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## Experience with cultured thymus tissue in 105 children

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### Abstract

**Background:** There are currently no approved therapies to treat congenital athymia, a condition of immune deficiency resulting in high early mortality due to infection and immune dysregulation. Multiple syndromic conditions, such as complete DiGeorge syndrome, 22q11.2 deletion syndrome, CHARGE (coloboma, heart defects, choanal atresia, growth or mental retardation, genital hypoplasia, and ear anomalies and/or deafness) syndrome, diabetic embryopathy, other genetic variants, and FOXP1 deficiency are associated with congenital athymia.

**Objective:** This report describes 105 patients treated with cultured thymus tissue (CTT) and focuses on the outcomes of 95 patients with treatment-naïve congenital athymia.

**Methods:** Ten prospective, single-arm open-label studies with patient enrollment from 1993 to 2020 form the basis of this data set. Patients were tested after administration of CTT for T cell development; all adverse events and infections were recorded.

**Results:** One hundred five patients were enrolled and received CTT (full analysis set). Ten patients had diagnoses other than congenital athymia and/or received prior treatments. Ninety-five treatment-naïve congenital athymia patients were included in the efficacy analysis set (EAS). The Kaplan-Meier estimated survival rates at year 1 and year 2 after administration of CTT in the EAS were 77% (95% CI, 0.670–0.844) and 76% (95% CI, 0.657–0.834), respectively. Twenty-one patients died in the first year before developing naïve T cells, and one died in the second year after receiving CTT. Three subsequent deaths were not related to immunodeficiency. A few patients developed alopecia, autoimmune hepatitis, psoriasis, and psoriatic arthritis after year 1. Infections, autologous graft-vs-host-disease manifestations, and autoimmune cytopenias all decreased approximately 1 year after administration of CTT.

**Conclusion:** Treatment with CTT led to development of naïve T cells with a 1-year survival rate of 77% and a median follow up of 7.6 years. Immune reconstitution sufficient to prevent infections and support survival typically develops 6 to 12 months after administration of CTT.

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**Clinical Implications:** Cultured thymus tissue (CTT) is an investigational treatment for congenital athymia. In 105 patients, survival was 77% at 1 year; no deaths considered related to CTT occurred after year 2.

**Capsule Summary:** Investigational cultured thymus tissue resulted in 1-year survival of 77% among 95 patients with congenital athymia (median follow-up, 7.6 years). Infections and autoimmunity decreased, and no immunodeficiency-related deaths occurred after year 2.

## Keywords

Congenital athymia; complete DiGeorge; CHARGE syndrome; 22q11.2; thymus transplantation; RVT-802; CTT

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## Introduction

Congenital athymia is a rare condition[1] in which children are born without a thymus resulting in profound immunodeficiency and immune dysregulation. Naïve T cells develop in the thymus and, after leaving the thymus, fight infection and help regulate the immune system. Without a thymus, children are susceptible to life-threatening infections and immune dysregulation. Children with congenital athymia generally do not survive beyond early childhood. While hematopoietic stem cell transplants (HSCTs) have been performed in patients with congenital athymia[2, 3] only 7 of 17 patients (41%) were reported as surviving by Janda et al.[2] Currently, the only available treatment for these patients, outside of a clinical trial, is supportive care, which may include strict infection-prevention measures including immunoglobulin replacement, prophylactic antimicrobials, and protective isolation.

Congenital athymia is associated with several underlying syndromic conditions that can be severe and, in some cases, can lead to death. Complete DiGeorge anomaly (cDGA) was used to refer to patients with congenital athymia in prior publications and encompassed 22q11.2 deletion syndrome (22q11.2ds), CHARGE (coloboma, heart defects, choanal atresia, growth or mental retardation, genital hypoplasia, and ear anomalies and/or deafness) syndrome, diabetic embryopathy, and other genetic defects associated with congenital athymia. FOXP1 deficiency was a separate category of athymic patients. Comorbidities of these associated syndromic conditions that are frequently experienced by patients with congenital athymia include hypoparathyroidism, congenital heart and kidney defects, esophageal and tracheal atresia, choanal atresia, hypothyroidism, hearing loss, and developmental delay.

Congenital athymia can be detected when newborns are screened for severe combined immunodeficiency (SCID). In the United States, SCID screening has become the standard method by which congenital athymia is detected. The diagnosis is confirmed using flow cytometry in conjunction with testing for genes causing SCID. Infants with congenital athymia have very low naïve T cell counts. There is no uniformly agreed upon level of naïve

T cells defining congenital athymia. Before the widespread use of newborn screening in the United States, congenital athymia was diagnosed when patients developed life-threatening infections that healthy infants are usually able to control without therapy. The diagnosis of 22q11.2ds was made in many children prior to heart surgery, but the children were rarely tested for T cell numbers, as congenital athymia is rare in 22q11.2ds.[4] Likewise, CHARGE syndrome was not associated with congenital athymia when our investigational program was initiated. More recently, newborn screening for SCID has led to early diagnosis in most patients in the United States.

Congenital athymia can present with a typical or an atypical phenotype. The typical phenotype has low T cell numbers, no rash or lymphadenopathy, and usually lack of T cell reactivity to mitogens. The atypical phenotype has oligoclonal T cell expansion, rash, lymphadenopathy, and T cell reactivity to mitogens such as phytohemagglutinin (PHA). This phenotype resembles Omenn syndrome or autologous graft versus host disease (GVHD) and typically presents at some point after birth. Patients with the atypical phenotype require treatment with immunosuppression to prevent the deleterious effects of their autoreactive T cells. Patients with the typical phenotype may transition to the atypical phenotype; although, it is unknown what triggers this transition.

Cultured thymus tissue (CTT), also known as allogeneic processed thymus tissue–agdc, is a tissue-based investigational product that is derived from donor thymus tissue and substantially manipulated to optimize viability of the tissue upon surgical implantation into the recipient.[5] The CTT is composed of many processed tissue slices; each slice is implanted in the recipient's quadriceps muscle. The administration of CTT, previously called thymus transplantation, has been investigated at Duke University since 1993.[5–10]

The goal of this research has been to design a treatment that enables the development of naïve T cells and T cell function in order to enhance survival. This report includes specific data from all patients treated with CTT to date since the inception of this program at Duke University in 1993.

## METHODS

Patients were enrolled and received CTT in 1 of 10 Institutional Review Board (IRB)–approved protocols between 1993 and 2020 after the parent(s)/guardian(s) signed an IRB–approved informed consent document (Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Statistical analyses were performed by Covance Inc. (Princeton, NJ).

Congenital athymia was defined in the protocols as any of the following: cDGA in which patients had athymia plus either a congenital heart defect or hypocalcemia/hypoparathyroidism, or FOYN1 deficiency. cDGA included: 22q11.2ds, CHARGE syndrome, other genetic defects associated with congenital athymia, and diabetic embryopathy. Key eligibility criteria included cDGA or FOYN1 deficiency, T cells  $<50/\text{mm}^3$  or naïve T cells ( $\text{CD3}^+\text{CD4}^+\text{CD45RA}^+\text{CD62L}^+$  or  $\text{CD3}^+\text{CD8}^+\text{CD45RA}^+\text{CD62L}^+$ )  $<50/\text{mm}^3$ , and the absence of genetic defects associated with SCID. Patients enrolled under the expanded access protocol could have naïve T cell counts  $>50/\text{mm}^3$ . Exclusion criteria

included heart surgery within 4 weeks before administration of CTT or anticipated within 3 months after administration of CTT, poor surgical candidate as determined by the surgeon or anesthesiologist, HIV infection, prior attempts at immune reconstitution, ventilator dependence, and cytomegalovirus (CMV) infection for patients requiring immunosuppression.

For inclusion, patients had athymia with circulating CD3<sup>+</sup>CD45RA<sup>+</sup>CD62L<sup>+</sup> T cells <50/mm<sup>3</sup> or <5% of the total T cell count on 2 separate flow cytometry analyses, 1 performed within 3 months and 1 performed within 1 month before administration of CTT, unless they were enrolled in the expanded access protocol, where naïve T cells could be above 50mm<sup>3</sup>. Patients with typical and atypical phenotypes were included. Patients with the typical phenotype were defined as having absence of rash and lymphadenopathy, and patients with the atypical phenotype were defined by presence of rash and lymphadenopathy.

Immunosuppression was given to patients based on the proliferative response to PHA, regardless of typical or atypical phenotype. Depending on the PHA response, patients received either rabbit anti-thymocyte globulin (RATGAM) alone or RATGAM plus a calcineurin inhibitor and/or steroids (see this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) for supplementary material on immunosuppression). Patients were assessed for HLA antigens. Only patients who had previously received a HSCT with a mismatched allele required HLA matching of the thymus to that allele.

### Administration

CTT is a fresh tissue-based investigational product currently manufactured at the Marcus Center for Cellular Cures (Duke University School of Medicine, Durham, NC) and administered at Duke University Medical Center in an operating suite. After manufacturing, CTT slices are inserted into pockets made in the quadriceps muscle of one or both legs.[11] Dosing was based on mm<sup>2</sup> of CTT per recipient body surface area in m<sup>2</sup> (See this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) for supplementary material). After administration of CTT, patients continued to receive immunoglobulin G (IgG) replacement, prophylactic antimicrobials, and immunosuppressant therapy, as indicated until immune reconstitution (see this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) for supplementary material on immune monitoring and safety monitoring).

### Adverse events

Adverse events (AEs) were defined as unrelated, unlikely, possibly, probably, or definitely related to either protocol procedures or the CTT. Examples of AEs related to procedures were fever or seizure with the use of RATGAM; complications of surgery such as wound dehiscence, wound infection, and inflammation around stitches; and side effects from immunosuppression therapy.

After administration of CTT, the primary investigator oversaw the weaning of patients off calcineurin inhibitors, intravenous IgG, and prophylactic antimicrobials. Patients were weaned off the calcineurin inhibitor over 8 to 10 weeks once the naïve T cell percentage was over 10% of CD3 T cells.[9] Prophylaxis to treat *Pneumocystis jiroveci* pneumonia was discontinued when the patient was at least 9 months out from administration of CTT,

no longer taking immunosuppressive therapy, had a CD4 count  $>200/\text{mm}^3$ , and had a PHA response of  $>100,000$  cpm. IgG replacement therapy could be stopped when the patient was at least 9 months out from administration of CTT, was weaned off immunosuppressive therapy, and had trough IgG levels in the normal range while receiving IgG replacement therapy. Two months after discontinuation of IgG replacement therapy, the patient's IgG level had to be in the normal range or IgG replacement therapy was resumed.

### Statistical analysis plan

The primary end point of the study was the assessment of patient survival 1 year after administration of CTT. Data on patient survival at 2 years and survival of patients who lived at least 1 year after receiving CTT were analyzed as supporting data. Secondary end points included CD3, CD4, CD8, naïve CD3, naïve CD4, and naïve CD8 T cell counts at 1 year. Proliferative responses to PHA, concanavalin A (Con A), and tetanus toxoid were also assessed. Frequency of AEs and characterization by system organ class were analyzed.

## RESULTS

### Patient characteristics

One hundred five patients were enrolled in the study and are included in the full analysis set (FAS), and 95 are included in the efficacy analysis set (EAS). Ten patients were excluded from the EAS: 2 because their ultimate diagnosis was SCID, 6 because they received previous hematopoietic stem cell transplant or fetal transplant, and 2 because their diagnoses were not definitive. The flow chart of patient disposition at end of year 2 is shown in Fig 1.

The baseline demographics, disease characteristics, and syndromic comorbidities of the EAS and FAS populations are shown in Table I. Heart defects associated with patients' syndromic comorbidities are listed in Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). The mean patient age at the time of CTT administration was 298 days and 493 days for the EAS and FAS, respectively. Of the 95 patients with congenital athymia in the EAS, 93 had the protocol-defined diagnosis of cDGA and 2 had a diagnosis of FOXN1 deficiency; the FOXN1 cases have previously been described in detail.[12]

Fifty of 93 patients with cDGA had the typical phenotype. Of these patients, 15 received RATGAM and 35 did not. Of the 42 patients with the atypical cDGA phenotype, 39 received RATGAM and 2 patients treated early in the program did not. One patient received deoxycoformycin as immunosuppression therapy. One patient was not categorized as either typical or atypical because of preexisting GVHD from unirradiated blood transfusions prior to transfer to our center; this patient received cyclophosphamide and RATGAM.[8, 9] The median dose of CTT administered was  $12,996 \text{ mm}^2$  of tissue surface area/ $\text{m}^2$  recipient body surface area (range,  $4902\text{--}23,755 \text{ mm}^2/\text{m}^2$ ) and  $12,675 \text{ mm}^2$  of tissue surface area/ $\text{m}^2$  recipient body surface area (range,  $4523\text{--}23,755 \text{ mm}^2/\text{m}^2$ ) for the EAS and FAS, respectively (data not shown).

## Outcomes

The Kaplan-Meier estimated survival rates of the EAS at year 1 and year 2 after receiving CTT are 77% and 76%, respectively (Fig 2, Table E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The median follow-up time for the EAS was 7.6 years and ranged from 0 to 25.5 years after receiving CTT. The Kaplan-Meier estimated survival rates for the FAS is in Fig E1 (in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) and mirrors the EAS results. For patients who were alive 1 year after receiving CTT, the estimated survival rate at a median follow-up time of 10.9 years is 93% (Fig E2 and Table E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

## T cell development and function after CTT Administration

Median lymphocyte cell count results are shown in Fig 3. As seen in Fig 3, C and Fig 3, D, the median T cell counts reached their peak at approximately 1 to 2 years after CTT administration. Fig 3, C and Fig 3, D show the median CD3, CD4, CD8,  $\alpha\beta$ , and  $\gamma\delta$  T cell numbers through time. The CD3, CD4, and CD8 T cells increased but remained below the 10th percentile for age. The  $\gamma\delta$  cell counts remained low. The CD8 T cell counts remained low through year 2. Normal ranges and detailed ranges for the EAS and FAS populations of the various cell populations are found in Table E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

Fig 3, E and Fig 3, F show the naïve T cell numbers. Naïve T cells increased from day 0 to year 2. The patients with atypical cDGA phenotypes tended to have lower numbers of T cells immediately after receiving CTT and increased into year 2. The naïve T cell numbers start at 0 in all patients and increase, reaching the highest numbers in year 2. More detailed graphs showing the minimum, maximum, first quartile, third quartile, median and mean for naïve T cells are shown in Fig E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

T cell function was studied by proliferative responses to mitogens and antigens. Fig 4, A and Fig 4, B show that proliferative responses to the mitogens PHA and Con A reach the normal range by 6 months after administration of CTT. The proliferative response to the antigen tetanus toxoid develops in year 2, after immunizations begin (Fig 4, C).

## B and NK cells

B- and NK-cell numbers remained in the normal range in most patients (Fig 3, A and Fig 3, B). Almost all patients were able to stop IgG replacement therapy.

## Donor chimerism

Three of the patients who were alive 1 year after administration of CTT had evidence of thymus donor T cell engraftment. The donor chimerism observed in one patient who had >98% donor cells at approximately 80 days after receiving CTT gradually decreased over the first year, and by the end of the second year, all T cells were from the recipient. The second patient with chimerism initially had 89% donor T cells at approximately 50 days after CTT administration, which decreased to 12% donor T cells 6 months after receiving CTT, and 23% donor T cells approximately 32 months following administration of CTT. The

third patient had 75% donor T cells at 2 months after receiving CTT, but none at 10 months. No AEs related to the donor chimerism were identified.

### Adverse events and infections

A summary of the severity of all AEs is presented in Table E6 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). AEs were reported as general AEs or infection-related AEs. Of 105 patients, there were 32 with at least 1 severe AE, 35 with at least 1 life-threatening event, and 26 AE-related deaths (discussed below). In addition, there were 2 deaths that occurred after study withdrawal in patients with SCID and therefore were not considered AE-related. Fifty-three patients had infections categorized as severe, 13 as life threatening, and 11 as fatal. AEs considered possibly related to CTT are reported in Table II and all AEs with 10% frequency are reported in Table E7 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

All AEs possibly related to CTT, or its administration, including autoimmune manifestations and signs of autologous GVHD, are reported in Table II. Cytopenias were reported for 15 patients (14.3%); a total of 22 events were reported (see Table E8 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Notably, 17 of 22 events occurred in year 1, and all of these cases resolved. Of the 5 events reported in year 2, only 2 events in 1 patient are ongoing. Except for alopecia [4 patients (3.8%)], other AEs possibly reflecting symptoms of autoimmunity were reported for only 1 or 2 patients.

The rate of infection decreased over time based on a comparison of the infection rates in different time frames after CTT was administered. There was a significant decrease in the number of infections when comparing the infections occurring within the first 6 months after receiving CTT to the number of infections occurring 6 to 12 months after receiving CTT ( $P < .001$ ; Table III). Infections also decreased when comparing the infections occurring within the first 12 months after receiving CTT to infections in months 12 to 24 ( $P < .001$ ). For inclusion in these analyses, the patients had to be alive at the end of year 1 or year 2, respectively.

A total of 78 new viral infections were reported after CTT administration. Of these, 47 infections were reported in the first year after receiving CTT, 15 were reported in the second year after receiving CTT, and a total of 16 were reported in subsequent years. There were 4 reports each of adenoviral upper respiratory infection, adenoviral gastroenteritis, and cytomegalovirus infection; 3 reports of Epstein-Barr virus; and 1 report of adenoviral urinary tract infection. No cases of BK virus were reported. An important caveat is that we cannot be certain all patients received viral testing after leaving Duke University Medical Center.

### Deaths

Among 105 patients in the FAS group, there were 28 deaths (Table IV); 26 were considered related to AEs and 2 others were reported after patients were withdrawn from the study due to SCID diagnosis following administration of CTT. Twenty-two of the 28 deaths, including 12 of the 13 infection-related deaths, occurred in the first year after administration of CTT, while the patients were still immunodeficient.

Multiple organisms were involved in the infection-related deaths. Parainfluenza virus 3, *Candida lucitanea* and *Candida albicans*, disseminated *Candida* infection, methicillin-resistant *Staphylococcus aureus*, mycobacterial infection, and respiratory syncytial virus were each responsible for 1 death each; 2 patients died of CMV; and 5 died of septic shock or sepsis.

Respiratory failure was responsible for 10 deaths. Conditions leading to respiratory failure and death included anasarca, superior vena cava syndrome, bronchopulmonary dysplasia, and blocked tracheostomy tube. Four of the deaths related to respiratory failure were associated with concomitant infections such as mycobacteria, CMV, and parainfluenza virus 3.

There were 5 patients for whom bleeding was a major concern and contributed to their deaths. Four of the patients had multiple recurrent bleeding events; of these, 1 patient had a cerebral bleeding event presumed to be related to an infection. The fifth patient in this category died on the day that CTT administration was planned, but prior to receiving CTT, secondary to hemorrhage at the site of a calcium accretion in the inferior vena cava extending into the left hepatic vein.

## DISCUSSION

CTT has been used to treat 105 pediatric patients over the past 27 years. Ninety-five of these patients were athymic, had no prior therapy, and are the focus of our reported efficacy. This group of patients had excellent survival results. Most patients in this study of CTT survived. For those who died, most deaths occurred in the first year after receiving CTT, before establishment of T cell immunity. In addition, most deaths were secondary to preexisting syndromic comorbidities, such as heart defects, or secondary to infections acquired before or shortly after receiving CTT. Immune reconstitution sufficient to support survival and prevent infections typically develops 6 to 12 months after administration of CTT.

The Kaplan-Meier survival curve for the FAS population is very similar to that of the EAS population and shows most deaths occurred in the first year. Four patients (not diagnosed with SCID and without lymphoma at the time of CTT administration) had previous HSCTs, including cord blood and peripheral blood infusions that did not result in T cell development sufficient to protect the child. All 4 patients subsequently developed naïve T cells after administration of CTT. Three of the 4 did not develop GVHD. The fourth, who was greater than 10 years old at the time of CTT administration, developed GVHD from the prior poorly matched female cord blood cells that had been given when the patient was an infant. The GVHD resolved in the second year after receiving CTT. These positive outcomes in 4 patients treated with prior HSCTs suggest that administration of CTT may be efficacious after an unsuccessful HSCT in athymic patients. However, undergoing a prior HSCT makes it more difficult to receive CTT because it requires matching of the donated thymus tissue to any alleles in the recipient that were not fully matched by the donor stem cells in the previous HSCT.



The Kaplan-Meier survival curve of the EAS analysis set for those patients who survived past 1 year of age shows only 1 death related to lack of naïve T cell development after the first year following CTT administration. The 3 deaths occurring after the second year following CTT administration were not related to immunodeficiency. These results imply that patients without life-threatening syndromic comorbidities who survive past 2 years will likely survive well into adulthood. Five patients in our cohort are greater than 18 years old and the oldest is over 25 years old. We look forward to following up with these patients over the next decades.

We have demonstrated that CTT can act similarly to normal thymus tissue to produce naïve T cells,  $CD3^+CD4^+CD45RA^+CD62L^+$  and  $CD3^+CD8^+CD45RA^+CD62L^+$ , that can then migrate into the periphery and fight infection as  $CD3^+CD4^+$  or  $CD3^+CD8^+$  T cells. We previously reported the results of immunohistochemistry staining of CTT biopsies 2 – 3 months after implantation. Among 30 biopsies examined, CTT was found in 25, and all but 2 showed thymopoiesis.[13]

Thymopoiesis in the CTT results in the appearance of naïve T cells in peripheral blood, typically between 6 and 12 months after receiving CTT. Our data demonstrate that lymphocyte counts are very similar after receiving CTT in the groups with typical and atypical cDGA phenotypes. The naïve CD4 counts are also quite similar in the groups with typical and atypical cDGA phenotypes, as are the naïve CD8 counts.  $CD3^+$  T cell counts increase over time, but the median  $CD3^+$  T cell counts remain below the 10th percentile for age. The same is true for the  $CD4^+$  T cells. It is noteworthy that T cell counts under the 10th percentile for age usually result in protection from infection in patients with cDGA, similar to the protection from infection afforded to children, such as those with less severe immunodeficiencies, who have T cell counts in the lowest 10% for age. Because of the low T cell numbers, the lymphocyte count is also low for age. Of note, the CD8 count is much lower relative to the 10th percentile for age compared with the CD4 count. Anecdotally, despite the low CD8 counts, live vaccines (measles, mumps, and rubella and varicella zoster virus) have been well tolerated by our patients.

We have previously studied the appearance of T cells in the blood before and after receiving CTT.[8, 14–16] After CTT administration, T cells emigrating from the CTT develop a broad diversity of TCR-V $\beta$  genes distributed over 20 families.[8, 15, 16] In 2 patients with the atypical cDGA phenotype that we studied in depth, T-regulatory cells were not evenly distributed across the 24 TCR-V $\beta$  families before administration of CTT, and some TCR-V $\beta$  families had no T-regulatory cells. Twelve months following administration of CTT, these 2 patients' T-regulatory cells had expanded to all of the TCR-V $\beta$  families with similar proportions as observed for CD4 T cells.[14] This distribution of T-regulatory cells after receiving CTT may explain how the rash and autoimmune cytopenias eventually resolve and allow for the discontinuation of immunosuppression in patients with the atypical cDGA phenotype.

T cell function was assessed using proliferative responses to the mitogens PHA, Con A and the antigen tetanus toxoid. Remarkably, the responses of PHA and Con A were normal by 6 months. In healthy children, 3 doses of a tetanus toxoid-containing vaccine are given

at 2, 4, and 6 months of life. An additional dose is given at 15 to 18 months to ensure a good response because initial doses are often not enough to maintain an adequate response. The same number of vaccinations were given to patients with congenital athymia although the vaccines started 1 year after CTT administration. After vaccination, the proliferative responses to tetanus toxoid were similar to those of healthy children.

We have published studies of B-cell function. Immunoglobulin levels are mostly normal for age.[8, 9] Specific antibodies form to tetanus toxoid, but only in low levels to pneumococcal serotypes.[8, 9]

The data presented herein provide support for the development of immunity in a patient's first year after receiving CTT. Infections at 6 to 12 months after CTT administration were decreased compared to the infections after 0 to 6 months. Consistent with this finding, after developing naïve T cells, patients have demonstrated the ability to overcome serious infections such as mycobacteria, parainfluenza virus 3, adenovirus, *Candida albicans*, varicella zoster virus, and CMV. Children with congenital athymia have immune dysregulation that leads to autoimmune phenomena such as autologous GVHD and cytopenias. Autologous GVHD with rash and lymphadenopathy usually decreases after CTT administration, likely secondary to treatment of these patients with RATGAM and calcineurin inhibitors, and does not reappear once immunosuppression is weaned, likely secondary to the reconstituted immune system. Cytopenias often develop in the first year after receiving CTT as naïve T cells begin to develop. However, as naïve T cell numbers increase toward the end of the first year after CTT administration, cytopenias resolve and the risk of new-onset cytopenias decreases.[6–10, 12, 13, 17]

AEs and infections were recorded whether or not they were related to implantation procedures or underlying syndromic comorbidities. These data show the full spectrum of challenges associated with congenital athymia.

The use of CTT administration in congenital athymia uniquely differs from the allogeneic transplants used to treat other primary immunodeficiencies in that HLA matching is not required. With mismatched hematopoietic transplants, mature donor T cells attack the mismatched allele in the recipient. In contrast, patients with congenital athymia do not have mature T cells. After receiving CTT, the recipient's bone marrow stem cells go to the CTT to develop into recipient T cells. The recipient dendritic cells delete any developing thymocytes that bind too tightly to recipient dendritic cells (self) or donor thymic epithelium in the CTT. No T cells leave the thymus that will attack the recipient or the CTT. We have previously published the lack of effect of HLA matching on outcomes post CTT administration.[17] All patients are assessed for HLA antibodies. The only patients that require HLA matching are those who have had a prior transplant in which the donor was not fully matched to the recipient. In these exceptional cases, the allele in the bone marrow donor that is not in the recipient must be present in the donated thymus tissue.

In summary, the development of CTT as an investigational therapy for children with congenital athymia has been lifesaving. Historically, children with congenital athymia who did not receive therapy died in the first few years of life. Based on these data, families now

have hope that their children, with a reconstituted immune system, will be able to live a full life.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- R01-AI54843, United States
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- R01-AI47040, United States
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- The Hartwell Foundation, United States
- Orphavant Sciences GmbH (later Enzyvant Therapeutics GmbH, ETG), Switzerland

Disclosure statement:

Cultured thymus tissue (CTT) is an investigational product implanted into patients under an Investigational New Drug (IND) application with the US Food and Drug Administration. M. L. Markert is the “sponsor” of the investigations. M. L. Markert developed the technology for CTT. Duke University has licensed the technology to Enzyvant Therapeutics GmbH. M. L. Markert and Duke University have received royalties from Enzyvant. Portions of M. L. Markert’s and her research team’s salaries are being paid by funding from Enzyvant. If the technology is commercially successful in the future, M. L. Markert and Duke University may benefit financially. The salary and other items needed to create CTT are paid at cost by insurance. Enzyvant Therapeutics, Inc. reviewed the manuscript for scientific accuracy and to ensure protection of their intellectual property. Editorial assistance, funded by Enzyvant Therapeutics, Inc., was provided by Lisa M Pitchford, PhD, of JB Ashtin.

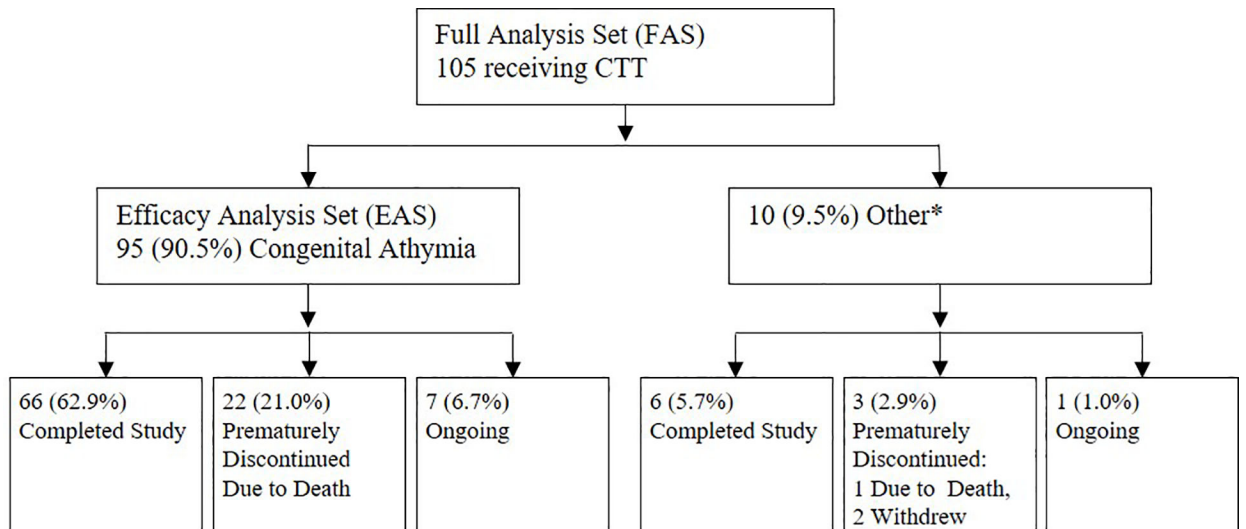
## Abbreviations used

<b>AE</b>	Adverse event
<b>22q11.2ds</b>	22q11.2 deletion syndrome
<b>cDGA</b>	Complete DiGeorge anomaly
<b>CHARGE</b>	Coloboma, heart defects, choanal atresia, growth or mental retardation, genital hypoplasia, and ear anomalies and/or deafness
<b>Con A</b>	Concanavalin A
<b>CTT</b>	Cultured thymus tissue
<b>CMV</b>	Cytomegalovirus
<b>EAS</b>	Efficacy analysis set
<b>FAS</b>	Full analysis set
<b>GVHD</b>	Graft versus host disease
<b>HSCT</b>	Hematopoietic stem cell transplant
<b>IgG</b>	Immunoglobulin G
<b>IRB</b>	Institutional Review Board
<b>PHA</b>	Phytohemagglutinin
<b>RATGAM</b>	Rabbit antithymocyte globulin

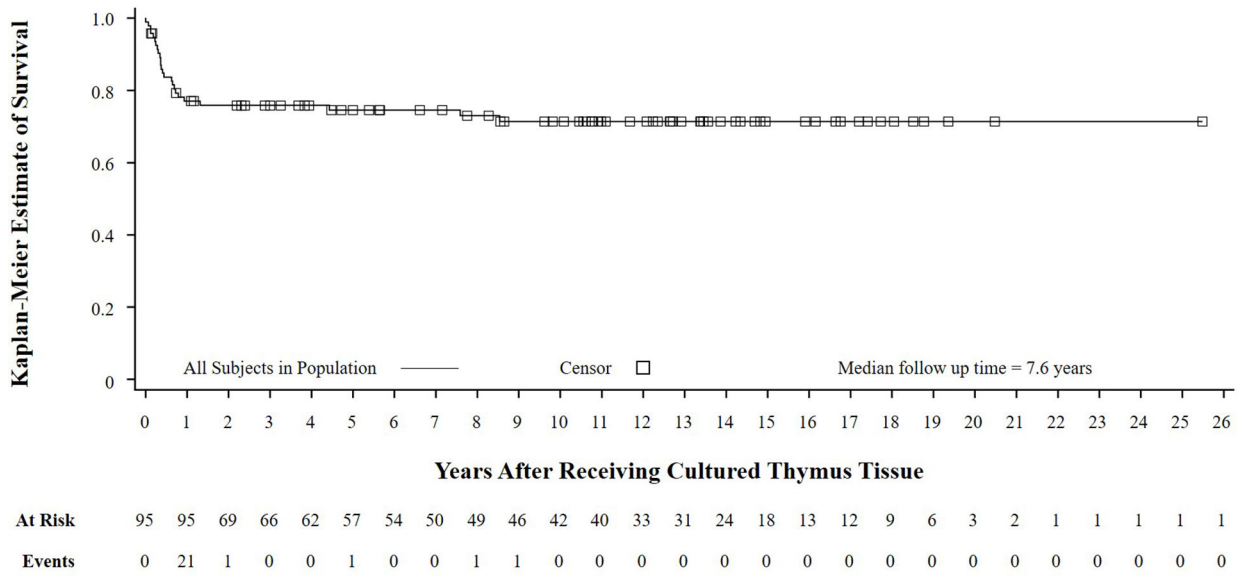
**SCID**                      Severe combined immunodeficiency

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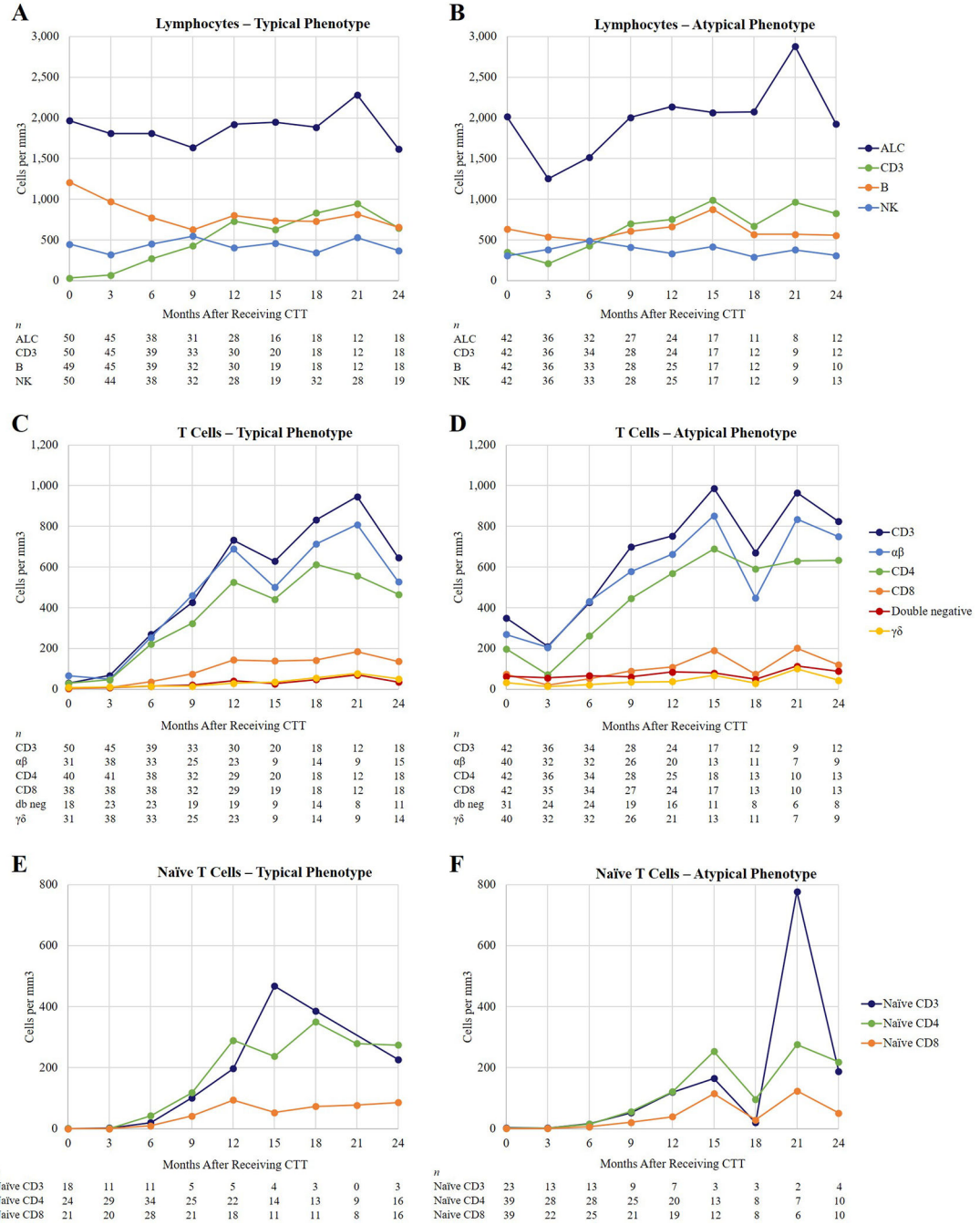
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**FIG 1.**

Flow chart of patient disposition at the end of year 2. \*"Other" includes 2 patients with SCID, 6 patients with previous hematopoietic or fetal thymic transplants, and 2 patients in which diagnosis was not definitive. *CTT*, cultured thymus tissue; *SCID*, severe combined immunodeficiency.



**FIG 2.** Kaplan Meier survival curve of EAS. This analysis shows that of the 95 patients, almost all deaths occurred in the first year. *EAS*, efficacy analysis set.



**FIG 3.** Flow cytometry. **A**, Median numbers of lymphocytes in patients with typical phenotype. **B**, Median numbers of lymphocytes in patients with atypical phenotype. **C**, T cell subsets in patients with typical phenotype. **D**, T cell subsets in patients with atypical phenotype. **E**, Naïve T cells in patients with typical phenotype. Note, there were no patient samples for month 21. **F**, naïve T cells in patients with atypical phenotype. Normal values for age and interquartile data are in Table E5,A and Table E5,B in this article’s Online Repository at



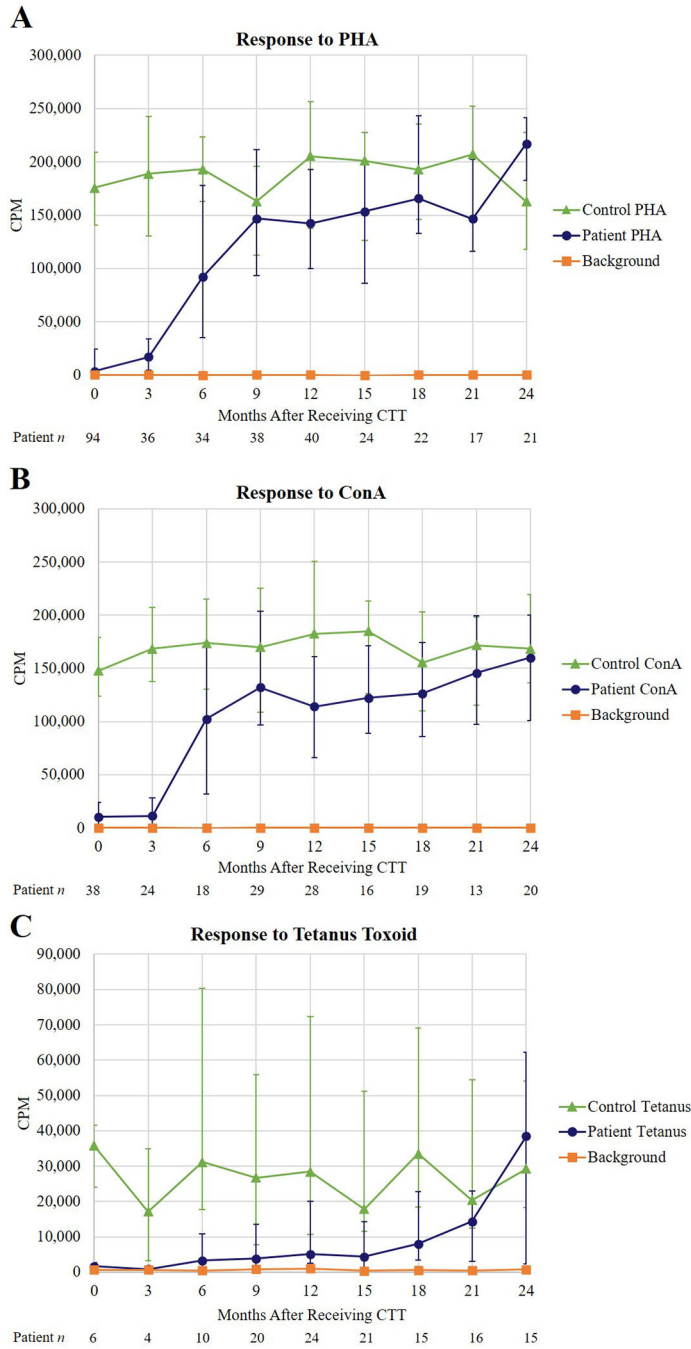
[www.jacionline.org](http://www.jacionline.org). *ALC*, absolute lymphocyte count; *CTT*, cultured thymus tissue; db neg, double negative; *NK*, natural killer.

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**FIG 4.** T cell proliferative responses to mitogens and antigens. Responses to **A**, PHA; **B**, Con A; and **C**, tetanus toxoid. The normal response to PHA is 85,000 to 286,636 cpm, to Con A is 75,000 to 224,079 cpm, and to tetanus toxoid is 11,273 to 106,085 cpm. *Con A*, concanavalin A; *cpm*, counts per minute; *PHA*, phytohemagglutinin.

**Table I.**

## Baseline characteristics and disease history

Characteristic	Analysis Population	
	EAS (N = 95)	FAS (N =105)
Age on day of transplant (days), mean (SD)	298 (214)	493 (924)
Male, n (%)	56 (58.9)	60 (57.1)
Race, n (%)		
White	66 (69.5)	76 (72.4)
Black/African American	21 (22.1)	21 (20.0)
Asian	3 (3.2)	3 (2.9)
American Indian/Alaska Native	2 (2.1)	2 (1.9)
Native Hawaiian/Other Pacific Islander	1 (1.1)	1 (1.0)
More than 1 Race	2 (2.1)	2 (1.9)
Ethnicity, n (%)		
Hispanic or Latino	18 (18.9)	20 (19.0)
Not Hispanic or Latino	77 (81.1)	85 (81.0)
Congenital athymia—gene mutations, n (%)		
<i>CHD7</i> mutation	12 (12.6)	12 (11.4)
22q11.2 deletion syndrome	36 (37.9)	38 (36.2)
<i>FOXN1</i> mutation	2 (2.1)	3 (2.9)
<i>TBX1</i> point mutation	0	1 (1.0)
<i>TBX2</i> point mutation	1 (1.1)	1 (1.0)
No genetic mutation identified <sup>*</sup>	37 (38.9)	41 (39.0)
Missing <sup>†</sup>	7 (7.4)	9 (8.6)
Age at diagnosis (days), mean (SD)	48 (79)	82 (173)
Diagnosis, n (%)		
SCID	0	2 (1.9)
FoxN1	2 (2.1)	3 (2.9)
Partial DiGeorge	0	1 (1.0)
Complete DiGeorge <sup>‡</sup>	93 (97.9)	97 (92.4)
Unknown	0	2 (1.9)
DiGeorge anomaly phenotype, n (%)		
Typical DiGeorge anomaly	50 (52.6)	52 (49.5)
Atypical DiGeorge anomaly	42 (44.2)	44 (41.9)
Unknown <sup>§</sup>	1 (1.1)	1 (1.0)
Fetal toxin exposure, n (%)		
Maternal diabetes	26 (27.4)	29 (27.6)
Syndromic comorbidities, n (%)		
Congenital cardiac anomaly or cardiothoracic vascular anomaly	85 (89.5)	91 (86.7)
Hypocalcemia	81 (85.3)	89 (84.8)
Diminished naïve T cell counts for age	95 (100.0)	105 (100.0)

Characteristic	Analysis Population	
	EAS (N = 95)	FAS (N =105)
Dysmorphic facies	44 (46.3)	44 (41.9)
Deafness or ear pinnae anomalies	49 (51.6)	53 (50.5)
Coloboma	18 (18.9)	18 (17.1)
Cleft lip	12 (12.6)	12 (11.4)
Cleft palate (frank clefting or submucous cleft)	17 (17.9)	17 (16.2)
Velopharyngeal insufficiency/hypernasal speech	3 (3.2)	4 (3.8)
Choanal atresia	10 (10.5)	10 (9.5)
Tracheal anomalies	25 (26.3)	25 (23.8)
Esophageal anomalies	10 (10.5)	10 (9.5)
Anal and/or rectal anomalies	6 (6.3)	6 (5.7)
Renal anomalies	26 (27.4)	27 (25.7)
Genital hypoplasia	11 (11.6)	13 (12.4)
Rib or vertebral anomalies	34 (35.8)	35 (33.3)
Limb anomalies	16 (16.8)	17 (16.2)
Growth or mental retardation	49 (51.6)	54 (51.4)
Other <sup>//</sup>	7 (7.4)	8 (7.6)

\* Includes 11 patients with CHARGE (coloboma, heart defects, atresia choanae, growth retardation, genital hypoplasia and ear abnormalities and/or deafness) who had no identified gene mutation.

<sup>†</sup>No data available for the patient.

<sup>‡</sup>Protocol-defined complete DiGeorge diagnosis included: complete DiGeorge anomaly in which patients had athymia plus either a congenital heart defect or hypocalcemia/hypoparathyroidism, 22q11.2 deletion syndrome, CHARGE syndrome, and infants of diabetic mothers.

<sup>§</sup>Patient presented with GVHD after 3 unirradiated blood transfusions, at least one of which was likely cytomegalovirus seropositive.

<sup>//</sup>Other conditions are swallowing difficulty; tube dependence; reflux and developmental delay; feeding intolerance; significant reflux; pyriform aperture stenosis central incisor; and CHARGE syndrome suspected based on phenotypic features of low-set ears, thin upper lip, micrognathia, significant hearing loss, micrognathia, and external ear abnormalities.

**Table II.**

Adverse events possibly related to CTT administration by system organ class and preferred term

	<b>FAS (N = 105)</b>	
	<b>n (%)</b>	<b>E</b>
<b>Number of Related Adverse Events</b>	<b>44 (41.9)</b>	<b>87</b>
<b>Blood and lymphatic system disorders</b>	<b>21 (20.0)</b>	<b>32</b>
Thrombocytopenia	11 (10.5)	13
Neutropenia	8 (7.6)	8
Coombs positive haemolytic anaemia	3 (2.9)	3
Autoimmune haemolytic anaemia	2 (1.9)	3
Haemolysis	2 (1.9)	2
Haemolytic anaemia	2 (1.9)	2
Immune thrombocytopenic purpura	1 (1.0)	1
<b>Skin and subcutaneous tissue disorders</b>	<b>9 (8.6)</b>	<b>10</b>
Alopecia	4 (3.8)	4
Rash	2 (1.9)	2
Granuloma skin	1 (1.0)	1
Psoriasis	1 (1.0)	1
Skin mass	1 (1.0)	1
Stephens-Johnson syndrome	1 (1.0)	1
<b>General disorders and administration site conditions</b>	<b>6 (5.7)</b>	<b>6</b>
Pyrexia	5 (4.8)	5
Oedema peripheral	1 (1.0)	1
<b>Renal and urinary disorders</b>	<b>5 (4.8)</b>	<b>6</b>
Proteinuria	5 (4.8)	5
Glomerulonephritis minimal lesion	1 (1.0)	1
<b>Gastrointestinal disorders</b>	<b>5 (4.8)</b>	<b>5</b>
Diarrhoea	3 (2.9)	3
Enteritis	1 (1.0)	1
Ileus	1 (1.0)	1
<b>Injury, poisoning and procedural complications</b>	<b>5 (4.8)</b>	<b>5</b>
Wound dehiscence	4 (3.8)	4
Graft haemorrhage	1 (1.0)	1
<b>Investigations</b>	<b>4 (3.8)</b>	<b>6</b>
Blood bicarbonate decreased	2 (1.9)	2
Lymphocyte morphology abnormal	1 (1.0)	2
Blood immunoglobulin E increased	1 (1.0)	1
Lymphocyte count abnormal	1 (1.0)	1
<b>Infections and infestations</b>	<b>4 (3.8)</b>	<b>4</b>
Cytomegalovirus infection	1 (1.0)	1
Staphylococcal skin infection	1 (1.0)	1
Stitch abscess	1 (1.0)	1

	FAS (N = 105)	
	n (%)	E
Wound infection staphylococcal	1 (1.0)	1
<b>Immune system disorders</b>	<b>2 (1.9)</b>	<b>3</b>
Graft versus Host Disease	1 (1.0)	1
Graft versus Host Disease in gastrointestinal tract	1 (1.0)	1
Graft versus Host Disease in skin	1 (1.0)	1
<b>Hepatobiliary disorders</b>	<b>2 (1.9)</b>	<b>2</b>
Autoimmune hepatitis	2 (1.9)	2
<b>Musculoskeletal and connective tissue disorders</b>	<b>2 (1.9)</b>	<b>2</b>
Juvenile idiopathic arthritis	1 (1.0)	1
Psoriatic arthropathy	1 (1.0)	1
<b>Endocrine disorders</b>	<b>1 (1.0)</b>	<b>2</b>
Basedow's disease	1 (1.0)	1
Hyperthyroidism	1 (1.0)	1
<b>Congenital, familial and genetic disorders</b>	<b>1 (1.0)</b>	<b>1</b>
Albinism	1 (1.0)	1
<b>Nervous system disorders</b>	<b>1 (1.0)</b>	<b>1</b>
Myelitis transverse	1 (1.0)	1
<b>Reproductive system and breast disorders</b>	<b>1 (1.0)</b>	<b>1</b>
Ovarian failure	1 (1.0)	1
<b>Vascular disorders</b>	<b>1 (1.0)</b>	<b>1</b>
Haematoma	1 (1.0)	1

Note: If a patient had multiple occurrences of an AE, the patient was included only once in the n 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 CTT or study procedures or with an unknown relationship based on investigator review.

AE, adverse event; E, number of events; FAS, full analysis set; MedDRA, Medical Dictionary for Regulatory Activities; PT, preferred term; SOC, system organ class

**Table III.**

Decreased infections 6 to 12 months compared with 0 to 6 months; 12 to 24 months compared with less than 12 months for FAS

	1-Year Analysis		2-Year Analysis	
	Onset Within 6 Months	Onset 6 to 12 Months	Onset Within 12 Months	Onset 12 to 24 Months
No. of patients analyzed*	N = 72		N = 69	
No. of patients with event during the interval	67	38	68	35
Mean (SD) No. of events per patient	3.5 (2.6)	2.3 (2.4)	4.4 (3.6)	2.7 (3.0)
Median (IQR <sup>†</sup> ) No. of events per patient	3.0 (1.0, 5.0)	1.5 (1.0, 3.0)	3.0 (2.0, 6.0)	2.0 (1.0, 3.0)
<i>P</i> value <sup>‡</sup>	< .001		< .001	

To be included in the 1-year analysis, patients must have been alive and on follow-up for at least 365 days after administration of cultured thymus tissue, and experienced at least one infection-related AE during one or both onset periods.

To be included in the 2-year analysis, patients must have been alive and on follow-up for at least 790 days after administration of cultured thymus tissue, and experienced at least one infection-related AE during one or both onset periods.

\* Number of patients who had events during the analysis period.

<sup>†</sup> *IQR*, interquartile range.

<sup>‡</sup> 2-sided *P* value based on a Wilcoxon signed-rank test of the mean difference in the number of infection-related AEs per patient between the first and second intervals.

**Table IV.**

Causes of death.

Day of life	Days after CTT	Causes of Death (N = 28)*
<b>Related to Infection (n = 13)</b>		
471	24	Progression of underlying PIV3 pneumonia
398	44	Sepsis
586	89	Death secondary to disseminated Candida infection
247	103	Progressive cerebral atrophy due to CMV
181	130	Sepsis
199	130	Sepsis
350	149	Respiratory failure from disseminated mycobacterial infection
264	160	Respiratory failure due to respiratory syncytial virus
416	234	Respiratory failure secondary to sepsis from Candida tropicalis and C parapsilosis
382	252	Respiratory complications from CMV infection
804	263	Multiorgan system failure as seen in severe septic shock
856	339	Methicillin resistant Staphylococcus aureus bacteremia
824	375	Presumed sepsis
<b>All Except for Infection (n = 15)</b>		
107	0	Hemorrhage with Nissen fundoplication surgery
172	45	Respiratory failure
162	66	Severe bilateral intraventricular hemorrhage
268	82	Hypoxia
537	108	Cardiorespiratory arrest related to anasarca
5879	116	Progression of EBV lymphoma resulting in intracranial bleeding and death
510	128	Large right parietal hemorrhage
256	137	Respiratory failure
667	229	Respiratory failure
392	289	Respiratory failure
982	480	Sudden catastrophic intracranial hemorrhage
1622	950	Not reported
1713	1617	Cardiopulmonary arrest
2918	2769	Respiratory failure
3311	3116	Respiratory failure

CMV, cytomegalovirus; EBV, Epstein-Barr virus; PIV3, parainfluenza virus type 3

\* Includes 2 deaths in patients who withdrew from the study prior to having a reportable adverse event.