CELL & GENE THERAPY INSIGHTS

RARE DISEASES – CLINICAL & COMMERCIAL DEVELOPMENTS

SPOTLIGHT

EXPERT INSIGHT

# Gene therapy for childhood cerebral adrenoleukodystrophy

Christine Duncan

Cellular therapy with allogeneic hematopoietic cell transplantation can stabilize disease and prevent progression in boys with early stage cerebral adrenoleukodystrophy. Gene therapy using modified autologous hematopoietic stem cells is emerging as a potential therapy and possible replacement for allogeneic transplant for children with cerebral adrenoleukodystrophy (ALD). This article examines the role of cellular therapy, allogeneic transplant and autologous gene therapy in boys with ALD. It highlights the published results of two investigations of gene therapy in this setting that show efficacy comparable to allogeneic transplant. Longer term follow-up is needed to fully understand the impact that this new treatment will have on the clinical care of boys with cerebral ALD.

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## INTRODUCTION

X-linked adrenoleukodystrophy (ALD) is a peroxisomal disorder caused by mutations in the *ABCD1* gene resulting in altered expression of the peroxisomal half-transporter ALD protein [1]. The ALD protein is needed to transport very long chain fatty acids (VLCFAs) into the peroxisome for degradation. Mutations in the *ABCD1* gene impair VLCFA transport into the peroxisome leading to their accumulation in multiple tissues, most notably the nervous system and adrenal glands [2,3]. Accumulation of VLCFAs in the adrenal glands can lead to cellular dysfunction by in part inhibiting the effect of ACTH on the adrenocortical cells [4,5]. The pathology in the central nervous system is complex and characterized by progressive cerebral and cerebellar inflammatory demyelination [6–8].

ALD affects approximately 1 in 21,000 males [9]. There is a range of clinical presentations including asymptomatic patients, adrenal insufficiency without nervous system manifestations, adult onset adrenomyeloneuropathy and cerebral ALD (cALD). There is no correlation between specific mutations and

clinical phenotype and individuals in the same family can have different presentations.

cALD is the inflammatory cerebral phenotype of the disease and affects 35–65% of males with ALD [8,10,11]. It presents most often in childhood and cALD may present with mild symptoms such as attention deficits, poor academic performance and behavioral change. Untreated the symptoms progress to moderate and functional disabilities including hearing loss, visual impairment/blindness, difficulty swallowing, need for enteral tube feeding, gait change with eventual wheelchair dependence, loss of continence, seizures and the inability to communicate [12,13]. Most untreated children with cALD die within 10 years of the onset of clinical symptoms [14]. Cellular therapy, traditionally hematopoietic cell transplantation (HCT), is the mainstay of therapy for boys with early stage cALD.

Gene therapy using modified autologous hematopoietic stem cells has emerged as a promising therapy for children with cALD. Gene therapy involves the insertion of a gene that is free of the defects of the disease being treated into the cellular DNA of the recipient using modified autologous stem cells. It is an attractive potential alternative to allogeneic transplant for those with cALD for multiple reasons. First, the risk for graft-versus-host disease (GVHD) and the need for related immune suppressing medications is eliminated likely decreasing the risk of transplant related mortality. Secondly, gene therapy eliminates the need to find an allogeneic stem cell donor, which may decrease the time from cALD diagnosis to the start of treatment. This is of vital importance as cALD can progress for months after successful cellular therapy [14,15]. Clinical manifestations present at the start of treatment are not expected to reverse and may worsen during the initial months following therapy [16]. This makes it important that candidates for treatment are identified early in the course of disease and begin cellular therapy as quickly as possible.

### Mechanism of effect

The precise mechanism by which cellular therapy halts progression of the inflammatory demyelination and clinical symptomology in patients with cerebral ALD is not fully elucidated. It could be hypothesized that the conditioning regimen plays a key role in arresting disease progression as it may alter the CNS inflammatory environment. However, patients who have primary failure to engraft following conditioning and stem cell infusion have continued neurologic deterioration indicating that conditioning chemotherapy alone is insufficient to control disease [10,17]. A hypothesized and debated potential mechanism of effect involves cells of myeloid origin crossing the blood–brain barrier and differentiating into donor-derived, long lasting macrophages and/or facilitating the correction of the abnormal functioning of the microglia [10,15,18–20].

## ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

The first report of allogeneic HCT for a child with cerebral ALD was in 1984 and was unsuccessful [21]. The recipient had rapidly progressive disease and received stem cells


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from an HLA-matched sibling donor. The child's disease continued to progress despite successful engraftment and he died of adenoviral infection 114 days following transplant. In the decades that followed, allogeneic HCT has become the standard of care for select boys with cALD in the USA and around the world [22]. This was due in part to advances in transplantation science and safety and to a better understanding of the determinants of outcome of HCT for cALD.

Allogeneic transplantation can arrest the progression of cALD when performed early in the disease process and is ineffective for those with advanced disease. Two tools used to determine the suitability of child with cALD for HCT are the MRI Loes score and the Neurologic Function Scale [23–26]. The Loes score is a radiographic tool that assigns a numeric score to a brain MRI based on the degree and extent of white matter changes and the presence of gadolinium contrast enhancement [25,27]. Scores range from 0 to 34 with higher scores indicating a greater extent of lesions and worse disease.

The Neurologic Function Scale is an ALD-specific functional tool used to evaluate the severity of gross neurologic dysfunction across multiple domains [26,27]. A point value is assigned to each of 15 different disabilities. A total score of 0 indicates the absence of clinical signs of cerebral disease and a higher total score indicates more severe deficits. Multiple studies have shown that lower Loes and NFS scores, indicating lower stage disease, are associated with superior survival and disease control following allogeneic HCT compared to those with higher scores [16,28–30]. Allogeneic transplantation using a well-matched donor and performed for early disease as assessed with Loes Score and NFS can have survival as great as 90% at 5 years, with lower survival rates reported when less well matched donors are used [14,28,31,32].

Despite the success of allogeneic transplant there remains an unmet need for alternative therapies. Allogeneic transplantation is associated with significant morbidity, particularly related to GVHD and the medications and complications associated with it. Additionally, there are patients for whom an acceptable donor cannot be identified in a timely fashion. Autologous gene therapy addresses these needs by eliminating the requirement to find an unrelated stem cell donor and the risk of GVHD.

## PRINCIPLES OF GENE THERAPY

The current process of gene therapy for cALD involves the introduction of an exogeneous *ABCD1* gene into the recipient's genome. The functional ABCD1 gene is introduced into the chromosomal DNA of the recipient's hematopoietic stem cells (HSCs), where it is incorporated and replicated with subsequent cell division. The exogenous gene is introduced to the host cell in a process called transduction using a viral vector. Transduction occurs either *in vivo* with the gene being directly introduced into the target tissue or *in vitro*. *In vitro* transduction followed by direct administration into the patient is the current standard in gene therapy for cALD. HSCs are collected from the patient, the exogeneous ABCD1 is transduced

into the HSCs using a viral vector, the recipient receives chemotherapy conditioning, and the genetically modified cells are infused into the patient intravenously. Conditioning therapy with chemotherapy is used to provide an engraftment advantage to the modified stem cells. The clinical trials highlighted in this work built on preclinical *in vitro* and murine models [15,33–36]. Future investigation may use other techiniques of gene modification including gene editing or tissue directed therapy.

## Vector systems

Viral vectors are used to deliver the gene therapy construct into the target cell. Multiple factors are considered when choosing the vector to use for an application including the ability to adequately carry the gene of interest (capacity) and to target the correct cell, the desired protein expression level, and goal duration of expression. Lentiviral vectors derived from HIV have been used for gene therapy in children with cALD. Lentiviral vectors are categorized as integrating, meaning that they insert into the host genomic DNA and are replicated with cell division. These are important features in the treatment of cALD as stable, long-term protein expression is essential to the success of treatment [37]. Additional advantages of lentiviral vectors are that they typically have high transduction rates and result in high levels of transgene expression, can infect non-dividing cells, and are not expected to elicit immunogenic responses in the host [10,38,39].

One of the greatest potential risks of integrating vectors is insertional mutagenesis, the development of mutations in the recipient DNA related to the chromosomal positioning of the genetic insertion, which can lead to abnormal cell phenotypes and malignant transformation [37,40]. This occurred in a study of gene therapy using a retroviral vector in children with severe combined immune deficiency. Five subjects in the study developed leukemia due to insertional mutagenesis attributed to interaction between the enhancer element of the viral vector and a cellular promoter of the host leading to the expression of an endogenous oncogene [37,40,41]. Since that time multiple strategies have been employed to reduce the risk of insertional mutagenesis including the development of self-inactivating (SIN) viral vectors which reduce the potential for interaction between the vector and host genome by disabling or eliminating the long terminal repeats of the viral enhancer sequences [41].

## Results of cALD gene therapy studies

There are currently no US Food and Drug Administration (FDA) or European Medicines Agency (EMA) approved gene therapy products for cALD and gene therapy is performed in the investigational setting only. Data has been published from two trials of hematopoietic stem cell gene therapy in boys with cALD to date [33,42]. The first trial was performed in France and reported in 2009 [33]. Two boys with cALD underwent granulocyte stimulating factor (G-CSF) stimulated mobilization and apheresis of peripheral blood mononuclear cells. CD34+ cells were selected by an immunomagnetic procedure, pre-activated with cytokines *ex vivo*, infected with a lentiviral vector expressing wild-type *ABCD1* cDNA, and cryopreserved. The vector was


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replication-defective HIV derived and SIN [10]. The products were thawed after quality and safety testing and infused intravenously following completion of myeloablative conditioning with busulfan and cyclophosphamide. The authors reported that the myeloablative regimen was used to remove resident non-transduced hematopoietic stem cells to increase the likelihood of engraftment of the transduced hematopoietic stem cells.

The mean vector copy number, the number of integrated provirus copies per cultured CD34+ cell, 5 days after transduction, was 0.7 and 0.6. The mean integrated vector copy number was 0.14 copies per cell at 30 months and 0.20 copies at 24 months in patients 1 and 2, respectively. At infusion 50% (patient 1) and 33% (patient 2) of the transduced cells expressed the ALD protein. The ALD protein expression at 30 days after infusion, 9 months and 24 months from infusion was 23, 13 and 10% in patient 1 and 25, 17 and 15% in patient 2.

With follow-up at 36 months the progressive demyelination observed by MRI stopped in both patients, gadolinium contrast enhancement resolved, and neurologic symptoms stabilized [10]. Both patients were reported as having normal neurologic examination at 36 months [10]. There was no evidence of insertional mutagenesis or clonal skewing in the hematopoietic stem cell population [33].

A multicenter study using an elivaldogene tavalentivec (Lenti-D) lentiviral vector drug product in the USA and Europe began enrolling patients in 2013 with interim results published in 2017 [42]. The primary aim of the study was to assess the safety and efficacy of gene therapy with the Lenti-D product in boys with cALD aged 17 years or younger. Key eligibility criteria included the presence of gadolinium enhancement on MRI attributed to cALD, an NFS score of 0 or 1, an MRI Loes score of 0.5-9.0, and lack of an HLA-matched sibling to serve as a stem cell donor for allogeneic HSCT. Hematopoietic stem cells were isolated from peripheral blood mononuclear cells collected by apheresis after G-CSF stimulation and transduced *ex vivo* with the Lenti-D lentiviral vector containing ABCD1 cDNA. The genetically modified cells were infused into the subjects following myeloablative conditioning with busulfan and cyclophosphamide.

A total of 17 boys were treated at the time of the published interim analysis. At the time of enrollment patients in the cohort had evidence of early cALD with a median Loes score of 2.0 (range 1.0–7.5). All treated patients had an NFS of 0. The median follow-up at the time of reporting was 29.4 months. Acute severe adverse events were consistent with those seen following myeloablative conditioning. The vector copy number at 24 months (assessed in 14 patients) ranged from 0.10 to 1.55. There was no evidence of insertional mutagenesis or preferential integration near genes previously associated with severe adverse events in other gene therapy trials including MDS1, EVI1, and LMO2. Expression of ALD protein was detected in peripheral blood leukocytes at all time points assessed. The median percentage of CD14+ cells expressing the ALD protein was 14.3% at 6 months, 16.1% at 12 months, and 19% at 24 months in all patients at the most recent follow-up.

**TABLE 1**

**Summary of transplant and gene therapy manuscripts.**

| Study | Year | N | Loes score | NFS | Conditioning intensity | Stem cell source | Neutrophil engraftment (median) | Platelet engraft-ment (median, days) | Survival | Graft failure | Acute GVHD | Chronic GVHD | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cartier *et al.* | 2009 | 2 | 2.25 and 7 | NR | Myeloablative Bu/Cy | Autologous HSCs | 13–15 days | NR | 100% at 36 months | 0% | 0% | 0% | [1,2] |
| Eichler & Duncan *et al.* | 2017 | 17 | 2 (median) | 0 (me-dian) | Myeloablative Bu/Cy | Autologous HSCs | 31 and 12* | 32 | 88% (median follow-up 29.4 months) | 0% | 0% | 0% | [3] |
| Fernandes *et al.* | 2018 | 9 | 11.5 (median) | 0.5 (medi-an) | Cy/Flu/TBI/ATG PTCY | Haploidentical PBSC | 16 | 19 | 89% (17–37 months follow-up) | 11% primary 33% late | NR | NR | |
| Chen *et al.* | 2018 | 3 | NR | NR | Bu/Flu/Cy/ATG | Haploidentical PBSC and bone marrow | 12 | 11 | 100% (63–294 day follow-up) | 0% | 100% | NR | |
| Miller *et al.* | 2011 | 60 | <10 = 30 ³10 = 30 | 0= 23 1 = 17 ³ 2 = 20 | Myeloablative Cy/TBI (n = 16) By/CY (n = 28) RIC Alem/Clo/Mel/TBI (n = 16) | Bone marrow | 15 (range 8–41) | 40 (range 15–146) | 78% (median 3.7 years, range 0.7–9.6 years | 23% | 18% | NR | [5] |
| Beam *et al.* | 2007 | 12 | 8 (median) | NR | Myeloablative Bu/Cy/ ATG | Umbilical cord blood | 22.9 days (range 13–49 days) | 86.8 (range 55–227) | 66.7% at 6.25 months | 8.3% | 33% | 17% | [4] |
| Resnick *et al.* | 2005 | 3 | NR | NR | RIC Bu/Flu/ATG | Bone marrow | 12 | NR | 100% (37–56 months) | 0 | 33% | NR | [8] |
| Baumann *et al.* | 2003 | 12 | 12¶ | NR | Myeloablative Bu/Cy +/- ATG | Bone marrow n = 10; PBSC n = 2 | NR | NR | 83% (0.3–5 years follow-up) | 8% | NR | NR | [7] |
| Shapiro, *et al.* | 2000 | 12 | 4 (median) | NR | Myeloablative Bu/Cy | Bone marrow | NR | NR | 50% (5–10 year follow-up) | NR | NR | 0 | [6] |

*Median time to neutrophil engrafted 31 days when G-CSF not used; 12 days when used.
¶14 transplanted, 12 available for long-term follow-up; two died shortly after transplant.
Alem: Alemtuzumab; ATG: Antithymocyte globulin; Bu: Busulfan; Clo: Clofarabine; Cy: Cyclophosphamide; HSCs: Hematopoietic stem cells; Mel: Melphalan; NR: Not reported; PBSC: Peripheral blood stem cells; PTCY: Post-transpalnt cyclophosphamide; RIC: Reduced intensity conditioning; TBI: Total body irradiation.

At the time of reporting 15 of 17 boys were alive and free of major functional disabilities. Two patients had disease progression; one withdrew from the study and later died following an allogeneic HCT. The other child died following progression of ALD. Of the 15 surviving patients, the scores on the neurologic function scale remained low (0–1) and the Loes score stabilized in 12 patients. Gadolinium contrast enhancement resolved at 6 months in 14 of the 15 surviving patients, but reemerged at later timepoints in 8 boys. The contrast enhancement later resolved in 3 boys and was present at most recent follow-up in the remaining 5 patients. The significance of the reemergence of contrast enhancement was not clear and the authors reported that the longer follow-up is needed to understand its importance.

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## TRANSLATIONAL INSIGHT

Gene therapy using hematopoietic stem cells is emerging as a potential alternative to allogeneic stem cell transplantation for boys with early stage cALD. Neither technique has been shown to be effective in boys who have later stage disease and early diagnosis of ALD is critical. In children who do not have a known family history of disease this may be best accomplished with newborn screening, which is not uniformly performed in the USA. Data from gene therapy studies published to date have shown disease control and acute toxicities similar to what has been reported following allogeneic transplantation (Table 1) [10,33,42]. There have been no reports of clonal dominance, insertional mutagenesis, therapy associated leukemia, or other serious, unexpected toxicities. Gene therapy for cALD remains investigational and widespread use of this therapeutic modality will require FDA and EMA approval in the USA and Europe, respectively.

There remain unresolved issues and unanswered questions regarding gene therapy for cALD. While the time needed to identify an allogeneic stem cell donor is eliminated, the process of gene therapy from mobilization to stem cell infusion takes approximately 4–6 weeks. Because of this the time to treatment with gene therapy may not be faster than allogeneic transplant in all cases. Questions remain about the needed intensity of the conditioning regimen used to promote engraftment of the genetically modified stem cells. The two published trials of cALD gene therapy used fully myeloablative chemotherapy conditioning regimens. The doses of busulfan and cyclophosphamide chemotherapy used these regimens are the same as those used in many leukemia-directedconditioning regimens. Myeloablative doses of busulfan and cyclophosphamide can cause significant short-term side effects and organ toxicities and long-term complications including infertility, the risk for treatment related malignancies, and chronic organ dysfunction. It is not clear if fully myeloablative conditioning is needed for the successful engraftment of the gene therapy product. Reducing the intensity of the conditioning regimen would be expected to decrease the risks of acute and chronic toxicities of treatment and likely to reduce the incidence of treatment related mortality. It can be hypothesized that myeloablative conditioning, particularly with CNS penetrant drugs are needed to reduce the intensity of conditioning chemotherapy must be balanced with the need for engraftment. Future investigations of gene therapy using reduced intensity conditioning regimens would shed light on this important topic.

Another topic for future discussion and investigation is whether the population eligible for therapy can be expanded to other populations including boys who have fully HLA-matched related donors, those with more advanced disease, and adult patients with cALD. Patients who had an ALD-free, HLA-matched sibling who could serve as a stem cell donor were excluded from participation in the reported gene therapy studies due to the reported safety and excellent overall survival reported following related donor transplant for boys with cALD [16]. If longer term follow-up of autologous gene therapy shows favorable results, then discussion of expanding treatment to children

who have related donors may be warranted. This would eliminate the risk of GVHD, need for immune suppressing medications, and other transplant complications of allogenecity in patients who chose gene therapy over related donor transplant.

The discussion of expanding cellular therapy with allogeneic transplant or gene therapy to children with advanced cALD is challenging. While the addition of N-acetylcysteine to the supportive care regimen allowed for the allogeneic HCT of boys with cALD more advanced than reported in the trials presented, transplant is ineffective and generally not accepted as a treatment option for advanced cALD [43]. There is no evidence that gene therapy would have superior efficacy over allogeneic HCT for advanced disease. While this could be the subject of future study, the expansion of gene therapy to those with advanced cALD cannot be recommended based on the currently available evidence.

Adult-onset cALD is a rare presentation of disease presenting in 1–3% of males with ALD and has been treated with allogeneic HCT [44,45]. This does not include adult patients with adrenomyeloneuropathy, who are not standardly treated with cellular therapy. Gene therapy has not been performed in adult patients and the potential efficacy and safety is not known. This is a possible area of investigation in the future.

Finally, the long-term durability of effect and safety of gene therapy using lentiviral vectors and myeloablative conditioning is unknown. The FDA recommends that gene therapy investigations performed in the USA have a minimum of 15 years of follow-up to monitor for delayed adverse effects [46]. Long-term data of this nature are not yet available for the studies presented. It is important moving forward that researchers demonstrate that recipients of gene therapy have long-term expression of the ALD protein and continued stabilization of disease, are free of toxicity specifically related to gene insertion, and do not experience chemotherapy-related late effects more than those expected following allogeneic HCT.



## FINANCIAL & COMPETING INTERESTS DISCLOSURE

*The author is a consultant with bluebird bio. No writing assistance was utilized in the production of this manuscript.*

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## AFFILIATIONS

**Christine Duncan**
Dana-Farber Cancer Institute and Boston Children's Hospital.


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