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REVIEW

Review of Treatment for Adenosine Deaminase Deficiency (ADA) Severe Combined Immunodeficiency (SCID)

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Abstract: Adenosine deaminase deficiency (ADA) is a purine salvage pathway deficiency that results in buildup of toxic metabolites causing death in rapidly dividing cells, especially lymphocytes. The most complete form of ADA leads to severe combined immune deficiency (SCID). Treatment with enzyme replacement therapy (ERT) was developed in the 1970s and became the treatment for ADA SCID by the 1980s. It remains an option for some infants with SCID, and a stopgap measure for others awaiting curative therapy. For some infants with ADA SCID who have matching family donors hematopoietic stem cell transplant (HSCT) is an option for cure. Gene therapy for ADA SCID, approved in some countries and in trials in others, is becoming possible for more infants with this disorder. This review covers the history of ADA SCID, the treatment options to date and particularly the history of the development of gene therapy for ADA SCID and the current state of the risks and benefits of the gene therapy option.

Keywords: adenosine deaminase deficiency, ADA, severe combined immunodeficiency, SCID, enzyme replacement therapy, ERT, gene therapy

Introduction

Adenosine deaminase deficiency (ADA) is a purine salvage pathway defect leading to toxic buildup of the substates adenosine (Ado) and deoxyadenosine (dAdo) and buildup of dAdo nucleotides (dAXP).[1] ADA is expressed in almost all cells but has very high activity in lymphocytes because they are rapidly dividing.[1,2] ADA deficiency can lead to sensorineural hearing loss, skeletal defects, and neurodevelopmental deficits, but the immunological manifestations are potentially life-threatening.[3,4] ADA severe combined immune deficiency (SCID) results from the most complete form of ADA deficiency where there is <1% ADA activity and usually presents near birth.[5] Late onset ADA can also be severe with <1% activity and lead to ADA SCID, or it can be less severe with partial activity causing a combined immune deficiency (CID), and either of these may be missed on T cell receptor excision circle (TREC) newborn screening (NBS) which is done in the first days of life.[6] Combined immune deficiency from ADA deficiency may present later in life with varying degrees of B cell, T cell, and NK cell dysfunction. To assure that no cases of ADA SCID are missed at least one state, Michigan, now adds ADA enzyme screening to its newborn screening panel.

History

ADA deficiency was described in 1972 by Eloise Gilbert when she discovered that the red blood cells (RBC) of two patients with CID contained no ADA enzyme on gel electrophoresis.[7] In initial studies looking toward possible treatments the addition of normal red blood cells to cultures of cells from ADA deficient patients was reported to replace the enzyme and restore function of patient lymphocytes and RBC transfusion was a first treatment for ADA SCID.[1] ADA enzyme conjugated to

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polyethylene glycol (PEG) was developed and utilized for therapy prior to the development of hematopoietic stem cell transplant (HSCT) therapy for ADA SCID.[8,9]

Most ADA deficiency leading to SCID can now be detected by NBS. ADA deficiency is the most common autosomal recessive form of SCID, and accounts for 15–20% of all SCID.[10,11] NBS for SCID relies on TRECs, by-products of competent T cell receptor formation, which are normally high near birth and nearly absent in SCID.[11–13] This PCR based screening has been utilized for more than a decade in the United States and has revolutionized SCID detection and treatment options. Treatment for SCID which has traditionally been HSCT is known to have the best outcome with lowest morbidity and mortality if initiated within the first 90 days of life before any infection occurs.[14–17] The TREC NBS test for SCID will detect all causes of T cell lymphopenia and will produce abnormal results for diseases such as prematurity, maternal immune suppression from steroids or other suppressive agents, congenital cardiac disease, non-SCID primary immunodeficiency, benign genetic defect/difference in primer area for PCR, or severe illness in the infant.[18] There has also been concern for and some case reports of false normal results particularly in infants who have deficiencies in *ZAP70*, MHC class II, NF-kappa -B essential modulator (NEMO), hypomorphic SCID or late onset ADA SCID.[18,19] Screening for the ADA enzyme on newborn screening should lower the false normals from ADA deficiency to near zero.[20,21]

Enzyme replacement therapy (ERT) has been utilized for ADA SCID since the 1970s and is still utilized in patients for whom transplant or gene therapy is not an option and is also utilized in other patients as a stopgap measure until a curative therapy of HSCT or gene therapy is available.[5,22]

Enzyme Replacement Therapy

Although red blood cell transfusion supplies exogenous ADA to the deficient host, it is only utilized for emergency situations and not for ongoing therapy. PEG-ADA is purified bovine ADA conjugated to PEG and was developed in the 1970s, and in general use by the 1980s.[1,9,22] Over time the product was reported to lose some efficacy secondary to immunogenicity and development of less immunogenic product which can be injected intramuscularly was developed (Adagen®, PEGylated purified bovine ADA).[23] Consensus guidelines recommend initiation of ERT after diagnosis of ADA SCID while definitive therapy with gene therapy or HSCT is planned. If gene therapy is not available, no acceptable HSCT donors are available, or firstline definitive therapy fails, it is recommended to restart ERT. In 2019 the FDA approved a PEGylated recombinant bovine ADA for ERT (elapegademase-lvlr [Revcovi®]; Chiesi Inc., Boston, MA, USA) as a replacement for Adagen®.

Hematopoietic Stem Cell Transplant

HSCT is a curative treatment for ADA deficiency. In patients with a matched sibling donor HSCT is the preferred option for ADA SCID.[23] A 2012 publication of outcomes for HSCT in ADA SCID reported best outcomes with matched sibling or family donors with overall survival of 86% for sibling donors and 81% for other family donors compared to only 66% survival with matched unrelated donors and 43% for haploidentical donors.[24] Unconditioned transplants were noted to have significantly higher survival compared to myeloablative conditioning (81% vs 54%) despite non-engraftment issues without myeloablation. Cellular and humoral immune recovery was noted as high for all survivors of HSCT regardless of conditioning.[24] HSCT remains the treatment of choice for those patients who have an HLA matched sibling or family donor, but this is only about 20% of ADA SCID patients.[1,10,11,23] An update to the 2012 publication was published in 2022 and reported that for ADA SCID patients treated by HSCT or gene therapy after 2000 who had no active infection at the time of treatment there was no difference in five-year event-free survival or overall survival regardless of ERT use or pretreatment regimen.[25]

Gene Therapy for ADA Deficiency

Because ADA is ubiquitously expressed and has little need for fine regulation, it is an ideal target for gene therapy. In addition, only low levels of ADA enzymatic activity are necessary for normal immune function and cells with an added correct gene copy with normal ADA activity have a significant selective advantage that improves implantation.[26] ADA SCID was the first disorder for which gene therapy was attempted. In 1990 a four-year-old with ADA SCID was given an autologous transfusion of peripheral lymphocytes transduced with a murine retroviral vector containing the ADA gene.[26] The immunological effect was unfortunately short-lived, although cells with corrected gene copies were detectable for

years.[27] This first attempt, and other initial gene therapy studies, centered around genetically modified peripheral blood leukocytes. Long-term follow-up showed no adverse events or toxicity with persistence of gene corrected cells, but ERT was continued during the studies, making therapeutic endpoints difficult to measure and, in retrospect, causing interference with engraftment by decrease of selective advantage.[26,27]

Gene therapy with use of hematopoietic stem/progenitor cells (HSPC) is now utilized in preference to the peripheral blood leukocytes utilized in early trials because of more stable engraftment and the ability to self-renew. The first trials of gene therapy with HSPC used bone marrow or unrelated cord blood followed by transduction and infusion without conditioning. Studies at San Raffaele Telethon Institute for Gene Therapy (SR-TIGET) demonstrated ability of engrafted HSPC to differentiate into multiple lineages. Despite this, bone marrow derived cells were not retained long-term.[27–29] Studies utilizing $CD34^+$ cells of umbilical cord origin showed detectable levels of virus transduced cells up to eight years later. The studies were performed with concurrent ERT, and ADA expression was not sufficient to provide immunologic correction. Despite not leading to immunologic correction, these studies did demonstrate safety and feasibility for further research.[30,31] Some major limitations of the early studies were the continuation of ERT post gene therapy and the lack of non-myeloablative chemotherapy.

In subsequent studies, SR-TIGET used gammaretroviral transduction into $CD34^+$ cells, ERT was stopped prior to bone marrow harvest, and non-myeloablative busulfan was utilized prior to infusion of modified cells. The first two ADA SCID patients treated with this protocol demonstrated bone marrow engraftment, differentiation, and a decrease in peripheral toxic metabolites.[32] Further studies with the same protocol were conducted with more ADA SCID patients and confirmed efficacy and long-term durability. A total of 18 patients were treated in a compassionate use trial, with a median follow-up of 6.9 years and 100% survival. Of these initial 18 patients, 15 remained off ERT long-term, there was no insertional mutagenesis, and no serious adverse events attributable to gene therapy were reported initially.[33,34] Gene therapy with gammaretroviral vector (brand name Strimvelis®, GlaxoSmithKline) was approved by the European Medicines Agency in May 2016 based largely on the success of these trials. It is currently available in the European Union for ADA SCID patients who lack an HLA-matched related donor.

The advantage of non-myeloablative conditioning was further substantiated by multiple studies. Melphalan was utilized as non-myeloablative conditioning and a gammaretroviral vector was utilized for transduction in eight patients; all survived and five of the eight had immunologic recovery with cessation of both ERT and immunoglobulin replacement therapy.[23,35,36] In a trial in the USA, 10 patients were reported to have received gene therapy for ADA with retroviral vectors. Four of the ten had no conditioning and remained on ERT while the remaining six patients had low dose (65–90 mg/m^2) busulfan conditioning and ERT was withdrawn. In the group with no busulfan only transient low levels of gene marking were observed, and patients remained on ERT due to lack of immunological improvement.[37] In the group with low dose busulfan conditioning and ERT withdrawal three patients were able to remain off ERT. Two of the remaining patients were restarted on ERT and another subsequently had an allogeneic hematopoietic stem cell transplant.[37] Further evidence supporting the need for pre-GT conditioning was show in a Japanese trial that infused autologous gene-therapy corrected $CD34^+$ bone marrow cells without cytoreduction. These patients only experienced partial immune reconstitution and needed to be restarted on ERT.[38]

While initial studies with gammaretroviral vectors did not show any insertional mutagenesis, in 2020 a case of T cell leukemia was reported 4.7 years after Strimvelis® treatment with retroviral insertion ~40 kb upstream of the LIM-only 2 transcriptional regulator (*LMO2*).[39] *LMO2* regulator activation has also been implicated in cases of mutagenesis in gene therapy trails for X-linked SCID.[40,41] At the time of this reported insertional mutagenesis, it was the only reported case in 33 patients (3%) treated for ADA SCID but remains a serious concern.

Self-inactivating lentiviral vectors have been under development since the 1990s because of their advantage of ability to transduce non-dividing cells and the improved biosafety from the self-inactivation component.[42] They may also be a preferable choice of vector due to more favorable genome insertion and higher gene transfer efficiency.[43] In 2014 an initial phase I/II trial for ADA-SCID with gene therapy via lentiviral vector showed promising results with immunologic and metabolic recovery.[44] Safety and efficacy of 50 patients who received busulfan conditioning and self-inactivating lentiviral vector gene therapy with 24-month follow-up data from the US and 36-month follow-up data from the UK. All patients received non-myeloablative busulfan conditioning and continued ERT for 30 days after infusion. No malignancies were reported during the reported follow-up period. Serious adverse event of immune reconstitution inflammatory syndrome was

reported in four patients between 3 and 22 months after infusion of gene therapy but all four recovered with supportive therapy. Survival was 100% at 24 months and 100% in the 20 patients followed for 36 months in the UK. Furthermore, event-free survival was 97% in the US at 12 and 24 months with one patient having re-initiation of ERT at 5.9 months due to lack of engraftment and subsequently receiving HSCT. In the UK, event-free survival was 100% at 12 months and 95% at 24 and 36 months with ERT re-initiated in one patient at 12.2 months due to lack of engraftment. In all other patients vector copies were detected in sufficient numbers at 24 and 36 months with sustained adequate mean ADA enzyme activity. By the end of study, 47 of 50 patients had humoral immune function adequate to stop IgG replacement therapy. All the UK patients received a fresh formulation while 10 of 30 patients in the US received a cryopreserved formulation but the difference in formulation rendered no differences in outcome.[45] The ability to successfully cryopreserve cells is key as it will ensure broad access to gene therapy as it is utilized in the future.[45]

Conclusions

After diagnosis of ADA SCID ERT is the first-line therapy, followed by evaluation for HSCT if an HLA matched relative donor is available. The newest treatment modality is gene therapy but is not universally available currently.

The treatment algorithm currently is (1) ERT until HSCT evaluation, (2) HSCT to proceed if HLA matched sibling identified, and if not, (3) consideration for gene therapy where available and if gene therapy not available continue ERT.

ERT for ADA SCID is not curative and effectiveness can wane with time. ADA SCID patients with an HLA matched relative to allow HSCT allows an option for cure in only approximately 20% of patients.[24] While gene therapy is a potential curative therapy for ADA SCID and substantial progress has been made in sustaining immunologic and metabolic corrections, safety from insertional mutagenesis remains a concern particularly with gammaretroviral vectors. Trials with lentiviral vectors report no mutagenesis to date with follow-up at 4–5 years post treatment.[25,45]

Disclosure

Dr Nicholas L Hartog reports speaker bureau for Binding Site, advisory board member for Regeneron and Genentech, speaker bureau and advisory board for Horizon Pharmaceuticals, Takeda, advisory board and steering committee for Pharming Healthcare, outside the submitted work. The authors report no other conflicts of interest in this work.

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