found acceptable. No new concerns were raised. Please refer to the DMPQ review memo for details.

Overall Reviewer's Assessment of CRL Item 12:

The item is considered resolved.

CRL item #13 (Labelling and Clinical Considerations):

Product labeling (package insert)

The following CMC items were noted in the review of the version of the package insert provided in the resubmission. The version provided with the resubmission already included comments provided by FDA during the original submission review.

Indications and Usage, and Section 1: Pharmacological class (product class) has yet to be defined. Once established the product label will need to be updated.

2.2 Administration instructions: Regarding "qualified surgeon", - since currently there is only one hospital that can perform treatment with this product, perhaps there should be additional language indicating that only "qualified hospitals" should be used. This language has been added.

2.2.1 Preparation for the Implantation Procedure:

#3: Current (b) (4) language states that (b) (4) personnel will inspect each dish and “if evidence of contamination, damage, spills, or leakage is noted following transport of product to the OR, the lot will be rejected and the surgical team will be informed. The product lot will be returned to (b) (4) and an investigation will be conducted.” The surgical team needs to be aware that they may not know until the time of surgery that a patient might not be able to be treated. This is an unlikely scenario, but that is how Module 3 is written. This section and Section 16 were updated to include language about the inspection of the product containers after transplant and will make a decision about the final disposition of the product at that time.

#6: “The operating room culture dish and saline are supplied by the operating room.” Item #7 suggests that more than one culture dish is used. The amount of materials needed for the surgical procedure is unclear. The wording has been revised to indicate the hospital is to supply polystyrene culture dishes and saline for the surgical procedure. The original wording referred to preservative-free saline, but different grades are available and not all grades would be appropriate for surgical procedures. This has been revised to saline provided by the hospital.

#7: Figure legend- Suggest clarifying these procedures are performed within the sterile field. The figure legend was modified as suggested.

3 DOSAGE FORMS AND STRENGTHS- “Each polystyrene culture dish...” Product container nomenclature should be consistent with other sections- other sections they say “product dish”. This also applies to Section 16. Also, it can be confusing whether the culture dish is the one used by the surgical team after removing the slices from the filter or the dish the product is supplied in. Need to have distinction between these two containers, even if they are the same material. When referring to the container the slices are provided in the term product dish is now used throughout the document.

5.3 “Final sterility test results are not available at the time of use...” This should read final sterility and mycoplasma test results are not available at the time of use...” These are (b) (4) separate assays with results available at different times. The suggested wording has been incorporated.

Section 12 and 16 should specify final product is in culture medium containing FBS. Sections 11 and 16 have been updated to include the formulation contains FBS.

12.1 Mechanism of Action: “RETHYMIC is intended to reconstitute immunity in patients who are athymic.” The word “reconstitute” suggests restoration, but the amount of tissue transplanted is a small fraction of normal thymus weight or volume and T cell counts are elevated, but not typically normal. Suggest “RETHYMIC is intended to reconstitute immunity in patients who are athymic by partially restoring normal naïve T cell levels.”

“RETHYMIC alters the positive and negative selection process...” Not sure “alters” is the right word here since the intention is for the thymus slices to act as a normal thymus using the normal thymic positive and negative selection process. As a normal consequence of the donor thymus being allo, tolerance to the thymus tissue would be achieved. The thymus normally produces and presents a large range of antigens, including self antigens,

which in this case would include allo antigens. But it's not really an altered cellular or molecular process, as might be the case if this was a gene therapy product. The mechanisms by which tolerance to host HLA-mismatched antigens is achieved has not been definitively determined, and it is not clear if there is some novel process that is occurring in the thymus, or perhaps APC from the donor migrate into the thymus transplant (as normally occurs in healthy individuals) and present antigen in the context of autologous MHC. I think it would be more accurate to state the MOA is to restore normal thymic function, and as a consequence of the TP being allo will also provide tolerance to donor thymus. Suggest replacing "alters" with "reconstitutes". After much internal discussion and multiple versions from the Applicant the agreed upon language was simplified to " RETHYMIC is intended to reconstitute immunity in patients who are athymic. The proposed mechanism of action involves the migration of recipient bone marrow stem cells 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 implantation." The revised wording addresses our concerns about the description of the product and its functions. However, it is not clear that "bone marrow stem cells" being recruited to the thymus slices is the most accurate terminology. Most scientific articles refer to "bone marrow-derived T cell precursors" migrating into thymus tissue for further maturation and selection, and not stem cells. It was recommended the Applicant consider the most appropriate wording.

16 HOW SUPPLIED/STORAGE AND HANDLING

This section should be updated to match handling procedures and personnel responsibilities described in (b) (4)-thy-010 -rev04" and (b) (4)-thy-010-frm1-rev06". Importantly, the surgical team should be aware that the lot could be rejected by manufacturing personnel within the OR (although very unlikely). This has been updated to describe the responsibilities of the manufacturing personnel who inspect the dishes after transport and who bring back unused slices to the (b) (4) facility. They also determine the final disposition of the product.

The description of the primary and secondary containers and the materials they are made of can be confusing. Recommend having a separate bullet describing how the product is packaged in the container closure system, and then putting dosing information in a separate bullet. A separate bullet was created that indicates how the product is supplied and the containers used. Information about dosing was moved to a different bullet.

Second bullet: "that adhere to circular filter membranes..." This should be changed to **adhered**, past tense, since they are already supplied adhered to the filters. Revised in the final copy.

Storage and Handling: "Do not refrigerate, freeze, agitate, or sterilize RETHYMIC." There's really no way for this to occur since (b) (4) manufacturing personnel will be handling the package in the operating room and handing the dishes to the assisting nurse just outside the sterile field. The language has been kept to be consistent with standard language for shipped biologics. The handling conditions on the product container labels have been updated to reflect the full wording.

The term “culture” should be removed in the description of the final container final container product dish because this could be confused with culture dish hospital uses and supplies. Once the product is transferred to the final container dish it should be referred to as the product container dish, even though the container is the same as the culture dish used during drug substance manufacturing. The final product container is now referred to as product dish throughout the document, other than indicating the type of material is polystyrene.

Recommended storage temperature (room temp) should be included in description. This has been added.

The surgical (b) (4) sponge is referred to in different ways throughout the document, such as sponge, surgical sponge, and (b) (4) sponge. The sponge should be referred to the same way throughout.

Product primary container and secondary container labels should be updated to match information in the package insert (e.g., handling conditions on label should include do not agitate or sterilize). The labels have been revised. Please refer to container label information below.

Product container labels

As documented in Amendment # 125685.80 the primary and secondary container labels have been updated as follows:

- Handling conditions now include “do not agitate” and “do not sterilize” to be consistent with the package insert.
- The route of administration has been added to the primary container label.
- The primary and secondary container label now indicate the product is formulated in medium containing FBS and that no preservative is used.
- The product is to be used “For intended recipient only”.
- The minimum product dose has been increased from (b) (4) to 5000 mm²/m².

These revisions are consistent with primary and secondary container biologics regulatory requirements.

In response to an information request the Applicant has clarified there is a patient-specific label in addition to the primary and secondary container labels that allows verification the product is matched to the intended patient. A copy was provided and the information on the patient-specific label is acceptable. Product labeling has been updated to reflect the use of the patient-specific label.

Overall Reviewer's Assessment of CRL Item 13:

The Applicant has made all the requested CMC revisions to the labeling and the container labels, or has removed text or wording that was of concern. Details on how the correct patient for implantation is verified, and a copy of the patient specific label have been provided and found acceptable. **The CRL item is considered resolved.**

POSTMARKETING COMMITMENTS

Two post-marketing commitments CMC were proposed by FDA and agreed upon by the Applicant during review of the BLA resubmission. Reasons for the (b) (4) assay PMC is detailed in the review of CRL item # 3, and for the new container closure system PMC in the review of CRL item # 7.

1. Enzyvant commits to develop a (b) (4) assay to facilitate the assessment of (b) (4) changes in product quality for stability and comparability. The new (b) (4) assay will either measure (b) (4) (b) (4) Enzyvant commits to submitting the proposed (b) (4) assay protocol in a product correspondence supplement by October 31, 2022. The assay method will be validated, and the sensitivity of the assay to detect shifts in (b) (4) will be established. Enzyvant will submit the final study report, which includes the validation report, as a Prior Approval Supplement by March 31, 2024.
2. Enzyvant commits to develop a (b) (4) . The level of comparability and stability data needed to support the (b) (4) will be commensurate with the degree of changes from the current (b) (4) . Enzyvant commits to conducting (b) (4) (b) (4) Enzyvant will submit the final study report, which includes the validation report, as a Prior Approval Supplement by October 31, 2024.

ADDITIONAL CONSIDERATIONS

Statistical comparison of current and previous manufacturing facilities. Module 5 has been updated with data on 105 subjects who have been treated with RETHYMIC. Of these, data on 93 subjects were included in the original BLA clinical data set. An additional 12 subjects treated from 2018 through 2020 have been added in this resubmission. All of the 12 new subjects received product that had been manufactured in the (b) (4) facility. Four of these subjects were treated under IND with product formulated and packaged in the (b) (4) final container. These are no longer in use under IND and will not be used for the commercial product. There have not been any notable changes to the safety or efficacy profile. The overall Kaplan-Meier estimated survival rates for the Efficacy Analysis Population (N=95) at Year 1 and Year 2 post- implantation were 77% (95% CI = 0.670, 0.844) and 76% (95 CI = 0.657-0.834), respectively. Comparable efficacy was seen between lots manufactured in the (b) (4) facility.

Thomas
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