BLA Clinical Review Memorandum

Application Type	Biologic License Application
STN	125685/0/60
CBER Received Date	April 9, 2021
PDUFA Goal Date	October 8, 2021
Division / Office	DCEPT/OTAT
Priority Review (Yes/No)	Yes
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Applicant	Enzyvant Therapeutics GmbH
Established Name	Allogeneic processed thymus tissue-agdc
(Proposed) Trade Name	RETHYMIC
Pharmacologic Class	

Formulation(s), including Adjuvants, etc.	RETHYMIC is composed of yellow to brown slices of processed and cultured thymus tissue supplied adhered to filter membranes.
Dosage Form(s) and Route(s) of Administration	RETHYMIC is supplied as up to 42 individual processed thymus tissues slices which are surgically implanted into the quadriceps muscle of the patient.
Dosing Regimen	RETHYMIC is administered in a single surgical procedure during which a dose of 5,000 to 22,000 mm^2 of processed thymus tissue / m^2 recipient body surface area (BSA) is implanted into the quadriceps muscle of the patient.
Indication(s) and Intended Population(s)	Immune Reconstitution in Pediatric Patients with Congenital Athymia
Orphan Designated (Yes/No)	Yes

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GLOSSARY

AE	adverse event
AESI	adverse event of special interest
ALC	absolute lymphocyte count
AR	Adverse reaction
BLA	biologics license application
BMTCTN	Blood and Marrow Transplant Clinical Trials Network
BSA	body surface area
BUN	blood urea nitrogen
BW	body weight
CA	Congenital Athymia
CBC	complete blood count
CBT	cord blood transplant
cDGA	Complete DiGeorge Anomaly
ConA	concavalin A
CHARGE	Coloboma, heart defect, choanal atresia, growth and development retardation, genital hypoplasia, ear anomalies/deafness
CI	confidence interval
CMV	cytomegalovirus
cpm	counts per minute
CSA	cyclosporine A
CSE	clinical summary of efficacy
CSR	clinical study report
CSS	clinical summary of safety
CTCAE	Common Terminology Criteria for Adverse Events
DGA	DiGeorge Anomaly
(b) (4)	
DNA	deoxyribonucleic acid
DUMC	Duke University Medical Center
EAS	efficacy analysis set
EBV	Epstein-Barr virus
eCRF	electronic case report form
EU	European Union
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HSPC	hematopoietic stem and progenitor cell
HSCT	hematopoietic stem cell transplant
ICH	International Council for Harmonization
IDM	infant of diabetic mother
Ig	Immunoglobulin
IVIG	Immunoglobulin intravenous
IND	investigational new drug
IS	immunosuppression
ISE	Integrated summary of efficacy
ISS	Integrated summary of safety

IU	international units
IV	intravenous
Kg	kilogram
KM	Kaplan Meier
L	liter
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MMF	mycophenolate
ml	milliliter
NH	Natural history
NK	cell natural killer cell
nm	nanometer
PCR	polymerase chain reaction
PH	proportional hazards
PHA	phytohemagglutin A
PJP	pneumocystis jirovicei pneumonia
PT	preferred term
Q	quartile
RATGAM	Rabbit anti-thymocyte globulin
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SMQ	Standardized MedDRA Query
SOC	System Organ Class (MedDRA)
SoC	standard of care
TEC	Thymic epithelial cells
TEAE	treatment emergent adverse event
TCR	T cell receptor
TREC	T cell receptor rearrangement excision circles
U	unit

1. Executive Summary

Enzyvant Therapeutics GmbH submitted a Biologics License Application (BLA), STN 125685, for licensure of allogeneic processed thymus tissue-agdc (RETHYMIC) for immune reconstitution in pediatric patients with congenital athymia (CA). The initial BLA was submitted on April 4, 2019 and the clinical data demonstrated improved survival and a favorable benefit-risk profile. However, a Complete Response (CR) was issued on December 4, 2019 for Chemistry, Manufacturing and Control (CMC) deficiencies. To address these CMC concerns, the Applicant made changes to the manufacturing facilities and procedures. This resubmission responds to the deficiencies and includes additional clinical data, including data from 18 subjects treated in the new manufacturing facility. The focus of the clinical review for this resubmission was to determine if the benefit:risk profile with the new manufacturing process remains favorable.

RETHYMIC is manufactured from tissue obtained from unrelated donors under the age of 9 months undergoing cardiac surgery. RETHYMIC is manufactured on demand and is for single administration via a surgical implantation procedure into the quadriceps of patients with CA.

RETHYMIC is processed from allogeneic-unrelated donor thymus tissue and processed to reduce viable thymocyte levels within the tissue slices to maintain similar overall tissue organization, viability and important cells. RETHYMIC is intended to function as normal endogenous thymus tissue. The proposed mechanism of action is migration of the recipient's common lymphoid progenitors (immature T cells) into the thymic allograft (RETHYMIC) where they are “educated” to produce mature, immunocompetent T cells that are tolerant of both donor and recipient tissues while maintaining the ability to respond to foreign antigens. The recommended dose of RVT-802 is 5,000 to 22,000 mm2 of total processed thymus tissue surface area/m2 recipient body surface area (BSA).

CA is a rare disease characterized by profound, life-threatening T cell immunodeficiency due to the absence of a functioning thymus at birth. Most children with CA die before 2 years of age. It is estimated that approximately 20 individuals are born with CA each year in the United States. The diagnosis of CA is based on documentation of extremely low number of naïve T cells by flow cytometry. CA most commonly occurs as part of Complete DiGeorge Anomaly (cDGA). The known genetic mutations associated with CA include 22q11.2 deletion, and mutations in chromodomain helicase DNA binding protein 7 (CHD7), Forkhead Box Protein N1 (FOXN1), T Box transcription factor 1 and 2 (TBX1), (TBX2). Currently, there is no approved treatment available for CA. Investigational hematopoietic cell transplantation (HCT) is of limited benefit. Management of CA consists of supportive care, intravenous immune globulin (IVIG) and antibiotic prophylaxis.CA is a rare disease with substantial unmet need.

To support the safety and effectiveness of RETHYMIC for CA, the applicant conducted 7 prospective, non-randomized, open-label studies and 3 single-patient clinical studies over a 28-year period. Because of the similarities in study population, study design, study procedures including safety monitoring and efficacy endpoints, concomitant medications, and duration of follow-up, data from these studies was pooled. These data

were compared to a retrospective natural history cohort of 49 patients with CA associated with cDGA who received only supportive care. We consider the integrated data from the subjects with CA treated in these single-arm studies compared to the historical control data to comprise a single adequate and well-controlled study. Based on the objective endpoint, mortality, and large treatment effect size, comparison to an external control is adequate to provide substantial evidence of effectiveness, consistent with the regulatory requirements of section 351 of the Public Health Act. The survival benefit of RETHYMIC was supported by clinically meaningful improvements in infection and additional biochemical and T-cell functional testing data to indicate immune reconstitution. Thus, these laboratory data combined with the biologic plausibility that the allogeneic thymus tissue-based product would induce immune reconstitution serve as confirmatory evidence.

The clinical studies with RETHYMIC included 105 subjects, of whom 95 were in the efficacy analysis set (EAS). The EAS was limited to subjects with CA who had not previously received hematopoietic stem cell transplants (HSCT) or fetal thymus transplant. The diagnosis of CA was based on naïve T cell numbers < 50 cells/mm^3 or naïve T cells accounting for < 5% of total T cells. In the EAS, CA was due to cDGA (n=93) and Forkhead box N1 (FOXN1) deficiency (n=2). Across the efficacy population, 59% were male and 41% were female; 70% were White, 22% were Black, 4% were Asian/Pacific Islander, 2% were American Indian/Alaskan native, and 2% identified as multi-race. The demographics of the EAS population is consistent with the demographics of CA within the United States. The age at treatment ranged from 33 days to 2.9 years with a median age of 8.5 months.

The primary efficacy endpoint defined in the statistical analysis plan (SAP) was survival at one-year after RETHYMIC. The survival rates at 1-year and 2-year after RETHYMIC transplant were 77% (95% Confidence Interval (CI) 0.67, 0.84) and 76% (95% CI .66, 0.83), respectively. The median age of all surviving subjects at last contact was 11.4 years (range 3 to 25.7 years) with most subjects censored due to data cut-off rather than death. Over half of the CA subjects treated with RETHYMIC have survived into the second decade of life. This is a substantial treatment effect compared to the natural history population, where 94% of subjects died by two years of age and all subjects died by three years of age.

The survival benefit of RETHYMIC was supported by decrease in frequency and severity of infection. During the first 6-months following RETHYMIC, there were 346 infections of which 135 were serious, considered the baseline rate of infections in the subjects as RETHYMIC does not provide protection against infections until at least 6 months after transplant. In the subsequent 6-month period following RETHYMIC, the number of infections declined by 69%; there were 109 infections, of which 51 were serious. The number of total infections, serious infections, and percentage of subjects with infections continued to decline through Year 2.

Efficacy of RETHYMIC is further supported by evidence of immune reconstitution. Thymopoiesis was noted on biopsies as early as 2 months after transplant. Increased numbers of naïve CD3, CD4 and CD8 T-cells in the EAS occurred during the first 2 years after transplantation. Achieving a naïve CD4 count $\geq$ 100 cells/mm^2 is generally considered sufficient to fight infection. In the EAS, median naïve CD4+ T cell counts were 1.0 (range: 0-38), 41.6 (range: 0-653), 212 (range: 1-751) and 274.5 (range: 33-858) cells/mm^3 at baseline, Month 6, Month 12, and Month 24 post-transplantation,

respectively. Median naïve CD8+ T cell counts were 0.2 (range: 0-45.9), 9.3 (range: 0-163), 57.9 (range 0-304.3) and 86 (range: 6.0-275) cells/mm^3 at baseline, Month 6, Month 12, and Month 24 post-transplantation, respectively. In addition, T cell proliferative responses to phytohemagglutinin (PHA), Concavalin A, Soluble CD3, Immobilized CD3, and tetanus toxoid were also increased and sustained through 2 years post-transplantation. The T cell receptor variable beta (TCRVβ) repertoire variability as assessed by immunoscope/spectratyping and flow cytometry demonstrated a diverse TCR repertoire through 2 years after transplantation. These data support the development and persistence of immune function through 2 years post-transplantation.

The safety population, full analysis set (FAS), consisted of all 105 subjects treated with RETHYMIC regardless of diagnoses and prior treatments. Demographics were similar between the safety and efficacy populations, except for age. The median age was 9 months (1 month-16.9 years), including 4 subjects over 3 years old.

Twenty-nine subjects (27.6%) died following RETHYMIC transplantation. The majority of deaths (23 of 29 deaths) were within the first-year post-transplantation, prior to adequate immune reconstitution. Most of these deaths were due to infections and/or complications associated with infection. Three of the deaths were considered possibly related to study treatment; two were attributed to immunosuppressive agents, and one was due to CMV that may have been acquired from the donor thymus tissue.

Within the FAS, there were 2 subjects with Severe Combined Immunodeficiency (SCID) and both died without receiving any benefit from RETHYMIC. Consequently, based on the mechanism of action of RETHYMIC, the clinical team believes RETHYMIC is not indicated for SCID.

Other risk factors for death include active pre-existing cytomegalovirus (CMV) infection, baseline renal impairment, and baseline CD3+ T cells >6000 cells/mm^3 and developing Graft versus Host Disease (GVHD). Atypical cDGA phenotype was associated with increased mortality during the first-year post-transplantation, but not long-term compared to typical cDGA subjects.

All 105 subjects treated with RETHYMIC had at least one adverse event (AE) and 89 (85%) had at least one serious adverse event (SAE). Most of these AEs and SAEs were attributed to underlying disease. Based on our adjudication of AEs, during the first 2-years after RETHYMIC transplantation, 80 subjects (76%) had 247 adverse reactions (ARs); these ARs included AEs related to RETHYMIC, transplant procedures or immunosuppression. The most common ARs (occurring in at least 10% of subjects) during the first 2 years after RETHYMIC transplantation included: hypertension, n=20 (19%); Cytokine Release Syndrome (CRS), n=19 (18%); rash, n=16 (15%); hypomagnesemia, n=17 (16%); thrombocytopenia, n=13 (12%); renal impairment/renal failure, n=13 (12%); and Graft versus Host Disease (GVHD), n=11 (10%). CRS was generally mild or moderate and only occurred in subjects treated with rabbit anti-thymocyte globulin (RATGAM). Treatment-related SAEs were reported in 37 subjects (35.2%) and were primarily due to autoimmune conditions, T cell dysregulation or complications of immunosuppression. Additionally, there were 2 cases of CMV, one that resulted in death, that may have been acquired from the donor thymus tissue.

The reviewed safety data does not warrant a Risk Evaluation and Mitigation Strategies (REMS), a clinical safety post-marketing requirement (PMR) study, or a safety post-

marketing commitment (PMC) study. The post-marketing risk mitigation plans include product labeling, applicant's pharmacy and surgical training, routine pharmacovigilance, and a voluntary observational post-marketing registry study of 75 CA patients.

The BLA contains substantial evidence of safety and effectiveness for RETHYMIC for immune reconstitution in pediatric patients with CA based on an adequate and well controlled study with confirmatory evidence. The primary clinical challenge in reviewing the data submitted in the BLA was that all studies were open-label single arm studies conducted at a single-center without a concurrent control group. However, the data provided were able to transcend these limitations as: 1) the applicant provided data from a large number of subjects, especially given the rarity of the disease, who had a long duration of follow-up (over 25 years), 2) the natural history is uniform and well-characterized, and 3) most importantly, there was a large survival effect that was consistent and persistent. The survival benefit was supported by data on decreased infections, biochemical immunologic data and well understood mechanism of action for RETHYMIC. Children treated with RETHYMIC experienced many AEs and SAEs, which were expected given the underlying disease, comorbidities, and concomitant use of immunosuppressive and nephrotoxic drugs. Adverse reactions were generally treatable, and risks associated with RETHYMIC can be mitigated through labeling, post-marketing pharmacovigilance and routine clinical practice. In conclusion, there is a favorable benefit risk profile in pediatric patients with CA. The clinical team recommends traditional approval of this BLA.

1.1 Demographic Information: Subgroup Demographics and Analysis Summary

The demographics of the subjects in the efficacy analysis set (EAS) and full analysis set (FAS) who were enrolled in the 10 open-label, non-randomized clinical trials that treated at least one subject with CA with RETHYMIC are summarized in Table 1.

Table 1: Subject Demographics

	FAS (N=105)	EAS (N=95)
Age on the day of implantation (days)		
Mean (SD)	493.34 (923.64)	297.9 (213.95)
Median (min, max)	269 (33, 6163)	256 (33, 1087)
Sex n (%)		
Female	45 (42.9%)	39 (41.1%)
Male	60 (57.1%)	56 (58.9%)
Race n (%)		
White	76 (72.4%)	66 (69.5%)
Black or African American	21 (20.0%)	21 (22.1%)
Other	8 (7.6%)	8 (8.4%)
Ethnicity n (%)		
Hispanic or Latino	20 (19.0%)	18 (18.9%)
Other	85 (81.0%)	77 (81.1%)

(Source: Statistical reviewer's table)

1.2 Patient Experience Data

Data Submitted in the Application

Check if Submitted	Type of Data	Section Where Discussed, if Applicable
☐	Patient-reported outcome	
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
☐	Patient-focused drug development meeting summary	
☐	FDA Patient Listening Session	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
	Other: Reports on infections from multiple data sources (including Observer and clinician reported)	Section 7.1.6.1
☐	**If no patient experience data were submitted by Applicant, indicate here.**	
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting	
☐	FDA Patient Listening Session	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
	Other: Caregiver Written Perspectives	Section 1.2

The BLA also included information on infections. Information on infections was collected from multiple sources including parents of trial participants (observer reported) and clinicians (clinician reported). As infections can be life-threatening in this population, this is meaningful clinical data. Parent provided information on infections increase the robustness of the data.

Although the FDA has not had meetings or listening sessions with the CA patient community, but we did receive letters independent of this BLA review and reviewed the literature that describes caregiver and family burdens associated with DGA. Specifically, families conveyed the challenges of prolonged and frequent hospitalizations, and the emotional and financial impacts for the entire family of living in isolation while awaiting disease modifying therapy. Families describe the care they need to provide as “all

consuming" and state "siblings bear a heavy burden…[and have to] sacrifice so much." Parents have reported needing to quit jobs in order to care for their child and better isolate them from potential infections. This patient experience data further demonstrates that CA is a serious disease that impacts the entire family.

2. Clinical and Regulatory Background

2.1 Disease or Health-Related Condition(s) Studied

CA is a rare disease characterized by profound, life-threatening T cell immunodeficiency due to the absence of a functioning thymus at birth. CA is associated with multiple genetic abnormalities interrupting embryonic thymus development including 22q11.2 deletion, mutations in Forkhead Box Protein N1 (FOXN1), Chromodomain-helicase-DNA-binding protein 7 (CHD7), T Box transcription factor 1 (TBX1) and Paired Box 1 (PAX1). Thymic aplasia has also been reported in infants with diabetic embryopathy and fetal exposure of retinoic acid. CA is extremely rare. The incidence of the most common genetic defect causing CA, 22q11.2 deletion, is estimated at 1:4000 -1:9700 live births. CA accounts for less than 1% of patients with 22q11.2 deletions. Although the overall incidence of CA is not fully established, it is estimated that approximately 20 children are born with CA each year in the United States.

CA is often first identified through newborn screening performed for SCID that demonstrates low or undetectable T cell receptor excision circles (TRECs) in CA. Diagnosis of CA typically includes lymphocyte phenotyping by flow cytometry demonstrating less than 50 naïve T cells/mm^3 or naïve T cells comprising less than 5% of the total T cells. Naïve T cells are identified by cell surface expression of CD45RA and L-selectin (CD62L). CA patients usually have normal number of B cells and natural killer (NK) cells and present with a $T^-B^+K^+$ phenotype. Genetic testing and clinical evaluation for comorbidities including congenital heart disease, hypocalcemia, and CHARGE syndrome (Coloboma, Heart defects, Atresia of the nasal choanae, Retardation of growth and development, Genitourinary anomalies, and Ear anomalies) can assist in identifying the underlying etiology of CA. CA most commonly occurs with cDGA.

The hallmark clinical feature of CA is infections. T cell immunodeficiency in CA leads to an increased susceptibility to bacterial, viral, and fungal infections. Patients with CA may present in the first few months of life with severe, recurrent, and persistent infections. Pneumonias occur at a particularly high rate in these patients. One multicenter survey of patients with CA found that ~ 30% of patients developed pneumonia. Pneumonias can be recurrent and severe, leading to development of chronic lung disease. Gastrointestinal infections are also frequent in this population, which can lead to failure to thrive, malabsorption, and diarrhea. Infections of the urinary tract from *K. pnuemoniae*, *E. faecium*, and echovirus have been reported. Additionally, CA patients may have meningitis, sinusitis, mastoiditis, and thrush. Most importantly, patients with CA experience life-threatening sepsis and life-threatening opportunistic infections, including Cytomegalovirus (CMV), Candida, Pneumocystis Carinii and Human Herpesvirus 6 infections. An unpublished case series of CA patients with cDGA revealed that the most common cause of death in CA patients was related to infection.

Manifestations related to T cell dysregulation have also been reported in CA patients. Extrathymic oligoclonal expansion of T cells have been reported in a subset of CA patients with cDGA, typically atypical cDGA. These cells confer little to no protective immunity and can infiltrate organs causing autologous GVHD. Patients with oligoclonal T cell expansion typically have a characteristic eczematous rash and associated lymphadenopathy. Autoimmune conditions such as hypothyroidism and coombs positive hemolytic anemia have also been reported in CA patients. Prognosis of CA is poor. These patients typically die within the first few years of life with only supportive care.

2.1.1 DiGeorge Anomaly

Complete DiGeorge Anomaly (cDGA) is the most common genetic syndrome associated with CA and accounts for < 1% of DiGeorge anomaly (DGA). DGA is a congenital disease estimated to occur 1 in 4,000 to 6,000 live births; DGA is characterized by defects in organs derived from the third and fourth pharyngeal pouches and the intervening third pharyngeal arch. Consequently, there is dysmorphogenesis of the thymus, thyroid, parathyroids, maxilla, mandible, aortic arch, cardiac outflow tract, and external/middle ear. The most common cardiac defects include interrupted aortic arches, truncus arteriosus, Tetralogy of Fallot, atrial or ventricular septal defects, and vascular rings. Hypocalcemia results from hypoplastic parathyroids and is potentially life-threatening. The thymus may be hypoplastic or completely absent resulting in a range of immune deficiencies. Amongst patients with cDGA, those with CA have the most impaired immune function.

Approximately 90% of those with DGA have a heterozygous chromosomal deletion at 22q11.2. The high incidence of chromosome 22q11.2 microdeletions may be attributed to homologous enrichment of low copy repeats in two areas of this region, which make it prone to homologous recombination deletion errors. The most common genetic deletion associated with DGA is a 1.5 to 3.0 Mb deletion in the 22q11.2 region (DGAI locus). This region of genomic DNA encodes ~30 genes (24 genes within the 1.5 Mb region). Another 2 to 5% of patients have heterozygous deletions in chromosome 10p13-14 (the DGAII locus). Phenotypic comparison of patients with DGAI and DGAII locus deletions demonstrate many similarities, although there is an increased incidence of sensorineural hearing loss in patients with the DGAII locus deletion. There have also been isolated case reports of patients with phenotypic features of DGA and a microdeletion in chromosome 17 or an isochromosome 18q. Patients with the DGA phenotype and the chromosome 22q11.2 deletion are more precisely referred to as having "DGA with chromosome 22q11.2 deletion" and those with other mutations are referred to as having "DGA without chromosome 22q11.2 deletion".

In addition, there are other patients who have no genetic or syndromic abnormalities. DGA has also been found to be associated with the CHARGE syndrome (coloboma, heart defects, choanal atresia, retardation of growth or development, genital hypoplasia, and ear anomalies/ deafness [most with mutations in CHD7]), variants in T-box transcription factor (TBX), and 10p deletion.

A spectrum of thymic abnormalities exists in DGA. The majority of patients have sufficient thymic tissue for the development of functional T cells (partial DGA). These patients have variable and non-life-threatening immunologic defects. T cell numbers and function may range from normal to immunodeficient. Thymic-derived CD25+ Treg are diminished in numbers and may account for the increased incidence of autoimmune and

atopic disease. B cells are usually normal or increased in number and mildly abnormal in function, consistent with defective T cell help. Although total B cell numbers are normal, the proportion of memory B cells is lower in patients with 22q11.2 deletions, especially in older patients. There is an increased prevalence of immunoglobulin A (IgA) deficiency and functional antibody defects (i.e., polysaccharide antibody deficiency). Most patients with partial DGA do not suffer from opportunistic or life-threatening infections although many suffer recurrent sinopulmonary infections. By comparison, thymic tissue is completely absent in ~1-2% of patients with complete DGA. Both T cell numbers and function are highly abnormal with peripheral blood CD3 T cells that are >3 standard deviations below the normal age adjusted range (T cell count <50/mm^3). Response to mitogens is absent or severely diminished. This form of DGA is fatal unless recognized promptly after birth and treated with thymic or bone marrow transplantation.

There are two phenotypes of complete DGA, typical and atypical. The latter is characterized by the presence of rash and lymphadenopathy with a peripheral blood T cell count <50/mm^3. Some patients with typical DGA will switch to an atypical phenotype sometime after birth. The atypical phenotype is characterized by lymphadenopathy, rash due to infiltration of the skin by T cells, circulating oligoclonal T cells and peripheral blood T cell count <50/mm^3. These T cells may proliferate in response to mitogens but are not protective against opportunistic infections. Immunosuppressive therapy with a calcineurin inhibitor is usually started when subjects transform to the atypical phenotype.

The pathogenesis of the thymic abnormalities in DGA patients remains poorly understood. One theory is that the T cell defects are secondary to an insufficient amount of thymic tissue that is otherwise functioning normally. A second, and more likely theory is that the T cell defects in DGA are due to an abnormal anatomic location of the thymus.

2.1.2 FOXN1 Deficiency

CA is also associated with FOXN1 deficiency (also known as nude severe combined immunodeficiency, winged helix deficiency, and alymphoid cystic thymic dysgenesis). FOXN1 deficiency is an exceptionally rare inherited disease with an estimated worldwide incidence <1 in 1,000,000 births. There have been only 10 cases reported in the literature. It is caused by homozygous autosomal recessive loss-of-function mutations in the FOXN1 gene which encodes a transcription factor essential for development of the thymus. Clinical manifestations include athymia, lack of hair, and dysplastic nails. The lack of T cell development in these patients, as in patients with cDGA, render them susceptible to infection and these children die from infection in the first few years of life.

2.2 Currently Available, Pharmacologically Unrelated Treatment(s)/Intervention(s) for the Proposed Indication(s)

Management of CA currently consists of supportive care, prophylaxis and treatment of infections, and management of comorbidities. Similar to other primary immunodeficiencies, as soon as CA is suspected, reverse isolation with air filtering systems such as high efficiency particulate air (HEPA) and positive pressure laminar air flow (LAF), is recommended. If an athymic patient is discharged, isolation and hygiene procedures are instituted at home. Antimicrobial prophylaxis is recommended to prevent bacterial, viral, and fungal infections. B cell function is usually reduced although B cell number is usually normal in CA patients. Therefore, CA patients should receive immunoglobulin replacement. All live vaccines are contraindicated. Blood products for

transfusion should be irradiated to prevent graft vs host disease (GVHD) and tested to ensure they are CMV negative.

CA patients also require management of comorbidities including hypocalcemia and cardiac defects. Ventilators due to underlying congenital heart disease, tracheostomy due to tracheomalacia, central line venous access and feeding tubes for nutritional supplementation may commonly be required.

The only potential curative therapy for CA is a hematopoietic cell transplantation (HCT) from bone marrow or cord blood. This requires the transfer of mature T cells from the donor to the recipient as the recipient does not have a thymus for T cell maturation from stem cells. HCT therapy without CD34 selection (a T cell replete implantation) may provide benefit in patients with CA, particularly in limited instances where the patient has a significant viral infection and access to a sibling's HLA matched cells. However, the quality of long-term immune reconstitution achieved with HCT is generally poor as the T cell receptor (TCR) repertoire that is transplanted is limited. Over time, with the death of CD4 T cells (because CD4 numbers depend on thymus production) and expansion of CD8 T cells due to viral infections, the T cell repertoire diminishes and the CD4 to CD8 ratio becomes markedly inverted. Consequently, immune reconstitution with HCT is characterized by circulating T cells that exhibit a memory phenotype, a restricted repertoire, no evidence of naïve T cell development and a lack of T-cell receptor excision circles (TRECs). Overall, survival rate with HCT is low. Accordingly, T cell immunodeficiency associated with CA is a condition with a high unmet medical need and no effective therapeutic options.

2.3 Safety and Efficacy of Pharmacologically Related Products

Not applicable. RETHYMIC is a first-in-class biologic product.

2.4 Previous Human Experience with the Product (Including Foreign Experience)

The product is not approved in any country. No foreign clinical data were submitted in the Biologics License Application (BLA).

2.5 Summary of Pre- and Post-submission Regulatory Activity Related to the Submission

Major regulatory milestones for the BLA are summarized in Table 2.

Table 2. Summary of Pre- and Post-submission Regulatory Activity

Date	Milestones
05/18/2001	IND 9836 submission
08/15/2003	Orphan Drug designation granted to RETHYMIC
04/14/2017	Breakthrough Therapy designation and Regenerative Medicine Advanced Therapy designation granted
05/01/2017	End-of-Phase 3 meeting
08/25/2017	Rare Pediatric Disease designation granted
10/30/2017	Orphan Drug designation transferred to Enzyvant Therapeutics GmbH
11/06/2017	Pre-BLA meeting
04/05/2019	BLA 125685 submission
06/04/2019	BLA filed, priority review
07/26/2019	120-day safety update
12/04/2019	PDUFA action: Complete Response due to CMC deficiencies
03/19/2020	Type A meeting to discuss deficiencies noted in complete response letter
04/09/2021	BLA resubmission
10/08/2021	PDUFA Action Date

(Source: Reviewer's table)
BLA, Biologics License Application; CMC, Chemistry, Manufacturing, and Controls; IND, Investigational New Drug application; PDUFA, Prescription Drug User Fee Act; RMAT, Regenerative Medicine Advanced Therapy

3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

3.1 Submission Quality and Completeness

The BLA submission was adequately organized and integrated to accommodate the conduct of a complete clinical review without unreasonable difficulty.

3.2 Compliance with Good Clinical Practices and Submission Integrity

Clinical trials were conducted in the United States under IND 9836, in accordance with the regulations specified in 21 CFR 312, and were compliant with Good Clinical Practice (GCP) international ethical and scientific quality standards for the design, conduct, recording, and reporting of clinical trials involving human subjects. The clinical trials included provisions for informed consent by all study subjects, and for ethical treatment of study subjects.

A Bioresearch Monitoring (BIMO) inspection of three clinical trials conducted by the IND sponsor did not reveal substantive problems that impact the data submitted in the application.

Table 3. BIMO Inspection Summary

Protocol	Location	FDA Form 483 Issued?	Inspection Classification
668-1	Duke University Health System Room 109 B Research Park IV Durham, North Carolina 27710	No	NAI = No Action Indicated
668-2	Duke University Health System Room 109 B Research Park IV Durham, North Carolina 27710	No	NAI = No Action Indicated
25966	Duke University Health System Room 109 B Research Park IV Durham, North Carolina 27710	No	NAI = No Action Indicated

(Source: BIMO inspection review memo)

3.3 Financial Disclosures

Covered clinical study:

- Study 668-1: Thymic implantation in complete DiGeorge syndrome.
- Study 668-2: Phase II study of thymus implantation in complete DiGeorge syndrome.
- Study 884: Thymus implantation with immunosuppression (includes data from a single subject enrolled in Study 884-1).
- Study 931: Parathyroid and thymus transplants in DiGeorge syndrome.
- Study 932: Dose study of thymus implantation in DiGeorge anomaly.
- Study 950: Phase I/II trial of thymus implantation with immuno-suppression (includes data from a single subject enrolled in 950-1).
- Study 25966: Safety and efficacy of thymus implantation in complete DiGeorge anomaly.
- Study (b) (6): Single subject treatment plan: Thymus implantation for Epstein-Barr virus (EBV) lymphoma.
- Study 51692: Expanded access protocol. Thymus implantation for immunodeficiency, hematologic malignancies, and autoimmune disease related to poor thymic function.
- Study 735: Thymic implantation in partial DiGeorge syndrome

Was a list of clinical investigators provided? x Yes ☐ No (Request list from applicant)

Total number of investigators identified: One principal investigator and 16 sub-investigators

Number of investigators who are sponsor employees (including both full-time and part-time employees): 0

Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): One (M. Louise Market, MD, PhD)

If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)):

Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Payments for the following

Upfront licensing (b) (4)

BLA submission (b) (4)

BLA approval) (b) (4)

Significant payments of other sorts:

Sale of priority review voucher: (b) (4)

Support for Duke clinical thymus transplant program: (b) (4) /year until commercial launch and then (b) (4) year until Dec 31, 2019

Support for Dr Markert's laboratory: (b) (4)

Proprietary interest in the product tested held by investigator: No

Significant equity interest held by investigator in sponsor of covered study: No

Is an attachment provided with details of the disclosable financial interests/arrangements? ☐ Yes ☐ No (Request details from applicant)

Is a description of the steps taken to minimize potential bias provided? ☐ Yes ☐ No (Request information from applicant)

Number of investigators with certification of due diligence (Form FDA 3454, box 3): 0

Is an attachment provided with the reason? ☐ Yes ☐ No (Request explanation from applicant)

Reviewer's comments: M. Louise Market, MD, PhD has received financial support from Enzyvant for her laboratory and will receive compensation upon BLA approval. This creates a financial conflict of interest as defined by 42 CFR part 50 and 45 CFR part 94, as the principal investigator has a significant financial interest in Enzyvant. Nonetheless, the clinical team does not believe that this compromises data integrity, particularly as BIMO inspections have not identified data integrity issues. Additionally, Dr. Markert did not have a financial interest when the early studies were conducted, and the treatment effect in CA is similar between the subjects enrolled in the earlier studies and those enrolled more recently when she was receiving funding from Enzyvant.

4. SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES

4.1 Chemistry, Manufacturing, and Controls

RETHYMIC is an allogeneic processed postnatal thymus tissue derived product. Thymus tissue is obtained as discarded tissue from unrelated donors less than or equal to 9 months of age undergoing cardiac surgery. This thymus tissue is aseptically processed and cultured under cGMP conditions to produce partially T cell-depleted thymus tissue slices.

The steps in the manufacturing of the RETHYMIC thymus tissue product were as follows: (b) (4) The physical and chemical characteristics of RETHYMIC drug substance (after 12-21 days in culture) are listed in Table 4.

Table 4. Physical and Chemical Characteristics of RETHYMIC Drug Substance

Appearance:	Yellow to brown slices of tissue with varying thickness and shape
Histology:	(b) (4) • The global overall histology assessment of the tissue is acceptable

(Source: Applicant's table)

Over the course of 28 years, manufacturing changes were introduced, test methods were changed, specifications were modified (and in some cases acceptance criteria widened during development), product sampling points were broadened, the manufacturing facility was changed, and for the commercial process, additional manufacturing flexibility was proposed.

The early products were manufactured in (b) (4) In 2016, the product began being manufactured at the intended commercial manufacturing facility, (b) (4) in the (b) (4) at (b) (4) At the time of the initial BLA, there was limited clinical data from the (b) (4) facility. To address the CMC CR issues, additional manufacturing processes were implanted at the (b) (4) facility.

Reviewer's comments: A complete response letter to the initial BLA submission was issued on December 4, 2019 citing several CMC deficiencies. The sponsor has adequately addressed the CMC deficiencies in this resubmission. Please see the CMC review for details.

Clinical data were pooled from all subjects who were treated with products manufactured by the (b) (4) or (b) (4) facilities. Most subjects (n=87, 83%) were treated with products manufactured by the (b) (4) facility. Additional clinical data on subjects treated with products manufactured by the (b) (4) facility were submitted in this resubmission including

longer follow up of previously treated subjects and 5 newly treated subjects after original submission. Together, clinical data from 18 subjects (17%) treated with products manufactured by the (b) (4) *facility were available. Subgroup analyses were performed to confirm that safety and efficacy were comparable between subjects treated with RETHYMIC manufactured by the* (b) (4) *and* (b) (4) *facilities (please see Section 7.1.7.8 for details).*

4.2 Assay Validation

Please see the CMC review.

4.3 Nonclinical Pharmacology/Toxicology

No significant safety or effectiveness issues were identified by the Pharmacology/Toxicology reviewer. Please see the Pharmacology/Toxicology review for details.

4.4 Clinical Pharmacology

There were no Clinical Pharmacology data submitted with this application.

4.4.1 Mechanism of Action

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 that are tolerant of both donor and recipient cells/tissues. Thymic epithelial (TE) cells are thought to be responsible for the recruitment of common lymphoid progenitors to the implanted RETHYMIC slices, and they shepherd the development of thymocytes into mature T cells. Tolerance to self (recipient) is thought to be achieved through the migration of recipient dendritic cells to the RETHYMIC slices, where they educate developing thymocytes to be tolerant to recipient tissues. In addition, donor TE cells remaining in the RETHYMIC slices teach tolerance to the donor tissue. RETHYMIC is thought to function as would a normal endogenous thymus with appropriate positive and negative selection of T cells, and therefore enables immune reconstitution.

4.4.2 Human Pharmacodynamics (PD)

The pharmacodynamic effects of RETHYMIC are tied to the mechanism of action and the treatment effect of RETHYMIC was evaluated in clinical trials as discussed elsewhere in this review.

4.4.3 Human Pharmacokinetics (PK)

The pharmacokinetic effects of RETHYMIC are not known; however, given the product type, PK data are not applicable.

4.5 Statistical

The statistical reviewer confirmed the statistical analysis. Please see the statistical review memo for details.

4.6 Pharmacovigilance

The reviewed safety data do not warrant a Risk Evaluation and Mitigation Strategies (REMS), a safety post-marketing requirement (PMR) study, or a safety post-marketing commitment (PMC) study. The post-marketing risk mitigation plans include product labeling, applicant's pharmacy and surgical training, routine pharmacovigilance plan.

The applicant proposes a voluntary, observational, registry study of 75 patients treated with RETHYMIC for CA. The proposed primary endpoints are survival and immune reconstitution. The impact of reconstitution markers, total and naïve CD3, CD4, and CD8 count individually and in combination, and timing of reconstitution on survival will be explored. Additionally, the occurrence and timing of adverse events of special interest (AESI) post treatment will be evaluated as secondary outcome measures.

5. SOURCES OF CLINICAL DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

5.1 Review Strategy

The original BLA application received a complete response letter due to CMC deficiencies. Dr. Tang, the clinical reviewer for the original BLA submission, recommended approval from the clinical perspective. In this resubmission, the applicant provided an updated summary of safety and efficacy and upon request, updated clinical datasets with data cutoff through April 30, 2021 (submitted on June 4, 2021) and a separate dataset of natural history (NH) with subject-level data. Individual clinical study reports (CSR) were assessed during the original BLA review and no updated CSRs were provided in this resubmission (Please refer to Dr. Tang's clinical review memo for details). Therefore, this review focuses on the integrated analyses of safety and efficacy based on the updated datasets provided in resubmission. Additionally, exploratory analyses were done by the clinical review team to ensure that the outcomes data in the new manufacturing process (b) (4) compared favorably to the original product (b) (4) safety and efficacy outcome data.

5.2 BLA/IND Documents That Serve as the Basis for the Clinical Review

Source documents for this review are: 1) documents filed under original application and resubmission of BLA 125685, and 2) documents under IND 9836 including meeting minutes, correspondence between the FDA and the applicant, serious adverse event (SAE) reports and study protocols.

5.3 Table of Studies/Clinical Trials

Table 5. Summary of Clinical Studies

Study	Objectives	Treatment Groups	Population	FAS	EAS
668-1	To assess the safety of thymus implantation	Subjects with typical cDGA received no immunosuppression Subjects with elevated T cell function received immunosuppression under individual treatment plan	SCID: 1 FOXN1: 1 cDGA: 24	14	14
668-2	To assess safety and efficacy as determined by survival; to assess thymopoiesis in the thymus graft and reconstitution of T cell function by flow cytometry and PCR			12	11

Study	Objectives	Treatment Groups	Population	FAS	EAS
884	To assess safety, tolerability, and efficacy of thymus transplant with immunosuppression	Subjects with typical cDGA with elevated T cell function and atypical cDGA received immunosuppression with RATGAM and cyclosporine	FOXN1: 1 cDGA: 11	12	11
931	To assess thymus tissue and parental parathyroid implantation	Subjects with typical and atypical cDGA and with hypoparathyroidism requiring calcium supplementation. Subjects received 1 of 2 immunosuppression regimens: Group 1: RATGAM Group 2: RATGAM plus cyclosporine (or tacrolimus) Subjects were also elig ble to receive a single parental parathyroid transplant. Four out of 5 received parathyroid transplants.	cDGA: 5	5	5
932	To evaluate correlations between dose of thymus tissue transplanted and immunological outcomes after transplant	Subjects with typical cDGA without immunosuppression	SCID: 1 cDGA: 6	7	6
950	Evaluate the safety and toxicity of thymus implantation with immune-suppression tailored to subject immune status	Subjects with typical or atypical cDGA and with varying PHA responses pre-implantation Group 1: subjects with typical cDGA with PHA response < 50,000 cpm but > 5,000 cpm or > 20-fold over background. These subjects received RATGAM. Group 2: subjects with typical cDGA with PHA response > 50,000 cpm or atypical cDGA with PHA response < 75,000 cpm when not on immunosuppression or a PHA response < 40,000 cpm when on immunosuppression. The subjects received RATGAM plus cyclosporine (or tacrolimus). Group 3: subjects with atypical cDGA with PHA response > 75,000 cpm when not on immunosuppression or PHA response > 40,000 cpm with immunosuppression. Subjects received RATGAM plus cyclosporine (or tacrolimus) and steroids.	cDGA: 14 Other: 1	15	14

Study	Objectives	Treatment Groups	Population	FAS	EAS
25966	To evaluate survival and the effect on immune function of thymus implantation with immunosuppression regimens tailored to the subject's immune status	Subjects with typical or atypical cDGA in 4 groups based on immune status and peri-implantation immunosuppression regimen: Group 1: Subjects with typical cDGA (PHA response < 5,000 cpm) - No immunosuppression Group 2: Subjects with typical cDGA (PHA response > 5,000 cpm but < 50,000 cpm) - RATGAM alone Group 3: Subjects with typical (pre-treatment PHA response > 50,000 cpm) or atypical cDGA (PHA response < 40,000 cpm on immunosuppression or < 75,000 cpm without immunosuppression) - RATGAM plus cyclosporine (or tacrolimus Group 4: Subjects with atypical cDGA (PHA response > 40,000 cpm on immunosuppression or > 75,000 cpm without immunosuppression) or maternal engraftment: RATGAM plus cyclosporine (or tacrolimus) plus basiliximab plus mycophenolate (Ongoing study: Subjects transplanted through 29 May 2020 with available data through April 30, 2021 were included in the analysis)	cDGA: 28	28	28
51962	Expanded access use in other conditions (hematologic malignancy, immunodeficiency, severe autoimmune disease related to poor thymic function, prior HCT)	Subjects with immunodeficiency, hematologic malignancies, or severe autoimmune disease related to poor thymic function. Adjunctive therapies (including bone marrow implantation with myeloablation, chemotherapy for malignancy, and cytotoxic lymphocyte infusions for viral infections) were allowed. Subjects received immunosuppression depending on their immune status. This is an ongoing study: Subjects transplanted through 29 May 2020 with available data through April 30, 2021 were included in the analysis.	cDGA: 8 FOXN1: 1 Other: 1	10	6
(b) (6)	Thymus Implantation for EBV Lymphoma	Subject with cDGA who had previously received infusions of peripheral blood mononuclear cells from a HLA-identical sibling.	cDGA: 1	1	0
735	Treatment of subjects with partial DGS	Subjects with partial DGS with prolonged significant T cell dysfunction and infections were treated with RETHYMIC implantation under immunosuppression.	Partial DGA: 1	1	0
NH	Retrospective natural history cohort	Supportive care only	cDGA: 49	N/A	N/A

(Adapted from Table 2-1 in Safety and Efficacy Clinical Update) Abbreviations: BLA = Biologics License Application; BM = bone marrow; BSA = body surface area; cDGA = complete DiGeorge anomaly; cpm = counts per minute; DGS = DiGeorge syndrome; EAS = efficacy analysis set; FAS = full analysis set; GVHD = graft versus host disease; HLA = human leukocyte antigen, PCR = polymerase chain reaction; PHA = phytohemagglutinin; RATGAM = rabbit anti-thymocyte globulin; SCID = severe combined immunodeficiency.

5.4 Consultations

5.4.1 Advisory Committee Meeting (if applicable)

No Advisory Committee meeting was held because initial review of information submitted in the BLA did not raise concerns or controversial issues that would have benefited from an advisory committee discussion.

5.4.2 External Consults/Collaborations

No external consultation was requested for the completion of clinical review.

5.5 Literature Reviewed (if applicable)

During review of the BLA, this reviewer consulted FDA regulatory guidance documents, as well as academic literature, for background and context regarding the targeted disease and the mechanism of action of the product. The literature consulted is provided in References.

6. DISCUSSION OF INDIVIDUAL STUDIES/CLINICAL TRIALS

The RETHYMIC clinical development program for the treatment of T cell immuno-deficiency resulting from CA has been ongoing at Duke University Medical Center for approximately 30 years. The clinical program consisted of 10 studies. The primary clinical efficacy data included in this BLA are derived from 7 single-center, open-label, non-randomized clinical studies in subjects with CA. Additional data to primarily support safety came from expanded access studies, including 2 single-patient studies. Please see section 5.3 for the relative contribution of subjects to the EAS and FAS for each of these studies.

In addition, the sponsor submitted a retrospective case series of subjects with CA associated cDGA who received only supportive care. This case series is the largest NH cohort of CA and serves as an external comparator for the RETHYMIC clinical trials.

- Core clinical studies:
    - IND 9836 Studies
        - Study 668-1: Thymic implantation in complete DiGeorge syndrome.
        - Study 668-2: Phase II study of thymus implantation in complete DiGeorge syndrome.
        - Study 884: Thymus implantation with immunosuppression (includes data from a single subject enrolled in Study 884-1).
        - Study 931: Parathyroid and thymus transplants in DiGeorge syndrome.
        - Study 932: Dose study of thymus implantation in DiGeorge anomaly.
        - Study 950: Phase I/II trial of thymus implantation with immunosuppression (includes data from a single subject enrolled in 950-1).
        - Study 25966: Safety and efficacy of thymus implantation in complete DiGeorge anomaly.
- Additional clinical studies:

  - IND 9836 Study
    - Study 51692: Expanded access protocol. Thymus implantation for immunodeficiency, hematologic malignancies, and autoimmune disease related to poor thymic function.
    - Study (b) (6): Single subject treatment plan: Thymus implantation for Epstein-Barr virus (EBV) lymphoma.
  - Study 735: Thymic implantation in partial DiGeorge syndrome
- Natural history cohort: retrospective case series of individuals with cDGA receiving supportive care only

Given the similarities in study design of the core studies, only the clinical protocol for Study 25966 is discussed below in detail in section 6.1. Study 25966 is the most recently conducted study and enrolled the most subjects (n=28). Please see section 5.3 for differences in enrolled population for the other studies. Table 6 summarizes the endpoints in the core clinical studies.

Study 931 differed from the other studies in that (4/5) subjects received parathyroid tissue from a parent immediately prior to RETHYMIC transplant. The study was otherwise similar to the other core studies.

Table 6. Summary Primary and Secondary Endpoints in Individual Clinical Trials

	668[a]	884	931	932	950	25966
Primary Endpoints						
Survival at Year 1	X	X	X	X	X	X
Survival at Year 2	X	X	X	X	X	X
Secondary Endpoints (at Year 1 and Year 2)						
CD3 cells	X	X		X	X	X
CD4 cells	X	X	X	X	X	
CD8 cells	X	X	X	X	X	
Naïve CD4 cells	X	X	X	X	X	X
Naïve CD8 cells	X	X	X	X	X	
Total TCRαβ cells						X
Total TCRγδ cells						X
Total B cells						X
Total NK cells						X
Proliferative T cell responses to mitogens, and antigens, including the following: PHA, ConA, sol CD3, immob CD3, TT, and *Candida*	X	X	X	X	X	X
Anti-tetanus toxoid antibody				X		
TCR repertoire variability	X	X	X	X	X	X
TREC/TREG[b]		X	X	X	X	X
Biopsy of implanted thymus	X	X	X	X	X	X

ConA = concanavalin A; immob = immobilized sol = soluble; NK =natural killer; PHA = phytohemagglutinin; TCR = T cell receptor; TT = tetanus toxoid; TREC = T cell receptor rearrangement excision circles; TREG = regulatory T cells
[a]Study 668 was initially opened as a Phase 1 study. In 2001, the study was amended to form a Phase 2 study. The initial Phase 1 study is Study 668-1 and the later Phase 2 study is Study 668-2.
[b]Summaries of TREC/TREG were done for trials where data were present in the database.

(Source: Applicant's Table, BLA 125685/003)

For a detailed summary of each clinical study protocol, please refer to Dr. Tang's clinical review.

6.1 Study 25966: Safety and Efficacy of Thymus Transplantation in Complete DiGeorge Anomaly

6.1.1 Objectives (Primary, Secondary, etc.)

The objectives of this study, as described in the study protocol, were to assess survival, naïve CD4 T cell counts, and the effect of thymus graft dose on naïve CD4 T cell counts at 1 year after transplantation in subjects with cDGA.

6.1.2 Design Overview

This is an ongoing Phase 1/2, single-site, open-label, non-randomized study conducted at Duke University Medical Center (DUMC). The study included subjects diagnosed as having the typical or atypical phenotype of cDGA. The initial institutional review board (IRB) approval was December 2010.

In this clinical study, enrolled subjects received RETHYMIC during a single surgical procedure. Subjects received appropriate immunosuppressive therapy prior to and after transplantation as pre-specified in the protocol based on baseline characteristics. Protocol-specified assessments were conducted for 2 years after transplantation, after which any follow-up assessments were to be performed by the subject's referring pediatric immunologist as part of routine medical care.

6.1.3 Population

Inclusion criteria

Male or female subjects of any age who met the following criteria were eligible to be included in the study:

1. A parent or guardian of the cDGA subject signed the consent form.
2. Medical screening was completed.
3. For a diagnosis of DiGeorge Anomaly (DGA), the subject had to have 1 of the following:
    - Congenital heart disease
    - Hypocalcemia requiring replacement
    - 22q11.2 hemizygosity or 10p13 hemizygosity
    - CHARGE association or CHD7 mutation
    - A subject with abnormal ears whose mother had diabetes (type I, type II, or gestational) had this risk factor recorded, but it was not sufficient to make the diagnosis of cDGA.
4. To meet criteria of typical cDGA, the subject had to have circulating CD3+ CD45RA+ CD62L+ T cell count < 50/mm3 or < 5% of the total T cell count.

    The phenotypic evaluation of T cells was done by flow cytometry, which was performed twice; the 2 studies had to show similar immunological findings for a subject to qualify. One assay had to be done within 3 months of transplantation, and 1 assay had to be within 1 month of transplantation.

    Phytohemagglutinin (PHA) proliferative response:
    - For Group 1: T cell proliferative response to PHA of < 5,000 cpm or < 20-fold above background.

- For Group 2: T cell proliferative response to PHA of > 5,000 cpm and < 50,000 cpm or > 20-fold over background.
- For Group 3: T cell proliferative response to PHA of > 50,000 cpm.

For all 3 groups, 2 assays of T cell numbers and PHA responses had to show similar immunological findings (e.g., both had to meet naïve T cell criteria) for a subject to qualify for this study. One assay had to be done within 3 months of transplantation and 1 assay had to be done within 1 month of transplantation. The latter assay was used to assign the subject to a group.

Optional tests in typical cDGA subjects:
T cell receptor rearrangement excision circles (TRECs) were not routinely assayed prior to transplantation in subjects; however, if done as part of newborn screening for SCID, TRECs were recorded.

5. To meet criteria of atypical cDGA:

The subject had a rash at screening or had previously had a rash. If a rash was present, a biopsy of the rash had to show T cells in the skin. If the rash and adenopathy had resolved, the subject had to still have > 50/mm^3 T cells and the naive T cell (CD45RA+ CD62L+ CD3+ T cells) count had to be < 50/mm^3 or < 5% of the total T cell population.

PHA proliferative response:
- Group 3: the PHA proliferative response had to be < 40,000 cpm on immunosuppression or < 75,000 cpm off immunosuppression.
- Group 4: the PHA proliferative response had to be > 40,000 cpm on immunosuppression or > 75,000 cpm off immunosuppression.

The assay had to be done twice. One assay had to be done within 3 months of transplantation and the 1 assay had to be done within 1 month of transplantation. The last assay was used to assign the subject to a group.

Circulating CD3+ T cells:
Circulating CD3+ T cells were expected to be > 50/mm3, but CD45RA+ CD62L+ CD3+ T cells had to be < 50/mm^3 or < 5% of the total CD3 count.
The phenotypic evaluation of T cells was done twice by flow cytometry. One assay had to be done within 3 months of transplantation and 1 assay had to be done within 1 month of transplantation.

Flow cytometry:
Flow cytometry examines T cell receptor variable beta (TCRVβ) repertoire. For this assay, which had to be performed once, there was no inclusion requirement regarding the results. A second assay could have been performed per Sponsor/Investigator discretion. This was to be done prior to transplantation if there were sufficient T cells to allow this to be done with the blood volumes permitted for research.

If there was an increase in T cell numbers or activation status, this assay was to be repeated at the discretion of the Investigator.

TRECs:
There was no requirement for a TREC assay or results from this assay. TRECs were assayed prior to transplantation in atypical subjects, if there were sufficient T cells to allow this to be done with the blood volumes permitted for research. If the TREC assay was done prior to transplantation, it was anticipated the subject would have TRECs less than 100 per 100,000 CD3+ cells. However, this was not a criterion for the protocol. A TREC assay done for newborn screening for SCID was acceptable.

Exclusion criteria
Potential subjects who met the following criteria were excluded from the study:

1. Heart surgery performed less than 4 weeks prior to projected transplantation date.
2. Heart surgery anticipated within 3 months after the proposed time of transplantation.
3. Rejection by the surgeon or anesthesiologist as surgical candidate.
4. Lack of sufficient muscle tissue to accept a transplant of 4 g/m2 body surface area (BSA).
5. Human immunodeficiency virus (HIV) infection.
6. Prior attempts at immune reconstitution, such as bone marrow transplantation or previous thymus transplantation.
7. CMV infection: For Groups 2, 3, and 4, CMV infection documented by > 500 copies/mL in blood by polymerase chain reaction (PCR) on 2 consecutive assays or by 2 positive urine cultures.
8. Ventilator support or positive pressure support such as continuous positive airway pressure (CPAP) or bi-level positive airway pressure (BiPAP) support for a condition that was deemed by the Investigator to be severe or irreversible or which renders the subject too clinically unstable to undergo the procedures.

6.1.4 Study Treatments or Agents Mandated by the Protocol

Pre-implantation:
If subjects had measurable T cell function (phytohemagglutinin [PHA] responses > 5,000 cpm) during screening, the plan was to deplete the T cells with thymoglobulin (Rabbit anti-thymocyte globulin [RATGAM]) and then perform allogeneic cultured postnatal thymus tissue product (RETHYMIC) transplantation. If autoantibodies were detected, the B cells were to be depleted with rituximab.

Pre-transplantation screening procedures were generally conducted over a period of approximately 2 to 12 weeks prior to transplantation; however, some subject information was collected up to 6 months prior to RVT-802 transplantation. Subject screening was done on an in-patient or outpatient basis, depending on the medical stability of the subject. Subjects were kept in reverse isolation and cared for by family or staff familiar with reverse isolation procedures. Subjects were not allowed to receive live vaccinations (for at least 2 years post-transplantation).

Subjects were treated with 1 of 4 different immunosuppression regimens depending on cDGA phenotype and T cell proliferative response to PHA pre-transplantation. The groups were as follows:

- Group 1: Subjects with typical cDGA (PHA response < 5,000 cpm); No immunosuppression
- Group 2: Subjects with typical cDGA (PHA response > 5,000 cpm but < 50,000 cpm); RATGAM alone
- Group 3: Subjects with typical (pre-treatment PHA response > 50,000 cpm) or atypical cDGA (PHA response < 40,000 cpm on immunosuppression or < 75,000 cpm without immunosuppression); RATGAM plus cyclosporine (or tacrolimus)
- Group 4: Subjects with atypical cDGA (PHA response > 40,000 cpm on immunosuppression or > 75,000 cpm without immunosuppression) or maternal engraftment; RATGAM plus cyclosporine (or tacrolimus) plus basiliximab and/or mycophenylate.

RETHYMIC Implantation:
All subjects received a single treatment with RETHYMIC. RETHYMIC slices were placed into the subject's quadriceps muscles by a pediatric surgeon in an open procedure under general anesthesia.

As specified in the protocol, the dose planned for transplantation was the number of grams of transplanted tissue divided by the weight of the infant in kilograms or per square meter of BSA of the infant. The minimum planned dose of RETHYMIC was 4 g/m^2 and the maximum dose was 18 g/m^2 of subject BSA. In 2015, the Investigational New Drug (IND) was updated to define a dose range of (b) (4) – 20,000 mm^2 of thymus tissue per recipient BSA in m^2 (IND 9836 Amendment Serial Number 0209). Based on the 2015 IND amendment, dosing was reported in mm^2/m^2.

6.1.5 Surveillance/Monitoring

After RETHYMIC implantation, subjects were followed for 24 months. Until January 2016, a thymus allograft biopsy was obtained in medically stable subjects approximately 2 months post-transplantation. The biopsied thymus tissue was examined for evidence of thymopoiesis and graft rejection by immunohistochemical staining. After January 2016 thymus allograft biopsies were not performed as subjects returned to their referring institution sooner.

Following thymus biopsy (~2 to 3 months post-transplantation), most subjects were discharged to home/referring medical center (medical care provided by referring pediatric immunologist), unless medically unstable. After January 2016, subjects returned to the care of the referring medical center approximately 1 to 2 weeks after transplantation or when they were stable enough to travel. After transfer back to the care of the referring pediatric immunologist, the subject was monitored for adverse events (AEs) by requesting information from the referring physician per the assessment schedule. Requests were also submitted to the referring pediatric immunologist for follow-up testing/blood samples per the protocol schedule of events; however, obtaining the testing/blood samples were dependent upon the parent(s), the referring/local physicians, and the subject's medical condition.

6.1.6 Endpoints and Criteria for Study Success

Efficacy
The primary efficacy endpoint as defined in the Statistical Analysis Plan (SAP) was survival at Year 1 and Year 2 post-transplantation.

As data permitted, secondary efficacy endpoints at Month 6, Year 1 and Year 2 post-transplantation included the following:

- Total CD3, CD4, and CD8 T cell counts
- Total naïve CD3, CD4, and CD8 T cell counts
- Proliferative T cell responses to stimulants including PHA, concanavalin A (ConA), soluble (Sol) CD3, immobilized CD3 (Immob CD3), tetanus toxoid, and Candida skin test antigen
- T cell receptor variable beta (TCRV) repertoire variability (as assessed by flow cytometry)
- TREC
- T regulatory cells (TREG)
- RETHYMIC biopsy evaluation.

Data on other flow cytometry parameters (double negative [CD4-CD8-; DB Neg], TCRa, TCRyo, B, and natural killer [NK] cells), serum immunoglobulins, isohemagglutinins, and B cell antibody responses to antigens were also collected as data permitted.

Safety

Safety assessments (AEs, laboratory evaluations, vital signs measurements, and physical examinations) were assessed according to the protocol schedule of events for 2 years following transplantation.

6.1.7 Statistical Considerations & Statistical Analysis Plan

Given the legacy data status and similarities across the clinical studies and given that the overall measure of efficacy of RETHYMIC is subject survival, the analyses of all RETHYMIC studies planned for inclusion in the Biologics License Application (BLA) were encompassed by a single SAP and were adapted to place focus on the common primary endpoint of subject survival. While the study objectives as stated in the protocol and this clinical study interim report are reflected in the analyses conducted for this study, the endpoints specified in the program wide SAP were given precedence over the analyses initially planned in the protocol.

Summary statistics (e.g., number of observations, mean, standard deviation [SD], median, quartiles, and ranges) were presented for continuous variables, and frequencies and percentages were presented for categorical variables. All statistical analyses were performed in SAS version 9.4 on a validated platform. Subjects who received RETHYMIC in this study were included in the efficacy and safety analyses.

For primary efficacy endpoints, survival at Year 1 and Year 2 > 50% was tested using the binomial exact test. Summary of Kaplan-Meier survival was calculated. Kaplan-Meier estimates of survival at Years 1, 2, 3, 4, and 5 post-transplantation, were presented with number at risk, number with events, and estimated survival probability.

Summary statistics of the secondary efficacy endpoints were calculated at baseline, Month 6, Year 1, and Year 2 post-transplantation. Summary statistics including the change from baseline to Month 6, Year 1 and Year 2 were calculated.

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

Safety assessments were collected for at least 2 years post-transplantation. Summaries of safety events included events happening within 2 years of transplantation. The number of AEs, number of subjects in whom AEs occurred, and the percentage of occurrence (%) were tabulated by system organ class (SOC), and by preferred term (PT). All reported events, regardless of time of onset, were included in the listings.

Analysis Populations:
All subjects who received RETHYMIC were included in the FAS. The EAS included all subjects with CA, who had no prior HCT. An EAS-cDGA analysis set included all EAS subjects except those that had FOXN1 deficiency.

6.1.8 Efficacy Analyses

Please refer to integrated review of efficacy, Section 7.

6.1.9 Safety Analyses

Please refer to integrated review of safety, Section 8.

6.2 Natural History Cohort

The Natural History (NH) data consist of a retrospective case series of 49 subjects collected between 1991 and 2017. The data are limited to date of birth, sex, race (available for 24/59 subjects) and cause of death (See Table 7). The exact dates of death were available for all but one subject (b) (6) whose date of death was estimated by the referring physician. All patients in the NH dataset had cDGA, which is consistent with this diagnosis being the most common etiology of CA and referral to Dr. Markert, who compiled the NH dataset. There were no imputation methods or censoring used for the natural history data.

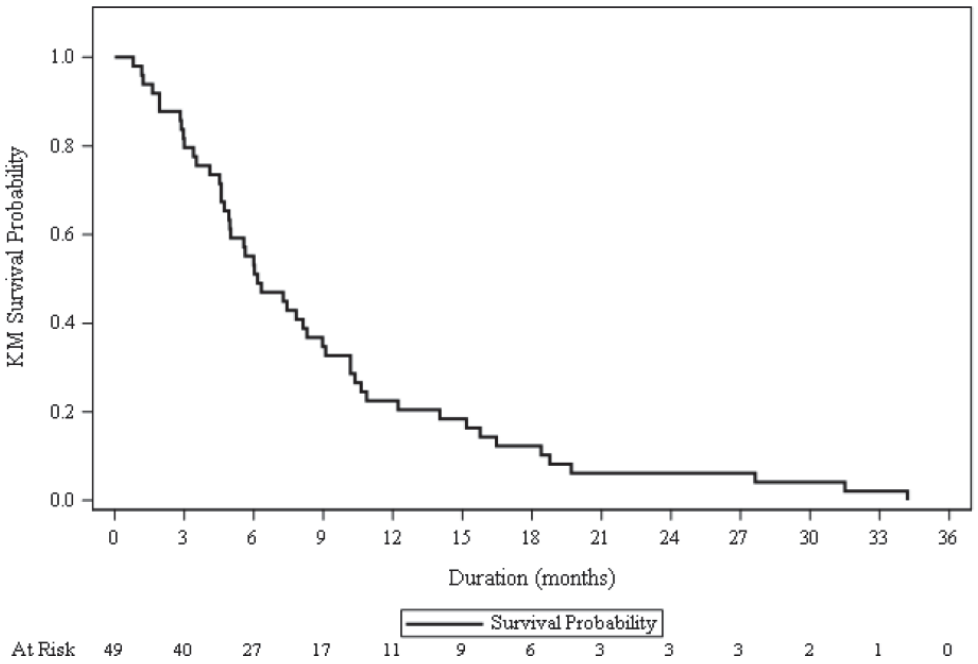
Table 7. Natural History Cohort Demographics and Disposition

		Number of Patients (n=49)	Percentage
Sex	Female	16	32.7%
	Male	33	67.3%
Race	Asian	1	2% (4.2% of available)
	Black or African American	2	4.1% (8.3% of available)
	White	21	42.9% (87.5% of available)
	Not Reported	25	51%
Diagnosis	cDGA	49	100%
Cause of Death	Non-infection	17	34.7%
	Infection Related	26	53.1%
	Unknown	6	12.2%

(Source: Reviewer's table based on Applicant amendment including natural history data)

As shown in Figure 1, One-year survival of cDGA patients was 22.4% and all patients died by 3 years of age. The median age of death was 185 days (6 months) with a range of 24 days to 34 months.

Figure1. Kaplan-Meier Survival of NH Cohort



(Source: BLA 125685/003 Summary of Clinical Efficacy)

Reviewer's comments: Data from the NH cohort is limited to only a few baseline covariates. For example, race information is missing for half of the subjects. Most importantly, aside from being diagnosed with cDGA, there is no information on phenotypes of cDGA, e.g. typical vs atypical cDGA, underlying genetic defects, co-morbidities, or supportive care received. While age at death is captured for each patient, clinical course (e.g., number or infections and severe infections) is not captured. Additionally, there is some missing information on exact cause of death.

It is advantageous that the natural history patients are contemporaneous to the RETHYMIC treated patients, suggesting that they received the same standard of care. However, there is a possibility that there may be selection bias between the subjects who were treated with RETHYMIC and those in the natural history cohort. However, this is unlikely to impact the interpretability of the data given the large size of the natural history database for this rare disease. Additionally, data from the medical literature supports the conclusion of this NH study, patients with CA die in the first few years of life.

7. INTEGRATED OVERVIEW OF EFFICACY

7.1 Indication #1

RETHYMIC is indicated for thymic implantation to support immune reconstitution in patients with primary immunodeficiency resulting from CA.

Reviewer's Comment: As only pediatric patients were studied, and to improve clarity, we recommend that RETHYMIC is indicated for immune reconstitution in pediatric patients with CA.

7.1.1 Methods of Integration

The 10 open-label studies with RETHYMIC were sufficiently similar to allow for a combined analysis of efficacy and safety. For integrated efficacy analyses, the program wide statistical analysis plan (SAP) specified a primary endpoint of survival rate at year 1 following transplant. Survival rate at Year 2 is a primary supportive efficacy endpoint in the SAP. Survival rate is estimated using KM method 95% CI.

7.1.2 Demographics and Baseline Characteristics

The age at treatment ranged from 33 days to 2.9 years with a median age of 8.5 months. The demographics of the EAS population is consistent with the demographics of CA within the United States (Please see Table 1 for additional details). The slight predominance in males is likely due to chance and the sample size. There were an adequate number of subjects from each sex to analyze.

All 95 subjects in the EAS had CA, although 4 subjects did not meet the protocol definition of CA based on < 50 naïve T cells/mm^3 ($CD3^+$, $CD45RA^+$, $CD62L^+$) in the peripheral blood or < 5% naïve T cells of T cell population. Within in the EAS population, 93 (98%) was due to cDGA and 2 (2%) had FOXN1 deficiency. Of the subjects with cDGA, 50 had typical cDGA phenotype, 42 had atypical cDGA phenotype, and one subject was unknown. The most common comorbidities were cardiac anomalies (90%) and hypocalcemia (85%) consistent with the cDGA diagnoses in this population. The genotypic and phenotypic features of the EAS at baseline are described in Table 8.

Table 8. Baseline Disease Characteristics of EAS Population

	EAS (N=95)
Day of life at diagnosis (days)	
Mean (SD)	48.2 (78.99)
Median (Minimum, Maximum)	22.0 (0, 537)
Diagnosis, n (%)	
SCID	0
FOXN1	2 (2.1)
Partial DiGeorge anomaly	0
Complete DiGeorge anomaly	93 (97.9)
DiGeorge phenotype, n (%)[a]	
Typical DiGeorge anomaly	50 (52.6)
Atypical DiGeorge anomaly	42 (44.2)
DGA gene mutation / Syndromic Association, n (%)	
Hemizygous deletion of chromosome 22q11.2	36 (37.9)
TBX point mutation	0
CHD 7 mutation[b]	12 (12.6)
CHARGE	23 (24.2)
None known	26 (27.4)
Missing[c]	10 (10.5)
Phenotypic features	
Congenital cardiac anomaly or cardiothoracic vascular anomaly	85 (89.5)
Hypocalcemia	81 (85.3)
Diminished T cell counts for age	95 (100.0)
Dysmorphic facies	44 (46.3)
Deafness or ear pinnae anomalies	49 (51.6)
Coloboma	18 (18.9)
Cleft lip	12 (12.6)

Cleft palate (frank ceiling or submucous cleft)	17 (17.9)
Velopharyngeal insufficiency / hypernasal speech	3 (3.2)
Choanal atresia	10 (10.5)
Tracheal anomalies	25 (26.3)
Esophageal anomalies	10 (10.5)
Anal and/or rectal anomalies	6 (6.3)
Renal anomalies	26 (27.4)
Genital hypoplasia	11 (11.6)
Rib or vertebral anomalies	34 (35.8)
Limb anomalies	16 (16.8)
Developmental delay / intellectual disability	49 (51.6)
Other	7 (7.4)
Fetal toxin exposure, n (%)	
Maternal diabetes	26 (27.4)
Type 1	5 (5.3)
Type 2	14 (14.7)
Gestational	7 (7.4)
Other toxin exposure[d]	5 (5.3)

Source: Adapted from Applicant's Table 2-5 in Safety and Efficacy Clinical Update for BLA Resubmission
[a]The cDGA phenotype could not be determined for one subject (subject (b) (6) in the EAS and is therefore missing from this table.
[b]All subjects with a *CHD7* mutation also had CHARGE.
[c]One subject (Subject (b) (6)) had a variant in TBX2 identified post-transplantation. This subject was counted as "missing"
as the genetic defect was not identified at the time of implantation.
[d]Other toxin exposure included: Subject (b) (6) - Group B Strep; Subject (b) (6) - marijuana and amphetamines; Subject (b) (6) - mother's antiepileptic drugs; Subject (b) (6) - mother treated for typhoid during pregnancy; Subject (b) (6) – pregnancy complicated by polyhydramnios.

7.1.3 Subject Disposition

Within the EAS, 67 (71%) subjects completed the planned two-year follow up; 23 (24%) subjects died within the two years; 5 (5%) were still alive at the time of June 4,2021 update but had not completed two-year follow up. Of these 5 subjects, 4 had completed 1-year follow up.

Median follow-up duration and 1-year, 2-year and 5-year survival by study is provided in Table 9.

Table 9. Summary of primary outcomes in efficacy studies

Study ID	Follow-up Duration (Median)	Survival Rate		
		1-year	2-year	5-year
668-1 and 668-2	12.1 years	72%	72%	72%
884	12.3 years	81.8%	81.8%	70%
931	10.6 years	80%	80%	80%
932	9.7 years	83.3%	83.3%	83.3%
950	8 years	71.4%	71.4%	71.4%
25966	4.9 years	78%	75%	75%*
51962	2 years	90%	90%	N/A
NH	N/A	22%	6%	0%

*Censored (Source: Reviewer's Table based on Applicant's June 4, 2021 datasets, SN0065)

7.1.4 Analysis of Primary Endpoint(s)

The program-wide SAP has a primary efficacy endpoint of survival at 1-year post-transplantation, and 2-year post-transplantation survival as supportive efficacy. One subject who did not have 1-year data was censored for the 1-year analysis and 5 subjects who did not have 2-year data were censored for the 2-year analysis. Binomial exact tests on the hypothesis that survival rates at 1-year and 2-year are >50% as specified in the SAP were both statistically significant ($p < 0.0001$). For the EAS, the estimated survival rates at Year 1 and Year 2 based on the Kaplan-Meier method were 76.8% (95% CI: 67%, 84.1%) and 75.7% (95% CI: 65.8%, 83.2%), respectively. (See Table 10).

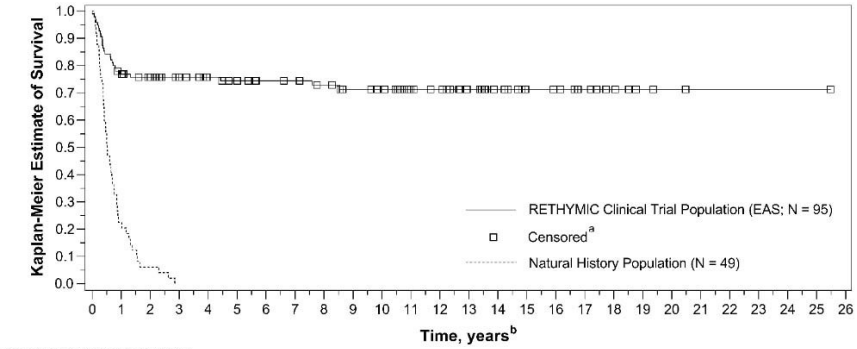
Table 10. Survival at 1-year and 2-year post-transplantation (EAS)

		1-year	2-year
Number of Subjects	Alive	72	67
	Censored	1	5
	Dead	22	23
Survival Rate		76.8%	75.7%

(Source: Reviewer's Table, Table based on Applicant's June 4, 2021 datasets, SN0065)

Kaplan-Meier estimates of overall survival were plotted comparing the EAS population to the NH population (Figure 2). For subjects treated with RETHYMIC who were alive at 1-year post-transplantation, the survival rate was 94% and was essentially unchanged thereafter. The median age of all surviving subjects was 11.4 years (3 years to 25.7 years), and censoring primarily occurred due to duration of follow-up rather than death. This is a large treatment effect size compared to what is observed in the natural history cohort where 94% of patients died by two-years and all subjects were dead by three years of age.

Figure 2. Kaplan-Meier Survival by Year (RETHYMIC Efficacy Analysis Population and Natural History Population)



[a]Patients were censored at the time of their most recent follow-up for the RETHYMIC clinical trial program. No patients in the natural history population were censored.
[b]Time is years after administration for the RETHYMIC clinical trial population and years of life for the natural history population.

(Source: Applicant's figure, PI)

Reviewer's comments: RETHYMIC demonstrated a substantial and persistent survival benefit. The survival benefit of RETHYMIC is consistent and persistent despite the heterogeneity of underlying genetic anomalies and diverse comorbid conditions.

7.1.5 Analysis of Secondary Endpoint(s)

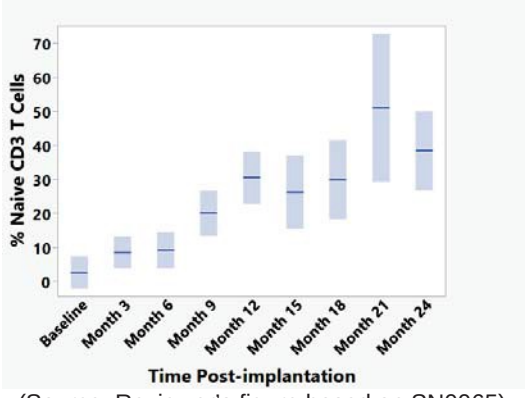
Key secondary efficacy endpoints include assessments of immune function via total and naïve T cell counts, T cell proliferation response to phytohemagglutinin (PHA) and diversity of T cell population, biopsy to confirm graft viability and thymopoiesis, as well as infection rate.

Several caveats were noted for data collection on these endpoints. Although the clinical study protocols included a schedule of assessments (every 3 months post-transplantation until 24 months), visit windows were not specified and, as such, study-related assessments were performed over a relatively wide timeframe. When a subject was discharged to the referring physician, requests were submitted to the referring pediatric immunologist for additional blood sampling/testing. While recommendations for the frequency of post-RETHYMIC follow-up activities were provided to the referring physician, obtaining follow-up blood samples/testing was dependent upon the subject's condition, subject's family, and referring/local physicians. Consequently, data collection for some of the planned secondary endpoints was limited. In addition, the specific subjects contributing data at any given timepoint may not have been the same across timepoints. In addition, naïve T cell count was not available for early studies.

7.1.5.1 Naïve and Total T cell number

Naïve $CD3^+$, $CD4^+$ and $CD8^+$ T cell numbers in peripheral blood were assessed via flow cytometry. Study subjects in the EAS were athymic at the time of study entry. The number and percentage of naïve $CD3^+$ cells overall showed marked and sustained increase over time following transplant. The median (range) naïve CD3+ T cell counts were 2.0 (0-98.0), 2.6 (0-262.0), 16.0 (0.5-159.0), 51.5 (3.0 -368.0), 124.0 (4.0 – 512.2), 326 (0 – 491.2), 110.6 (0-671.4), and 777.5 (98-1457.0) cells/mm^3 at baseline, 3-month, 6-month, 9-month, 12-month, 15-month, 18-month, and 24-month post-transplantation respectively. As shown in Figure 3, naïve CD3+ T cell percentage also increased overtime after RETHYMIC implantation and reached ~10% between 3- and 6-months post-transplantation. Immunosuppressive therapies were generally discontinued once subjects achieved at least 10% naïve CD3+ T cells.

Figure 3. Percentage of Naïve CD3 T Cells (EAS)



(Source: Reviewer's figure based on SN0065)

Naïve $CD4^+$ and $CD8^+$ T cell count exhibited similar trends of increase post-transplantation. Median naïve CD4+ T cell counts were 1.0 (range: 0-38), 41.6 (range: 0-

653), 212 (range: 1-751) and 274.5 (range: 33-858) cells/mm^3 at baseline, Month-6, Month-12, and Month-24 post-transplantation, respectively. Median naïve CD8+ T cell counts were 0.2 (range: 0-45.9), 9.3 (range: 0-163), 57.9 (range 0-304.3) and 86 (range: 6.0-275) cells/mm^3 at baseline, Month-6, Month-12, and Month-24 post-transplantation, respectively. Throughout RETHYMIC clinical development program, a naïve CD4 count $\geq$ 100 cells/mm^3 was generally considered sufficient to fight infection. Usually, it took 6-12 months after RETHYMIC implantation to achieve naïve CD4 T cell level of $\geq$ 100 cells/mm^3.

Numbers of total CD3+, CD4+, CD8+ T cells pre- and post-transplantation are listed in Table 11. Total CD3, CD4, and CD8 cell counts increased over time as expected with the development of thymic function during the study.

Table 11. Total T Cell Count (EAS)

		Number CD3 (cells/mm^3)	Number CD4 (cells/mm^3)	Number CD8 (cells/mm^3)
Baseline	N	95	85	82
	Mean ± Stdev	657 ± 1366	252 ± 472	241 ± 695
Month 3	N	87	83	78
	Mean ± Stdev	456 ± 1149	225 ± 503	198 ± 669
Month 6	N	78	77	77
	Mean ± Stdev	750 ± 2080	575 ±1685	92 ± 235
Month 9	N	66	65	64
	Mean ± Stdev	625 ± 503	432 ± 357	129 ± 186
Month 12	N	58	58	57
	Mean ± Stdev	719 ± 450	545 ± 357	152 ± 133
Month 18	N	32	33	33
	Mean ± Stdev	726 ± 525	609 ± 500	151 ±129
Month 24	N	31	32	32
	Mean ± Stdev	778 ± 418	564 ± 319	159 ± 111

(Source: Reviewer's Table based on SN0065)

Reviewer's Comments:

Immune reconstitution after RETHYMIC transplantation:
The numbers and percentages of naïve CD3+, CD4+, CD8+ T cells all exhibited similar trends of increase overtime after RETHYMIC transplantation compared to baseline. Definitive increase of naïve T cell number and percentages were typically achieved between 6 and 12 months after RETHYMIC transplantation and persisted throughout the two-year follow up period. These findings support immune reconstitution and improvement in immune function following RETHYMIC transplantation in CA patients.

Correlation between post-transplantation naïve T cells levels and survival
Changes in naïve CD4+ T cell numbers during the first year after RETHYMIC transplant were compared between subjects who were dead and those who were alive. The two groups were comparable at baseline (alive vs death: 4.6 ± 8.7 cells/mm^3 vs 3.6 ± 5.8 cells/mm^3). Naïve CD4+ T cell count increased on average of 2-fold at 3 months (11.9 ± 24.0 cells/mm^3) post-transplantation in subjects who were alive, whereas it remained similar to baseline in subjects who died (5.0 ± 6.9 cells/mm^3). The increase in naïve CD4+ T cell numbers continued in subjects who were alive and were consistently higher than those in subjects who were dead. It appears an early increase in naïve CD4 T cell count (month 3) after transplantation is associated with better survival outcome.

In addition, Naïve CD4+ T cell level of ≥ 100 cellslmm3 was considered sufficient against infection throughout the clinical development program and typically achieved by 1-year post-transplantation. Consistently, most deaths (23 out of 26) occurred during the first-year post-transplantation and prior to establishment of a sufficient level of immune reconstitution.

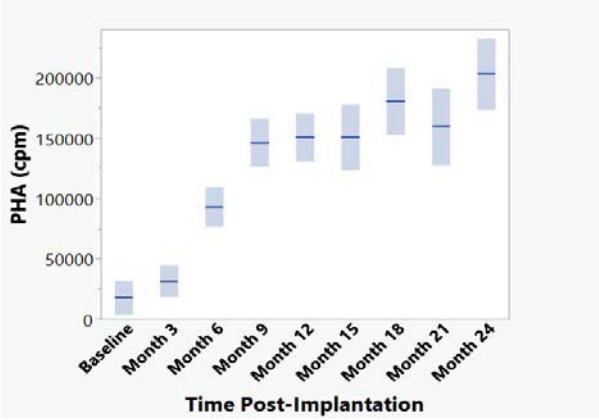
Survival was analyzed by maximal naïve CD4+ T cell number achieved post-transplantation. Naïve CD4+ T cell numbers were available for 89 of the 95 EAS subjects. Maximal naïve CD4+ T cell numbers ranged between 0 and 1836 cells/mm3 with a median of 225 cells/mm^3. 52 subjects developed naïve T cell number of ≥100 cells/mm^3 of whom all but one subject (subject (b) (6) were alive at last contact date. 61 subjects developed naïve T cell number of ≥ 50 cellslmm^3 and only one (subject (b) (6) of the 61 subjects died. Subject (b) (6) died 9 years after RETHYMIC implantation. Year 1 and year 2 survival for subjects who achieved naïve T cell number of ≥ 50 cells/mm^3 were 100%. In contrast, of the 25 EAS subjects who did not develop naïve CD4+ T cell level of ≥ 50 cells/mm^3, 16 died (64%). Post-transplantation naïve CD4+ T cell count was not available for 9 EAS subjects. 7 of the 9 subjects with unknown CD4+ count post-transplantation died.

In conclusion, naïve CD4+ T cell count after RETHYMIC implantation is correlated with better survival outcome. Naïve CD4+ T cell count of ≥ 50 cellslmm3, although lower than the pre-defined threshold of ≥ 100 cellslmm3, may be sufficient to improve survival in patients with CA.

7.1.5.2 Proliferative T Cell Response

Proliferative T cell response to PHA were measured at baseline and every three months post-transplantation during the two year follow up. The median of PHA response in EAS subjects were 3688 cpm (n=94), 65197 cpm (n=34), 136500 cpm (n=40), 21936 cpm (n=24) at baseline, month 6, month 12 and month 24 respectively. Median PHA response of normal controls were 175676 cpm, 196800 cpm, 203361 cpm, 162619 cpm at baseline, month 6, month 12 and month 24 respectively. A PHA response of ≥ 75,000 was considered normal throughout the study. As shown in Figure 4, the proliferative T cell response to PHA increased steadily and normalized through Year 2 post-transplantation.

Figure 4. PHA Response Overtime (EAS)



(Source: Reviewer's figure based on SN0065)

Only a small subset of EAS subjects had data on T cell response to candida, ConA, Sol CD3, Immob CD3, Tetanus toxoid. Nevertheless, a steady increase of the median of the proliferative T cell response to ConA, sol CD3, immob CD3, and tetanus toxoid from baseline to Year 1 and Year 2 post-transplantation was observed. With the exception of Candida, which was likely low due to a lack of Candida exposure in these subjects, the median T cell proliferative response normalized for all stimulants tested within 2 years post-transplantation. For ConA, the Year 2 response was normal (i.e., > 75,000 cpm). For sol CD3, the stimulation index (ratio of the median of patient response value/median of patient background value) was 155, which is considered a normal response (i.e., > 15). A normal response (stimulation index > 50) was also observed for immob CD3 (stimulation index of 219). The tetanus toxoid stimulation index was 54 which was also considered a normal response (stimulation index > 2).

7.1.5.3 Biopsy

Immune outcomes were also assessed after RETHYMIC transplantation by examining biopsies of the allografts approximately 2 to 3 months post-transplantation. Histologic evidence of thymopoiesis in biopsies was defined as the presence of a lacy pattern of cytokeratin-positive thymic epithelial cells and the presence of CD3+, CD1a+, and Ki-67+ cells.

Biopsy results are summarized in Table 12. RETHYMIC biopsies were performed for 50 subjects in the EAS. Evidence of thymopoiesis was observed in 40 subjects (80%). Evidence of RETHYMIC rejection was reported in 1 subject (2.0%). For this subject, Subject (b) (6), rejection at both RETHYMIC implantation sites (bilateral quadriceps) was indicated by the presence of “many T cells surrounding a small area with cytokeratin”, per site report. The subject was a black male with atypical cDGA with severe rash who was enrolled under an individual treatment plan. Per the Investigator, on admission to DUMC at 289 days of life his weight was below birthweight. The subject received RETHYMIC on Day 354 of life. The subject had received immunosuppressive treatment with 1.12 mg IV pentostatin (16 days prior to implantation and 10 days prior to implantation). Pentostatin was used because the subject had too much respiratory compromise to safely give RATGAM; however, pentostatin may not have been sufficient to control the oligoclonal T cell population associated with the atypical phenotype of cDGA. The subject died due to sepsis 44 days after RETHYMIC implantation.

Table 12. Biopsy Summary

RVT-802 Biopsy	EAS N =85 n (%)
n[a]	50
Presence of CD1a	30 (60.0)
Presence of CD3	43 (86.0)
Presence of Ck14	35 (70.0)
Presence of cortical medullary distinction	8 (16.0)
Presence of Hassall body	11 (22.0)
Presence of Ki-67	40 (80.0)
Presence of other	3 (6.0)
Evidence of thymopoiesis	40 (80.0)
Evidence of rejection	1 (2.0)

(Source: Applicant's table, BLA 125685/003)

Nine subjects had a biopsy performed with no evidence of thymopoiesis or rejection observed. These subjects are briefly described below:

- Subject (b) (6) had a biopsy performed, but no evidence of thymopoiesis or rejection was noted. This may have been secondary to the use of pulse steroids when this subject first developed what is now known as atypical cDGA shortly after implantation. This subject never developed naïve T cells prior to death due to sepsis at 130 days after transplant.
- Subject (b) (6) did not have definitive evidence of thymopoiesis reported at the time of biopsy. However, this subject later developed naïve T cells; it is possible the biopsy sample did not show thymic function by chance (sampling artifact).
- Subject (b) (6) did not have definitive evidence of thymopoiesis observed at the time of biopsy; this subject died prior to confirmation of the development of thymic function.
- Subject (b) (6) did not have definitive evidence of thymopoiesis reported at the time of biopsy although the presence of Ki-67 and CK14 suggested that early thymopoiesis may have been occurring.
- Subject (b) (6), from whom the presence of Hassall bodies and CK14 was reported, was not considered to have definitive evidence of thymopoiesis at the time of biopsy. However, the Investigator commented that the area used for biopsy was too small to allow a thorough evaluation.
- Subject (b) (6) had a biopsy performed during autopsy; per the biopsy report comments, the lack of thymopoiesis was secondary to CMV infection.
- The biopsy report for Subject (b) (6) had a comment that a very small area with cytokeratin was observed and that subsequent slides were not in the area of thymus; subsequently, there may have been thymopoiesis elsewhere. This subject went on to develop naïve T cells.
- Subject (b) (6) had CD3 and CK14 identified during biopsy; however, definitive evidence of thymopoiesis could not be confirmed. This subject died 229 days post-transplantation before the development of thymic function could be confirmed.
- Subject (b) (6) did not have definitive evidence of thymopoiesis observed at the time of biopsy; however, this subject did develop naïve CD4 T cells at 1 year indicating thymopoiesis was occurring.

7.1.5.4 Other Secondary Endpoints

TCRV Repertoire Variability

TCRV  repertoire variability was assessed by immunoscope/spectratyping and flow cytometry. Immunoscope/spectratyping results were available for a subset of subjects. Results were quantified using the Kullbach-Leibler divergence (D_{KL}) statistic. A lower DKL score indicates a more normal (Gaussian-like) repertoire. In the EAS, 16 subjects had the CD4 D_{KL} value reported at baseline (median = 1.09), 9 subjects at Year 1 (median = 0.137) and 3 subjects at Year 2 (median = 0.122). At Year 1 and Year 2, the small value of DKL suggests the development of a Gaussian-like repertoire for CD4 receptors in these subjects.

Evaluation on the TCRV variability by measuring the percent Gaussian, percent oligoclonal, and percent skewed was also performed. A higher percent Gaussian as compared with oligoclonal was indicative of a more normal Gaussian-like repertoire. Table 13 shows the result of the median of percent Gaussian, percent oligoclonal and percent skewed at baseline, Year 1 and Year 2 post-baseline on CD4. A Gaussian-like repertoire of CD4 was not observed at baseline and the median of the percent oligoclonal was reported as 97.5%. At Year 1 post-transplantation, the median of percent Gaussian increased to 43% while the percent oligoclonal decreased to 4%. The median of percent Gaussian at Year 2 was 29% with only 6% oligoclonal. While data were limited, the median percent oligoclonal CD4 decreased at Year 1 with low levels sustained through Year 2. Conversely, the median percent Gaussian increased at Year 1 with results maintained at Year 2. These data indicate the development of a more diverse repertoire over time which is needed to respond to a variety of foreign antigens.

Table 13. TCRVJ3 Repertoire by Immunoscope/Spectratyping

CD4 Cells	Gaussian (%)		Oligoclonal (%)		Skewed (%)	
	n	median	N	median	n	median
Baseline	19	0	20	97.5	19	5.0
Year 1	11	43.0	11	4.0	11	52.0
Year 2	4	29.0	4	6.0	4	59.0

(Source: Applicant's table, BLA 125685/003)

In the thymus, a diverse and polymorphic T cell repertoire is generated by random recombination of discrete TCR - Variable (V), Diversity (D), and Joining (J) gene segments for the TCR chain and V and J segments for the TCRa chain. This repertoire is subsequently shaped by intra-thymic selection events to generate a peripheral T cell pool of self-major histocompatibility complex restricted, non-auto aggressive T cells. T cell receptor diversity was assessed using flow cytometry and spectratyping. The median of the tested TCRV families were compared with the reference normal range for each family. Twenty-four V families of CD4 were tested. In the EAS, 18 (75%) V families were within normal range at baseline. At Year 1 and Year 2 post-transplantation, 24 (100%) V families were within normal range, which indicated the development of a more diverse T cell repertoire within 2 years implantation.

T Cell Receptor Rearrangement Excision Circles (TRECs)

Data collected for TRECs were limited. The presence of TRECs indicates the presence of recent thymic emigrants and thus active thymopoiesis. Prior to implantation with RETHYMIC, subjects were athymic. Given the low levels of T cells in the athymic subjects prior to treatment with RETHYMIC, TRECs were either not measurable or very low at baseline for most subjects. For CD3, 17 subjects had reported baseline values, with a median of 100 TRECs/100,000 cells. At Year 1 post-transplantation, 4 subjects had reported values with a median of 6770 TRECs/100,000 cells. At Year 2, the median was reported as 5610 TRECs/100,000 cells (n = 4). From the available data on CD3, an increase in TRECs following RETHYMIC implantation was observed. Data on CD4 and CD8 was scarce, and thus no corresponding conclusions regarding TREC results for these cell types can be drawn.

Regulatory T Cells (TREGs)

Data collected regarding TREGs were sparce with only a few subjects tested. Given the limited data available, no conclusion regarding TREG can be drawn.

B Cell Function

B cell function was evaluated through the analysis of serum immunoglobulins, isohemagglutinins, and vaccine titers (as data permitted). Subjects were maintained on monthly immunoglobulin replacement therapy for up to 2 years post-transplantation until they met protocol-specified criteria for discontinuation. Immunoglobulin synthesis and antibody formation develops after RETHYMIC implantation and the development of immunocompetent naïve T cells. Because subjects were receiving immunoglobulin replacement, values for IgG were expected to be higher due to the immunoglobulin replacement, and thus not representative of the subjects' endogenous IgG levels. Immunoglobulin replacement does not impact the development of IgA, IgE, and IgM so these values can be considered representative of the subject's endogenous immunoglobulin levels. Because IgA and IgM values are commonly low in partial DGS patients, immunoglobulin values were expected to be low in cDGA patients. A summary of IgG, IgA, IgM, and IgE antibody results is presented in Table 14.

Table 14. Summary of Serum Immunoglobulins

Serum Ig Levels	Baseline n (%)			Year 1 n (%)			Year 2 n (%)		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
IgG antibody	2 (2.4%)	32 (38.1%)	50 (59.5%)	1 (2.4%)	19 (45.2%)	22 (52.4%)	1 (4.3%)	17 {73.9%)	5 (21.7%)
IgA antibody	55 (66.3%)	15 (18.1%)	13 (15.7%)	6 (15.8%)	16 (42.1%)	16 (42.1%)	2 (9.1%)	14 (63.6%)	6 (27.3%)
IgM antibody	30 (35.7%)	46 (54.8%)	8 (9.5%)	10 (26.3%)	16 (42.1%)	12 (31.6%)	6 (27.3%)	14 (63.6%)	2 (9.1%)
IgE antibody	0	36 (43.9%)	46 (56.1%)	0	19 (86.4%)	3 (13.6%)	0	12 (70.6%)	5 (29.4%)

(Source: Applicant's table, BLA 125685/003)

7.1.6 Other Endpoints

7.1.6.1 Infections

Although not pre-specified in the SAP, post-transplantation infections over time were analyzed. Pre-implantation infection history was often missing; therefore, pre- and post-transplantation infections were not compared. The analyses were based on reported infection related adverse events (AEs). Infection-related AEs and serious adverse events (SAE) were compared by the time of onset. A Wilcoxon signed-rank test was performed to compare the number of infections reported in the first 6 months after implantation versus > 6 to < 12 months post-transplantation. This analysis was also performed to compare the number of infection-related AEs with onset ::5 12 months versus > 12 to ::5 24 months after implantation. Percentage of subjects with infections and number of

infections per subject were also analyzed. For <6 months and 6-12 months comparison, the first 6 months period included all 95 EAS subjects, the 6-12 months period included 80 subjects who were alive after 6 months post-transplantation. For year 1 and year 2 comparison, year 1 included all 95 EAS subjects and year 2 included 73 subjects who were alive 1 year post-transplantation. The results are summarized in Table 15. Based on the Wilcoxon signed-rank test, there was a significant difference in the frequency of infection AEs with an onset within 6 months versus an onset within 6 to :: 12 months post-transplantation ($p < 0.001$) for the EAS population. Reduction in the frequency of infections and serious infections (SAE) in year 2 compared to year 1 was also significant.

Table 15. Infections Post-RETHYMIC Transplantation (EAS)

	< 6 months (n=95)	**6-12 months (n=80)**	**Year 1 (n=95)**	**Year 2 (n=73)**
# infection AE	346	109	455	99
#subject w/ infection AE	83	45	89	34
%subject w/ infection AE	87.4%	56.3%	93.7%	46.6%
# infection SAE	135	51	186	58
#subject w/ infection SAE	55	25	63	23
%subject w/ infection SAE	57.9%	31.3%	66.3%	31.2%

(Source: Reviewer's table based on SN0065)

Reviewer's comments: Infections are clinically meaningful, proximal manifestations of CA. Therefore, a decrease in frequency of infections and severe infections provides data to support that clinically meaningful immune reconstitution has occurred. Although extent of social-distancing, seasonality of various infections, concomitant medical problems (e.g. catheters, surgery, malnutrition), medications can impact frequency of infections, the consistent and dramatic trend in a decrease in overall infections and serious infections following treatment with RETHYMIC, especially 6-12 months after RETHYMIC transplantation, when immune reconstitution has occurred. Independent of the survival benefit, a decrease in infections, especially serious infections that cause hospitalization is a clinically meaningful benefit.

Immunosuppression could be a confounding variable. To examine if change in immunosuppressant regimen was confounding the RETHYMIC treatment effect, infections during the 2-year follow up period were compared between subjects with and without immunosuppression (62 and 33 subjects respectively). As shown in Table 16, immunosuppression did not affect infection related AEs and SAEs. The two groups were comparable in number of infections and serious infections and percentage of subjects with infections and serious infections. This analysis provides support for the hypothesis that the improving infection rates were most likely attributable to RETHYMIC's effect on the underlying immunodeficiency, rather an effect from the tapering dose of immunosuppression.

Table 16. Infection by Immunosuppression Group (EAS)

	Immunosuppression	
	Yes (n=62)	**No (n=33)**
# infection AE	361	193
#subject w/ infection AE	59	31
%subject w/ infection AE	95.2%	93.9%
# infection SAE	159	85
#subject w/ infection SAE	47	23
%subject w/ infection SAE	75.8%	69.7%

(Source: Reviewer's table based on SN0065)

7.1.7 Subpopulations

Subgroup analyses of the primary efficacy endpoint were performed for 16 study subgroups, including demographics (gender, race, age at treatment), baseline disease characteristics (cDGA phenotype, 22q11.2 hemizygosity, CMV infection, underlying genetic mutations), immunosuppression, RETHYMIC dosage, culture time, manufacturing facility, study protocol, baseline renal function, baseline hepatic function, and maximum naïve CD4 count post-transplantation.

7.1.7.1 Baseline Demographics (gender, race, age at treatment)

Gender
The EAS subjects included 39 females and 56 males. Overall survival rates were 79.5% and 67.9% for females and males respectively.

Reviewer's Comments: Considering the relatively small sample size of the study, this difference is likely due to chance.

Race
The EAS included 66 white subjects, 21 black subjects and 8 other races including Asian, Pacific Islander, American Indian or Alaska Native and multi-race. Overall survival rates were 75.8%, 66.7% and 62.5% for white, black, and other races respectively.

Reviewer's Comments: These differences are likely due to chance given the small sample size.

Age
The EAS subjects were divided to four age groups based on age at RETHYMIC transplant: < 6 months, 6-12 months, 12-18 months and > 18 months. Overall survival rates were 62.9%, 86.7%, 60%, 90% for age groups < 6 months, 6-12 months, 12-18 months and > 18 months, respectively.

Reviewer's Comments: The lack of a trend between the age cohorts suggests that the differences are due to chance. However, since immune reconstitution and functional protection from life-threatening infections can take 6-12 months to develop, it may be

prudent to consider treating patients as early as feasible when considering other factors in their clinical care.

7.1.7.2 Baseline Disease Characteristics (cDGA phenotype, 22q11.2 hemizygosity, CMV infection)

cDGA Phenotype

The EAS included 50 typical cDGA subjects and 42 atypical cDGA subjects. Overall survival for typical and atypical cDGA subjects were generally comparable, 76% and 69%, respectively. However, there was a notable difference in mortality rates during the first year.

Reviewer's comments: The long-term survival was substantial in both phenotypes of typical and atypical cDGA. The relative increased mortality in the short-term with atypical cDGA may be due to atypical cDGA being a risk factor for other complications, such as GVHD. (See section 8 for a discussion of these risks.

22q11.2 hemizygosity

There were 36 subjects had 22q11.2 hemizygosity in EAS, 59 subjects did not. Survival rates were comparable between the two groups with 72.2% for those with 22q11.2 hemizygosity and 72.8% for those without.

Pre-implantation CMV infection

Six subjects (Subjects (b) (6) had at least one positive CMV culture during pre-implantation screening. Three of the six subjects died post-transplantation. The applicant considered pre-implantation CMV findings false positive for Subjects (b) (6) as these subjects had only 1 positive test out of 2, 5, and 5 pre-implantation CMV tests respectively; and no post-transplantation CMV related adverse events were reported for this subject.

In addition, Subject (b) (6) had medical history of CMV systemic infection and was treated with foscarnet. At the time of screening, Subject (b) (6) had 3 pre-implantation CMV tests, and all were negative. Following implantation, the subject had 19 post-transplantation CMV PCR blood tests, up to day 328 post implantation of which 9 tests detected CMV. Specifically, CMV was detected in the blood on post-transplantation Days 3, 10, 16, 31, 175, 178, 181, 185, and 245. In addition, the subject had 10 CMV PCR blood tests across this same time period that were negative. Additional information on CMV testing in this subject has recently been provided that on day 712 post-transplantation, the subject had a reactivation of CMV on day 676 post-transplantation with a peak of 200 international units/ml day on day 682 post-transplantation. The subject was started on induction valganciclovir on day 680 post-transplantation with transition to maintenance valganciclovir around day 709 post-transplantation. The last completed CMV PCR test (day 711 post-transplantation) detected <100 IU/ml.

Reviewer's comments: Given that Subject (b) (6) had CMV tests immediately after transplant and a medical history of CMV viremia, it is likely that the subject had pre-existing CMV infection before treatment with RETHYMIC. Including Subject (b) (6), 4 subjects had pre-implantation history of disseminated CMV infection/CMV viremia,

(subject (b) (6) and three of these subjects died. Pre-implantation CMV infection appeared to be associated with higher post-transplantation mortality in subjects with CA, although only one subject's death was directly attributed to CMV (Subject (b) (6).

- *Subject (b) (6): history of cardiac arrest, disseminated intravascular coagulation, respiratory arrest who died of respiratory failure on Day 45.*
- *Subject (b) (6): history of respiratory failure, ventilator dependent, GVHD who died of hypoxia on Day 82; received cyclosporine, methylprednisolone, cyclophosphamide, prednisolone and RATGAM;*
- *Subject (b) (6): died due to CMV infection on Day 103; received cyclosporine, MMF, methylprednisolone, prednisolone, RATGAM, and tacrolimus.*

The appropriateness of RETHYMIC in patients with pre-existing CMV viremia could be determined on a case-by-case basis in the context of the fatal nature of the disease and the time it will take to establish immune reconstitution.

7.1.7.3 Baseline Renal and Liver Function

Overall survival for subjects with elevated serum creatinine (SCr), AST, or ALT pre-implantation was lower compared to those with normal pre-implantation levels. Specifically, overall survival was 40% (n=10) and 77.4% (n=65) for subjects with elevated pre-transplantation SCr and those without respectively. Overall survival was 64.9% (n=37), 77.6% (n=58), 61.5% (n=26), 76.8% (n=69) for subjects with elevated ALT, normal ALT, elevated AST, and normal AST respectively. The differences in survival between subjects with elevated liver enzyme at baseline and those without were not statistically significant.

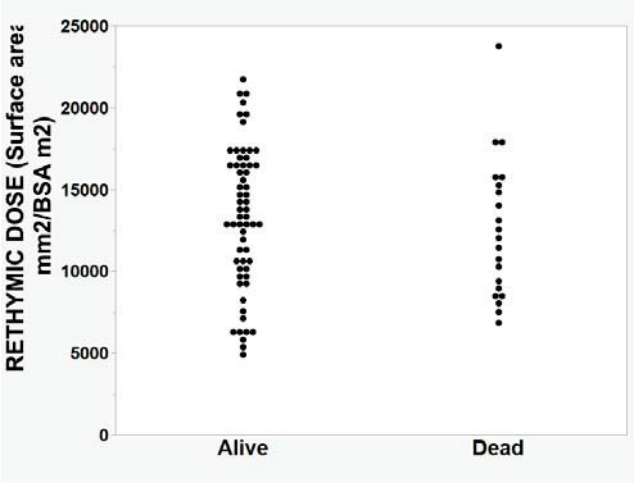
Reviewer's comments: Although only a small number of subjects had elevated SCr (n=10) pre-transplantation, the mortality rate is much higher (60%) compared to those with normal SCr levels. Impaired renal function may result in sub-optimal immunosuppression and consequently functioning of RETHYMIC. Use of nephrotoxic medications such as calcineurin inhibitors and antivirals may result in further kidney injury even acute or chronic kidney failure.

7.1.7.4 RETHYMIC dose

REHTYMIC doses administered in efficacy population were grouped in four quartiles:Q1 (4522.7<-9069.9 mm2/m2), Q2 (9069.9<-12674.6 mm2/m2), Q3 (12674.6<-16052.0 mm2/m2) and Q4 (16052.0<-23754.5 mm2/m2). Survival and naïve T cell count were analyzed by dose groups. There was no apparent dose response.

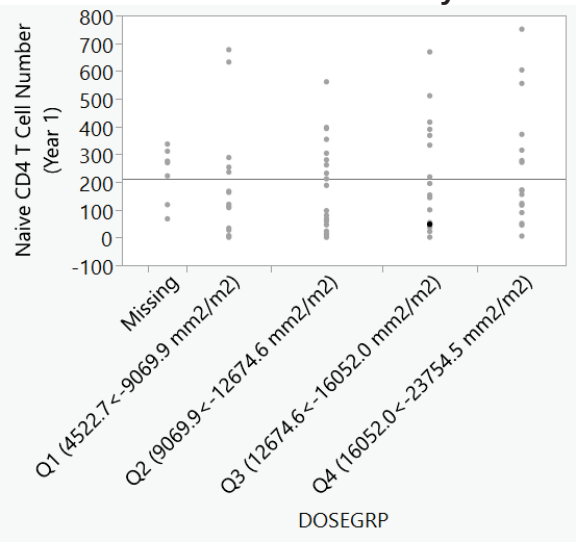
Reviewer's comments: As shown in Figure 5, dose ranges were similar between subjects who died and those who were still alive. Dose ranges were also overlapping for total and naïve CD3+, CD4+ (Figure 6), CD8+ counts and PHA response (data not shown). However, there did tend to be a trend that subjects treated with doses in the highest quartile (16052.0<-23754.5 mm2/m2) had the highest overall survival 86.4%.

Figure 5. RETHYMIC Dose by Vital Status (EAS)



(Source: Reviewer's figure based on SN0065)

Figure 6. Year 1 Naïve CD4 T Cell Counts by Dose Groups (EAS)



(Source: Reviewer's figure based on SN0065)

Recommended dose for product label

In the earliest clinical studies (prior to 2002), a recommended dose range was not specified in the clinical study protocols and the dose of RETHYMIC was not captured. In a November 2015 amendment to the IND (SN 0209), a dose range of 2,000 to 20,000 mm^2/m^2 was implemented. Assuming a density of 1 g/cm3 and a thickness of 1 mm, doses of 4 to 18 g/m^2 are approximately equal to a dose of 4,000 to 18,000 mm^2/m^2. Since the November 2015 IND amendment, the area of the thymus tissue was determined by photograph using software analysis. In particular, a digital image was taken of the cultured thymus tissue with a ruler in the same focal plane. The thymus tissue was selected by contrast with the surrounding area using imaging software. The surface area of the thymus tissue was calculated using imaging software. The BSA was determined according to the DuBois and DuBois formula using the patient's height (cm) and weight (kg): BSA=0.007184 × [height in cm] 0.725 × [weight in kg] 0.425. While

clinical studies allowed for a dose range of 2,000 to 20,000 mm^2 BSA, the lowest dose administered in clinical studies was 4,523 mm^2/m^2 BSA, and for EAS subjects the lowest dose administered was 4901 mm^2/m^2 BSA. There were 5 patients that received greater than 20,000 mm^2/m^2 BSA. The highest dose administered in the EAS subjects was 23754.5 mm^2/m^2 BSA, however, this subject (Subject (b) (6)) died 234 days post-transplantation due to infection. The second highest dose administered in EAS subjects was 21734.1 mm^2/m^2 BSA, the subject (*Subject* (b) (6) was still alive at the time of this resubmission.

Reviewer Comment's: Based on data from the EAS population, the recommend dose range for the label is 5,000 – 22,000 mm^2 RETHYMIC/m^2 BSA. Within this dose range, there may be a benefit of implanting more RETHYMIC slices, but this needs to be considered in the context of an individual patient's muscle mass, number of implantation sites, longer surgery, and other patient specific considerations.

7.1.7.5 Immunosuppression

There were 62 subjects in EAS who received at least one immunosuppression medication throughout the study; 33 subjects were not treated with any immunosuppression regimen. Overall survival was comparable between subjects with and without immunosuppression treatment, 71% (44/62) and 76% (25/33), respectively.

Reviewer's comments: Once the CA diagnosis was made, based on a subject's cDGA phenotype and T cell response to PHA, the subject was assigned to different conditioning regimens (summarized in Table 17).

Table 17. Summary of Conditioning Regimens

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

Subject Characteristics		Immunosuppression Utilized During Clinical Studies with RETHYMIC
Complete DiGeorge Anomaly Phenotype	**Phytohemagglutinin (PHA) Response[1]**	
	Evidence of maternal engraftment	• Basiliximab[3] • MMF[4]

(Source: Applicant's table) Abbreviations: ATG-R: anti-thymocyte globulin [rabbit] (Thymoglobulin); cpm: counts per minute; MMF: mycophenolate mofetil; PHA: phytohemagglutinin;

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

As noted above, Cyclosporine (CSA) could be changed to tacrolimus if the subject did not tolerate CSA. There were four levels of conditioning regimens: 1) none, 2) ATG-R + corticosteroid, 3) ATG-R + corticosteroid + CSA and/or Tacrolimus, and 4) ATG-R + corticosteroid + CSA + Basiliximab and/or MMF. For level 4, MMF and/or Basiliximab could be added if the ATG-R + corticosteroid + CSA/Tacrolimus regimen was considered not sufficient. Overall survival was compared between the four conditioning regimens (Table 18). It is noted that four subjects in EAS did not receive any of the four conditioning regimens, but received penstostatin, cyclophosphamide or fludarabine alone or daclizumab in combination with ATG-R + CSA, these subjects were collectively referred to as "other" in the analysis (Table 18).

Table 18. Overall Survival by Conditioning Regimens

	EAS	FAS
Group 1: No Conditioning	76.3% (29/38)	75% (30/40)
Group 2: RATGAM	73.7% (14/19)	76.2% (16/21)
Group 3: RATGAM + CSA and/or Tacrolimus	83.3% (25/30)	84.8% (28/33)
Group 4: RATGAM + CSA + Basiliximab/MMF	0% (0/4)	0% (0/4)
Other	25% (1/4)	16.7% (1/6)

(Source: Reviewer's table based on SN0065)

The four subjects in group 4 were Subjects (b) (6) *. Three of the four subjects died within 6 months of RETHYMIC transplantation, and one died in the first year. The narratives of the four subjects are summarized below. Even though the*

mortality rate was 100% within the first year for this most intensive conditioning group, there were only four subjects, a number too small to draw any conclusion. It is hard to determine whether deaths may be indirectly related to immunosuppression in this group. Furthermore, it appears that deaths were either due to underlying disease or infection or both. It is challenging to know whether deaths may be indirectly related to immunosuppression in this group. Furthermore, these conditioning regimens were based on that the subjects were athymic and had none or little recipient T cells at baseline. However, these subjects had relatively higher PHA response at baseline. The possibility cannot be ruled out that these atypical cDGA subjects with high T cell proliferation response might need more intensive conditioning than they received.

- *Subject* (b) (6) *was a Black or African female subject with atypical cDGA and was 753 days old at informed consent. The subject was the infant of a diabetic mother. The subject received cyclosporine (from 777 days prior to transplantation to 49 days prior to transplantation), mycophenolate mofetil (from 1 day after transplantation and ongoing); methylprednisolone (from 4 days prior to transplantation until day of transplantation), and 3 doses of RATGAM (from 4 days prior to transplantation to 2 days prior to transplantation). The subject had significant congenital heart defects. She had undergone multiple previous cardiac surgeries and additional surgery was anticipated in the summer of 2021. The subject died on day 293 post-transplantation after being released to home. The cause of death was recorded as cardiopulmonary arrest.*

- *Subject* (b) (6) *was a white male subject with atypical cDGA and was 460 days old at informed consent. The subject received immunosuppressive treatment with cyclosporine (ongoing from 30 days prior to transplantation), methylprednisolone (from 30 days to 25 days prior to transplantation, from 6 days to 1 day prior to transplantation, and from 8 days to 75 days after transplantation), prednisolone (from 21 days to 6 days prior to transplantation), RATGAM (3 doses from 4 days to 1 day prior to transplantation) and basiliximab (from 6 days to 7 days after transplantation). The subject had history of open-heart surgery, and it was noted that the thymus was a "very small thymic remnant". The subject had parainfluenza 1 virus which was treated with ribavirin. He was stable but was ventilator dependent. During the transplant admission he was noted to have severe oral candidiasis which was treated with gentian violet because the subject had significantly elevated liver function tests. A liver biopsy was performed but was not informative. An echocardiogram was done while at the transplant center which showed moderate to severe pulmonary artery conduit insufficiency and moderate to severe pulmonary artery conduit stenosis. He was transferred from the transplant center back to the referring hospital where he had an acute sepsis event shortly after transfer and was treated with sulfamethoxazole trimethoprim for BAL positive for Stenotrophomonas maltophilia. The subject died 89 days after RYTHYMIC implantation due to disseminated Candida infection.*

- *Subject* (b) (6) *was a white male subject with atypical cDGA and was 358 days old at informed consent. The subject received immunosuppression treatment with MMF (from 284 days to 1 day prior to transplantation), prednisolone (from 296 days to 41 days prior to transplantation, from 23 days to 19 days prior to transplantation, and from 47 days 81 days after transplantation), cyclosporine (from 71 days prior to 21 days after transplantation), methylprednisolone (from 40 days to 24 days prior to transplantation, from 19 days to 1 day prior to*

transplantation, Day 3, and from 30 days to 46 days after transplantation), RATGAM (3 doses from 5 days to 3 days prior to transplantation), and tacrolimus (from 21 days to 106 days after transplantation). The subject experienced Grade 4 respiratory failure 103 days after transplantation (serious due to life-threatening). Prior to the event the subject had sepsis and respiratory distress on Day 92 after transplantation and required oxygen and intubation on Day 100 after transplantation. On the day of the event, the subject self-extubated and arrested with bradycardia and desaturation. The subject was sent to the operating room to change the endotracheal tube to a larger size. During this time there were complications and the subject had multiple codes, including a prolonged code. On Day 105 after transplantation, the subject experienced Grade 4 generalized edema (serious due to other medically important event). Prior to the event the subject had an atrial line placed. The subject developed significant head and neck swelling and had a complex airway that required an emergency tracheostomy. The edema was possibly related to blockage of vessel emptying into the heart. On Day 108 after transplantation, the subject's generalized edema became Grade 5 (serious due to death). The subject was diuresed with 7 mg furosemide by IV every 12 hours, however the fluid was unable to be removed and the subject died. The cause of death was reported as cardiorespiratory arrest related to anasarca. The events of respiratory failure and generalized edema were ongoing at the time of death.

- *Subject* (b) (6) *was a white male subject with atypical cDGA and was 87 days old at informed consent. The subject received treatment with cyclosporine (from 9 days prior to 159 days after transplantation), daclizumab (1 day prior to transplantation), methylprednisolone (from 6 days prior to 69 days after transplantation and from 79 days after to 159 days after transplantation), mycophenolate mofetil (from 5 days after to 42 days after transplantation and from 52 days after to 59 days after transplantation), and RATGAM (3 doses from 5 days prior to 3 days prior to transplantation). The subject experienced Grade 5 viral upper respiratory tract infection 151 days after transplantation (serious due to fatal). The subject had an elevated WBC, cough, and fever associated with RSV infection. The subject was treated with inhaled ribavirin. A chest X-ray showed no pneumothorax. On Day 154 after transplantation, the subject's respiratory status worsened, and the subject was transferred to the PICU where the subject experienced Grade 4 respiratory failure (serious due to life-threatening). The subject was intubated, and IV ribavirin was started. Respiratory support was continued and despite treatment, the subject experienced worsening of pneumonia and became more acidotic. The subject's creatinine increased, urine output decreased, despite the use of diuretics, and the subject developed anasarca. The subject's condition worsened daily. On Day 160 after transplantation, the subject died. The cause of death was determined to be respiratory failure due to respiratory syncytial virus infection.*

7.1.7.6 Maximum Naïve CD4 Counts Post-transplantation

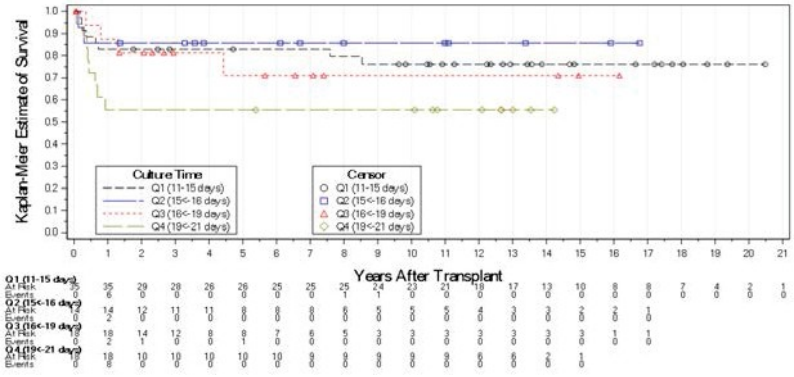
As discussed in Section 7.1.5.1, higher post-transplantation naïve CD4+ T cell counts appeared to be associated with better survival.

7.1.7.7 RETHYMIC Culture Time

An analysis of the primary and secondary efficacy outcomes with regards to the duration of time between harvesting the thymus and implantation of RETHYMIC (culture time) was generated using the most recent dataset (i.e., clinical dataset updated through 30 April 2021). The analysis divided time after harvest to transplant in 4 quartiles and analyzed survival. In the efficacy analysis set (EAS), there were 35 subjects (recipients) in the quartile representative of the shortest culturing period (11-15 days), 14 subjects in the next quartile (16 days), 18 subjects in the next quartile (17 to 19 days), and 18 subjects in the quartile representative of the longest culturing period (20 to 21 days).

In the EAS, the Kaplan-Meier estimated survival rates at Year 1 were 83% (95% CI [0.658, 0.919]), 86% (95% CI [0.539, 0.962]), 89% (95% CI [0.624, 0.971]), and 56% (95% CI [0.305, 0.748]) for subjects receiving tissue cultured from 11 to 15 days, 16 days, 17 to 19 days, and 20 to 21 days, respectively. Similarly, the Kaplan-Meier estimated survival rates at Year 2 were 83% (95% CI [0.658, 0.919]), 86% (95% CI [0.539, 0.962]), 83% (95% CI [0.568, 0.943]), and 56% (95% CI [0.305, 0.748]) for subjects receiving tissue cultured from 11 to 15 days, 16 days, 17 to 19 days, and 20 to 21 days, respectively. Most deaths (18 of 19 deaths in the EAS) occurred within the first year after implantation with the majority of deaths occurring in subjects receiving tissue cultured for the longest time period (8 deaths among 18 subjects receiving tissue cultured between 20 and 21 days) and the shortest period of time (6 deaths among 35 subjects receiving tissue cultured from 11 to 15 days). Overall, the survival rate was lowest among subjects receiving RETHYMIC cultured from 20 to 21 days with a Kaplan-Meier estimated survival rate 56% (95% CI [0.305, 0.748]), with all deaths in this quartile occurring in the first-year post-transplantation. Similar results were also observed when analyzed by the full analysis set (FAS). Overall survival was ≥ 77% in the EAS for all tissue culture times except for subjects receiving tissue cultured for the longest duration of 20 to 21 days. Subjects in this group had an overall survival of 55.6% with 10 of 18 subjects surviving (Figure 7). A Cox proportional hazards (PH) model was performed to fit the survival data to time in tissue culture to see if it had a significant effect on survival. While there was a trend towards decreased survival with increasing time in tissue culture, this analysis did not find a statistically significant difference when survival was compared against subjects receiving tissue cultured for 16 days, which was the most common duration of tissue culture used for implantation.

Figure 7. Kaplan-Meier Survival by Year and Time in Tissue Culture (EAS)



(Source: Applicant's figure 2 in clinical amendment SN0071)

An analysis of the total and naïve T cell counts with regards to the duration of time between harvesting the thymus and implantation of RETHYMIC (culture time) is

provided in Table 19. While data were limited and thus variable, median naïve CD4 T cell counts were above 100 cells/mm^3 at Year 1 and Year 2 post-transplantation in all tissue culture time quartiles. This threshold is considered sufficient to fight infection and supports the use of RETHYMIC cultured for up to 21 days, as the longer culture time does not delay the effective development of thymic function.

Table 19. Naïve CD4+ Cell Count by Quartile of Time in Tissue Culture Subgroup

		EAS Naïve CD4 (cells/mm^3)					
Subgroup		Baseline		Year 1		Year 2	
		n	Median	n	Median	n	Median
Time in Tissue Culture	Quartile 1 11 to 15 Days (N=35)	21	1.000	21	222.300	12	323.000
	Quartile 2 16 Days (N=14)	10	0.825	10	170.835	7	273.000
	Quartile 3 17-19 Days (N=18)	14	1.000	6	217.000	3	202.000
	Quartile 4 20-21 Days (N=18)	14	1.250	6	297.410	4	135.695

(Source: Applicant's table from clinical amendment SN0071)

Reviewer's comments: While the highest proportion of deaths occurred in subjects receiving RETHYMIC manufactured between 20 and 21 days, these deaths were most commonly associated with pre-existing infections or comorbidities present prior to treatment with RETHYMIC. Specifically, 6 of the 8 subjects (Subjects (b) (6) who died in the EAS following receipt of RETHYMIC cultured from 20 to 21 days died from infections that were present prior to implantation with RETHYMIC. The presence of pre-existing infections in combination with significant disease-related comorbidities likely contributed to the higher incidence of deaths reported in this quartile. The two subjects who did not die from complications associated with pre-existing infections were Subjects (b) (6) . Both of these subjects developed serious viral infections in the early post-transplantation period, prior to the expected development of thymic function, and were unable to overcome these infections. Furthermore, the majority of deaths occurred within the first year following implantation, including all 8 of the deaths reported in the longest tissue culture time quartile. It is difficult to determine the impact of tissue culture time on survival because of the small sample size, confounders and deaths occurring prior to the expected development of immune reconstitution. In addition, from a CMC perspective, there is no evidence to suggest that thymus slices cultured 20-21 days were of lower quality or differ significantly from those in the next quartile down (please see Dr. Finn's CMC review for details).

7.1.7.8 Manufacturing Facility

Manufacturing initially occurred in (b) (4) and was subsequently changed to the (b) (4) facility (b) (4). The EAS includes 87 subjects treated at (b) (4) and 18 treated at (b) (4). The primary difference in (b) (4) and (b) (4) populations are that the (b) (4) subjects have had longer follow-up. Additionally, the (b) (4) subjects are

generally older as children were unable to be treated while CMC manufacturing changes were being implemented to respond to the CR. (No subjects were treated between January 2018 and February 2019). The (b) (4) subjects had a mean age at time of RETHYMIC transplant of 7.9 +/- 4.9 months and the (b) (4) subjects had a mean age of 19.9 months +/- 8.3 months.

Overall survival was 81% and 83% in the EAS and FAS populations treated with product manufactured in the (b) (4) facility and about 70% for patients treated with product manufactured in the (b) (4) facility. Although there are a limited number of subjects treated with the (b) (4) product, a Kaplan-Meier survival curve compared survival of the (b) (4) and (b) (4) treated subjects in the EAS, and the curves are similar (Figure 8). A Cox proportional hazard (PH) analysis for survival by manufacturing site found the difference was not statically significant (p=0.68).

Figure 8. Kaplan-Meier Survival by Year (EAS Population)

(b) (4)						
At Risk	79	79	60	59	59	57
Events	0	19	1	0	0	1
(b) (4)						
At Risk	16	16	12	9	3	
Events	0	3	0	0	0	

(Source: Applicant's figure from clinical amendment SN0065)

A binomial exact test for survival (evaluating against >50% survival) by manufacturing site at year 1 and at year 2 post-transplantation showed a significant likelihood of >50% survival at Year 1 for subjects treated with RETHYMIC from either the (b) (4) or (b) (4) facilities (Table 20). At Year 2 the likelihood of >50% survival does not achieve significance for subjects treated with RETHYMIC from the (b) (4) facility while the likelihood of survival >50% is highly significant for subjects treated with RETHYMIC from the (b) (4) facility (Table 21). The results at Year 2 for subjects treated with the (b) (4) product are likely not statistically significant due to the limited number of patients at Year 2, and the number of patients censored from this analysis due to short follow-up duration.

Table 20. Survival at Year 1 Post-transplantation by Manufacturing Facility

Manufacturing Facility	(b) (4)		(b) (4)	
	EAS N=16	FAS N=18	EAS N=79	FAS N=87
Alive at Year 1: n (%)	12 (75.0)	14 (77.8)	60 (75.9)	67 (77.0)
Dead at Year 1: n (%)	3 (18.8)	3 (16.7)	19 (24.1)	20 (23.0)
Censored at Year 1: n (%)	1 (6.3)	1 (5.6)	0	0
Number of subjects alive or dead at Year 1	15	17	79	87
Survival Rate	80.0%	82.4%	75.9%	77.0%
95% CI	0.52, 0.96	0.57, 0.96	0.65, 0.85	0.67, 0.85
One sided p-value[a]	0.0176	0.0064	<0.0001	<0.0001

Source: Table 14.2.1.3.28
Abbreviations: EAS = efficacy analysis set; FAS = full analysis set; CI = confidence interval
Manufacturing facility: (b) (4)
[a] Based on exact binomial test of survival rate > 50%.

(Source: Applicant's table from clinical amendment SN0065)

Table 21. Survival at Year 2 Post-transplantation by Manufacturing Facility

Manufacturing Facility	(b) (4)		(b) (4)	
	EAS N=16	FAS N=18	EAS N=79	FAS N=87
Alive at Year 2: n (%)	8 (50.0)	9 (50.0)	59 (74.7)	65 (74.7)
Dead at Year 2: n (%)	3 (18.8)	3 (16.7)	20 (25.3)	22 (25.3)
Censored at Year 2: n (%)	5 (31.3)	6 (33.3)	0	0
Number of subjects alive or dead at Year 2	11	12	79	87
Survival Rate	72.7%	75.0%	74.7%	74.7%
95% CI	0.39, 0.94	0.43, 0.95	0.64, 0.84	0.64, 0.83
One sided p-value[a]	0.1133	0.0730	<0.0001	<0.0001

Source: Table 14.2.1.3.29
Abbreviations: EAS = efficacy analysis set; FAS = full analysis set; CI = confidence interval
Manufacturing facility: (b) (4)
[a] Based on exact binomial test of survival rate > 50%.

(Source: Applicant's table from clinical amendment SN0065)

Reviewer's comments: The analyses of survival for subjects treated with RETHYMIC from the (b) (4) facility compared with the (b) (4) facility are limited by the relatively small number of subjects and accompanying relatively short duration of follow-up for subjects treated with RETHYMIC from the (b) (4) facility. Nonetheless, the overall data demonstrated a similar pattern and frequency of survival for subjects treated with RETHYMIC from either manufacturing site. The Cox PH analysis further supports the conclusion that survival is similar regardless of the manufacturing facility and with accumulating clinical information the binomial exact analysis finds significant likelihood of >50% survival 1 year after treatment with RETHYMIC manufactured at the (b) (4) facility consistent with the data from subjects treated with RETHYMIC manufactured at the (b) (4) facility.

Flow cytometry data evaluating T-cell types was available for subsets of patients treated with RETHYMIC manufactured at the (b) (4) or (b) (4) facilities. Descriptive statistics of the data are shown for Naïve CD3+, CD4+, and CD8+ cell counts (Table 22) and for Total CD3+, CD4+, and CD8+ cell counts (Table 23) collected at baseline, Month 6, Month 12, Month 18, and Month 24 after RETHYMIC implantation for the EAS population. The available information after 12 months is sparse but is included for completeness. It should be noted that there are few subjects with data available at all time points and, though the number of subjects evaluated at different time points may be

similar or equal, the descriptive statistics may not represent evaluation of the same subjects at any two timepoints.

Consistent with the diagnostic criteria for CA, naïve T cells of all types were very low at baseline, with mean values of <15 cells/mm^3 in all cases. Maximum naïve T cell counts were less than 50 cells/mm^3 except for naïve CD3 cells, which had a maximum of 77 for subjects treated with RETHYMIC from the (b) (4) facility and a maximum of 98 for subjects treated with product from the (b) (4) facility. In general, there was little change in the numbers of naïve T cells from baseline to Month 6 although apparent increases in naïve CD3+ and naïve CD4+ cell counts were observed in subjects treated with RETHYMIC from the (b) (4) facility. Though the Month 12 observations are limited, the mean number of naïve T cells of all types was increased from baseline and exceeded 50 cells/mm^3 regardless of the manufacturing facility for RETHYMIC excepting the naïve CD8+ cell counts (mean 31.3 cells/mm^3) in subjects treated with RETHYMIC from the (b) (4) facility.

Table 22. Naïve T Cell Counts (cells/mm^3) by Manufacturing Facility (EAS)

		Naïve CD3 Cells		Naïve CD4 Cells		Naïve CD8 Cells	
Visit	Statistic	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Baseline[a]	n	14	27	13	52	13	46
	Mean (SD)	14.241 (24.727)	7.214 (18.656)	7.523 (13.084)	3.675 (6.377)	4.231 (11.352)	5.930 (12.037)
	Median	1.000	2.130	1.000	1.250	0.000	0.530
	Q1, Q3	0.000, 30.000	0.220, 6.000	0.000, 10.000	0.000, 4.175	0.000, 0.000	0.000, 3.180
	Min, Max	0.00, 77.00	0.00, 98.00	0.00, 38.00	0.00, 35.10	0.00, 41.00	0.00, 45.94
Month 6	n	11	16	11	56	11	45
	Mean (SD)	13.636 (13.140)	51.259 (54.436)	13.364 (20.982)	71.493 (106.953)	2.364 (2.461)	22.364 (30.537)
	Median	10.000	25.500	5.000	28.220	1.000	12.610
	Q1, Q3	3.000, 22.000	8.500, 81.000	1.000, 16.000	8.880, 93.900	0.000, 5.000	4.000, 29.000
	Min, Max	1.00, 44.00	0.52, 159.00	0.00, 73.00	0.00, 653.00	0.00, 7.00	0.00, 163.00
Month 12	n	6	8	6	39	6	34
	Mean (SD)	120.167 (171.464)	207.825 (153.091)	88.000 (120.632)	271.692 (205.302)	31.333 (51.899)	88.985 (81.419)
	Median	68.500	162.410	41.000	261.820	13.500	61.095
	Q1, Q3	17.000, 103.000	101.810, 280.480	7.000, 123.000	80.000, 390.160	4.000, 21.000	20.560, 142.000
	Min, Max	4.00, 460.00	61.00, 512.20	1.00, 315.00	5.50, 751.00	0.00, 136.00	2.68, 304.30
Month 18	n	2	5	2	21	2	19
	Mean (SD)	51.500 (72.832)	286.396 (255.828)	37.500 (53.033)	259.574 (171.449)	13.000 (18.385)	66.304 (56.519)
	Median	51.500	244.000	37.500	294.360	13.000	49.000
	Q1, Q3	0.000, 103.000	110.610, 386.000	0.000, 75.000	119.850, 363.000	0.000, 26.000	21.060, 122.130
	Min, Max	0.00, 103.00	20.00, 671.37	0.00, 75.00	9.00, 511.00	0.00, 26.00	7.00, 186.05
Month 24	n	NA	7	1	25	1	25
	Mean (SD)	NA	295.260 (247.224)	94.700 (NA)	281.873 (200.787)	45.510 (NA)	93.641 (69.890)
	Median	NA	227.000	94.700	273.000	45.510	84.000
	Q1, Q3	NA	76.000, 484.530	94.700, 94.700	113.000, 361.000	45.510, 45.510	44.820, 131.000
	Min, Max	NA	42.80, 746.17	94.70, 94.70	33.00, 858.00	45.51, 45.51	6.04, 275.00

(Source: Applicant's table from clinical amendment SN0065)

Table 23. Total T Cell Counts (cells/mm^3) by Manufacturing Facility (EAS)

		Total CD3 Cells		Total CD4 Cells		Total CD8 Cells	
Visit	Statistic	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Baseline[a]	n	16	79	15	70	15	67
	Mean (SD)	281.125 (209.806)	732.826 (1485.224)	145.467 (82.827)	274.932 (516.302)	80.733 (102.019)	276.350 (763.736)
	Median	276.500	135.000	156.000	87.140	47.000	13.000
	Q1, Q3	123.500, 437.000	17.000, 378.000	68.000, 185.000	11.000, 247.000	19.000, 83.000	1.000, 81.690
	Min, Max	0.000, 700.000	0.000, 7684.000	0.00, 278.00	0.00, 2458.00	0.000, 366.000	0.000, 4594.000
Month 6	n	13	65	12	65	12	65
	Mean (SD)	372.231 (371.993)	524.003 (939.307)	282.417 (325.821)	381.844 (763.862)	51.250 (46.406)	72.665 (110.664)
	Median	252.000	347.000	164.500	261.000	35.000	40.000
	Q1, Q3	160.000, 393.000	213.000, 519.000	56.000, 423.000	152.000, 349.000	17.000, 74.000	20.000, 77.000
	Min, Max	37.000, 1383.000	30.000, 7532.000	21.00, 1146.00	10.00, 6187.00	5.000, 158.000	1.420, 651.160
Month 12	n	11	47	10	48	10	47
	Mean (SD)	394.545 (346.008)	824.354 (413.633)	277.500 (262.715)	609.494 (342.191)	98.000 (98.751)	162.407 (122.788)
	Median	271.000	771.000	207.500	565.800	54.500	140.000
	Q1, Q3	148.000, 578.000	550.000, 1045.000	85.000, 365.000	351.500, 738.415	24.000, 180.000	68.750, 250.700
	Min, Max	5.000, 1196.000	119.000, 1946.580	4.00, 875.00	93.00, 1780.00	1.000, 276.000	14.000, 624.850
Month 18	n	4	28	4	29	4	29
	Mean (SD)	1090.750 (1113.657)	789.032 (378.139)	823.750 (858.401)	596.216 (336.193)	212.750 (230.841)	150.590 (101.794)
	Median	830.000	832.000	609.000	592.000	168.000	141.000
	Q1, Q3	371.500, 1810.000	466.925, 1078.500	320.000, 1327.500	360.990, 712.000	32.000, 393.500	62.000, 199.000
	Min, Max	45.000, 2658.000	105.000, 1606.000	32.00, 2045.00	72.00, 1530.00	4.000, 511.000	18.000, 390.000
Month 24	n	3	28	3	29	3	29
	Mean (SD)	538.000 (73.655)	803.778 (432.000)	363.667 (60.575)	588.674 (330.045)	102.667 (29.535)	166.999 (116.805)
	Median	523.000	768.000	337.000	566.000	101.000	141.000
	Q1, Q3	473.000, 618.000	451.015, 1031.375	321.000, 433.000	307.160, 768.000	74.000, 133.000	97.390, 209.000
	Min, Max	473.000, 618.000	104.000, 1701.000	321.00, 433.00	76.00, 1255.00	74.000, 133.000	23.000, 580.000

(Source: Applicant's table from clinical amendment SN0065)

T cell proliferation in response to PHA stimulation results by RETHYMIC manufacturing facility are presented in Table 24. These results were collected for both manufacturing facilities only at baseline, Month 12, Month 15, Month 18, and Month 24. Data are shown for change from baseline and percent change from baseline. These data are variable and there are few observations from patients treated with RETHYMIC manufactured at the (b) (4) facility. There is a large increase from baseline observed in the PHA proliferation response that is apparent in the change from baseline and percent change from baseline in subjects treated with RETHYMIC from either manufacturing facility. Variability and sparsity of data from the (b) (4) manufacturing facility limit further conclusions from these data.

Table 24. PHA Response by Manufacturing Facility (EAS)

		(b) (4) (EAS = 16)		(b) (4) (EAS = 79)	
Visit	**Statistic**	**Change from baseline[a]**	**Percent Change from baseline (%)**	**Change from baseline[a]**	**Percent Change from baseline (%)**
Month 12	n	4	4	36	36
	Mean (SD)	105510.0 (6216.84)	7703.378 (7540.519)	144091.1 (94355.83)	14363.777 (31834.03)
	Median	106674.5	7199.752	135500.0	3166.661
	Q1, Q3	101303.0, 109717.0	1473.032, 13933.724	85006.5, 189923.0	920.694, 9481.135
	Min, Max	96987, 111704	86.23, 16327.78	-95651, 350722	-91.76, 169395.51
Month 15	n	3	3	20	20
	Mean (SD)	122497.7 (34982.52)	33060.148 (46025.20)	133063.9 (85005.21)	8901.391 (15250.35)
	Median	114007.0	13159.771	143774.0	2682.023
	Q1, Q3	92542.0, 160944.0	333.637, 85687.037	75630.0, 192429.5	520.438, 9482.034
	Min, Max	92542, 160944	333.64, 85687.04	-59659, 275656	-84.81, 63400.37
Month 18	n	2	2	20	20
	Mean (SD)	321617.0 (450729.66)	296539.136 (419116.24)	166043.5 (63189.88)	25647.525 (49350.34)
	Median	321617.0	296539.136	141524.5	6056.717
	Q1, Q3	2903.0, 640331.0	179.198, 592899.074	108664.5, 235435.0	835.529, 23128.905
	Min, Max	2903, 640331	179.20, 592899.07	87324, 258225	129.52, 175344.72
Month 24	n	1	1	19	19
	Mean (SD)	45788.0 (NA)	1289.077 (NA)	189889.2 (105331.97)	24391.500 (48402.37)
	Median	45788.0	1289.077	203645.0	4829.769
	Q1, Q3	45788.0, 45788.0	1289.077, 1289.077	154440.0, 236224.0	488.662, 24059.139
	Min, Max	45788, 45788	1289.08, 1289.08	-55656, 381832	-90.53, 165104.07

Reviewer's comments: Aside from naïve CD8+ T cell counts not reaching the 50 cells/mm³ threshold at month 12, naïve CD4+ T cell counts in (b) (4) group (mean, 88 cells/mm³, median 41 cells/mm³) were significantly lower than those in the (b) (4) group (mean, 272 cells/mm³, median, 262 cells/mm³) by ANOVA test (p=0.015). From product perspective, culture time was not longer for RETHYMIC manufactured in (b) (4) facility. There was only one subject in (b) (4) group who received product cultured 20-21 days. For detailed discussion on product quality, please refer to Dr. Finn's CMC review memo.

From the clinical perspective, there were two factors that could potentially have contributed to the lower naïve T cell counts at year 1 in (b) (4) subjects, dosage, and age. As previously discussed in Section 7.1.7.4, there were no significant dose effect on the number of naïve T cells. That said, 72% of (b) (4) subjects received doses in the lower two quartiles, while 45.2% of (b) (4) subjects received doses in the lower two quartiles (Table 25). In addition, as previously discussed in the demographics section, (b) (4) subjects were on average older than (b) (4) subjects at treatment. The majority of (b) (4) subjects were older than 1 year of age at treatment. This difference in age may

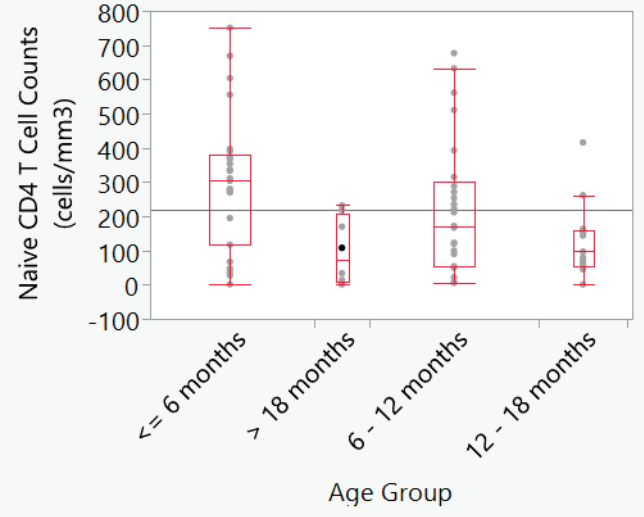
contribute to the low naïve T cell counts at year 1. As shown in Figure 9, naïve CD4 T cell counts at year 1 were significantly lower in subjects older than 1 years of age at treatment (12-18 months and > 18 months) compared to those younger than 1 years of age (ANOVA, p = 0.011).

Table 25. Doses by Manufacturer Facility (EAS)

DOSEGRP	(b) (4)	(b) (4)
Missing	14	0
Q1 (4522.7<-9069.9 mm2/m2)	17	6
Q2 (9069.9<-12674.6 mm2/m2)	16	7
Q3 (12674.6<-16052.0 mm2/m2)	21	2
Q4 (16052.0<-23754.5 mm2/m2)	19	3

(Source: Reviewer's table based on SN0065)

Figure 9. Naïve CD4 Cell Counts by Age at Treatment (EAS)



(Source: Reviewer's figure based on SN0065)

Although there was a relatively lower naïve T cell number in the (b) (4) *group, the PHA response and survival were comparable between two manufacturing facilities.*

7.1.8 Persistence of Efficacy

Long-term survival was evident as discussed previously. While data collection for secondary efficacy parameters was limited to 2 years post-transplantation in this application, Markert and colleagues have published on the immune outcomes of these subjects beyond 2 years. In particular, naïve T cell counts have been reported to peak around 1 to 2 years post-transplantation and then stabilize. With use of general estimating equations analysis, it was shown that the naïve CD4+ T cells begin to decrease 2 years after implantation while the numbers of naïve CD8+ T cells remain stable after Year 2. Naïve CD4 and CD8 T cell counts generally remained below the 10th percentile for age but were considered sufficient to fight infection and enable survival [Markert, 2010]. In addition, the time course of the development and persistence of T cells over time for a single subject with data through 14.8 years was investigated. At the last assessment (14.8 years of age), the subject had normal CD3, CD4, naïve CD4, and naïve CD8 counts [Lee, 2014]. T cell diversity has also been shown to persist over

time, indicating the long-term maintenance of T cell diversity. Finally, T cell proliferative responses have also been shown to persist over time [Markert, 2007; Markert, 2009; Markert, 2010]. These results complement the long-term survival benefit by demonstrating that, following emergence of immune function at approximately 6 months after RETHYMIC implantation, the immune reconstitution is durable and persists long term. Furthermore, in a previous analysis, evidence of long-term tolerance of the newly developed T cells towards RETHYMIC was confirmed using a mixed lymphocyte cultures test. Specifically, in a subset of 12 subjects ranging from 10.5 months to 6.4 years post-transplantation (median 4.2 years), tolerance of recipient T cells toward their HLA-non-matched thymus (RETHYMIC) grafts was observed [Chinn, 2008].

7.1.9 Product-Product Interactions

There were no studies of potential drug-drug or drug-food interactions performed with RETHYMIC.

7.1.10 Additional Efficacy Issues/Analyses

None.

7.1.11 Efficacy Conclusions

The RETHYMIC development program for the treatment of T cell immunodeficiency resulting from CA has been conducted at Duke University Medical Center during the past 30 years. The primary clinical efficacy data included in this Application are derived from 7, single-site, open-label, non-randomized clinical studies in subjects with CA (Studies 668-1, 668-2, 884 [includes 884-1], 931, 932, 950 [includes 950-1], and 25966), three supporting single-subject/expanded access studies (Studies 735, (b) (6), and 51692) and a 49-patient natural history cohort. The 10 clinical trials cumulatively treated 105 subjects with RETHYMIC. Of these, 95 subjects met the criteria for inclusion in the EAS. The EAS included subjects with CA (93 associated with cDGA and 2 with FOXN1 deficiency) who had not previously received a transplant (HCT or thymus).

The 1-year survival for EAS subjects was 76.8% (95% Confidence Interval (CI): 67%, 84.1%) at 1 year and 75.7% (95% CI: 65.8%, 83.2%) at 2 years. The lower limits of 95% CIs at Year 1 and Year 2 far exceeded the specified survival rate of 50% under the null hypothesis. For subjects treated with RETHYMIC who were alive at 1-year post-transplantation, the survival rate was 94% and was essentially unchanged thereafter. The median age of all surviving subjects was 11.4 years (3 years to 25.7 years), and censoring primarily occurred due to duration of follow-up rather than death. This is a large treatment effect size compared to what is observed in the natural history cohort, where 94% of subjects died by two years and all subjects died by three years of age.

The survival benefit was further supported by secondary efficacy endpoints for immune function. The immune function established following RETHYMIC was shown to persist for at least 2 years post-transplantation. Median naïve CD3, CD4, and CD8 cell counts increased through 2 years post-transplantation. T cell proliferative responses to PHA, ConA, Sol CD3, Immob CD3, and tetanus toxoid were also increased and sustained through 2 years post-transplantation. TCRVβ repertoire variability as assessed by immunoscope/spectratyping and flow cytometry demonstrated a diverse TCR repertoire through 2 years after implantation. In addition, the incidence of infections was shown to

significantly decline 12 to 24 months post-transplantation as compared to the first 12 months post-transplantation. These data support the development and persistence of immune function through 2 years post-transplantation.

In summary, treatment with RETHYMIC reconstituted an immunocompetent T cell population in subjects with CA. This resulted in a reduction in infections that enabled long-term survival in a population with an otherwise rapidly fatal disease.

8. INTEGRATED OVERVIEW OF SAFETY

8.1 Safety Assessment Methods

The analysis of safety was based on the clinical integrated safety datasets that were submitted to the Agency on June 4, 2021 (BLA 125685/SN0065). The safety datasets contained data that were pooled from the 10 studies.

The results are presented as descriptive statistics and summary tabulations. Continuous variables were presented as minimum and maximum values, mean, median and standard deviation (SD). Categorical data (frequency tabulations) were presented by the number and percentage of subjects. The denominator for all percentages was the number of subjects that were pooled for the analysis set of interest, unless otherwise indicated.

Baseline was defined as the last value obtained prior to RETHYMIC implantation. If multiple values were recorded on the same day, the average of all measurements taken on that day was used as the baseline value. The day of RETHYMIC implantation was defined as Day 0 and was used to calculate days relative to implantation. Imputation of missing dates was done prior to entering the data into the database according to the eCRF completion guidelines. Consequently, no programmatic imputation was performed.

In the pooled analysis datasets, the severities of all AEs were reported as collected in the individual studies using the CTCAE version used in that study. A tabular summary of AEs with severity ≥ Grade 3 was generated. Adverse events were summarized separately by presenting the number and percentage of subjects having any event; having a related event; having an event in each MedDRA system organ class (SOC) and preferred term (PT); and having each individual event and the severity, relationship, and outcome of each event. The number of events was also presented. Missing severities, relationship or outcomes were classified as unknown. For the purposes of summarization, all AEs including infection-related AEs were included in AE summary tables, unless otherwise specified.

A subject with more than one occurrence of the same AE in a particular SOC was counted only once in the total of subjects experiencing AEs in that particular SOC. If a subject had the same AE at more than one severity, or with more than one relationship to study drug, the most severe rating or the stronger causal relationship to study drug was given precedence. Any missing severities, causalities, or outcomes were not imputed and were classified as unknown.

Safety assessments were generally conducted weekly for the first 12 weeks post-transplantation while the subject was still hospitalized. Further safety assessments were summarized at Months 3, 6, 9, 12, 18, and 24. Additional AEs reported beyond 2 years were also summarized. However, the reporting of AEs beyond 2 years was limited given differences in the duration of subject follow-up and the return of subjects to their referring institution.

While the original protocols for studies 735, (b) (6), 668-1, 668-2, 884, 931, 932, and 950 did not include an end date for subject follow-up, a program-wide protocol addendum (dated 31 July 2010) limited subject follow-up to the first 2 years after implantation. This follow-up period was selected because immune reconstitution is usually achieved within 1 to 2 years after implantation. In addition, subjects returned to their referring institution shortly after implantation and, as such, were followed by their referring physician as part of standard clinical care. Studies 25966 and 51692 also utilized a 2-year follow-up period. Once subjects returned to the referring institution, adverse event (AE) reporting was dependent upon the referring physician. For consistency across the study protocols and in alignment with the 2-year follow-up period, AEs were summarized within 2 years post-transplantation. Additional AEs reported beyond 2 years were evaluated. However, given the change in the duration of subject follow-up post-transplantation and the return of subjects to their referring institution, the reporting of AEs beyond 2 years was limited.

8.2 Safety Database

8.2.1 Studies/Clinical Trials Used to Evaluate Safety

Safety data are derived from all 10 clinical studies including study (b) (6) (single subject treatment plan: thymus implantation for EBV lymphoma) and study 735 (treatment of subjects with partial DGA). All 105 subjects exposed to RETHYMIC are included in safety analyses and are referred to as Full Analysis Set (FAS). There were 10 subjects who were included in the FAS but not included in the EAS (please refer to Section 7.1.3 for a list of the 10 subjects).

8.2.2 Overall Exposure, Demographics of Pooled Safety Populations

Demographics

Subjects in the FAS had a median age at implantation of 269 days (range: 33-6163): 35 subjects (33%) were younger than 6 months old; 30 subjects (29%) were 6-12 months old; 21 (20%) subjects were 12-18 months old; and 19 (18%) subjects were more than 18 months old. Four subjects who were included only in the FAS (b) (6) were more than three years old. Subjects (b) (6) were 4741, 5763, 1017 and 6163 days old, respectively, at the time of implantation. FAS subjects included 45 females and 60 males. There were 76 White, 21 black or African American, 3 Asian, 2 American Indian or Alaska Native and 2 multiple race FAS subjects.

The diagnosis of DGA was confirmed based on the presence of characteristic clinical features, including congenital cardiac anomalies, a small or missing thymus, and hypocalcemia secondary to hypoparathyroidism. The diagnosis of athymia, and subsequently cDGA, was confirmed via flow cytometry based upon <50 naïve T cells/mm^3 (CD45RA+, CD62L+) in the peripheral blood or <5% of total T cells being naïve in phenotype. The median age of diagnosis was 24 days (range, 0-1067 days). 98

subjects had cDGA, of which 52 had typical cDGA and 44 had atypical cDGA. a cDGA phenotype classification was missing for two subjects (Subjects (b) (6) with cDGA as it could not be determined at the time of enrollment. In addition, Subject (b) (6) had what was thought to be partial DGA at the time of study enrollment as this subject was treated prior to the availability of reagents to assess naïve T cell markers. Given this diagnosis, this subject was included only in the FAS. However, in reviewing this subject's presentation at the time of this application, the Sponsor/Investigator considered it likely this subject had what is now considered to be atypical cDGA, and thus this subject was reported as such. Three subjects had the diagnosis of FOXN1 deficiency. Two subjects had SCID.

One subject (Subject (b) (6) had athymia of unknown etiology. Specifically, Subject (b) (6) (included in the initial BLA filing) had exposure to maternal diabetes (gestational) and exhibited phenotypic features that included diminished T cell count for age and hypocalcemia; however, the Sponsor/Investigator did not think that this subject had athymia due to cDGA.

Subject (b) (6), who was treated after the initial BLA filing, had a diagnosis of athymia associated with a TBX1 point mutation. This subject was reportedly healthy until she received the varicella vaccine at one year of age and, 2 weeks later, the subject developed cutaneous chickenpox that spread over her body. At 15 months of age, the subject developed significant autoimmune hemolytic anemia. One year later at 27 months, the subject developed granulomatous skin lesions that likely were secondary to vaccine strain rubella (as rubella was detected in her nasopharyngeal secretions on admission for treatment with RETHYMIC). At 3.7 years of age, the subject developed Sweet's syndrome requiring a prolonged intensive care unit admission for treatment of the lesions. The subject was considered by the Sponsor/Investigator to have a unique presentation of athymia as compared to historically treated subjects. This subject also had a variant in the TNFRSF13B gene (TACI). This TACI mutation has not been reported to be associated with athymia and in the opinion of the Sponsor/Investigator this this mutation was playing no role in the underlying immunodeficiency of this subject.

Consistent with criteria used in diagnosing or characterizing athymia, gene mutations and syndromic associations relating to deletion of 22q11.2 (36.2%) and CHARGE (coloboma, heart defect, choanal atresia, growth and development retardation, genitourinary defects, ear defects including deafness) syndrome (21.9%) were commonly observed. Twelve of 23 subjects diagnosed with CHARGE had documentation of a chromodomain helicase DNA binding protein 7 (CHD7) mutation, which is known to be associated with the development of CHARGE syndrome. Thirteen subjects were missing data on cDGA gene mutations/syndromic associations as it was not applicable (subjects diagnosed with FOXN1 deficiency or SCID) or genetic sequencing was not performed. Of note, genetic sequencing was often not performed in the early years of the program as it was not widely available. One subject (Subject (b) (6) had a variant in TBX2 identified post-transplantation. This subject was counted as "missing" as the genetic defect was not identified at the time of implantation. The mothers of 29 subjects were diabetic. Of these, 6 were Type 1, 14 were Type 2, and 9 had gestational diabetes.

As expected, given the disease heterogeneity, a wide range of phenotypic features associated with athymia were observed. All subjects had a naïve T cell count that was low for their age and the majority of subjects reported a congenital cardiac or

cardiothoracic vascular anomaly (86.7%), hypocalcemia (84.8%), growth/mental retardation (51.4%), or deafness or ear pinnae anomalies (50.5%). In addition, gastroesophageal reflux disease (GERD), developmental delays, pyrexia (likely associated with infections with no identified organism), and diarrhea/vomiting were also common in these subjects. Most subjects (>50%) entered the studies with pre-existing central venous catheters and G tubes, with over a third of subjects requiring parenteral nutrition. A quarter of subjects had pre-existing oxygen requirements, including a medical history of mechanical ventilation.

Exposure by Dose

In the FAS, the median dose of RETHYMIC implanted was 12,675 mm2/m2 (range: 4,523 to 23,755 mm2/m2) using a median of 30 slices (range: 10 to 108 slices; Table 26). Two subjects were implanted with 108 slices with the next greatest number of slices implanted being 56. In one instance of 108 slices (Subject (b) (6) in Study 668-1) the individual slice areas were much smaller than the typical area of slices. In the other subject who had 108 slices implanted (Subject (b) (6) in Study 884), the subject was about 13 years of age with a greater body surface area (BSA) than other subjects treated with RETHYMIC. As for exposure to immunosuppressive therapy, immunosuppressive medications were administered to 71 subjects (67.6%) in the FAS.

Table 26. Subjects Exposure by Dose

	EAS N=95	FAS N=105
Total dose implanted (mm^2/m^2)		
n	82	91
Mean (SD)	13053.4 (4345.43)	12579.2 (4444.44)
Median	12996.3	12674.5
Minimum, Maximum	4901.6, 23754.5	4522.7, 23754.5
Missing	13	14
Recipient BSA (m^2)		
n	93	102
Mean (SD)	0.334 (0.0782)	0.374 (0.1874)
Median	0.326	0.339
Minimum, Maximum	0.213, 0.567	0.213, 1.410
Missing	2	3
Thymus slices		
n	81	89
Mean (SD)	29.9 (13.51)	30.8 (15.57)
Median	30.0	30.0
Minimum, Maximum	10, 108	10, 108
Missing	14	16

Source: Table 14.1.5
Abbreviations: BSA = body surface area; EAS = efficacy analysis set; FAS = full analysis set; n = number of subjects included in the analysis; N = number of subjects included in the analysis set; SD = standard deviation

(Source: Applicant's table)

8.2.3 Categorization of Adverse Events

Medical history and AEs were coded according to MedDRA version 19.1. The severities of non-infection-related AEs and SAEs were graded (Grades 1 to 5) according to CTCAE version 3.0 and was used for Studies 668-1/668-2, 884/884.1, 931, 932, 950/950.1, 25966, (b) (6) and 735. CTCAE version 4.0 was used for Study 51692. Infection-related AEs were evaluated using either CTCAE criteria or criteria defined in the Blood and Marrow Transplant Clinical Trials Network (BMTCTN) definitions of infection severity. Infection-related AEs with BMTCTN severity ≥ severe were included in the analysis of AEs of Grade ≥ 3. Life-threatening infection-related AEs with an outcome of fatal were reported as Grade 5 events.

8.3 Caveats Introduced by Pooling of Data Across Studies/Clinical Trials

This integrated safety analysis was the only feasible way to do a comprehensive safety analysis. There were no concerns regarding the pooling of the data.

8.4 Safety Results

8.4.1 Deaths

Of the 105 subjects who received RETHYMIC, 29 subjects were dead at the last follow up. Cause of deaths and time of death are summarized in Table 27.16 subjects died within 6 months after implantation. 7 died between 6 and 12 months; 2 died during year 2, and four died more than two years after implantation at year 3, year 5, year 8 and year 9 respectively. The causes of deaths were categorized into to four groups, infection, cardiopulmonary arrest, respiratory failure/hypoxia and hemorrhagic events. One subject's cause of death was not reported to the sponsor and listed as unknow. Infection is the most common cause of death accounting for 48.3% of the total death (14/29). Of all subjects died of infection, 57% (8/14) died within the first six month after receiving RETHYMIC; and all but one died within first year. This is consistent with that sufficient level of immune reconstitution was typically reached between 6- and 12-months post-transplantation.

Table 27. Cause of Death Summary

Cause of death		Number of Subjects	Subject ID
Infection	Year 1	13	(b) (6)
	Year 2 and after	1	(b) (6)
Cardiopulmonary arrest	Year 1	2	(b) (6)
	Year 2 and after	1	(b) (6)
Respiratory failure/hypoxia	Year 1	5	(b) (6)
	Year 2 and after	2	(b) (6)
Hemorrhagic events	Year 1	3	(b) (6)
	Year 2 and after	1	(b) (6)
Unknown	Year 1	0	
	Year 2 and after	1	(b) (6)

(Source: Reviewer's table based on SN0065)

A brief description of all deaths is provided below.

- Subject (b) (6): This male subject with typical cDGA received RETHYMIC on day 96 if life and died 66 days post-transplantation. On day 62 after implantation, the subject experienced central nervous system hemorrhage. A CT scan revealed severe intraventricular and intraparenchymal hemorrhage. A ventriculostomy was inserted and tapped 2 to 3 times per day. The subject had multiple tonic clonic seizures, respiratory acidosis and elevated blood pressure which continued until death. On day 66 after implantation, the subject's parents opted to change the subject's status to do not resuscitate and withdraw ventilatory support. The subject was extubated and died shortly thereafter with the cause of death listed as severe bilateral intraventricular hemorrhage.

- Subject (b) (6) This male subject with typical cDGA received RETHYMIC on day 51 of life and died on day 130. On Day 125 after transplantation, the subject experienced Grade 4 sepsis. On Day 126 after transplantation, due to the subject's poor prognosis, a do not resuscitate order was written. On Day 130 after transplantation, the subject died. Respiratory failure and sepsis were considered ongoing at the time of the subject's death. The cause of death was listed as sepsis.
- Subject (b) (6): This female subject with typical cDGA received RETHYMIC on day 127 of life and died on post-operative Day 45. The subject experienced Grade 4 enterococcal bacteremia 34 days after transplantation. On Day 35 after transplantation, the subject experienced Grade 4 hypotension and metabolic acidosis. On Day 39 after transplantation, the subject developed a left pneumothorax with hypotension and was briefly treated with pressors. On the same day a left chest tube was inserted. The subject remained in disseminated intravascular coagulation with a markedly distended abdomen. On Day 41 after transplantation, the subject developed a right pneumothorax. On Day 45 after transplantation, the subject experienced Grade 5 respiratory failure. The cause of death was listed as respiratory failure.
- Subject (b) (6): This male subject with typical cDGA received RETHYMIC on day 69 of life and die on day 130 post-transplantation. On Day 130 after transplantation, the subject experienced Grade 5 sepsis. The subject suddenly became tachypneic, and bronchospastic and developed cardiac arrhythmia. The subject was unable to be resuscitated. The cause of death was listed as sepsis.
- Subject (b) (6) This male subject with typical cDGA received RETHYMIC on day 107 of life and died on the same day of implantation. Following an uneventful RETHYMIC implantation, a Nissen/gastrostomy placement was undertaken. On opening the abdomen, bleeding developed and could not be controlled. The autopsy concluded that calcium accretion in the inferior vena cava, extending into the left hepatic vein, had ruptured at the time of Nissen / gastrostomy placement. The cause of death was listed as hemorrhage with Nissen fundoplication surgery.
- Subject (b) (6): The female subject with typical cDGA received RETHYMIC on day 149 of life and died on 2769 post-transplantation. The subject had a medical history of multiple respiratory infections, including influenza. The subject was admitted to hospital with mitral valve insufficiency and recurrent superior vena cava syndrome/bleeding and was placed on a ventilator. The subject received palliative care. The cause of death was listed as respiratory failure.
- Subject (b) (6) The female subject with atypical cDGA received RETHYMIC on day 195 of life and died approximately 3116 days after implantation. The subject had respiratory distress at home, likely secondary to tracheal occlusion, which resulted in death.
- Subject (b) (6): The male subject with atypical cDGA received RETHYMIC on day 354 of life and died on day 44 post-transplantation. The subject experienced Grade 4 alveolar lung disease 15 days after transplantation (serious due to caused/prolonged hospitalization). The subject had continued high fevers requiring treatment with vancomycin and tobramycin. Numerous attempts were made to identify a source of infection, including a tagged WBC study which went to the alveolar area of the lung. The subject was too sick for a lung biopsy. On Day 39 after transplantation, the subject experienced Grade 5 enterococcal sepsis. On Day 43 after transplantation, the subject experienced Grade 5 pseudomonal bacteremia. The autopsy report concluded that the clinical cause of

death was sepsis. Worsening anasarca was secondary to sepsis and multiorgan system failure.

- Subject (b) (6): The male subject received RETHYMIC on day 119 of life and died on day 137 after implantation. On Day 130 after transplantation, the subject experienced Grade 5 viral upper respiratory tract (RSV) infection (serious due to death). Per the Sponsor/Investigator, the subject had RSV since Day -6. The subject had been treated with ribavirin since Day 50 under eIND 63,474. Venous blood gases included pH 7.16, pCO2 of 118 mmHg, pO2 of 49 mmHg, and bicarbonate at 40 mmol/L. The subject was intubated. On Day 132 after transplantation, the subject was placed on an oscillator. Despite full support, respiratory failure continued. On Day 137 after transplantation, the subject's family requested the ventilator be withdrawn and the subject died. The cause of death was listed as respiratory failure; however, the autopsy report showed RSV strongly present throughout the lung and death was completely attributed to RSV infection.
- Subject (b) (6): This female subject with SCID received RETHYMIC on day 449 of life and died on day 375 after implantation. The subject experienced Grade 3 GVHD in skin 165 days after transplantation. The subject died due to presumed sepsis 375 days after RETHYMIC transplantation. The subject experienced Grade 3 Stevens-Johnson syndrome 173 days after transplantation (serious due to other medically important event). The subject had a toxic epidermal necrolysis-like pattern which was suggestive of acute graft versus host disease. However, viral or drug etiology could not be excluded, such as the mycoplasma infection detected and treated on Day 175 after transplantation. The subject was withdrawn from the study by treating physician and died on day 375 after implantation. The cause of death was presumed sepsis.
- Subject (b) (6): The female subject with typical cDGA received RETHYMIC on day 96 of life and died on day 1617 after implantation. The subject collapsed at home. CPR was performed and the emergency medical service called but the subject was pronounced dead after 45 minutes of resuscitation at the local hospital. The cause of death was listed as cardiopulmonary arrest.
- Subject (b) (6): The female subject with cDGA (phenotype missing) received RETHYMIC on day 186 day of life and died on day 82 after implantation. The subject experienced Grade 5 hypoxia 66 days after transplantation. The subject entered the study with GVHD from unirradiated blood transfusions, CMV infection, also presumed to be related to unirradiated blood transfusions, and total paralysis secondary to GVHD. The subject's clinical course included hypotension and was thought to be consistent with sepsis. Despite full support, the subject could not maintain adequate oxygen levels and died on Day 82 after transplantation. The cause of death was listed as hypoxia.
- Subject (b) (6): The male subject with atypical cDGA received RETHYMIC on day 104 of life and died on day 160 after implantation. The subject experienced Grade 5 viral upper respiratory tract infection 151 days after transplantation (serious due to fatal). The subject had an elevated WBC, cough, and fever associated with RSV infection. The subject was treated with inhaled ribavirin. A chest X-ray showed no pneumothorax. On Day 154 after transplantation, the subject experienced worsening of pneumonia and became more acidotic. The subject's creatinine increased, urine output decreased, despite the use of diuretics, and the subject developed anasarca. On Day 160 after transplantation,

the subject died. The cause of death was determined to be respiratory failure due to respiratory syncytial virus infection.

- Subject (b) (6): The male subject with typical cDGA received RETHYMIC on day 103 of life and died on day 289 after transplantation. After enrollment, the subject received an RETHYMIC and paternal parathyroid transplant, and a thymus graft biopsy was attempted on 79 days post-transplantation, but the biopsy did not include any thymus tissue. The subject was transferred back to the referring hospital a few months later. Subsequently, blood samples were obtained showing that normal-appearing naive T cells had developed, as well as normal parathyroid hormone levels. For several months that followed, the subject remained ventilator-dependent, developed more bronchopulmonary dysplasia, and was kept on very high oxygen levels and pressures. The subject had episodes of fevers that were treated with antibiotics and steroids. On Day 289 after transplantation, the subject died at the local medical center. The cause of death was determined to be respiratory failure.
- Subject (b) (6): The male subject received RETHYMIC on day 382 of life and died on day 510 after implantation. The subject experienced Grade 5 central nervous system hemorrhage 127 days after RETHYMIC transplantation (serious due to hospitalization and death). The subject, who had a medical history of intraventricular hemorrhage, presented with grunting and shortness of breath and was seen in a local emergency room. The subject was found to be minimally responsive in respiratory distress. The subject was intubated. Blood pH was 6.8. A head CT showed a large right parietal intracranial hemorrhage with extension into right temporal lobe, subarachnoid space, and ventricular system, along with communicating hydrocephalus with diffuse cerebral edema and a 1.0 cm right-to-left midline shift. A ventriculostomy catheter was placed. A follow-up CT showed interval stability to slight increase in the right-to-left shift. Blood was observed in the catheter. A neurologist performed 2 evaluations over 24 hours and declared the subject brain dead on the following day. Life support was withdrawn. On Day 510 after transplantation, the subject died. The cause of death was determined to be right parietal hemorrhage.
- Subject (b) (6): The female subject was diagnosed with Artemis-deficient SCID and received RETHYMIC on day 672 of life and died on day 950 after implantation. The subject prematurely discontinued from the study. Cause of death was not reported.
- Subject (b) (6): This male subject with atypical cDGA received RETHYMIC on day 144 of life and died on day 103 after implantation. The subject experienced Grade 5 CMV infection 77 days prior to transplantation (serious due to death). The subject had CMV levels in urine > 200,000 copies/mL. The subject was treated with IV human CMV immune globulin and ganciclovir. On Day -1 prior to transplantation, ganciclovir was discontinued and foscarnet was started, to prevent suppression of thymopoiesis. On approximately Day 30 after transplantation, foscarnet was stopped and treatment with ganciclovir was restarted, in addition to treatment with IV human CMV immune globulin. Despite the change in therapy, CMV levels increased further during the second month after transplantation. On Day 81 after transplantation, a head CT showed progressive Grade 3 cerebral atrophy (serious due to medically important event) and new basal ganglia calcifications. CMV levels continued to increase to more than 300,000 copies/mL. The subject was on a ventilator with worsening liver function tests and thrombocytopenia. The subject became unresponsive. On Day

103 after transplantation (Day 247 of life), the parents elected to withdraw support. The cause of death was determined to be progressive cerebral atrophy due to CMV.

- Subject (b) (6): This female subject with atypical cDGA received RETHYMIC on day 182 of life and died on day 234 after implantation. The subject experienced two events of Grade 5 device related infection 229 days after transplantation. A blood culture from the central line was positive for Candida parapsilosis and Candida tropicalis. The subject experienced Grade 5 lower respiratory tract fungal infection 234 days after transplantation had respiratory acidosis and was intubated. On the same day, the subject died. Autopsy results showed yeast in the lungs. The cause of death was reported as respiratory failure secondary to sepsis from Candida tropicalis and Candida parapsilosis.
- Subject (b) (6): The male subject with atypical cDGA received RETHYMIC on day 130 of life and died on day 252 after implantation. The subject experienced Grade 5 Cytomegalovirus infection 38 days after transplantation (serious due to life-threatening and death) with symptoms of fever and increased respiratory rate. Elevated IgE, decreased platelets, and decreased white blood cells were also reported. The urine culture on Day 38 was positive for CMV. Treatment with ganciclovir was started. On Day 252 after transplantation, the subject died due to respiratory complications from CMV infection. Both Subject (b) (6) and the thymus donor tested negative for CMV pre-implantation; however, the subject tested positive for CMV in the urine on Day 38. Per the Investigator, at 49 days post-transplantation the donor urine culture for CMV was negative, and the CMV antibodies had not changed, but a CMV PCR test was positive. The positive CMV PCR in the donor was too low to quantitate or to amplify for a sequencing comparison to the CMV in Subject (b) (6). Though the origin of the CMV infection in Subject (b) (6) remained unknown, it was concluded that the potential risk of CMV transmission from the donor could not be excluded, and thus the death of Subject (b) (6) was considered by the sponsor to be possibly related to RETHYMIC.
- Subject (b) (6) The female subject with atypical cDGA received RETHYMIC on day 438 of life and died on day 229 after implantation. The subject experienced Grade 4 neutropenia 168 days after transplantation. Laboratory results showed a neutrophil count of 470/mm3. The subject improved with filgrastim treatment. The event was deemed likely related to an adenovirus infection, but the possibility of an immunological AE could not be ruled out. On Day 180 after transplantation, ANC had dropped to 0. On Day 182 after transplantation, the subject was diagnosed with Grade 3 tachypnea. On Day 183 after transplantation, the subject showed increasing proteinuria and was diagnosed with Grade 3 renal failure. On Day 186 after transplantation, the subject was diagnosed with Grade 3 hemolysis (serious due to medically important event) and received treatment with rituximab and steroids. On Day 195 after transplantation, creatinine was elevated to 4.1 mg/dL. The subject had continued proteinuria and Fanconi's syndrome. The subject became massively edematous and total parenteral nutrition had to be stopped. The subject recovered; creatinine dropped to 0.3 mg/dL 2 weeks later. The etiology of the renal failure was thought to be the cidofovir therapy, but an immunological AE could not be ruled out. On Day 204 after transplantation, the subject was extubated, and the events of tachypnea and respiratory failure were considered resolved. The subject experienced Grade 5 respiratory failure 223 days after transplantation (serious due to life-threatening, caused/prolonged hospitalization, and death) and was transferred back to the pediatric intensive care unit and intubated. Later, the subject was changed to oscillator support. Six

days before onset of the event, adenovirus load per polymerase chain reaction was found at 397,000 copies/mL and cidofovir was restarted (adenovirus load ranged from undetectable to 8900 copies/mL during the previous 8 months). On Day 229 after transplantation, life support was withdrawn. The cause of death was determined to be respiratory failure due to adenovirus infection.

- Subject (b) (6) This male subject with atypical cDGA received RETHYMIC on day 502 of life and died 480 days after transplantation. The subject experienced grade 4 GVHD on day 62 after implantation. On Day 242 after transplantation, the subject began immunosuppression treatment with cyclosporine and methylprednisolone. On Day 348 after transplantation, the subject was admitted to Duke University Medical Center (DUMC) for continue immunosuppression treatment, which included methylprednisolone, tacrolimus, azathioprine, prednisolone, and MMF. The subject experienced Grade 4 respiratory failure 459 days after transplantation. The event of GVHD was ongoing at the time of the subject's death. On Day 478 after transplantation, the subject experienced Grade 5 cerebral hemorrhage (serious due to death). the subject was transferred to the PICU and placed on the oscillator. Diuretics were removed to spare the kidneys, peritoneal losses were replaced with plasma protein fraction (human), and the subject required daily platelet transfusions due to bone marrow suppression. There was no known trigger, however the cerebral hemorrhage was likely due to the uremia and resulting increase risk of bleeding due to platelet dysfunction. The cause of death was determined as sudden catastrophic intracranial hemorrhage after autologous GVHD.
- Subject (b) (6) This male subject with typical cDGA received RETHYMIC on day 201 of life and died 149 days after implantation. The subject experienced Grade 5 respiratory failure 120 days after transplantation. The subject required Bi-level positive airway pressure on Day 120, was intubated on Day 126 and transitioned to an oscillator on Day 127. The subject continued on various types of mechanical ventilation until death on Day 149 due to disseminated mycobacterial infection. The cause of death was respiratory failure from disseminated mycobacterial infection.
- Subject (b) (6): This male subject with atypical cDGA received RETHYMIC on day 541 of life and died 263 days after implantation. The subject experienced Grade 4 renal failure and respiratory failure 234 days after transplantation (both serious due to life-threatening). The subject had an abnormally low glomerular filtration rate (GFR) (38.6 mL/min/1.73m2) 42 days prior to transplantation. The low GFR likely was secondary to prolonged usage of cyclosporine which began 224 days prior to transplantation. The subject developed renal failure and required near daily dialysis in the month prior to death. The subject also had increasing CO2 levels (range 200 to 250) despite increased ventilator settings to control gas exchange. The events of renal failure and respiratory failure were ongoing at the time of death. The subject experienced Grade 5 multiple organ dysfunction syndrome 263 days after transplantation (serious due to death) and died. The previous day the subject had been withdrawn from pressors. An autopsy was performed. The cause of death was multiorgan system failure as seen in severe septic shock.
- Subject (b) (6): This male subject with atypical cDGA received RETHYMIC on day 447 of life and died 24 days after transplantation. The subject experienced Grade 3 hypoxia 3 days after transplantation (serious due to life-threatening). The subject had been extubated the previous day and had a history of parainfluenza

virus 3 (viral upper respiratory tract infection) and Varicella-zoster virus. The subject required supplemental oxygen. On Day 17 after transplantation, the subject experienced Grade 4 respiratory failure (serious due to life-threatening). The subject also developed severe fluid retention, anasarca, and worsening renal function. The subject showed no improvement on maximum support and was made “do not resuscitate”. On Day 24 after transplantation, the subject experienced Grade 5 parainfluenza viral pneumonia (serious due to death). The family elected to transfer the subject to a conventional ventilator, and he died shortly thereafter. The cause of death was progression of underlying parainfluenza virus 3 pneumonia.

- Subject (b) (6): This male subject with atypical cDGA received RETHYMIC on day 429 of life and died 108 days after transplantation. The subject experienced Grade 4 respiratory failure 103 days after transplantation. Prior to the event of respiratory failure, the subject had sepsis. The subject self-extubated and arrested. The subject was sent to the operating room to change the endotracheal tube to a larger size. A prolonged code ensued. On Day 105 after transplantation, the subject experienced Grade 4 generalized edema. Prior to the event the subject had an atrial line placed. The subject developed significant head and neck swelling and had a complex airway that required an emergency tracheostomy. The subject soon died. The cause of death was reported as cardiorespiratory arrest related to anasarca.
- Subject (b) (6): This male subject with atypical cDGA received RETHYMIC on day 497 of life and died 89 days after implantation. The subject experienced Grade 4 fungemia 64 days after transplantation (serious due to life-threatening event). A skin culture of the diaper rash was obtained on Day 63 and grew rare Candida albicans and rare gram negative rods. The subject experienced Grade 5 systemic Candida 89 days after transplantation. A pancreatic tissue biopsy was done on autopsy which was positive for Candida lucitenia and Candida albicans. Cause of death was determined to be systemic Candida.
- Subject (b) (6): This male subject with typical cDGA was 15 years of age with history of EBV lymphoma when informed to consent to participate in the study and died 117 days after implantation. An AE of neutropenia was observed on post-transplantation Day 10. On post-transplantation Day 24, the subject’s EBV lymphoma progressed, resulting in a number of symptoms related to the progression of EBV lymphoma. Progression of EBV lymphoma continued and the subject’s clinical condition deteriorated over several weeks. The subject died due to progression of EBV lymphoma (resulting in intracranial bleeding) approximately 4 months after implantation. The Sponsor/Investigator considered the fatal SAE to be possibly related to study treatment.
- Subject (b) (6): This male subject with typical cDGA received RETHYMIC on day 517 of life and died 336 days after transplantation. The subject experienced Grade 5 Staphylococcal bacteremia 336 days after transplantation. Per the Sponsor/Investigator, the subject’s death was not unexpected due to numerous medical issues including the development of adenovirus and methicillin resistant Staphylococcal infection. The cause of death was pulmonary failure due to the event of Staphylococcal bacteremia which could not be treated adequately given the subject’s ongoing renal failure and the inability to remove multiple infected lines due to critical illness.
- Subject (b) (6): This female subject with atypical cDGA received RETHYMIC on day 811 of life and died 293 days after implantation. On day 293 post thymus

implantation, parents of the subject noted she was in respiratory arrest. She was transported to the emergency department (ED) in cardiopulmonary arrest and was unable to be revived. The subject had significant congenital heart defects. She had undergone multiple previous cardiac surgeries and additional surgery was anticipated in the summer of 2021. She did not have any other significant prodromal symptoms and had reportedly been doing well overall. The cause of death was reported as cardiopulmonary arrest.

Three deaths were considered by the sponsor/investigator as related or possibly related to study treatment. All three deaths were due to infections or complications from infections and included: one event (Subject (b) (6)) of EBV lymphoma progression resulting in an intracranial hemorrhage considered possibly related to the use of immunosuppression, one event (Subject (b) (6)) of CMV infection considered related to RATGAM, and one event (Subject (b) (6) of CMV infection considered possibly related to RETHYMIC. Specifically, while the thymus donor tested negative for CMV prior to tissue donation, both the subject and donor tested positive for CMV post-RETHYMIC. Though the origin of the CMV infection in Subject (b) (6) remained unknown, it was concluded that the potential risk of CMV transmission from the donor could not be excluded, and thus the CMV infection in Subject (b) (6) was considered possibly related to RETHYMIC.

Reviewer's comments: The most common cause of death (n=14) was infection/infection related complications which was consistent with T cell immunodeficiency in patients with CA, especially before immune function was established.

The second most common cause of death was respiratory failure/hypoxia. Among the 7 subjects reported to have died due to respiratory failure/hypoxia, all 7 had medical histories for prior respiratory distress and/or multiple prior respiratory related infections. Similarly, cardiopulmonary arrest was most likely related to the underlying condition of these subjects (n=3). The majority of subjects of subjects entered RETHYMIC studies with medical histories in the respiratory, thoracic, and mediastinal disorder. In addition, subjects entered studies with pre-existing cardiovascular disorders associated with the disease under study, which, may have increased the risk of cardiovascular events after RETHYMIC treatment.

Of the four hemorrhagic deaths, three were cerebral hemorrhage and one was hemorrhage during Nissen fundoplication surgery. The underlying conditions for the four hemorrhagic events varied. It is noted that in addition to the four subjects, subject (b) (6) had intracranial bleeding due to progression of EBV lymphoma.

The early post-operative period is a treacherous period for subjects with CA who receive RETHYMIC, with deaths occurring in ~1/4 of the subjects for a variety of reasons. Most of the reasons were not unexpected given their underlying disorders. The subjects were particularly vulnerable to begin with for respiratory infections, but being in the hospital to receive the therapy, being under anesthesia, and intubated, and possibly receiving IS therapy puts them into an even more vulnerable status. Particularly because it takes RETHYMIC ~6 months or more to start working well, the peri-operative time and up to a year later is a particularly vulnerable time.

Risk Factors for deaths are discussed below.

Pre-implantation active viral infection:
There were three subjects appeared to have active viral infection prior to RETHYMIC implantation, all three died of the viral infection after implantation. Subjects (b) (6) had RSV infection since day -6 and died of upper respiratory tract RSV infection on day 137 after implantation despite being treated with ribavirin since day 50 under eIND 63,474. Subject (b) (6) had CMV infection on day -77 and was treated with IV human CMV immune globulin and ganciclovir which was switched to foscarnet on day -1 to prevent suppression of thymopoiesis. The CMV infection continued, and the subject died of progressive cerebral atrophy due to CMV 103 days after receiving RETHYMIC. The third subject was subject (b) (6) who had a history of parainfluenza virus 3 (viral upper respiratory tract infection) and Varicella-zoster virus. On day 24 post-transplantation, the subject died of parainfluenza virus 3 pneumonia. Given the 100% death in subjects with pre-existing active viral infection, it will be beneficial to control any existing viral infection before proceeding to RETHYMIC treatment. For CMV, reactivation should be closely monitored particularly in subjects who receive immunosuppressive medications. Furthermore, RETHYMIC and immunosuppressive treatment was considered to be related to the progression of EBV lymphoma and ultimate death in subject (b) (6). Subject (b) (6) died of CMV infection, however, origin of CMV infection remained unclear.

CMV infection:
Six subjects had at least 1 positive pre-implantation CMV culture. Per the sponsor's response to the agency's information request (IR), three of the six subjects tested positive for only once (1 out 2 tests, 1 out of 5 tests, and 1 out of 5 tests respectively) pre-implantation and the sponsor considers these were false positive. Another subject, subject (b) (6), had history of systemic CMV infection but was tested negative during screening. However, CMV was detected in the blood on post-transplantation day 3, 10, 16, 31, 175, 178, 181, 185, and 245 and ultimately the subject had a reactivation of CMV on day 676 which required treatment with valgnciclovir. Subject (b) (6) was alive at last follow up. Based on pre- and post-transplantation of CMV findings, this reviewer considers subject (b) (6) as having pre-existing CMV infection. Therefore, four subjects are considered to have had preexisting CMV infection, three died. However, it is noted that only one subject died of CMV infection. The other two deaths were unrelated to CMV. In addition, pre-existing infection, regardless of pathogen, is associated with death due to infection prior to establishment of sufficient immune function.

cDGA phenotype:
Mortality rates of typical and atypical cDGA phenotypes were 19.2% (10/52) and 27.3% (12/44) respectively for year 1, 21.2% (11/52) and 27.3% (12/44) respectively for year 2. The overall mortality rates were 26.9% (14/52) and 29.5% (13/44) for CA patients with typical and atypical cDGA phenotypes respectively. It appears that patients with atypical cDGA had a slightly higher risk of death overall and were more likely to die during the first year after RETHYMIC implantation. The underly cause of this higher mortality rate in CA patients with atypical cDGA phenotype may be multifactorial. T cell dysregulation were more evident in atypical cDGA patients which was manifested as autologous graft versus host disease (aGVHD) prior to and after RETHYMIC implantation. GVHD complications may have contributed to the higher death rate in patients with atypical cDGA. For example, death of subject (b) (6) was at least in part due to intracranial hemorrhage due to GVHD complications. In fact, subjects with GVHD AE had a mortality rate of 54.5% (6/11) which is much higher than those without GVHD AE post-

transplantation (24.5%). Furthermore, immunosuppression required to control GVHD would have rendered these patients more susceptible to infections and infection related complications. Immunosuppression medications were more commonly used in patients with atypical cDGA phenotype.

Immunosuppression
Of the 105 subjects, 71 received immunosuppression and 34 did not. Overall, 23.5% (8/34) of subjects who did not receive immunosuppression died and 29.6% of subjects who received immunosuppression died. There was no statistically significant difference between overall mortality rates of the two groups.

Pre-implantation renal impairment
10 subjects had elevated pre-implantation serum creatinine, of whom 6 died. Most of these subjects (70%) were younger than 6 months at the time of RETHYMIC implantation. Half of the subjects had typical cDGA, the other half had atypical cDGA. 70% of the subjects did not receive immunosuppression. It appears that elevated pre-implantation serum creatinine increased risk of death in subjects received RETHYMIC.

Total T cell number at baseline
CD3+ T cell number at screening ranged between 0 and 49,827 cells/mm^3. Table 28 shows mortality rate in FAS subjects by total T cell number at screening. Shearer et al. showed that median CD3+ T cell count was 3680 (10%-90%: 2500-5500) cells/mm^3 in infants of 0-3 months of age who did not have immunodeficiency. Accordingly, total T cells counts in subjects received RETHYMIC were divided into three groups: <2000 cells/mm^3, 2000-6000 cells/mm^3 and >6000 cells/mm^3. Mortality rates were comparable between subjects with total T cells count of < 2000 cells/mm^3 and 2000-6000 cells/mm^3. However, 4 of 8 subjects with total T cell counts of more than 6000 cells/mm^3 died. High T cell counts while naïve T cells count was low at screening appear to be a risk factor for death. All 8 subjects had atypical cDGA phenotype. One possibility could be that the higher baseline total T cell numbers were due to oligoclonal expansion of T cells which do not function normally. In addition, total CD3+ cell count and naïve CD4+ cell counts were compared between subjects who die and who were alive. Average CD3+ cell number were ~60% higher in subjects who died, while average baseline naïve CD4+ T cell numbers were relatively similar between subjects who died and those who were alive. This further confirms that in this patient population, higher baseline total T cells number may represent a risk for worse prognosis after RETHYMIC treatment.

Table 28. Mortality by T Cell Number at Screening

CD3 T cell (cells/mm3)	Number of Subjects	Mortality
< 2,000	90	25.6% (23/90)
2,000 – 6,000	7	28.6% (2/7)
> 6,000	8	50% (4/8)

(Source: Reviewer's table based on SN0065)

Naïve CD3+ T cell number at baseline
There were 4 subjects who had baseline naïve CD3+ T cells of more than 50 cells/mm^3: Subjects (b) (6) *. As shown in Table 29, 50% of the subjects died within one year after RETHYMIC transplantation. Subject* (b) (6) *died of sepsis and multi-organ failure. Subject* (b) (6) *appeared to have died of underlying cardiac condition. Neither subject achieved immune reconstitution prior to death as reflected by the low number of*

naïve CD4+ T cells post-transplantation. Other than death, the numbers of AEs, SAEs, and infection related AEs in these 4 subjects were similar to average findings in the safety population. As the number of subjects with elevated baseline naïve T cells is small and the dire underlying condition of these subjects, it is inconclusive as to whether the baseline naïve CD3+ T cell count of >50 cells/mm^3 truly represents a risk for death after RETHYMIC treatment.

Table 29. Summary of Subjects with Elevated Baseline Naïve T Cells

	Subject (b) (6)	Subject (b) (6)	Subject (b) (6)	Subject (b) (6)
Baseline Max Number Naive CD3 (cells/mm3)	98	55	112	77
Post-transplantation Max Number Naive CD4 (cells/mm3)	11.48	75	21	218.68
Vital Status	Dead (within 3 months)	Alive (in year 3 posttransplant)	Dead (within 10 months)	Alive (in year 2 posttransplant)
Age at treatment	354	809	811	1087
Diagnosis	atypical cDGA	atypical cDGA	atypical cDGA	typical cDGA
SCr	not elevated	not elevated	elevated	not elevated
AST	not elevated	elevated	elevated	elevated
ALT	elevated	elevated	elevated	not elevated
Immunosuppression	yes	yes	yes	yes
#AEs	7	14	2	1
#SAE	5	10	1	0
#Infection AEs	2	8	0	0

(Source: Reviewer's table based on SN0065)

Prior HCT or Thymus transplant
Six subjects received prior HSCT before RETHYMIC transplantation. Subjects (b) (6) *Two of the six subjects died (Subjects* (b) (6) *and* (b) (6)*. The mortality rate in subjects who had prior HCT was 33% which was in line with the safety population. One subject, Subject* (b) (6)*, received fetal thymus transplant prior to RETHYMIC treatment and was alive at last follow up. The number of subjects with prior HSCT or thymus transplant is too small to draw any conclusion on efficacy and safety in the population, however, based on mortality rates, there is no apparent safety concern.*

8.4.2 Nonfatal Serious Adverse Events

In the two years following implantation, the majority of subjects (89 [84.8%]) reported at least one serious adverse event (SAE). There was a total of 525 SAEs of which 496 were nonfatal. The most common non-fatal SAEs affecting at least 3% of subjects are summarized in Table 30.

Table 30. Nonfatal SAEs Affecting ≥ 3% of Subjects

AE Preferred Term	# subjects (n=105)	% subjects
All nonfatal SAEs	85	81%
Device related infection	46	44%
Pyrexia	18	17%
Respiratory failure	15	14%
Hypoxia	14	13%
Pneumonia	8	8%
Thrombocytopenia	8	8%
Graft versus host disease	8	8%
Diarrhea	7	7%
Hypotension	6	6%
Lower respiratory tract infection bacterial	6	6%
Neutropenia	6	6%
Renal failure	6	6%
Respiratory distress	6	6%
Staphylococcal bacteremia	5	5%
Viral upper respiratory tract infection	5	5%
Cytokine release syndrome	4	4%
Enterococcal bacteremia	4	4%
Hypersensitivity	4	4%
Pancreatitis	4	4%
Coombs positive hemolytic anemia	3	3%
Cystitis escherichia	3	3%
Enterobacter bacteremia	3	3%
Gastroenteritis rotavirus	3	3%
Hypocalcemia	3	3%
Influenza	3	3%
Parainfluenza virus infection	3	3%
Seizure	3	3%
Viremia	3	3%

(Source: Reviewer's table based on SN0065)

Nonfatal SAEs most frequently reported in the infections and infestations SOC were device-related infection (46 [44%]), pneumonia (8 [8%]), lower respiratory tract infection (6 [6%]), viral upper respiratory tract infection (5 [5%]) and staphylococcal bacteremia (5[5%]). SAEs were also frequently reported in the respiratory and mediastinal disorders SOC, the most frequently reported being respiratory failure (15[14%]), hypoxia (14 [13%]) and respiratory distress (6 [6%]). Pyrexia was also commonly (18 [17%]) reported.

After two years there were 97 nonfatal SAEs reported in 27 subjects. The most commonly reported nonfatal SAEs were infections with 50 events being reported in 16

subjects including device related infections, upper and lower respiratory tract infections and pneumonia. Respiratory failure and hypoxia were reported in 3 and 2 subjects respectively. Hypocalcemia was reported in 4 subjects which is consistent with parathyroid deficiency found in patients with CA.

8.4.2.1 Treatment related non-fatal SAEs

Any AE or SAE related to the RETHYMIC implantation procedure or biopsy, RETHYMIC itself, protocol required immunosuppression, or supportive care associated with these procedures were considered to be related to study treatment. Treatment related SAEs were reported in 37 subjects (35.2%). SAEs that were considered to be related to study treatment or procedure and affecting more than one subject are summarized in Table 31.

Table 31. Treatment or Procedure Related SAEs affecting > 1 Subject

AE Preferred Term	Number of subjects (n=105)	% subjects
All treatment related SAE	37	35%
Graft versus host disease	8	8%
Hemolytic anemia	8	8%
Thrombocytopenia	7	7%
Neutropenia	6	6%
Respiratory distress	5	5%
Pancreatitis	3	3%
Cytokine release syndrome	3	3%
Hypothyroidism	2	2%
Autoimmune hepatitis	2	2%
Cytomegalovirus infection	2	2%
Seizure	2	2%
Proteinuria	2	2%
Renal failure	2	2%
Rash	2	2%

(Source: Reviewer's table based on SN0065)

Reviewer's comments: The most common SAEs that were considered attributable to study treatment falls into the category of autoimmune conditions which includes hemolytic anemia (8%), thrombocytopenia (7%), neutropenia (6%), hypothyroidism (2%) and autoimmune hepatitis (2%). The other common treatment related SAE category was related to T cell dysregulation and 8% of subjects had serious GVHD AE related to RETHYMIC. These findings are consistent with the mechanism of action of RETHYMIC and immune dysregulation in patients with CA. It is also noted that there were two subjects who had serious CMV infections post-transplantation that were attributed to product because of the possibility of transmission. It is unknown how much of a contribution autoimmune neutropenia may have in infection incidence and even death.

8.4.3 Study Dropouts/Discontinuations

Of the 105 subjects who received RETHYMIC, one (1.0%) subject (Subject (b) (6) with cDGA who received prior fetal thymus transplants withdrew from study follow-up due to

a physician decision 6 years post-transplantation. Subject (b) (6) showed evidence of thymus implant rejection on RETHYMIC biopsy 77 days post-transplantation. Two subjects with SCID (Subjects (b) (6) died after withdrawing from the study due to physician decision. No subjects have withdrawn from study participation since the initial BLA filing.

8.4.4 Common Adverse Events

A total of 2187 AEs were reported as of April 30, 2021 of which1858 AEs were reported in the first two years. All 105 subjects (100%) reported at least one AE in the first 2 years after RETHYMIC implantation. The majority of subjects (93 [88.6%]) experienced events that were Grade ≥ 3 and this included 25 (23.8%) subjects who died within the first 2 years after implantation.

The most common adverse events affecting at least 10% of FAS subjects are summarized in Table 32.

Table 32. Most Common Adverse Events Affecting ≥ 10% Subjects

AE Preferred Term	# subjects	% subjects
All AEs	105	100%
Pyrexia	66	62%
Device related infection	54	51%
Rash	37	35%
Diarrhea	33	31%
Hypertension	30	28%
Alanine aminotransferase increased	28	26%
Thrombocytopenia	27	25%
Hypoxia	27	25%
Anemia	26	25%
Aspartate aminotransferase increased	25	24%
Viral upper respiratory tract infection	23	22%
Hypothyroidism	21	20%
Hypomagnesaemia	21	20%
Respiratory failure	21	20%
Neutropenia	20	19%
Cytokine release syndrome	19	18%
Clostridium difficile colitis	17	16%
Vomiting	16	15%
Staphylococcal bacteremia	16	15%
Pneumonia	15	14%
Seizure	15	14%
Ear infection	14	13%
Urinary tract infection enterococcal	14	13%
Hepatomegaly	13	12%
Oropharyngeal candidiasis	13	12%

AE Preferred Term	# subjects	% subjects
Otitis media	13	12%
Upper respiratory tract infection	13	12%
Lower respiratory tract infection bacterial	12	11%
Blood creatinine increased	12	11%
Cough	12	11%
Hypersensitivity	11	10%
Urinary tract infection bacterial	11	10%
Graft versus host disease	11	10%

(Source: Reviewer's table based on SN0065)

The most common AE category was infection related AEs which were reported by 97 (92.4%) subjects. The top three infection related AEs were device related infections (51%), viral upper respiratory tract infection (22%), and *C. Difficile* infection (16%). These were consistent with the underlying conditions of these patients and the resulting prolonged hospital stays. The second most common AE category was general disorders and administration site conditions (67 [63.8%]) which was followed by gastrointestinal disorders (66 [62.9%]), skin and subcutaneous tissue disorders (65 [61.9%]) and investigations (62 [59.0%]).

The top 10 common AEs during the two year follow up were pyrexia (66 [62.3%]), device related infection (54 [50.9%]), rash (37 [34.9%]), diarrhea (33 [31.1%]), hypertension (30 [28.3%]), alanine aminotransferase increased (28 [26.3%]), thrombocytopenia (27 [25.5%]), hypoxia (27 [25.5%]), anemia (26 [24.5%]) and aspartate aminotransferase increased (25 [23.6%]).

8.4.4.1 Treatment related adverse events

Adverse events considered to be related to study treatment or procedure and affected at least 5% subjects are summarized in Table 33. Of the 105 subjects, 80 subjects (76%) reported at least one treatment related AE in the first two years following RETHYMIC transplantation.

Table 33. Adverse Events Related to Study Treatment or Procedure (≥ 3%)

Adverse Event	# subjects (n=105)	% subjects
All treatment related AEs	80	76%
Hypertension	20	19%
Cytokine release syndrome	19	18%
Hypomagnesemia	17	16%
Rash[1]	17	15%
Thrombocytopenia[2]	13	12%
Renal impairment/Renal failure[3]	13	12%
Graft verse host disease[4]	11	10%
Hemolytic anemia[5]	9	9%

Adverse Event	# subjects (n=105)	% subjects
Neutropenia	9	9%
Respiratory Distress[6]	8	8%
Proteinuria	7	7%
Pyrexia	6	6%
Acidosis[7]	6	6%
Diarrhea[8]	5	5%
Seizure[9]	5	5%
Alopecia	4	4%
AST/ALT	4	4%
Hyperglycemia	4	4%
Wound dehiscence	4	4%
Blood creatinine increased	3	3%
Pancreatitis	3	3%

(Source: Reviewer's table based on SN0065)
[1] Rash includes rash, granuloma skin, rash popular, urticaria.
[2]Thrombocytopenia includes thrombocytopenia and Immune thrombocytopenic purpura.
[3]Renal impairment/Renal failure includes renal failure and acute kidney injury, proteinuria and blood creatinine increased.
[4]GVHD includes GVHD, GVHD-gut, GVHD-skin, Omenn syndrome.
[5]Hemolytic anemia includes autoimmune hemolytic anemia, coombs positive hemolytic anemia, hemolysis, hemolytic anemia.
[6]Respiratory distress includes respiratory distress, hypoxia, respiratory failure.
[7]Acidosis includes acidosis, renal tubular acidosis and blood bicarbonate decreased
[8]Diarrhea includes diarrhea and hemorrhagic diarrhea
[9]Seizures include infantile spasms, seizures and febrile convulsion.

Reviewer's comments: Based on the clinical datasets submitted on June 4, 2021 (BLA125685/SN0065), this reviewer re-adjudicated and compiled a list of adverse events related to study treatment or procedure. As footnoted in Table 36, AE preferred terms were combined when applicable. This reviewer generally agrees with the sponsor's assessment of AE relatedness except for GVHD. All GVHD adverse events are considered by this reviewer as related to study treatment for the following reasons. These adverse events are directly related to T cell function and mechanism of action of RETHYMIC. All GVHD AE onsets were within six months of implantation (subacute), therefore had a consistent temporal relationship with RETHYMIC implantation. Per the Applicant's IR response submitted in SN0067, patients who developed GVHD all seemed to have pre-implantation history of autoreactive T cells, maternal engraftment, or donor T cells (e.g., from pre-implantation cord blood transfusion). However, based on data submitted in this BLA and medical literature, the possibility cannot be ruled out that RETHYMIC may have exacerbated pre-existing condition and/or enabled development of new autoreactive host T cells due to altered negative selection. In essence, there is too much unknown information to be able to easily adjudicate the SAEs, AEs, and deaths.

Adverse events related to study treatment affecting at least 10% of subjects in the first two years following RETHYMIC treatment are the following: Hypertension (19%), Cytokine Release Syndrome (18%), Rash (15%), Hypomagnesemia (16%), Thrombocytopenia (12%), Renal Impairment/Renal Failure (12%) and Graft versus Host

Disease (10%). CRS, hypertension, and hypomagnesemia were considered to be related to immunosuppression medications. CRS is a known AE associated with RATGAM which was widely used in the studies. Hypertension and hypomagnesemia are well known AEs associated with calcineurin inhibitors.

8.4.4.2 Adverse reactions

The applicant identified a subset of AEs considered to be related to either the RETHYMIC implantation procedure itself or to the implanted RETHYMIC drug product, and not to concomitant immunosuppressive medications or other study procedures. These events are summarized in Table 34 (Applicant's table 3-4 in Safety and Efficacy Clinical Update for BLA, submitted in SN0060). There were relatively few events considered related to the RETHYMIC implantation procedure or RETHYMIC itself. A total of 44 (42%) subjects reported 87 events considered by the Sponsor/Investigator to be possibly related to RETHYMIC. This accounted for 4% of all reported AEs. Overall, RETHYMIC related events generally were distributed into 3 main categories: autoimmune diseases, complications associated with the implantation procedure itself, and events considered specifically related to T cells.

Table 34. Adverse Reactions (by Applicant)

System Organ Class Preferred Term	EAS (N=95) n (%)	E	FAS (N=105) n (%)	E
Number of Related Adverse Events	**39 (41.1)**	**67**	**44 (41.9)**	**87**
Blood and lymphatic system disorders	**17 (17.9)**	**24**	**21 (20.0)**	**32**
Thrombocytopenia	10 (10.5)	12	11 (10.5)	13
Neutropenia	5 (5.3)	5	8 (7.6)	8
Coombs positive haemolytic anaemia	2 (2.1)	2	3 (2.9)	3
Autoimmune haemolytic anaemia	1 (1.1)	1	2 (2.0)	3
Haemolysis	1 (1.1)	1	2 (2.0)	2
Haemolytic anaemia	2 (2.1)	2	2 (1.9)	2
Immune thrombocytopenic purpura	1 (1.1)	1	1 (1.0)	1
Skin and subcutaneous tissue disorders	**8 (8.4)**	**9**	**9 (8.6)**	**10**
Alopecia	4 (4.2)	4	4 (3.8)	4
Rash	2 (2.1)	2	2 (1.9)	2
Granuloma skin	1 (1.1)	1	1 (1.0)	1
Psoriasis	1 (1.1)	1	1 (1.0)	1
Skin mass	1 (1.1)	1	1 (1.0)	1
Stevens-Johnson syndrome	0	0	1 (1.0)	1
General disorders and administration site conditions	**4 (4.2)**	**4**	**6 (5.7)**	**6**
Pyrexia	3 (3.2)	3	5 (4.8)	5
Oedema peripheral	1 (1.1)	1	1 (1.0)	1
Renal and urinary disorders	**5 (5.3)**	**6**	**5 (4.8)**	**6**
Proteinuria	5 (5.3)	5	5 (4.8)	5
Glomerulonephritis minimal lesion	1 (1.1)	1	1 (1.0)	1
Gastrointestinal disorders	**3 (3.2)**	**3**	**5 (4.8)**	**5**
Diarrhoea	1 (1.1)	1	3 (2.9)	3
Enteritis	1 (1.1)	1	1 (1.0)	1
Ileus	1 (1.1)	1	1 (1.0)	1
Injury, poisoning and procedural complications	**4 (4.2)**	**4**	**5 (4.8)**	**5**
Wound dehiscence	4 (4.2)	4	4 (3.8)	4
Graft haemorrhage	0	0	1 (1.0)	1
Investigations	**3 (3.2)**	**4**	**4 (3.8)**	**6**
Blood bicarbonate decreased	1 (1.1)	1	2 (1.9)	2
Lymphocyte morphology abnormal	1 (1.1)	2	1 (1.0)	2
Blood immunoglobulin E increased	1 (1.1)	1	1 (1.0)	1
Lymphocyte count abnormal	0	0	1 (1.0)	1
Infections and infestations	**4 (4.2)**	**4**	**4 (3.8)**	**4**
Cytomegalovirus infection	1 (1.1)	1	1 (1.0)	1
Staphylococcal skin infection	1 (1.1)	1	1 (1.0)	1
Stitch abscess	1 (1.1)	1	1 (1.0)	1
Wound infection staphylococcal	1 (1.1)	1	1 (1.0)	1
Immune system disorders	**0**	**0**	**2 (1.9)**	**3**
Graft versus host disease	0	0	1 (1.0)	1
Graft versus host disease in gastrointestinal tract	0	0	1 (1.0)	1
Graft versus host disease in skin	0	0	1 (1.0)	1
Hepatobiliary disorders	**1 (1.1)**	**1**	**2 (1.9)**	**2**
Autoimmune hepatitis	1 (1.1)	1	2 (1.9)	2
Musculoskeletal and connective tissue disorders	**2 (2.1)**	**2**	**2 (1.9)**	**2**
Juvenile idiopathic arthritis	1 (1.1)	1	1 (1.0)	1
Psoriatic arthropathy	1 (1.1)	1	1 (1.0)	1
Endocrine disorders	**1 (1.1)**	**2**	**1 (1.0)**	**2**
Basedow's disease	1 (1.1)	1	1 (1.0)	1
Hyperthyroidism	1 (1.1)	1	1 (1.0)	1
Congenital, familial and genetic disorders	**1 (1.1)**	**1**	**1 (1.0)**	**1**
Albinism	1 (1.1)	1	1 (1.0)	1

Nervous system disorders	**1 (1.1)**	**1**	**1 (1.0)**	**1**
Myelitis transverse	1 (1.1)	1	1 (1.0)	1
Reproductive system and breast disorders	**1 (1.1)**	**1**	**1 (1.0)**	**1**
Ovarian failure	1 (1.1)	1	1 (1.0)	1
Vascular disorders	**1 (1.1)**	**1**	**1 (1.0)**	**1**
Haematoma	1 (1.1)	1	1 (1.0)	1

Source: Table 14.3.1.8.9
Abbreviations: AE = adverse event; E = number of events; EAS = efficacy analysis set; FAS = full analysis set; MedDRA = Medical Dictionary for Regulatory Activities; N = number of subjects included in the analysis set; n = number of subjects with events; PT = preferred term; SOC = system organ class
Note: If a subject had multiple occurrences of an AE, the subject was presented only once in the subject count for a given SOC and PT. Adverse events were coded using MedDRA version 19.1. Related events were defined as events that were definitely, probably or possibly related to RVT-802 or with an unknown relationship based on investigator review.

(Source: Applicant's table, SN0060)

Reviewer's Comment: After reviewing the clinical datasets submitted on June 4, 2021 (BLA 125685/0065), Section 3.1.3.2 of Safety and Efficacy Clinical Update (BLA 125685/0060) and Section 3.1.5.2 of Clinical Summary of Safety (BLA 125685/0003), it remains unclear how relatedness of these adverse reactions was adjudicated by the sponsor. Limited narratives were provided for some adverse events that were considered as adverse reactions. However, for most adverse events, there was no explanation on why they were considered related to RETHYMIC or not.

All clinical trials in REHTYMIC development program were open label without concurrent controls to help identify adverse reactions. Because of the unique nature of RETHYMIC, being cultured thymus tissue product and long lasting, it is hard to interpret temporal relationship between RETHYMIC and adverse events. While adverse events occurred immediately or shortly after treatment are generally considered to be more likely related to study product or implantation procedure, adverse events occurred weeks, months or even years after RETHYMIC treatment may still be related to the product as RETHYMIC would still be viable and sequalae or complications of RETHYMIC treatment may not be evident until some time after implantation. Without an established temporal relationship and without concurrent control, it is not always feasible to distinguish the etiology of adverse events.

Furthermore, immunosuppression medications are required for RETHYMIC treatment for the majority of patients. Adverse events related to immunosuppression should be communicated in the label as well. Therefore, this reviewer recommends use treatment related adverse events in place of adverse reactions. For RETHYMIC label, the Agency and the Applicant agreed to use treatment related adverse events (affecting ≥ 5% of subjects) in Table 33 of this review for AR table (Table 1 in RETHYMIC label).

8.4.4.3 Adverse Events by Manufacturer

Of the 105 subjects, 87 received RETHYMIC manufactured in the (b) (4) facility and 18 subjects received RETHYMIC manufactured in the (b) (4) facility. AEs by manufacturer affecting at least 15% of subjects in two years post-transplantation are listed in Table 35.

Table 35. Adverse Events by Manufacturer Facilities

(b) (4) (n=87)			(b) (4) (n=18)		
AE Preferred Term	#subjects	%subjects	AE Preferred Term	#subjects	%subjects
Pyrexia	53	61%	Pyrexia	9	50%
Device related infection	46	53%	Diarrhea	7	39%
Rash	27	31%	Rash	7	39%
Hypertension	26	30%	Device related infection	6	33%

Hypoxia	24	28%	Acute kidney injury	5	28%
Alanine aminotransferase increased	23	26%	Viral upper respiratory tract infection	5	28%
Anemia	23	26%	Aspartate aminotransferase increased	4	22%
Diarrhea	22	25%	Hypertension	4	22%
Thrombocytopenia	21	24%	Alanine aminotransferase increased	3	17%
Aspartate aminotransferase increased	19	22%	Hepatomegaly	3	17%
Cytokine release syndrome	18	21%	Hyperglycemia	3	17%
Hypomagnesaemia	17	20%	Hypomagnesaemia	3	17%
Hypothyroidism	16	18%	Hypoxia	3	17%
Respiratory failure	16	18%	Lower respiratory tract infection bacterial	3	17%
Clostridium difficile colitis	15	17%	Neutropenia	3	17%
Neutropenia	15	17%	Pneumonia	3	17%
Viral upper respiratory tract infection	15	17%	Proteinuria	3	17%
Staphylococcal bacteremia	14	16%	Respiratory distress	3	17%
Vomiting	13	15%	Thrombocytopenia	3	17%

(Source: Reviewer's table based on SN0065)

The most frequent AEs in both groups were pyrexia which affected 61% of (b) (4) subjects and 50% of (b) (4) subjects. Rash and device related infection were among the top 5 AEs and affected more than 30% of subjects in both group while the frequencies were lower in subjects received RETHYMIC manufactured in the (b) (4) facility. Frequency of diarrhea was higher in (b) (4) subjects (39%) compared to (b) (4) subjects (22%). Acute kidney injury only occurred in (b) (4) subjects (5 [28%]).

Treatment related adverse events comparison between (b) (4) and (b) (4) subjects are shown in Table 36. 77% of (b) (4) subjects had at least one treatment related AE which is comparable to that 72% of (b) (4) subjects had at least one treatment related AE. The most common treatment related AEs occurred in ≥ 10% of (b) (4) subjects were: cytokine release syndrome (21%), hypertension (20%), hypomagnesemia (17%), thrombocytopenia (11%) and Rash (10%). The most common treatment related AEs occurred in ≥ 10% (b) (4) subjects were: acute kidney injury (17%), hypertension (17%), blood creatinine increased (11%), diarrhea (11%), hypomagnesemia (11%), proteinuria (11%) and rash (11%). There were some differences between the most common treatment related AEs between the two manufacturer groups, although the numbers of (b) (4) subjects were too small to make meaningful conclusion. Acute kidney injury (AKI) was again a treatment related AE occurred only in (b) (4) subjects.

Table 36. Treatment Related Adverse Events by Manufacturer Facilities

(b) (4) (n=87)	#Subjects	%Subjects	(b) (4) (n=18)	#Subjects	%Subjects
All Related AEs	67	77%	All related AEs	13	72%
Cytokine release syndrome	18	21%	Acute kidney injury	3	17%
Hypertension	17	20%	Hypertension	3	17%
Hypomagnesemia	15	17%	Blood creatinine increased	2	11%
Thrombocytopenia	10	11%	Diarrhea	2	11%
Rash	9	10%	Hypomagnesemia	2	11%
Neutropenia	8	9%	Proteinuria	2	11%
Pyrexia	6	7%	Rash	2	11%
Hypoxia	5	6%	Abdominal distension	1	6%
Proteinuria	5	6%	Acidosis	1	6%
			Alanine aminotransferase increased	1	6%
			Aspartate aminotransferase increased	1	6%
			Cellulitis staphylococcal	1	6%
			Cytokine release syndrome	1	6%
			Febrile convulsion	1	6%
			Gingival hypertrophy	1	6%
			Hemolytic anemia	1	6%
			Hyperglycemia	1	6%
			Hypoalbuminemia	1	6%
			Ileus	1	6%
			Irritability	1	6%
			Lipase increased	1	6%
			Neutropenia	1	6%
			Oedema peripheral	1	6%
			Pancreatitis	1	6%
			Thrombocytopenia	1	6%
			Urticaria	1	6%

(Source: Reviewer's table based on SN0065)

Nine SAEs were reported by 4 (b) (4) subjects (22.2%) including hemolytic anemia, thrombocytopenia, neutropenia, pancreatitis, proteinuria, cellulitis staphylococcal, diarrhea and ileus.

Reviewer's comments: AKI AEs occurred in the five (b) (4) subjects are reviewed below. None of these subjects had elevated serum creatinine before receiving RETHYMIC.

- *Subject (b) (6): This case was multifactorial because the subject had severe intercurrent infections and was receiving multiple potentially nephrotoxic antibiotics and immunosuppression. In addition, the subject had multiple organ*

systems that were failing at the time of the AKI. The events of AKI and subsequent renal failure were unrelated to study treatment. Renal failure was likely secondary to treatment of multiple infections and multi-system organ failure. The subject died of systemic candida infection.

- *Subject (b) (6): This event of AKI was likely secondary to supratherapeutic levels of cyclosporine; the event resolved with changes in the subject's medications.*
- *Subject (b) (6): This case was multifactorial because the subject had severe intercurrent infections and was receiving multiple medications that may have been nephrotoxic. AKI in this subject progressed to renal failure and the subject died from pulmonary failure due to the event of Staphylococcal bacteremia and renal failure.*
- *Subject (b) (6): This AKI event appears to have been related to dehydration due to inadequate intake following disruption of the subject's gastro-jejunal tube. The event resolved promptly with replacement of the gastro-jejunal tube and hydration.*
- *Subject (b) (6): This AKI event appears to have been related to poor hydration related to the use of RATGAM and the RETHYMIC implantation procedure or to treatment with cyclosporine. The event resolved promptly with hydration.*

Overall, the AKI events appeared to be related to clinical manifestations of the disease and concomitant medication (antibiotics and immunosuppression) and not directly related to the RETHYMIC. The causes of the events were multifactorial and included concomitant nephrotoxic medications and dehydration.

Overall, there was no significant safety concerns with the (b) (4) facility.

8.4.4.4 Adverse Events by Immunosuppression

Of the 105 subjects, 71 subjects received immunosuppressive medications and 34 did not. The top 5 common AEs in subjects received immunosuppression were pyrexia (46 [64.8%]), device related infection (39 [54.9%]), diarrhea (28 [39.4%]), rash (27 [38.0%]) and hypertension (26 [36.6%]). The top 5 common AEs in subjects without immunosuppression were pyrexia (20 [58.8%]), device related infection (15 [44.1%]), rash (10 [29.4%]), hypoxia (10 [29.4%]) and alanine aminotransferase increased (9 [26.4%]).

Adverse events with more than 2-fold increase in percentage of subjects who received immunosuppression are listed in Table 37.

Table 37. Adverse Events by Immunosuppression

Adverse Event	w/o immunosuppression (n=34)		w/ immunosuppression (n=71)	
	#Subjects	%Subjects	#Subjects	%Subjects
Diarrhea	5	15%	28	39%
Hypertension	4	12%	26	37%
Thrombocytopenia	5	15%	22	31%
Cytokine release syndrome	0	0%	19	27%
Hypomagnesemia	3	9%	18	25%
Neutropenia	4	12%	16	23%
GVHD	1	3%	10	14%
Fungal skin infection	0	0%	9	13%
Urinary tract infection bacterial	2	6%	9	13%
Proteinuria	2	6%	8	11%
Renal failure	1	3%	7	10%
Pharyngitis streptococcal	1	3%	6	8%
Hyponatremia	0	0%	6	8%
Abdominal distension	1	3%	5	7%
Hematochezia	1	3%	5	7%
Ileus	1	3%	5	7%
Sinusitis bacterial	1	3%	5	7%
Blood bicarbonate decreased	1	3%	5	7%
Hyperkalemia	1	3%	5	7%
Thrombosis	1	3%	5	7%
Sinus bradycardia	0	0%	5	7%
Gastroenteritis norovirus	0	0%	5	7%
Sinusitis	0	0%	5	7%
Staphylococcal skin infection	0	0%	5	7%
Urinary tract infection	0	0%	5	7%
Erythema	0	0%	5	7%
Pneumatosis intestinalis	0	0%	4	6%
Blood urea increased	0	0%	4	6%
Acidosis	0	0%	4	6%
Rash maculo-papular	0	0%	4	6%

(Source: Reviewer's table)

The majority of AEs that occurred more frequent in subjects who received immunosuppression were either known side effects of immunosuppressive medications, e.g., CRS associated with RATGAM, or adverse events that require immunosuppression, e.g., GVHD and rash in atypical cDGA patients. CRS and fungal skin infections were only reported in subjects who received immunosuppression.

Frequency of infection adverse events were compared between subjects with and without immunosuppression and were found to be similar.

8.4.5 Clinical Test Results

Clinical laboratory assessments included the following:

- Hematology: hemoglobin, hematocrit, platelet count, white blood cell count (WBC), neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, atypical lymphocytes, and other myelocytes.
- Chemistry: sodium, potassium, chloride, CO2 (bicarbonate), glucose, blood urea nitrogen (BUN), and creatinine.
- Liver function tests: AST, ALT, alkaline phosphatase (ALP), bilirubin, lactate dehydrogenase (LDH), lipase, amylase, albumin, total protein, gamma-glutamyl transpeptidase (GGT), and triglycerides.
- Endocrine: calcium, ionized calcium, magnesium, phosphorus, urine calcium, urine creatinine, ratio, thyroxine (T4), free T4, thyroid stimulating hormone (TSH), intact parathyroid hormone (iPTH), anti-TGB (anti-thyroglobulin), and anti-TPO (anti-thyroperoxidase).

While significant changes in clinical laboratory assessments in individual subjects were reported in adverse events, e.g., elevated ALT, AST, hypothyroidism etc., the wide variation in the number of subjects assessed at each timepoint did not permit a meaningful conclusion as to the effect of RETHYMIC implantation on these parameters. Overall, there were no apparent clinically meaningful trends in change from baseline through 2 years post-transplantation.

8.4.6 Adverse Events of Special Interest

Adverse events of special interest (AESI) defined in the study protocols included infection-related AEs, cancers, autoimmune diseases, GVHD, rashes, and granulomas.

- Infection-related AEs were of interest as the ability to respond and control infections is indicative of the development of thymic function.
- Autoimmune diseases, GVHD, and granulomas were potential AEs that may have been related to RETHYMIC given its ability to reconstitute the immune system. Granulomas were of interest as they may have been indicative of the development of sarcoidosis. If granulomas were found, assessment of angiotensin converting enzyme (ACE) and eye examinations were performed.
- Rashes were of interest as these may have been indicative of new development or flare of pre-existing rashes associated with atypical cDGA. Rashes persisting more than 2 weeks were biopsied to assess the etiology of the rash.
- Cancer: subjects were followed for the development of cancers given the risk of malignancy in subjects with poor T cell function.

8.4.6.1 Infection related AEs

Given the disease under study, infection-related AEs were of interest as the ability to immunologically respond to and control infections was indicative of the development of thymic function. A total of 600 infection related AEs were reported in the first two years by 97 subjects (92.4%). Infection related AEs affecting at least 5% of subjects are listed in Table 38. The most common infection related AEs affecting ≥ 10% of subjects were: device related infection (50%), viral upper respiratory tract infection (19%), *Clostridium Difficile* colitis (15%), straphylococcal bateremia (14%), urinary tract infection enterococcal (12%), oropharyngeal candidiasis (11%), lower respiratory tract infection bacterial (10%), pneumonia (10%) and urinary tract infection bacterial (10%).

Table 38. Infection Related Adverse Events Affecting ≥ 5% of Subjects

AE Preferred Term	#subjects (n=105)	%subjects
Device related infection	52	50%
Viral upper respiratory tract infection	20	19%
Clostridium difficile colitis	16	15%
Staphylococcal bacteremia	15	14%
Urinary tract infection enterococcal	13	12%
Oropharyngeal candidiasis	12	11%
Lower respiratory tract infection bacterial	11	10%
Pneumonia	11	10%
Urinary tract infection bacterial	11	10%
Ear infection	9	9%
Otitis media	8	8%
Cystitis escherichia	7	7%
Enterococcal bacteremia	7	7%
Fungal skin infection	7	7%
Pneumonia pseudomonal	7	7%
Viraemia	7	7%
Cystitis klebsiella	6	6%
Eye infection bacterial	6	6%
Gastroenteritis rotavirus	6	6%
Sinusitis bacterial	6	6%
Stoma site infection	6	6%
Eye infection staphylococcal	5	5%
Gastroenteritis norovirus	5	5%
Parainfluenza virus infection	5	5%
Staphylococcal skin infection	5	5%
Upper respiratory tract infection	5	5%

(Source: Reviewer's table based on SN0065)

Infection-related AEs in most subjects (n=78 [74.3%]) were of Grade ≥ 3, were assessed as serious in 75 (71.4%) subjects, and as related to study treatment in 8 (7.6%) subjects. The pathogens associated with infections (other than device-related infections) reported in ≥ 10% of subjects included:

- Viral upper respiratory tract infection: rhinovirus, rhinovirus / enterovirus unknown, coronavirus, RSV and human metapneumovirus;
- Clostridium difficile colitis: Clostridium difficile;
- Staphylococcal bacteremia: Staphylococcus;
- Urinary tract infection bacterial: Enterobacter cloaecae / asburiae, Enterobacter cloacae, Serretia marcescens, Burkholderia cepacian (Subject (b) (6), Enterobacter Unknown, Citrobacter freundii and Proteus mirabilis;
- Oropharyngeal candidiasis: Candida;
- Lower respiratory tract infection bacterial: Neisseria, unknown, Stenotrophomonas, Citrobacter, Delftia acidovorans, Serratia Marcescens, Mixed

Gram-Negative Rods, Enterococcus Faecalis, Enterococcus, Proteus, Sphingobacterium, Acinetobacter, Flavobacteruim menigosepticum and Nocardia.

There were 8 subjects (7.6%) with infection-related AEs that were assessed as related to study treatment. These included CMV infection (n=2 [2.2%]), device-related infection (n=1 [1.1%]), staphylococcal skin infection (n=1 [1.1%]), stitch abscess (n=1 [1.1%]), varicella-zoster virus infection (n=1 [1.1%]), staphylococcal cellulitis (n=1 [1.1%]) and staphylococcal wound infection (n=1 [1.1%]). There were 4 treatment-related infectious SAEs including 2 SAEs of CMV infection, 1 SAE of device-related infection and 1SAE of staphylococcal cellulitis. These SAEs are described below:

- Subject (b) (6): This subject developed a Grade 5 CMV infection which was first detected 77 days prior to implantation (serious due to death) that was considered related to the use of RATGAM.
- Subject (b) (6) This subject developed a Grade 5 CMV infection 38 days after implantation (serious due to life-threatening and death) with symptoms of fever and increased respiratory rate. This SAE was possibly related to RETHYMIC as both the donor and recipient tested positive for CMV post-transplantation although the donor had tested negative prior to thymic donation.
- Subject (b) (6): This subject reported a Grade 2 device-related infection 4 days prior to RETHYMIC implantation which was considered related to study treatment (serious due to other medically important event). The subject developed a coagulase negative Staphylococcus infection following insertion of a central line infection for RATGAM administration. This SAE was not considered related to RETHYMIC.
- Subject (b) (6): This subject developed a Grade 3 cellulitis Staphylococcal infection on the right axilla and at the thymus graft biopsy site 143 days after transplantation (serious due to other medically important event). On Day 160, the right axilla cellulitis Staphylococcal infection was considered recovered/resolved (axilla lymph nodes were small and non-tender). On Day 174, the cellulitis Staphylococcal infection at the thymus graft biopsy site was considered recovered/resolved. The event of cellulitis Staphylococcal infection on the right axilla was unrelated to study treatment and the event of cellulitis Staphylococcal infection at the thymus graft biopsy site was related to study treatment.

As previously discussed in Section 7.6.7.1, Infection-related AEs were compared by the time of onset with a Wilcoxon signed-rank test that compared the number of infections with an onset <6 months after implantation before normal T cell function had developed versus those reported between >6 to ::12 months post-transplantation. This analysis was also conducted to compare the number of infection-related AEs with onset ::12 months versus >12 to ::24 months after implantation. There were more infection-related AEs with an onset ::6 months vs onset >6 to ::12 following implantation. Similarly, the Wilcoxon signed-rank test on the difference between the number of infection-related AEs with an onset ::12 months versus >12 to ::24 months after implantation was also statistically significant with fewer infection related AEs at the later timepoints. These data support the claim that the incidence of infection-related AEs decreased over time with the development of immune function.

8.4.6.2 Graft versus Host Disease

GVHD is a potentially serious complication following the transplantation of thymic tissue into an unmatched recipient. GVHD results when an immunocompetent donor's T cells recognize the recipient as foreign and mount an immune response against the recipient's tissues. This results in significant morbidity most commonly impacting the skin, liver, and gastrointestinal tract. Symptoms may include rash, nausea/vomiting, diarrhea, elevated bilirubin, and liver enzymes, and can be fatal in severe cases. Subjects were also at risk for the development of maternal engraftment and associated GVHD given the significant T cell immunodeficiency present at birth. In addition to the risks of externally mediated GVHD, subjects with the atypical cDGA may develop autologous GVHD which is associated with oligoclonal "host" T cells that are autoreactive. When possible, chimerism testing was performed to determine the etiology of GVHD events.

11 subjects developed GVHD after receiving RETHYMIC (Table 39), of whom 8 had atypical cDGA, 2 had SCID, and 1 had FOXN1 deficiency. All but two subjects were more than 1 year old at the time of RETHYMIC implantation. 6 of the 11 subjects (54.5%) died.

Table 39. Summary of Graft vs Host Disease (GVHD)

Subject ID	Diagnosis	Sex	Age at Treatment (days)	RETHYMIC Dose	AE Preferred Term	AE onset (days post-transplantation)	AE Grade	SAE	Vital Status
(b) (6)	FOXN1 Deficiency	F	424	12,675 mm2/m2	aGVHD	56	2	N	Alive
	Artemis SCID	F	672	13,721 mm2/m2	GVHD maternal	150	3	Y	Dead
	Atypical cDGA	F	182	23,755 mm2/m2	aGVHD	146	4	Y	Dead
	Atypical cDGA	F	290	21,734 mm2/m2	GVHD maternal	83	4	Y	Alive
	Atypical cDGA[1]	M	502	15,731 mm2/m2	aGVHD	62	4	Y	Dead
	Atypical cDGA	M	541	10,277 mm2/m2	aGVHD	36	3	N	Dead
	Atypical cDGA	M	470	7,104 mm2/m2	aGVHD	150	3	Y	Alive
	Atypical cDGA[1]	M	517	8,459 mm2/m2	aGVHD	128	3	Y	Dead
	Atypical cDGA	M	6163	4,522.7 mm2/m2	GVHD cord blood HSCT	12	3	Y	Alive
	Atypical cDGA[2]	F	671	Missing	aGVHD	39	3	N	Alive
	SCID	F	449	8,042 mm2/m2	GVHD maternal	165	3	Y	Dead

[1]At the time of enrollment Sponsor/Primary Investigator assessed the phenotype as typical complete DiGeorge Anomaly (cDGA). The Sponsor/Primary investigator has reassessed the phenotype and considers the phenotype to be atypical cDGA in response to the Agency's information request on GVHD adverse events.
[2]At the time of enrollment Sponsor/Primary Investigator assessed the phenotype as partial DGA. The Sponsor/Primary investigator has reassessed the phenotype and considers the phenotype to be atypical cDGA.

The GVHD events are descried below.

- Subject (b) (6): This subject had autologous GVHD and an atypical phenotype prior to transplantation. However, the diagnosis of atypical phenotype was not made

until after enrollment as the total and naïve T cells counts were extremely low. A skin biopsy on day 42 after transplantation was interpreted as possible Gianotti Crosti syndrome, atypical DiGeorge syndrome and a drug reaction. Chimerism studies on day 58 after transplantation showed no detectable thymus donor or maternal T cells; recipient alleles were detected. This finding is consistent with autologous GVHD. A thymus graft biopsy was done on day 61 post-transplantation. At the time of the graft biopsy the subject also had another skin biopsy, and the results were consistent with atypical DiGeorge syndrome. The subject experienced Grade 4 autologous GVHD 62 days after transplantation (serious due to other medically important event). The Sponsor/PI did not believe this to be the correct diagnosis because lymphocyte enumeration on day 62 after transplantation showed the total T cell count was only 68 cells/mm^3. At the time, the Sponsor/PI had never seen autologous GVHD with such a low T cell count. The diagnosis of Gianotti Crosti syndrome was continued, therefore no immunosuppression was given. It is important to note that a T cell chimerism study on day 58 showed < 2% thymus donor or maternal T cells. On day 242 after transplantation the subject developed a “very impressive full-body erythematous papular and plaque-type rash with significant pruritus, excoriation, and scale” and the PI decided to treat the subject as if the problem was autologous GVHD. The subject improved with prednisolone, and with cyclosporine which was later changed to tacrolimus on day 267 post transplantation. On Day 348 after transplantation, due to the complexity of immunosuppression treatment required to suppress the autologous GVHD symptoms, the subject was admitted to Duke University Medical Center (DUMC) for continued immunosuppression treatment, which included methylprednisolone, tacrolimus, azathioprine, prednisolone, and MMF. Chimerism studies on day 349 after thymus transplantation showed no detectable thymus donor T cells; recipient specific alleles were detected. The event of autologous GVHD was ongoing at the time of the subject’s death. The patient had a number of comorbidities and died 480 days post thymus transplantation.

- Subject (b) (6): This subject had grade 3 autologous GVHD on day 36 after RETHYMIC implantation. Chimerism of the blood CD3+ fraction was conducted 56 days post-transplant. There were <2% thymus donor cells and <2% maternal cells present with recipient alleles detected. Within the sensitivity of the assay no definite donor thymus or maternal cells were present. On day -225 relative to transplantation this subject had a skin biopsy showing CD3+ T cell infiltration consistent with autologous GVHD as seen in an atypical complete DiGeorge phenotype. The subject’s poor renal function made it difficult to use sufficient calcineurin inhibitors to control the autologous GVHD.
- Subject (b) (6): This subject experienced Grade 3 autologous GVHD 150 days after transplantation (serious due to medically important event). A gut biopsy on Day 154 was consistent with autologous GVHD.). Because T cells chimerism studies of Days 136 and 156 both showed only recipient T cells, without evidence of maternal nor thymus donor T cells. The event is consistent with autologous GVHD which he had prior to transplantation. On day 160 after transplantation, the loose stools that he had throughout the hospitalization were determined by gut biopsy to be secondary to autologous GVHD. The subject was treated with steroids starting at 2 mg/kg but this was not successful. The autologous GHVD was controlled after the subject received alemtuzumab starting on day 431 post-transplantation and naïve CD3+ T cells increased to 112 cells/mm^3 and with naïve CD4+ T cells at 89 cells/mm^3. The B cell number was below normal and

the NK number was normal. On Day 512 after transplantation, the event was considered resolved after therapy with alemtuzumab.

- Subject (b) (6): The subject experienced guaiac positive stools at Day 56 after transplantation. Oral feeding was placed on hold. A kidney, ureter, and bladder x-ray was negative for pneumatosis. Endoscopy/colonoscopy on Day 99 after transplantation showed, per the finding noted in the pathology report, a lack of disaccharidases in the small bowel, and scattered apoptotic cells in the gut consistent with mild GVHD. Cyclosporine and steroids were increased, and total parenteral nutrition (TPN) was initiated around Day 92 after transplantation. Autologous GVHD was considered resolved 105 days after transplantation and TPN was stopped 116 days after transplantation. On Day 99 after transplantation, a thymus graft biopsy showed thymopoiesis. Chimerism on Day 101 after transplantation showed no thymus donor or maternal T cells. Recipient specific alleles were detected.
- Subject (b) (6): The subject experienced Grade 4 graft versus host disease 83 days after transplantation (serious due to medically important event). The subject presented with a flare of severe maternal GVHD symptoms on skin and in the gut. Maternal GVHD had been present prior to transplantation with symptoms of rash. The maternal T cells had been 29% on Day -44. Prior to (Day 70) and after the onset of Grade 4 maternal GVHD (Day 111), the T cell chimerism had increased to 97% and 94% respectively. Per the Sponsor/Investigator, the CD4:CD8 ratios on those dates were 0.05 and 0.07, respectively. The subject was treated with cyclosporine and then tacrolimus. The maternal GVHD flared 83 days post-transplantation, with slight apoptosis on sigmoid colon and rectal biopsies on Day 107. Prior to this gut biopsy, it had not been known that the gut was affected by the maternal GVHD. On Day 177 after transplantation, the maternal GVHD symptoms reappeared, but were mild in intensity. Chimerism studies on day 195 after thymic transplantation showed 64.7% maternal T cells. On Day 280 after transplantation, the subject's rash worsened. Per the Sponsor/Investigator, the skin and gut maternal GVHD flared and the patient was given alemtuzumab, 5 doses from Day 407 to 415 and again 4 doses given Days 462 to 464. Only with this therapy did the maternal GVHD finally come under control.
- Subject (b) (6): The subject experienced Grade 3 event of autologous GVHD in gastrointestinal tract 128 days after transplantation (serious due to other medically important event). Biopsies on Day 128, Day 161, Day 177 and Day 232 showed apoptosis. A T cell chimerism test was performed on Day 51 post-transplantation which found 100% recipient T cells with no donor or maternal T cells supporting the diagnosis of autologous GVHD. The Sponsor/Investigator considered it likely that the autologous GVHD may have recurred because, with the poor renal function from prior treatment with calcineurin inhibitors (for what was thought to be autoimmune renal disease at the time), it was not possible to use sufficient dosing of cyclosporine to treat the autologous GVHD (because of the risk of renal failure.) Note that the patient was on cidofovir for adenovirus at the same time (which greatly increased the risk of renal insufficiency). The event of autologous GVHD in the gastrointestinal tract was considered ongoing at the time of the subject's death.
- Subject (b) (6): This athymic patient originally received a cord blood transplant on day 80 of life with cyclosporine, steroids and ATG. The transplant was from a female cord blood that was mismatched for HLA-B and HLA-C to patient 802. The patient did well but developed adenopathy on CT at age 7.5 years. At year

8.65 the patient was found to be EBV positive. The patient was found to have mediastinal B-cell lymphoma at age ~age 13.8, ileocecal B cell lymphoma s/p chemo 2 years later, and then recurrent diffuse B lymphoma being treated with RCHOP one year later. The patient received a transplantation at approximately age 16.9 years. The subject experienced grade 3 GVHD on Days 12 to 544 after transplantation. The GVHD was considered to have been triggered from the female cord blood cells attacking the patient as the percentage of T cells that were cord blood was 92% prior to transplantation and 97% at about 6 months later. The GVHD from cord blood donor cells resulted in diarrhea, rash, decreased platelets, anemia, decreased WBC, alopecia and abdominal pain. The subject was initially treated with steroids. However, as a consequence of this treatment with steroids, the subject also experienced treatment-related hyperglycemia on Days 84 to 104 after transplantation. The subject recovered following hospitalization and treatment with insulin. Steroids were stopped because of the side effects and infliximab was used to control the diarrhea from the GVHD through Day 523. The SAEs of GVHD and hyperglycemia were assessed as being possibly related to treatment. Subsequently, in the next year and a half the cord blood dropped to 82% then 22% (then <2%) as the T cell counts increased and the naïve T cell counts increased.

- Subject (b) (6): Chimerism 15 days prior to thymic transplantation showed 96% maternal cells in a blood CD3 cell sample. Recipient alleles were detected. The subject experienced Grade 1 abnormal lymphocyte count (presence of thymus donor T cells) and Grade 3 GVHD 150 days after RETHYMIC transplantation. Repeated chimerism studies showed thymus donor T cells in the peripheral blood (range 79-91%). A bone marrow biopsy on day 150 after thymic transplantation showed pure red blood cell aplasia consistent with GVHD. It could not be ascertained if maternal cells or thymus donor cells were responsible for GVHD. On Day 169 after thymic transplantation, the neutropenia improved and the event of GVHD was considered resolved. The event of abnormal lymphocyte count (presence of thymus donor T cells) was ongoing and considered not resolved. The subject was hospitalized with Grade 2 GVHD in gastrointestinal tract 358 days after thymic transplantation. A biopsy of the gut on day 192 after thymic transplantation showed some apoptosis. Both thymus donor T cells and maternal cells were present. It could not be determined which cells caused the GVHD. The event was not resolved during the study.
- Subject (b) (6): On Day 39 after transplantation, the subject experienced a non-serious AE of Grade 3 GVHD in the gastrointestinal tract that the Sponsor/Investigator initially considered possibly related to study treatment. She underwent a gut biopsy on this day and on Day 44 after transplantation. By Day 218 after transplantation, the subject had stopped total parenteral nutrition and was tolerating internal feeds without vomiting and diarrhea and the event was considered recovered/resolved.
- Subject (b) (6): The subject experienced Grade 3 GVHD in skin 165 days after thymic transplantation (serious due to other medically important event). At approximately 5 months after RETHYMIC transplantation, maternal T cells appeared in the circulation; GVHD developed at the same time. A skin biopsy on Day 167 had mild changes that were not diagnostic of GVHD. However, a biopsy 6 days later on Day 173, showed a toxic epidermal necrolysis–like pattern that was suggestive of acute GVHD. The SAE was ongoing and resolved only with chemotherapy after withdrawal of the subject from the study.

- Subject (b) (6): the subject experienced Grade 4 autologous GVHD (previously reported as enteritis) 146 days after transplantation (serious due to life-threatening, caused/prolonged hospitalization) with fever, diarrhea, and upper respiratory symptoms and was admitted to the local hospital. The diarrhea caused the subject's cyclosporine levels to drop and the subject developed a rash. Enteral cyclosporine and steroid doses were increased, and the subject was discharged. On Day 158 after transplantation, the diarrhea continued. The subject developed Grade 2 pyrexia (serious due to caused/prolonged hospitalization) with a body temperature of 101ºF, vomiting, hyponatremia and hypoalbuminemia. The subject was readmitted to local hospital. The subject was treated with antibiotics. Cultures were negative. TCRVβ repertoire on Day 159 after transplantation was markedly normalized from pre-transplantation. Flow cytometry on Day 159 showed T cells were predominately CD4, alpha beta+ (pre-transplant majority were double negative, suggesting T cells matured in the thymus). No naïve T cells were present. On Day 181 after transplantation, a colonoscopy and endoscopy showed extensive T cell infiltration of the gut and enteritis. A biopsy of the large and small bowel showed enteritis and colitis. The subject was treated with tacrolimus (starting Day 186, when the cyclosporine was stopped, until Day 206), infliximab (weekly starting Day 185 until Day 206 for a total of 4 doses), mycophenolate (Day 194 to Day 216), and steroids. T cell chimerism on day 187 after transplantation showed no evidence of thymus donor or maternal or T cell clones. Recipient alleles were detected. The chimerism findings are consistent with autologous GVHD.

Reviewer's comments: 11 subjects reported at least one GVHD AE after receiving RETHYMIC. In three subjects, maternal T cells were thought to be responsible for the GVHD AEs, of whom two subjects experienced flare up of pre-implantation GVHD symptoms. However, in one of the three cases (subject (b) (6), maternal T cells first became detectable around 5 months post-transplantation coinciding with GVHD onset. One subject, Subject (b) (6), experienced GVHD due to donor T cells from pre-implantation cord blood transplantation. 7 of the 11 subjects had autologous GVHD due to autoreactive recipient T cells. All of the 7 subjects had pre-implantation history of rash and/or lymphadenopathy rendering them with history of aGVHD. When available, pre-implantation oligoclonal expansion of recipient T cells were reported and thought to be responsible for aGVHD symptoms. 6 of the 7 subjects had diagnosis of atypical cDGA and one had diagnosis of CA due to FOXN1 deficiency.

All GVHD AE onset were within the first six months after RETHYMIC implantation, ranging between 12 days and 165 days. In at least four subjects, GVHD AEs presented as rashes (GVHD-skin) and progressed rapidly to GVHD-GI. Four subjects presented with GVHD-GI or GVHD-skin and GVHD-GI. Two subjects presented with bone marrow GVHD. In one subject, GVHD description was so limited that it was not clear what the presenting symptoms were; 8 of the GVHD AEs were SAEs and 6 of the 11 subjects died. The higher mortality rate in these subjects was not surprising in part because GVHD required aggressive immunosuppressive treatment which may have render these patients more susceptible to serious infections, sepsis and other complications.

Subjects with GVHD AEs had low total and naïve T cell counts at baseline and throughout the first six months. Total T cells counts were 385±546, 338±673 and 1082±2520 cells/mm^3 (mean ± stdev) at baseline, 3 months and 6 months post-transplantation. Naïve CD4+ cell counts for subjects with GVHD AEs were 0.4±0.7,

0.1±0.2 and 3.1±3.1 cells/mm^3 (mean ± stdev) at baseline, 3 months and 6 months post-transplantation. Autologous GVHD AEs developed after RETHYMIC may symptomatically resemble Omenn syndrome which is caused by RAG1 and RAG2 mutations. However, the very low total and naïve T cells numbers in these AEs differed from the normal or high T cell counts observed in Omenn syndrome cases. It is unclear why subjects with such low T cell numbers experienced serious GVHD AEs. It is unclear what role RETHYMIC played in these GVHD events.

These adverse events are directly related to T cell function and mechanism of action of RETHYMIC as positive and negative selection process is altered. All GVHD AE onset were within six months of implantation (subacute), therefore has a consistent temporal relationship with RETHYMIC implantation. Based on data submitted in this BLA and medical literature, the possibility cannot be ruled out that RETHYMIC may have exacerbated pre-existing condition and/or enabled development of new autoreactive host T cells due to altered negative selection. Therefore, this reviewer considers all GVHD AEs as likely related to RETHYMIC. GVHD appears to be a risk factor for death as well.

8.4.6.3 Autoimmune Conditions

While autoimmune diseases have been commonly reported in subjects with partial DGS and were expected events in subjects with cDGA, given the mechanism of action of RETHYMIC and its ability to reconstitute the immune system, the role of RETHYMIC in the development of autoimmune diseases could not be excluded. Autoimmune AESIs included cytopenias (such as thrombocytopenia, neutropenia and anemia), hypothyroidism, alopecia, autoimmune hepatitis, and transverse myeltitis. Cytopenias are expected events prior to the development of thymic function. In particular, autoimmune cytopenias have been reported in immunocompromised patients when T cell control of autoreactive B lymphocytes is reduced. Because these subjects lack T cells, they do not have T cells to help control autoreactive B cells which may result in the development of autoimmune cytopenias. Cytopenias have also been observed in subjects with partial DGS.

Autoimmune conditions observed aftertreatment with RETHYMIC included thrombocytopenia (27%), hypothyroidism (20%), neutropenia (19%), hemolytic anemia (10%), alopecia (10%), hyperthyroidism (8%), autoimmune hepatitis (2%), transverse myeltitis (1%), juvenile idiopathic arthritis (1%), and albinism (1%) and primary ovarian failure (1%). Thirty-seven patients (35%) in the RETHYMIC clinical program experienced autoimmune- related adverse reactions. These events included: thrombocytopenia (including idiopathic thrombocytopenic purpura) in 13 subjects (12%), neutropenia in 9 subjects (9%), proteinuria in 7 subjects (7%), hemolytic anemia in 5 subjects (5%), alopecia in 4 subjects (4%), hypothyroidism in 2 subjects (2%), autoimmune hepatitis in 2 subjects (2%), and autoimmune arthritis (juvenile idiopathic and psoriatic arthritis) in 2 subjects (2%). One subject (1%) each experienced transverse myelitis, albinism, hyperthyroidism, and ovarian failure.

8.4.6.3.1 Autoimmune Cytopenia

The most common cytopenia involved the platelet lineage with 28 subjects (26.7%) developing thrombocytopenia. The thrombocytopenia was assessed as treatment-related in 13 (12.4%) subjects, serious in 10 (9.5%), and as serious and treatment-

related in 7 (6.7%) subjects. Most of the cases were reported in the first 12 months following RETHYMIC implantation.

Neutropenia was the second most common cytopenia and was reported in 21 (20%) subjects. While some events of neutropenia were associated with infection or other unrelated events, 9 subjects (8.6%) experienced events of neutropenia considered related to study treatment. Neutropenia in 6 (5.7%) subjects were assessed as serious; and all were considered related to study treatment. Finally, 1 subject (1%) developed febrile neutropenia (SAE) but was considered not autoimmune in nature or related to treatment.

Eleven events of hemolytic anemia were reported in 10 (9.5%) subjects, of which 10 events in 9 subjects (8.6%) were SAEs. All 10 hemolytic anemia SAEs were considered related to study treatment.

In general, autoimmune cytopenias occurred in the first-year post-implant prior to the development of a normal T cell repertoire. It is likely that the absence of T cells contributed to the development of autoimmune cytopenias since autoreactive B cells are left unchecked. One hypothesis is that the T cell repertoire had not fully developed resulting in the absence of regulatory $FOXP3^+$ CD4 T cells as well as total CD4+ T cells. CD4+ T cells and $FOXP3^+$ T regulatory cells do not populate the various TCRBV families equivalently in the first year. For instance, some TCRBV families have high levels of CD4+ T cells but no $FOXP3^+$ T regulatory cells and vice versa. Thus, in the first year after RETHYMIC, there are no/insufficient regulatory T cells to control autoreactive B lymphocytes.

8.4.6.3.2 Autoimmune Thyroid Disease

Autoimmune thyroid disease manifesting as hypo- or hyperthyroidism was reported. The former was the most common presentation and developed in 21(20%) subjects in the FAS. It was assessed as related to study treatment in only 2 (1.9%) subjects and both were SAEs. Most hypothyroidism occurred during the second year after RETHYMIC implantation. Four subjects developed hypothyroidism after two years and three within the first year. The first 2 subjects who developed hypothyroidism was considered as related to study treatment as these were the first two cases developing this condition after RETHYMIC implantation. However, all subsequent cases of hypothyroidism were not considered treatment-related, as hypothyroidism is common in patients with partial DGA (20%) who do not receive RETHYMIC.

Hyperthyroidism was reported in 7 subjects. The first subject treated with RETHYMIC, subject (b) (6) developed hyperthyroidism SAE (Graves disease) 5239 days after implantation which was assessed as related to study treatment. However, all other hyperthyroidism AEs were assessed as not related to study treatment. All hyperthyroidism AEs occurred after the first-year post-transplantation. These events were expected as hyperthyroidism including Graves' disease has been reported following HSCT to treat other primary immunodeficiencies and following treatment with alemtuzumab.

8.4.6.3.3 Autoimmune Hepatitis

Autoimmune hepatitis was reported in 1 (1.1%) subject within 1 year of implantation. Subject (b) (6) developed Grade 3 SAE of autoimmune hepatitis 245 days after implantation (serious because “other medically important event”). On Day 245, the subject was diagnosed with autoimmune hemolytic anemia, which was treated with blood transfusion, steroids, and rituximab. The subject developed elevated liver enzymes approximately 2 months later. The subject was negative for the following antibodies: anti-mitochondrial, anti-LKM (liver, kidney, microsomal), anti-smooth muscle, anti-reticulin, anti-gastric parietal cells, and anti-ribosome. The maximal value for AST was 606 U/L, and ALT was 861 U/L. The subject underwent a liver biopsy, which showed lymphocytic infiltrate consistent with autoimmune hepatitis. The subject was treated with steroids with liver enzyme levels decreasing by Day 385 after (AST was 70 U/L, and ALT was 103 U/L). The subject was in remission on Day 3156 and the SAE was considered resolved on Day 3202 after implantation.

A second case of autoimmune hepatitis was reported in Subject (b) (6), >2 years after RETHYMIC. This subject developed Grade 3 autoimmune hepatitis 949 days after implantation with liver enzymes (ALT and AST) up to 20 times normal values. Otherwise, the subject was clinically well. Treatment with immunosuppressives was given with liver enzymes returning into the normal range on Day 1116.

8.4.6.3.4 Autoimmune Myelitis

Subject (b) (6) reported an event of transverse myelitis 283 days post-implant. While the etiology of the transverse myelitis is unknown, it was potentially autoimmune in nature. The subject also had a C77G polymorphism in the protein tyrosine phosphatase, receptor type, C (PTPRC) gene, or CD45 gene. This mutation has been reported to be associated with multiple sclerosis, autoimmunity, and infectious diseases. The contribution of this gene mutation to this subject’s disease process is unknown. The event of transverse myelitis was ongoing at the time of last follow-up (1992 days post-transplantation).

8.4.6.4 Skin Conditions

Rashes were an AESI, especially those persisting more than 2 weeks as they may be related to T cell infiltration of the skin. T cells are commonly found in skin biopsies of subjects with atypical cDGA and is an expected AE in these subjects. Rashes may also be related to maternal engraftment prior to the development of thymic function. Thus, subjects were closely monitored for rashes post-implant and were biopsied when possible.

Rash was reported in 42 (40%) subjects after RETHYMIC implantation. Rashes in 39 subjects occurred within the first year after implantation and in one subject occurred in the second-year post-transplantation. Two subjects had rashes after two years post-transplantation. 12 events were considered related to study treatment. One rash was assessed as SAE and treatment related. Subject (b) (6) with atypical cDGA developed a Grade 4 rash resembling GVHD on Day 218 after RETHYMIC implantation. A biopsy of the rash showed dyskeratotic cells and focal interface dermatitis, which is a rash of atypical cDGA. The subject was also diagnosed with Grade 4 hypoxia (oxygen saturation of 89%) secondary to GVHD. Oxygen was administered via nasal cannula, but the subject was subsequently intubated for respiratory failure. Steroids (15 mg/kg IV every 12 h for a total of 4 doses) and tacrolimus were also administered. The rash

improved with therapy but did not resolve and was ongoing, as was hypoxia, at the time of the subject's death due to respiratory failure secondary to sepsis from C. tropicalis and C. parapsilosis, 234 days after implantation

Skin conditions that were potentially autoimmune in nature included urticaria, alopecia, eczema, atopic dermatitis. Alopecia is an autoimmune condition which can occur at any time after implantation and may have been related to the atypical phenotype. Alopecia was reported in 10 (10%) subjects. 4 (3.8%), 2 (1.8%) and 4 (3.8%) subjects had alopecia in year 1, year 2 and after two years post-transplantation respectively. The AE was assessed as treatment-related in 4 (3.8%) subjects.

Other skin conditions that are common in young children including atopic dermatitis was reported in 2 (2.2%) subjects and eczema in 5 (4.8%) subjects. They were not related to RETHYMIC. None was SAE.

8.4.6.5 Malignancies

Malignancies were considered AESIs since poor T cell function leads to the loss of immune surveillance. Cancers have been reported in patients with partial DGA. Furthermore, subjects were considered at risk for the development of lymphoproliferative disorders associated with EBV or CMV. Five neoplasms were reported as AEs in 4 (4.3%) subjects within 2 years of RETHYMIC implantation. These included Grade 1 benign hepatic neoplasm and Grade 1 benign splenic tumor (Subject (b) (6), Grade 3 myelodysplastic syndrome (Subject (b) (6) and Grade 2 squamous cell carcinoma (Subject (b) (6)). They were not related to treatment. In Subject (b) (6), the benign growths were hemangiomas. Subject (b) (6) had 3 episodes of EBV lymphoma prior to implantation that were treated with chemotherapy. Notably, Subject (b) (6) EBV lymphoma entered remission post-treatment with RETHYMIC. The event of squamous cell carcinoma also resolved. This subject remains alive and in remission 1057 days post-transplantation.

A SAE of Grade 5 EBV associated lymphoma was reported in Subject (b) (6) and was assessed as related to treatment. This subject had pre-existing EBV lymphoma prior to implantation the RETHYMIC implantation protocol (specifically the use of fludarabine and dexamethasone) may have contributed to the progression of lymphoma; however, no mechanism by which the study treatment could have hastened the progression of the lymphoma was identified. The lymphoma would have likely progressed and led to the subject's death irrespective of the subject's participation in this study.

Subject (b) (6) reported a Grade 1 nodule over the site of thymus implantation (preferred term: skin mass) 2031 days post-transplantation. This event was considered possibly related to RETHYMIC and resolved 594 days after onset. The clinical significance of this finding is unknown.

8.4.6.6 Cytokine Release Syndrome/Hypersensitivity/Urticaria

Cytokine release syndrome, an expected effect of RATGAM administration, was reported as an AE in 19 (18.1%) subjects (21 events). Of these 21 events, 15 were moderate in intensity and 6 were severe and Grade 3 with 20 events assessed as related to treatment. All of the latter were reported at the time of RATGAM administration. Four events in 4 (3.8%) subjects were assessed as SAEs including 3 events related to RATGAM administration and 1 event reported 43 days post-

transplantation that was unrelated to study treatment and likely an infusion reaction to IVIG administration (Subject (b) (6)). In all 3 subjects in which the event was assessed as serious and related to treatment with RATGAM, the SAE resolved after the completion of RATGAM administration.

Hypersensitivity was reported in 11 (10.5%) subjects and occurred :: 12 months after implantation in 9 (8.6%). These AEs were assessed as treatment-related in only 1 (1.1%) subject (Subject (b) (6)) but was related to a blood transfusion and not RETHYMIC. It was considered related to treatment because the blood transfusion was required for anemia secondary to study mandated phlebotomy. Hypersensitivity was assessed as an SAE in 4 (4.3%) subjects but were not related to study treatment. The significance of these hypersensitivity reaction is unknown since they are common and expected events in immunocompromised subjects.

Urticaria was reported in 10 (9.5%) subjects and were possibly related to study treatment in 4 (3.8%) subjects (5 events). None was SAE. Subject (b) (6) reported one event related to cyclosporine and one event related to basiliximab. The other 3 events were related to the anti-coagulant lovenox (enoxaparin sodium, Subject (b) (6), a food allergy (Subject (b) (6) and an unknown cause (Subject (b) (6). The significance of the urticaria AEs is unknown since the thymus is not known to play a role in the etiology of this event.

8.5 Additional Safety Evaluations

8.5.1 Dose Dependency for Adverse Events

Adverse events were summarized by RETHYMIC dose quartiles. Subjects received doses of 4522.7 to :: 9068.9 mm^2/m^2 were in the first quartile (N = 23), 9069.9 < to 12674.6 mm^2/m^2 in the second quartile (N = 23), 12674.6 < to 16052.0 mm^2/m^2 in the third quartile (N = 23) and 16052.0 < to 23,754.5 mm^2/m^2 in the fourth quartile (N = 22). The dose administered was not reported in the first 14 treated subjects in the early studies.

The pattern of AEs reported was similar in all dose groups and consistent with the AE profile in the overall population. There was no apparent effect of subjects' dose on the pattern of AEs reported after RETHYMIC implantation. Serious AEs were also evaluated by RETHYMIC dose quartile. The pattern of SAEs reported was similar in all dose groups and consistent with the SAE profile in the overall population. There was no apparent effect of subjects' dose on the pattern of SAEs reported after RETHYMIC implantation.

8.5.2 Time Dependency for Adverse Events

Occurrence of AEs were compared between < 6 months and 6-12 months. All 105 subjects (100%) experienced at least one AE during the first 6 months, and 66 subjects (62.9%) had at least one AE between 6 an d12 months after RETHYMIC implantation. The most common AEs occurred in < 6 months were pyrexia (n=60 [57.1%]), device related infection (n=39 [37.1%]), rash (n=29 [27.6%]) and hypertension (n=26 [24.8%]). The most common AEs occurred in 6-12 months post-transplantation were device related infection (n=13 [12.4]), thrombocytopenia (n=8 [7.6%]), pyrexia (n=7 [6.7%]) and rash (n=7 [6.7%]). Overall, fewer subjects experienced AEs in 6-12 months compared to

in < 6 months post-transplantation. Pyrexia, device related infection and rash were among the most frequent AEs for both time periods.

AEs were also compared between year 1 and year 2 post-transplantation (Table 40). All 105 subjects had at least one AE within the first year of RETHYMIC implantation, while 58 subjects (55.2%) had at least one AE during year 2 post-transplantation. The most common AEs in < 1 year were pyrexia (n=61 [58.1%]), device related infection (n=44 [41.6%]), rash (n=33 [31.4%]), hypertension (n=29 [27.6%]), diarrhea (n=28 [26.7%]) and hypoxia (n=26 [24.8%]). The most common AEs occurred in year 2 were device related infection (n=15 [14.3%]), hypothyroidism (n=14 [13.3%]), pyrexia (n=7 [6.7%]) and viral upper respiratory tract infection (n=5 [4.8%]). Fewer subjects experienced AEs in year 2 compared to year 1. Device related infection remained the most frequent AE in year 2. Hypothyroidism occurred more frequently in year 2 (n=14 [13.3%]) compared to year 1 (n=3 [2.9%]).

Table 40. Adverse Events < 12 months vs 12-24 months (in at least 10% subjects)

AE onset < 12 months			AE onset 12-24 months		
AEDECOD	Number of subjects	%subjects	AEDECOD	Number of subjects	%subjects
All AEs	105	100.0%	All AEs	58	55.2%
Pyrexia	61	58.1%	Device related infection	15	14.3%
Device related infection	44	41.9%	Hypothyroidism	14	13.3%
Rash	33	31.4%			
Hypertension	29	27.6%			
Diarrhea	28	26.7%			
Hypoxia	26	24.8%			
Alanine aminotransferase increased	25	23.8%			
Thrombocytopenia	24	22.9%			
Anemia	22	21.0%			
Aspartate aminotransferase increased	21	20.0%			
Hypomagnesaemia	20	19.0%			
Cytokine release syndrome	19	18.1%			
Neutropenia	16	15.2%			
Viral upper respiratory tract infection	16	15.2%			
Respiratory failure	15	14.3%			
Clostridium difficile colitis	14	13.3%			
Vomiting	14	13.3%			
Hepatomegaly	13	12.4%			
Staphylococcal bacteremia	12	11.4%			

(Source: Reviewer's table based on SN0065)

A total of 411 SAEs occurred in 86 subjects (81.9%) in year 1. 114 SAEs occurred in 33 subjects (31.4%) in year 2. Frequency of SAE also decrease over time.

The decreasing trend of AEs and SAEs overtime after RETHYMIC treatment was consistent with immune reconstitution being achieved in these subjects and reflected the overall improvement of the subjects' condition.

8.5.3 Product-Demographic Interactions

8.5.3.1 Age at Implantation

AEs and SAEs were compared by four age at implantation groups: :: 6 months (N = 35), > 6 to :: 12 months (N = 30), > 12 months to :: 18 months (N = 21) and > 18 months (N = 19). The most commonly reported AEs in each age category are summarized below:

- :: 6 months: 680 AEs were reported in 35 subjects (100%), of which 160 were SAEs. The most frequently reported AEs were pyrexia (20 [57.1%]), device-related infection (16 [45.7%]), rash (13 [37.1%]), ALT increased (12 [34.3%]), hypoxia (10 [28.6%]), and AST increased, anemia, and hypertension, which were reported in 9 (25.7%) subjects each.
- 6 to :: 12 months: 444 AEs were reported in 30 subjects (100%), of which 116 were SAEs. The most frequently reported AEs were pyrexia (18 [60%]), device-related infection (13 [43.3%]), rash (11 [36.7%]), hypoxia (10 [33.3%]), hypertension (10 [33.3%]), and hypomagnesemia (8 [26.7%]).
- 12 months to :: 18 months: 480 AEs were reported in 21 (100%) subjects, of which 167 were SAEs. The most frequently reported AEs were device-related infection (17 [81.0%]), pyrexia (14 [66.7%]), diarrhea (10 [47.6%]), thrombocytopenia (9 [42.9%]), rash (7 [33.3%]), respiratory failure (7 [33.3%]) and anemia, AST increased, and oropharyngeal candidiasis were reported in 6 (28.6%) subjects each.
- > 18 months: 254 AEs were reported in 19 [100%]) subjects, of which 82 were SAEs. The most frequently reported AEs were pyrexia (10 [52.6%]), device related infection (6 [31.6%]), diarrhea (6 [31.6%]), neutropenia (6 [31.6%]) and hypertension (6 [31.6%]).

Overall AE and SAE patterns were similar among age groups with pyrexia and device related infection being most common. There was no apparent effect of subjects' age at implantation on the pattern of AEs and SAEs reported within 2 years after RETHYMIC implantation. However, it is noted that in older age groups thrombocytopenia (12 to :: 18 months) and neutropenia (> 18 months) were more frequent affecting 42.9% and 31.6% of subjects respectively. Clinical significance of this increased cytopenia frequency in older subjects was unclear due to the limited number of subjects.

Reviewer's comments: Mortality rates were 37.1%, 13.8%, 42.9% and 21.1% for age at implantation groups < 6 months, 6-12 months, 12-18 months and >18 months respectively. The clinical significance of higher mortality rate in subjects who were 12-18 months of age at implantation was unclear. There was no consistent improving or worsening pattern as age at implantation increases. However, the two older age groups combined (>1 year) appeared to have higher death rate than the two younger age groups (< 1 year). The percentages of SAEs were higher in subjects who were older

than 1 year of age at implantation. 34.8% and 32.3% of AEs were serious in subjects who were 12-18 months and > 18 months old, while 23.5% and 26.1% of AEs were serious in subjects who were < 6 months and 6-12 months old respectively. Therefore, treating CA subjects with RETHYMIC in the first year of life may lead to a better outcome.

8.5.3.2 Sex

There were 60 male subjects and 45 female subjects (FAS). The pattern of reported AEs was similar in male and female subjects and was consistent with the overall adverse event profile. There did not appear to be any clinically relevant differences between males and females with respect to the nature of AEs reported within 2 years of implantation.

- 1082 AEs were reported in 60 male subjects (100%), of which 310 were SAEs. The most frequently reported AEs (affecting > 30% subjects) in male subjects were pyrexia (40 [66.7%])), device-related infection (34 [56.7%])), and rash (19 [31.6%]).
- 776 AEs were reported in 45 female subjects (100%), of which 215 were SAEs. The most common AEs (affecting > 30% subjects) in female subjects were pyrexia (22 [48.9%]), device related infection (18 [40%]), hypertension (15 [33.3%]), rash (15 [33.3%]) and diarrhea (14 [31.1%]).

Serious AEs within 2 years of implantation were reported in 53 (88.3%) male subjects and in 36 (80%) female subjects. In general, the number and types of SAEs reported were similar regardless of sex. Consistent with the SAE profile in the overall population, the most frequently reported SAEs included device-related infection (males: 29 [48.3%], females: 17 [37.8%]), hypoxia (males: 10 [16.7%], females: 5 [11.1%]), and pyrexia (males: 7 [11.7%], females: 11 [24.4%]). The number of subjects who reported respiratory failure SAEs appeared to be higher in males than in females (males: 14 [23.3%], females: 4 [8.9%]). This may have been related to a higher number of male subjects entering the study with pre-existing conditions of the respiratory, thoracic, and mediastinal conditions, including hypoxia and a ventilator requirement. These differences are not considered to be clinically relevant. No other SAEs were reported at clinically relevant differences in frequencies in males and females after RETHYMIC implantation. Based on these data, there was no apparent effect of subjects' gender on the pattern of AEs reported within 2 years after RETHYMIC implantation.

8.5.3.3 Race

There were 76 white subjects, 21 black subjects, 3 Asian subjects and 5 subjects of other or multiple races. Non-white subjects were combined for analyses since the number of each race group is small for meaningful interpretation. The pattern of AEs reported were similar in white and non-white subjects and was consistent with the overall AE profile.

8.5.4 Product-Disease Interactions

8.5.4.1 cDGA Phenotype

Adverse events reported within 2 years of implantation were compared by subjects' cDGA phenotype. There were 52 subjects with typical cDGA and 44 with atypical cDGA.

In both atypical and typical cDGA subjects, the most commonly reported AEs were device-related infection (atypical: 27 [61.3%]), typical: 20 [38.5%]), pyrexia (atypical: 27 [61.3%]), typical: 30 [57.7%]), and rash (atypical: 15 [34.1%], typical: 18 [34.6%]).

The 2 groups differed with respect to the frequencies of AEs such as hypertension, hypomagnesemia and cytokine release syndrome, which are known to be associated with use of immunosuppressant medications. These were administered per protocol to subjects with atypical cDGA but were not required in most subjects with typical cDGA. In particular, symptomatic subjects with atypical cDGA required immunosuppressive therapy not only to prevent the rejection of RETHYMIC, but also to treat the complications associated with the atypical phenotype of cDGA. Consequently, hypertension (atypical cDGA: 17 [38.6%]); typical cDGA: 9 [17.3%]), hypomagnesemia (atypical cDGA: 9 [20.5%], typical cDGA: 6 [11.5%]), and cytokine release syndrome (atypical cDGA: 13 (29.5%); typical cDGA: 4 (7.7%)]) were all reported at higher frequencies in subjects with atypical cDGA. These differences in immunosuppressant-related AE frequencies between subjects with atypical and typical cDGA were expected.

GVHD AEs were only reported in atypical cDGA subjects. There were 6 autologous GVHD, 1 maternal GVHD and 1 GVHD from prior cord blood transplant reported in 8 typical cDGA subjects (18.2%). No GVHD was reported in typical cDGA subjects (0%). It appears that atypical cDGA subjects with history of rashes and lymphadenopathy are more susceptible to develop GVHD AEs after receiving RETHYMIC.

240 SAEs were reported in all 40 typical cDGA subjects (76.9%). 240 SAEs were reported in 41 atypical cDGA subjects (93.2%). SAE frequency was slightly higher in atypical cDGA subjects.

Overall, there were significant differences in immunosuppression related AEs and GVHD between atypical and typical cDGA phenotypes. SAE frequency was higher in atypical cDGA phenotype. It is also noted that subjects with atypical GVHD had a slightly higher risk of death overall and were more likely to die during the first year after RETHYMIC implantation as discussed in Section 8.4.1.

8.5.4.2 Disease Etiology/Gene Mutation

There did not appear to be any clinically relevant differences between subjects with and without 22q11.2 deletion with respect to the nature of AEs and SAEs reported within 2 years of implantation. The adverse event profile in subjects with 22q11.2 deletion, CHARGE/CHD7 mutation and no known mutation / no reported mutation were similar and consistent with the overall AE profile.

8.5.5 Overdose, Drug Abuse Potential, Withdrawal, and Rebound

The maximum recommended dose is 22,000 mm^2 of RETHYMIC/m^2 recipient body surface area (BSA). Standard clinical care is recommended for patients receiving a dose > 22,000 mm^2 of RETHYMIC/m^2 recipient BSA.

During clinical development one subject received a dose higher (23,755 mm^2/m^2) than the maximum recommended dose. This subject developed an AE of enteritis. A biopsy showed T cell, B cell, and neutrophil infiltration of the gut which resolved after treatment with immunosuppression, 146 days after treatment with RETHYMIC. The AE of enteritis

may have been related to the high dose of RETHYMIC. The subject died 234 days after treatment with RETHYMIC due to complications from a lower respiratory tract fungal infection that was not considered related to RETHYMIC.

8.5.6 Immunogenicity (Safety)

The Applicant referenced an analysis by the Principal Investigators and her colleagues that found no correlation between HLA-matching on immune outcomes in 30 subjects treated with RETHYMIC at 1-year post-transplantation [Markert 2008]. This was reviewed and accepted by the Agency during the original submission. HLA matching was not required for RETHYMIC implantation.

8.6 Safety Conclusions

In the RETHYMIC clinical development program, 105 subjects received RETHYMIC including 98 subjects with cDGA, 3 subjects with FOXN1 deficiency, 2 subjects with SCID, 1 subject with athymia of unknown origin, and 1 subject with athymia due to TBX1 mutation. The subject's medical histories and baseline conditions were consistent with that expected in CA. The most common medical conditions were related to the underlying disease including congenital cardiac or cardiothoracic vascular anomalies, hypoparathyroidism, hypocalcemia, and deafness/ear pinnae anomalies. Most subjects (>50%) entered the studies with pre-existing central venous catheters and G tubes, with over a third of subjects requiring parenteral nutrition. These findings confirm the medical complexity and serious clinical condition of study subjects consistent with the disease under study.

The median dose of RETHYMIC transplanted in the FAS, was 12,675 mm^2/m^2 (range: 4,523 to 23,755 mm^2/m^2) using a median of 30 slices (range: 10 to 108). At the time of transplantation, the median age of subjects was 269 days ranging from 33 days to 6163 days (16.9 years) in the FAS. The oldest subject was (Subject (b) (6) was transplanted in adolescence after the development of EBV lymphoma following a prior CBT. Twenty-nine subjects (27.6%) died following RETHYMIC transplantation; 16 subjects died within 6 months after transplantation. 7 died between 6 and 12 months; 2 died during year 2, and four died more than two years after transplantation at year 3, year 5, year 8 and year 9 respectively. The majority of deaths (23 of 29 deaths) were within the first-year post-transplantation, prior to the development of thymic function. Most of these deaths were due to infections, complications associated with infection or respiratory failure/hypoxia and were therefore consistent with the disease under study. Three death were considered to be related to REHYMIC. Risk factors for death included pre-existing CMV infection and active viral infection, renal insufficiency, atypical cDGA phenotype, use of immunosuppression and high total T cell number pre-transplantation.

All 105 subjects who received RETHYMIC had at least one AE with a total of 1858 AEs reported within the first 2 years and a total of 2187 AEs reported at any time following RETHYMIC transplantation. The most common (>25% of subjects) AEs in the first two years were pyrexia (66 [62.3%]), device related infection (54 [50.9%]), rash (37 [34.9%]), diarrhea (33 [31.1%]), hypertension (30 [28.3%]), alanine aminotransferase increased (28 [26.3%]), thrombocytopenia (27 [25.5%]), hypoxia (27 [25.5%]). These AEs were consistent with the subject's reported medical histories, use of immunosuppression, and disease under study. The most frequent AEs in the two years following RETHYMIC transplantation were infection related; and 97 (92.4%) subjects reported a total of 600 infection-related events. The most common (>15% of subjects) infections included

device related infections (51%), viral upper respiratory tract infection (22%), and *C. Difficile* infection (16%). The high incidence of infection-related AEs was expected given the immunocompromised population under study.

The clinical trials in RETHYMIC development program considered any AE associated with the RETHYMIC transplantation procedure or biopsy, supportive care associated with these procedures including protocol required immunosuppression, or RETHYMIC as related to study treatment (adverse reactions). In the first two years post-transplantation, there were 80 subjects (76%) reporting 247 adverse reactions (13.3% of reported AEs). Adverse reactions affecting at least 10% of subjects are the following: Hypertension (19%), Cytokine Release Syndrome (18%), Rash (15%), Hypomagnesemia (16%), Thrombocytopenia (12%), Renal Impairment/Renal Failure (12%) and Graft versus Host Disease (10%). The most common adverse reactions were considered to be related to immunosuppression, T cell dysregulation or autoimmune conditions which is expected and consistent with the disease being treated and mechanism of action of RETHYMIC.

The AE profile was generally found to be consistent across demographic and disease groups. There were no clinically relevant effects of gender, race, 22q11.2 hemizygosity, disease etiology/gene mutation. AE patterns differed by age at transplantation. SAE and cytopenia (thrombocytopenia and neutropenia) were more frequent in subjects who were older than 1 year of age at RETHYMIC transplantation. Mortality rate was slightly higher in subjects who were older than 1 year (32.5%) compared to subjects who were younger than 1 years of age (26.5%) at transplantation. Difference in AEs by age at transplantation suggest that it may be beneficial to treat subjects early.

Differences in the AE profile among subjects with typical and atypical cDGA were expected and can be attributed to differences in the use of immunosuppressive medications. The latter medications were administered per protocol in subjects with atypical cDGA but were only required in a small subset of those with typical cDGA who had an elevated immune response at baseline. In contrast, the former subjects required immunosuppressive therapy not only to prevent the rejection of RETHYMIC but also to treat the complications associated with pre-existing oligoclonal T cell proliferations associated with atypical cDGA. Consequently, hypertension (atypical cDGA: 17 [38.6%]); typical cDGA: 9 [17.3%]), hypomagnesemia (atypical cDGA: 9 [20.5%], typical cDGA: 6 [11.5%]), and cytokine release syndrome (atypical cDGA: 13 (29.5%); typical cDGA: 4 (7.7%)]) were all reported at higher frequencies in subjects with atypical cDGA. In addition, GVHD AEs were only reported in subjects with atypical cDGA and not those with typical cDGA, which may be partially related to presence of autoreactive T cells pre-transplantation in subjects with atypical cDGA.

Subjects with renal insufficiency at baseline had a higher death rate (60%). There was no significant difference in death and AEs in subjects with elevated liver enzymes prior to RETHYMIC transplantation.

The pattern of AEs reported was similar in all dose groups and consistent with the AE profile in the overall population. The highest dose quartile appeared to have lower death rate suggesting that a higher dose within the recommended range may be beneficial.

Five subjects treated with products manufactured in the (b) (4) facility had AKI while no AKI was reported in subjects treated with products manufactured in the (b) (4) facility.

The AKI events appeared to be related to clinical manifestations of the disease and concomitant medication (antibiotics and immunosuppression) and unrelated to the RETHYMIC. There were no significant safety concerns on the (b) (4) facility.

As expected, AEs related to immunosuppression such as CRS, hypertension, hypomagnesemia were more frequent in subjects who received immunosuppression. Frequency of infection adverse events were comparable between subjects with and without immunosuppression.

In addition to infections, AESI defined in the study protocols included autoimmune diseases, GVHD, rashes and cancers. Autoimmune conditions observed after RETHYMIC treatment included thrombocytopenia (27%), hypothyroidism (20%), neutropenia (19%), hemolytic anemia (10%), alopecia (10%), hyperthyroidism (8%), autoimmune hepatitis (2%), transverse myeltitis (1%), juvenile idiopathic arthritis (1%), and albinism (1%) and primary ovarian failure (1%). While autoimmune diseases have been commonly reported in subjects with partial DGS and were expected events in subjects with cDGA, given the mechanism of action of RETHYMIC and its ability to reconstitute the immune system, the role of RETHYMIC in the development of autoimmune diseases could not be excluded.

GVHD was reported in 11 subjects (10%) of whom three had maternal GVHD, 1 had GVHD due to pre-transplantation CBT, and 7 had autologous GVHD. All GVHD AEs onset were within the first six months after RETHYMIC, ranging between 12 days and 165 days. In at least four subjects, GVHD AEs presented as rashes (GVHD-skin) and progressed rapidly to GVHD-GI. Four subjects presented with GVHD-GI or GVHD-skin and GVHD-GI. Two subjects presented with bone marrow GVHD. In one subject, GVHD description was so limited that it was not clear what the presenting symptoms were. 8 of the GVHD AEs were SAEs. 6 of the 11 subjects (54.5%) died. Although pre-existing autoreactive T cells may contribute to these GVHD events, the possibility cannot be ruled out that RETHYMIC may have exacerbated pre-existing condition and/or enabled development of new autoreactive host T cells due to altered negative selection.

Rashes were of interest as these may have been indicative of new development or flare of pre-existing rashes associated with atypical cDGA. Rash was reported in 42 (40%) subjects after RETHYMIC transplantation.

Five neoplasms were reported as AEs in 4 (4.3%) subjects within 2 years of RETHYMIC transplantation. These included Grade 1 benign hepatic neoplasm and Grade 1 benign splenic tumor, Grade 3 myelodysplastic syndrome and Grade 2 squamous cell carcinoma, which were all considered not related to treatment. A SAE of Grade 5 EBV associated lymphoma was reported in Subject (b) (6) and was assessed as related to treatment. This subject had pre-existing EBV lymphoma prior to transplantation the RETHYMIC transplantation protocol (specifically the use of fludarabine and dexamethasone) may have contributed to the progression of lymphoma; however, no mechanism by which the study treatment could have hastened the progression of the lymphoma was identified.

The clinical laboratory and vital signs data from these studies did not raise any safety concerns for RETHYMIC when administered to subjects with CA.

In conclusion, the safety data support the use of RETHYMIC in subjects with CA, an otherwise fatal disease.

9. ADDITIONAL CLINICAL ISSUES

9.1 Special Populations

9.1.1 Human Reproduction and Pregnancy Data

This section is not applicable since CA is congenital diseases where the children die prematurely before reaching reproductive age.

9.1.2 Use During Lactation

This section is not applicable since CA is congenital diseases where the children die prematurely before reaching reproductive age.

9.1.3 Pediatric Use and PREA Considerations

CA manifests at birth. The clinical data to support the safety and efficacy of RETHYMIC was obtained solely in children, median age of 9 months, ranging from 33 days – 16 years. All subjects in the EAS population were less than 3 years of age, as survival without RETHYMIC treatment is unlikely past 3 years of age. There was no trend in survival based on age at transplant. There were 4 subjects older than 3 years of age; 2 of these subjects had prior HSCT, 1 subject had prior thymic transplants, and 1 subject had a TBX1 mutation. There were no new safety signals identified in children over 3 years of age. Given the rarity of the disease and that the condition is fatal in early childhood without treatment, we believe that there are sufficient clinical data to support the indication of RETHYMIC for all children with CA.

PREA requirement was waived as REHYMIC holds an orphan drug designation for treatment of CA. A PeRC meeting was not held as there were no issues that needed to be discussed with PERC for this disease with orphan drug designation.

9.1.4 Immunocompromised Patients

CA manifests as a severe combined T-cell immunodeficiency due to the absence of a functional thymus. Therefore, the clinical development program was conducted in infants and newborns who were severely immunocompromised.

9.1.5 Geriatric Use

There is no data on the use of RETHYMIC in the geriatric population since the afflicted individuals generally do not survive beyond the age of 2 years.

10. CONCLUSIONS

Despite the heterogenous underlying genetic anomalies and diverse comorbid conditions, the significant survival benefit of RETHYMIC is consistent and persistent in this otherwise fatal disease. The effectiveness of RETHYMIC is further supported by the decreased frequency of infections, increased numbers of naïve T-cells, emergence of a diverse TCRVβ repertoire, and evidence of thymopoiesis on biopsy after RETHYMIC transplantation. The adverse reactions associated with RETHYMIC were generally related to autoimmune disorders, T cell dysregulation or complications from the

immunosuppression. These adverse reactions could generally be mitigated through clinical care, but even the serious risks were acceptable when compared to the risk of death within the first few years of life without RETHYMIC therapy. The review of the submitted clinical data supports traditional approval for RETHMIC for immune reconstitution in pediatric patients with CA.

11. RISK-BENEFIT CONSIDERATIONS AND RECOMMENDATIONS

11.1 Risk-Benefit Considerations

Risk-benefit considerations for RETHYMIC are summarized in Table 41: Benefit/Risk Considerations.

Table 41. Summary of Benefit / Risk Considerations

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Congenital athymia is a rare pediatric condition in which children suffer recurrent, severe infections and usually die by 3 years of age from an infection. • Congenital athymia most commonly is due to cDGA but can occur with FOXN1 deficiency.	• Congenital athymia is a serious and life-threatening disease that is associated with recurrent and severe infections due to T cell immunodeficiency.
Unmet Medical Need	• There are no approved therapies that treat congenital athymia.	• There is a substantial unmet medical need.
Clinical Benefit	• 105 patients were treated with RETHYMIC in 10 open-label, single-center clinical trials conducted over 3 decades. • Efficacy database included 95 patients under 3 years of age with congenital athymia who did not receive prior hematopoietic cell transplantation or thymus transplantation. • There was no concurrent control. However, a retrospective natural history case series of 49 patients with congenital athymia receiving only supportive care showed that all patients died by 3 years of age. • The survival rates at 1-year and 2-year after RETHYMIC transplantation were 77% and 76% respectively. • For subjects treated with RETHYMIC who were alive at 1-year post transplantation, the survival rate was 94% and was essentially unchanged thereafter, the median age of surviving subjects was 11.4 years (3-25.7) at last follow-up. • Frequency and severity of infections decreased significantly overtime following RETHYMIC transplantation. • Most treated patients had an increase in naïve CD3, CD4, and CD8 cell numbers and T cell proliferative response to antigen and mitogen by 12 months after RETHYMIC.	• Despite the heterogenous underlying genetic anomalies and diverse comorbid conditions, the significant survival benefit of RETHYMIC is substantial and persistent in this otherwise fatal disease. • The effectiveness of RETHYMIC is further supported by the decreased frequency and severity of infections and increased numbers of naïve T-cells after RETHYMIC transplantation.
Risk	• Most common treatment related adverse events were autoimmune diseases, transplantation procedure complication, or T cell related such as graft vs host disease (GVHD). • AEs and SAEs were common due to the serious underlying condition and use of immunosuppressive medications in patients with congenital athymia. • Following RETHYMIC, it may take 6 to 12 months to achieve sufficient level of immune reconstitution during which time patients are at risk of serious infections.	• Most AEs are due to underlying medical condition and concomitant medications and expected. • Overall, these risks are manageable with routine medical practice.
Risk Management	• No REMS.	• Warnings and Precautions to include information about risks of acquiring transmissible infectious disease, autoimmune disorders, malignancy, GVHD, pre-existing CMV infection and baseline renal impairment in patients treated with RETHYMIC. • Routine Pharmacovigilance

11.2 Risk-Benefit Summary and Assessment

Congenital athymia is a rare disease characterized by profound, life-threatening T cell immunodeficiency due to the absence of a functioning thymus at birth. There is a substantial unmet medical need as there is no approved therapy for the treatment of congenital athymia. Based on data from 10 open-label studies conducted over 3 decades compared to a large natural history cohort, RETHYMIC improved survival of children with congenital athymia. During the first two years following RETHYMIC treatment, 76% survived, whereas without treatment only 6% were alive at 2 years of age. The survival benefit from RETHYMIC persisted. This large treatment effect overcomes any methodologic study design concerns with the use of an external control group. The effectiveness of RETHYMIC is further supported by the decreased frequency of infections, increased numbers of naïve T-cells, emergence of a diverse TCRV repertoire, and evidence of thymopoiesis on biopsy after RETHYMIC transplantation. The risks include autoimmune disorders, T cell dysregulation or complications from immunosuppression that can generally be managed with routine medical care.

In conclusion, the overall risk-benefit is favorable for RETHYMIC for immune reconstitution in pediatric patients with congenital athymia.

11.3 Recommendations on Regulatory Actions

Based on analyses of the clinical safety and efficacy data contained in the BLA submission, the clinical team considers the benefit/risk profile favorable in support of full approval for RETHYMIC for immune reconstitution in pediatric patients with congenital athymia. The clinical team recommends regular approval for RETHYMIC for immune reconstitution in pediatric patients with congenital athymia.

11.4 Labeling Review and Recommendations

FDA made substantial changes to each section of the Prescribing Information (PI), based on available clinical trial data, as well as FDA guidance on product labeling. The Clinical Reviewer considers the revised PI to be acceptable. Major labeling changes made by the Agency included adding GVHD and baseline renal impairment to Warnings and Precautions, revising the adverse reaction table, and adding additional patient counseling information.

The overall content of the PI suitably conveys known information regarding safety and efficacy results demonstrated in clinical trials of RETHYMIC. The PI contains adequate warnings for healthcare providers and caregivers for the treatment with RETHYMIC for immune reconstitution in pediatric patients with congenital athymia.

11.5 Recommendations on Postmarketing Actions

The reviewed safety data do not warrant a Risk Evaluation and Mitigation Strategies (REMS), a safety postmarketing requirement (PMR) study, or a safety postmarketing commitment (PMC) study. The postmarketing risk mitigation plans include product labeling, applicant’s pharmacy and surgical training, and routine pharmacovigilance plan. The Applicant independently proposed to conduct a 75-subject post-marketing study to assess survival, T-cell markers of immune reconstitution, and adverse events.

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