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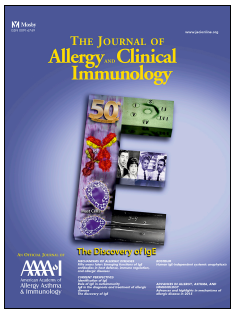

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Consensus approach for the management of severe combined immune deficiency caused by adenosine deaminase deficiency

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Abstract

Inherited defects in adenosine deaminase (ADA) cause a subtype of severe combined immunodeficiency (SCID), known as ADA-SCID. Most affected infants can be diagnosed while still asymptomatic by a SCID newborn screening test, allowing early initiation of therapy. We reviewed the evidence currently available and propose a consensus management strategy. In addition to the treatment of the immune deficiency of ADA-SCID, patients should be followed for specific non-infectious respiratory, neurological and biochemical complications associated with ADA deficiency. All patients should initially receive enzyme replacement therapy (ERT), followed by definitive treatment with either of two equal first line options. If an HLA matched sibling donor (MSD) or matched family donor (MFD) is available, allogeneic hematopoietic stem cell transplantation (HSCT) should be pursued. The excellent safety and efficacy observed in over 100 ADA-SCID patients who received gamma-retrovirus or lentivirus mediated autologous hematopoietic stem cell gene therapy (HSC-GT) since 2000 now positions HSC-GT as an equal alternative. If MSD/MFD HSCT or HSC-GT are not available or have failed, ERT can be continued or re-instituted, and HSCT using alternative donors should be considered. The outcomes of novel HSCT, ERT and HSC-GT strategies should be evaluated prospectively in "real life" conditions to further inform these management guidelines.

Key words

Adenosine deaminase deficiency

Enzyme replacement therapy

Gene therapy

Hematopoietic stem cell transplantations

Lentivirus

Severe combined immune deficiency

Abbreviations

Ado: adenosine

ADA: Adenosine deaminase

ADA-SCID: Severe combined immune deficiency caused by adenosine deaminase defects

dAdo: deoxyadenosine

dATP: deoxyadenosine triphosphate (dATP)

dAXP: total deoxyadenosine nucleotides

ERT: enzyme replacement therapy

HSC-GT: hematopoietic stem cell gene therapy

HSCT: hematopoietic stem cells transplantations

MFD: HLA matched family donors

MUD: HLA matched unrelated donors

MSD: HLA matched sibling donors

PEG-ADA: ADA coupled to PEG, pegylated ADA

PJP: *Pneumocystis jirovecii* Pneumonia

RBC: red blood cells

SAHase: S-adenosylhomocytsteine hydrolase

SCID: severe combined immunodeficiency

TREC: T-cell receptor excision circles

Introduction

Inherited deficiency of adenosine deaminase (ADA, now often referred to as ADA1) causes a subtype of severe combined immunodeficiency (SCID) characterized by unique effects on lymphoid and non-lymphoid cells. The pathogenesis of ADA deficiency has been extensively studied in humans and in a highly representative murine model, as reviewed recently [1]. The absence of functional ADA leads to increased concentrations of its substrates adenosine (Ado) and 2'-deoxyadenosine (dAdo) and their phosphorylated derivatives, (dAXP) and the inactivation of *S*-adenosylhomocysteine hydrolase (SAHase) in cells [2]. Excessive levels of deoxyadenosine triphosphate (dATP) can block DNA synthesis by inhibiting ribonucleotide reductase, and inactivation of SAHase can interfere with processes dependent on transmethylation. ADA deficiency has been associated with preferentially with abnormalities in lymphoid development and function [3-10]. Additional defects with varied clinical significance, and attributed to diverse mechanisms, have been observed in myeloid cells [11-13], lungs [14-17], brain [18-23], skeleton [24-26], liver [27-29] and kidneys [30-32], as well as increased risk for development of tumors [33-37] (Table 1). ADA associated abnormalities have been reviewed previously [1; 38-39], and will not be detailed here.

Since the original description of the condition in 1972 [40], more treatment options have been developed for ADA than for other genetic forms of SCID (Figure 1). Current treatments include enzyme replacement therapy (ERT), allogeneic hematopoietic stem cell transplantation (HSCT), *ex vivo* corrected autologous hematopoietic stem cell gene therapy (HSC-GT), or combinations of these options. Almost a decade ago, experts in ADA-deficient SCID reviewed the pathogenesis of this condition and provided guidelines for its management [41]. Since then, there have been significant advances in the management of ADA deficiency.

Long-term outcomes for ADA-deficient patients receiving different therapies have been reported from single- [17; 42-43; H.B.G., unpublished data, 27 June 2018] and multi-center studies [44-45]. HSC-GT has been approved for clinical use in the EU [46] and promising results from lentivirus vector-based HSC-GT studies are emerging [47]. Therefore, it is timely to review the new information and provide updated guidance for management of affected patients. The authors, together with clinicians and scientists, as well as patient advocacy groups, the pharmaceutical industry and USA government representatives interested in ADA deficiency convened in Toronto, Ontario, Canada on April 29, 2018. The group reached a consensus regarding new treatment guidelines and a treatment algorithm that are described here.

Management of adenosine deaminase deficiency

Newborn screening for SCID uses DNA from infant dried blood spots to detect T-cell receptor excision circles (TRECs) as a surrogate marker for new T cell production. The introduction of newborn screening in all but 3 states in the USA and in an increasing number of countries worldwide has led to significant changes in the diagnosis of SCID and ADA deficiency [48]. Currently, where newborn screening for SCID or positive family history are available, ADA-deficient patients might be asymptomatic when initially evaluated [49, J Puck et al, manuscript submitted]. Clues to the diagnosis of ADA deficiency include an associated neutropenia, characteristic bone abnormalities, and in some cases elevated liver enzymes. Diagnosis of ADA deficiency is usually established by demonstrating absent or very low (<1% of normal) ADA activity in red blood cells (RBC), which is accompanied by elevated levels of Ado and dAdo in plasma, urine, or dried blood spots. An elevated level of dATP (also measured as total dAdo nucleotides, dAXP) in RBC is pathognomonic for ADA deficiency. Demonstrating bi-allelic mutations in the *ADA* gene should also be done to further confirm ADA deficiency, permit genetic counseling for the family and possibly help predict the phenotype [50]. A minority of affected patients carry hypomorphic *ADA* mutations, which result in diminished ADA enzyme activity and confer a delayed or late onset phenotype [51]. In rare instances, such patients may have newborn TREC values above the “cut-off” levels in population-based screening and therefore may not be identified [52-53]. Hence, healthcare providers should maintain vigilance for the possibility of delayed or late presentation of ADA deficiency.

The availability of newborn screening for SCID has also changed the initial management of ADA-deficient patients. While previously patients were often sick with infections and required prolonged hospital admissions upon presentation prior to definitive therapies, currently some patients may be maintained in protective isolation at home, following guidelines suggested for other forms of SCID [54], and with the added consideration of providing immediate enzyme replacement while planning definitive therapy (see below). Immediate management guidelines include immunoglobulin supplementation appropriate for age and weight and prophylactic antibiotics for *Pneumocystis jirovecii* Pneumonia (PJP). Trimethoprim-sulfamethoxazole is considered the most effective method to prevent PJP and is usually initiated after 30 days of life to avoid risks of kernicterus and bone marrow suppression. Neutropenia is common among ADA-SCID patients; therefore, neutrophil counts should be monitored frequently. Persistent or severe neutropenia has often led to the replacement of sulfa-based PJP prophylaxis with alternative medications, such as pentamidine or atovaquone, although the role of trimethoprim-sulfamethoxazole in the development of the neutropenia is still not clear [11]. Many centers also initiate anti-fungal prophylaxis to prevent development of candida-related thrush, diaper

dermatitis or more severe fungal infections. Avoidance of herpesvirus infections is also important, and CMV exposure from breastmilk from CMV IgG seropositive mothers may require suspending nursing and using prophylactic antiviral therapy. Patients should be monitored closely for the development of infectious and non-infectious complications associated with ADA deficiency. The monitoring of ADA-deficient patients should include hematological indexes, analysis of cellular and humoral immunity, respiratory status, liver and kidney function, endocrine evaluation, skeletal, neurological and hearing assessment, infectious diseases and tumor surveillance, etc. Pulmonary alveolar proteinosis can cause respiratory distress with rapid onset and must be differentiated from infectious pneumonia and promptly treated with ADA ERT, to which it responds with rapid resolution [J.M.P., unpublished data, 27 June 2018].

Enzyme replacement therapy

Appreciation that ADA deficiency is a systemic metabolic disease and that nucleosides can cross biological membranes led to an attempt to provide the missing enzyme by transfusing RBC from healthy donors [55]. This approach carried substantial risks, failed to restore antigen-specific immunity, and was eventually abandoned. In 1987, weekly intramuscular injection of a PEGylated bovine ADA was reported to maintain ADA activity in plasma at a level far higher than total blood ADA activity achievable by RBC transfusion. By acting extracellularly on the nucleoside substrates of ADA, “ectopic” PEG-ADA reversed dAXP accumulation and SAHase inactivation in RBC, leading to improved lymphocyte counts in 2 patients who had failed both transfusion therapy and transplantation [56]. A clinical trial in these 2 patients and 4 others led to FDA approval of PEG-ADA (Adagen®, pegademase bovine) in 1990. Trial patients, only 1 of whom was a newly diagnosed infant, were enrolled serially, and each was started at a low weekly dose of Adagen, which was then increased to 15-20 U/kg until RBC dAXP normalized and T lymphocyte counts began to improve. The package insert for Adagen retained the trial’s dose escalation schema.

The FDA mandated a 2-year post-approval monitoring of all ERT patients, with biochemical parameters assayed at Duke University, and clinical status and immune function followed locally. This yielded a better picture of the response to ERT, including evidence that infants with SCID and failure to thrive might require higher dosing than had been used in the clinical trial, and that neutralizing antibodies could develop in about 10% of patients during the first year of treatment [57-58]. Monitoring of plasma ADA activity, RBC dAXP, and anti-ADA antibodies has been performed without charge by MSH to patients in >20 countries.

Numerous case reports have described the first 1-2 years of ERT, and also significant events such as the development of anti-ADA neutralizing antibody and malignancies; these reports have periodically been reviewed [41; 59-61]. At the last workshop on ADA deficiency management, it was estimated that overall probability of survival among ~180 ERT patients over the previous 2 decades was 78%, and that a patient alive 6 months after starting ERT had a 90% probability of surviving for the next 12 years [41]. Most deaths occurred during the first 6 months, in patients who were severely ill at diagnosis. Later mortality was due to refractory hemolytic anemia, progression of chronic pulmonary insufficiency and malignancies. Lymphomas, often EBV-related, have developed in at least 10 patients, after as few as 3 years of ERT, but mostly beyond 8 years [33-35; 37]. This may be related to a progressive decline in lymphocyte counts and function during long-term ERT, which has been documented at several treatment centers [17; 42-43; 64-67]. The reasons for this decline are uncertain, but were apparently not related to loss of the biochemical action or a change in the pharmacokinetics of Adagen, or the development of neutralizing antibody.

Given the experience of almost 3 decades, our current workshop consensus is that ERT should be given to all patients newly diagnosed with ADA-SCID as an immediate stabilizing measure. In addition to the benefit from restoring immune function, ERT has also been reported to improve the hepatocellular abnormalities [27-28], pulmonary alveolar proteinosis [15; 52] and the bone dysplasia [26] that are associated with untreated ADA deficiency. As ERT acts systemically, it may have the potential to protect from neurologic injury caused by elevated levels of adenosine and deoxyadenosine. However, clear evidence that ERT reverses already existing neurologic involvement is lacking. The panel noted that a marked general improvement in patient alertness, well-being and nutritional status has been observed after initiating ERT. This may be due to systemic metabolic detoxification, as it occurs prior to restoration of immune function.

ERT leads to rapid increase in ADA activity in the plasma, and over a period of 4-8 weeks results in the return of RBC dAXP to nearly undetectable levels and a significant increase in SAHase activity. An increase in B cell numbers is evident within the first month of therapy in some patients, while T cells numbers typically begin to increase by 2-4 months [58]. Production of antibodies also normalizes [68]. Early treatment may reverse metabolic toxicity to the thymus and non-lymphoid organs, further stabilizing patients before HSCT or HSC-GT [44]. Whether early initiation of ERT protects the developing brain and auditory system is uncertain, but it may

be possible to document such benefit in patients discovered by newborn screening, who are well at the time ERT is begun.

In recent years many physicians have initiated ERT at a dose of 30 units/kg based on ideal body weight, administered by intramuscular injections twice weekly (total weekly dose 60 units/kg). This regimen was first employed in two respirator-dependent SCID patients in whom dosing per the package insert maintained insufficient plasma ADA activity to completely normalize metabolic abnormalities in RBC, or to restore immune function [58]. The twice-weekly higher dose regimen was biochemically effective and led to resolution of life-threatening viral infections. Because Adagen is supplied in single-dose vials, and as dosing twice a week is inconvenient for patients who must travel long distances to receive injections, some centers have administered 60 U/kg once weekly, which may require using two injection sites. After 4-6 months, the dose may be reduced to 30 U/kg once weekly, provided that clinical status has stabilized and there is evidence of protective immunity based on T cell counts and antigen-specific responses.

In most patients, ERT should be used a “bridge” for relatively short periods (a few months to ~2 years) prior to undergoing HSCT or HSC-GT [41]. The optimal time to discontinue ERT before HSCT or HSC-GT has not been systematically studied. Concern that the immunity provided by ERT could interfere with engraftment, especially in the non-conditioned setting, led to the former practice of stopping ERT 2-4 weeks before transplant. However, the potential benefits of systemic detoxification, particularly when conditioning is employed, have led some to suggest continuation of ERT until and possibly for a time after HSCT [47]. For HSC-GT, the approach used in the initial gamma-retrovirus vector trials in Milan [69], and now for the approved Strimvelis, has been to stop ERT 2-3 weeks before HSC-GT to avoid blunting the selective advantage for ADA-replete lymphocytes over ADA-deficient cells. In contrast, studies in the ADA-deficient mouse model showed improved engraftment and thymus reconstitution when ERT was continued for one month after HSC-GT, compared to mice for whom ERT was stopped a week before HSC-GT [70]. The approach of continuing ERT was adopted in subsequent clinical trials (Figure 2) that used lentiviral vector [47]. Continuing ERT for one month after the infusion of gene-replete cells did not prevent the rapid increase in ADA activity in RBC (Figure 3A) yet delayed the rise in RBC dAXP (Figure 3 B) and the decline of T and B cell numbers (Figure 3C and 3D) that typically occurred following ERT cessation, associated with remarkable increase in T and B cells numbers to near normal values [71]. However direct comparison of the effects of ERT discontinuation timing relative to HSC-GT is not available, As

Strimvelis is a commercial licensed product and the lentiviral vector is advancing towards registration, ERT cessation relative to HSC-GT must presently follow its current guidelines.

Over the almost 3 decades since its approval, the number of patients in whom ERT has been employed long term has steadily decreased, and there have been no systematic studies of long-term survivors. Continued good health after 25 years has been reported in one patient [67], whereas 2 others experienced increased susceptibility to infections and other non-infectious complications over time [17]. The deterioration in lymphocyte counts and function over time, noted above, may eventually lead to a decline in antiviral immunity and immune tumor surveillance, contributing to an increasing risk of malignancies. For all of these reasons, ERT longer than 5-8 years should be avoided, and employed on a continuous basis only when neither HSCT nor HSC-GT have been available or effective, and in older patients with a delayed or late onset phenotype who may be poor candidates for those definitive procedures.

Regular assessment of the effects of ERT should include metabolic and immune testing. Ideally, trough plasma ADA activity and RBC dAXP should be measured monthly until immune function improves, then every 2 months in the 1st year, every 3-4 months in the 2nd year of treatment, and twice yearly thereafter. Monitoring frequency should be increased when doses of ERT are changed, a new formulation is used, compliance might be compromised, or antibodies to PEG-ADA are detected. An unexplained decrease in plasma ADA activity, particularly when associated with increase in RBC dAXP, should lead to testing for neutralizing antibodies to ADA. Increasing the dose of PEG-ADA or dividing a weekly dose into two administrations have been proposed as measures to overcome ADA-neutralizing antibodies [42; 57]. Immune testing, including enumeration of lymphocyte subpopulations should be done monthly until T cell reconstitution is evident, then every 3 months for the initial year of treatment. Additional functional testing of immune reconstitution should follow the guidelines established for patients with SCID after allogeneic HSCT, such as those published by the Pediatric Blood and Marrow Transplant Consortium [72]. Immunoglobulin supplementation should be continued until B cell function is evident by increased B cell numbers, normalization of IgA and IgM levels and appearance of isohemagglutinin antibodies. After discontinuing immunoglobulin supplementation, specific antibody responses to vaccination must be documented.

Adagen was the first PEGylated drug to receive FDA approval. The need to obtain bovine tissue as a source of purified ADA has posed significant challenges to reliable and consistent production of Adagen, and use of bovine products has raised concerns about safety. Therefore, the manufacturer has developed a recombinant version of bovine ADA conjugated to

PEG, which is now in late stages of clinical evaluation (NCT01420627, Clinicaltrials.gov). Once US Food and Drug Administration approval is obtained, the recombinant protein-based PEG-ADA will replace Adagen. The performance of the new drug will then be evaluated in regular clinical use, and no doubt will be discussed at a future workshop on the management of ADA SCID.

Allogeneic hematopoietic stem cell transplantation

Single- and multi-center studies have demonstrated the ability of HSCT from HLA matched sibling donors (MSD) or matched family donors (MFD) to provide long-term correction of the metabolic and immune abnormalities in ADA-deficient patients. The outcome of HSCT further improved after the year 2000, reflecting improved supportive care [73; H.B.G., unpublished data, 27 June 2018]. Therefore, once the diagnosis of ADA-SCID is verified, HLA typing of the patient, all full siblings and the parents must be performed. In highly consanguineous pedigrees, it would also be important to undertake HLA typing of close relatives and to see if a matched family donor can be identified. If a MSD/MFD is available, HSCT may be attempted as soon as feasible. Nearly all MSD/MFD HSCT for ADA-SCID have been undertaken without cytoreductive or immune ablative conditioning. A multicenter study demonstrated that among 54 ADA-deficient patients who received MSD/MFD HSCT, there were 46 survivors (85.2%), with 3 patients (5.6%) dying from treatment-related causes [44]. Donor engraftment was reported in 100% of the patients who did not receive any conditioning, and >90% in patients who did receive conditioning. Only 1 of 27 patients (3.7%) required continuing immunoglobulin replacement. Restoration of T, B and NK cell function and engraftment of donor HSC was reported in 85-95% of ADA-deficient SCID patients receiving non-conditioned MSD HSCT. Recently, data from a single center's experience with unconditioned MSD/MFD HSCT for ADA deficiency showed that 4 of 16 patients (25%) required a repeat procedure [H.B.G., unpublished data, 27 June 2018]. The reasons for the high rate of HSCT failure are not clear, but may relate to the level of immune function established by ERT at the time of HSCT, which may have mediated rejection or non-engraftment of donor cells. These data suggest that if there is significant immune reconstitution with ERT, then ERT should be discontinued prior to HSCT or that mild conditioning be used to deplete cells generated while on ERT. Yet, while reduced intensity conditioning might further improve donor engraftment [74], the expert panel concluded that currently there is insufficient evidence to recommend the routine use of conditioning in most patients with ADA-SCID receiving MSD HSCT.

Autologous hematopoietic stem cell gene therapy

ADA deficiency was the first human disease to be treated with autologous gene therapy [75-77]. Studies using gamma-retrovirus vectors to deliver the ADA gene demonstrated the safety and efficacy of this strategy as well as the importance of conditioning in ensuring long-term persistence of adequate multi-lineage gene corrected cells [78-79]. Since the year 2000, major modifications have led to marked progress in HSC-GT for ADA deficiency (Figure 4), with more than 100 ADA-deficient patients having received HSC-GT (Table 2). Remarkably, all ADA-deficient patients who received HSC-GT are reported to be alive, although approximately 10-20% had to either restart ERT or receive subsequent HSCT/HSC-GT. Most patients had near normal T, B and NK cell numbers, with adequate response of T cells to stimulation, and were able to discontinue immunoglobulin replacement [80-85].

To reduce potential adverse effects from the conditioning on early growth and development and achieve adequate detoxification, most newly diagnosed ADA-SCID patients are treated with ERT until they are at least 3-6 months old and able to undergo HSC-GT. Since the groundbreaking study in The San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), Milan, Italy, the importance of low dose busulfan conditioning in ensuring engraftment and expansion of sufficient ADA-corrected cells has become well established. Indeed, such strategy of reduced intensity conditioning was subsequently adapted for HSC-GT trials for both ADA and non-ADA defects [86]. The pharmacokinetic-adjusted busulfan dosage typically used for ADA HSC-GT is well tolerated by ADA-deficient patients with essentially no acute symptoms except for transient grade 3 to 4 neutropenia and grade 2 to 3 thrombocytopenia. No serious adverse events related to gene therapy or events indicative of clonal proliferation were reported in a recent comprehensive review of the initial SR-TIGET experience [87]. The success of the SR-TIGET trial has led to its commercialization as Strimvelis, which has been approved for clinical use in the European Union since 2016.

Although none of the ADA-deficient patients who received HSC-GT experienced leukemia, in contrast to patients enrolled in the X-linked SCID, Wiskott-Aldrich Syndrome and Chronic Granulomatous Disease HSC-GT trials using gamma-retroviral vectors [86], concerns about the safety of the delivery vector led to the development of a newer self-inactivating lentivirus vectors. Several studies (NCT01852071, NCT02999984, NCT01380990) with more than 50 ADA-deficient patients have demonstrated the safety and efficacy of this approach [85].

Patients may be ineligible to undergo HSC-GT in cases of insufficient amounts of BM HSC collected, which could be particularly challenging for older patients. This may be circumvented by the use of mobilized peripheral blood or repeated collections. Another potential

limitation is related to active infections with specific viral pathogens that could prevent HSC manipulation in the manufacturing facility. Recent experience in an HCV infected infant suggests that the use of new antivirals can bring about sufficient clearance of the HCV infection to allow successful subsequent gene therapy treatment [88]. Similarly, another patient, who presented with CMV disease as a neonate and was treated with antiviral medications, successfully received HSC-GT [D.B.K., unpublished data, 27 June 2018].

One of the limitations of current HSC-GT is the need to infuse the cells shortly after the transduction and to maintain the patient in hospital isolation pending T cell recovery, which has required the patients and their caregivers to travel to the few centers capable of performing such procedures in an effective and safe manner. Strimvelis requires patients to remain in Milan, Italy, for 4-6 months. To overcome these limitations, a current study using the lentiviral vector is evaluating the possibility of cryopreserving transduced cells (NCT02999984). Cryopreservation will provide the time needed for full characterization of the product prior to the infusion; furthermore, in the case of lentivirus gene therapy, pharmacokinetic adjustment of busulfan levels may be performed in the recipient prior to thawing and infusion of the gene corrected cells (Figure 5). Cryopreservation may also allow patients to remain at their home hospital, where stem cells can be collected and shipped to a central facility for processing, transduction and freezing. Subsequently, the cryopreserved transduced cells can be shipped back to the transplant center closer to the patient's home for thawing and infusion.

Data on the outcome of HSC-GT for ADA deficiency was acquired from few prospective clinical trials with carefully selected patients, yet evaluation of such procedures demonstrated remarkable safety profile and success. Moreover, although direct scientific comparison of HSC-GT with HSCT is not possible as prospective randomized studies are not available, HSC-GT provides important advantages, such as avoidance of severe graft versus host disease (Table 3). Accordingly, there was a consensus that HSC-GT should now be considered alongside MSD/MFD-HSCT as one of the first line treatment choices for ADA-deficient patients (Figure 6). This recommendation represents a major change from previous guidelines, such as the recent guidelines by the European Society for Blood and Marrow Transplantation /European Society for Immunodeficiencies guidelines for treatment of ADA-SCID [89], and reflects the promising results of HSC-GT. Nevertheless, it should be noted that Strimvelis is currently indicated in the European Union only for patients for whom no suitable MSD is available. Also, Strimvelis is currently considered more expensive than the estimated costs of HSCT, at least in the USA [90], although additional clinical costs as well as travel and accommodations expenses

associated with HSCT need also to be factored. As additional data from “real life” experience accumulates, the role of HSC-GT for ADA deficiency will become clearer.

HSCT using alternative donors for ADA-deficient patients

The management for patients with ADA deficiency who do not have a MSD/MFD or access to HSC-GT is particularly challenging. In contrast to the success of MSD/MFD HSCT for ADA deficiency, survival after alternative HSCT with alternative donors has been disappointing [44]. In many instances, patients lacking MSD/MFD have continued ERT for extended periods. However, due to the frequent inability of ERT to support long-term immunity, as well as its high cost, it is recommended that ERT should not be used indefinitely, particularly for new patients. Accordingly, the possibility of HSCT using alternative donors needs to be considered in all newly diagnosed patients.

Among alternative donors, HLA matched unrelated donors (MUD) historically provided better outcomes than haplo-identical HSCT [44], although it is possible that earlier identification of ADA-deficient patients by newborn screening for SCID, prior to acquiring infections, might improve the outcome from both groups. The intensity of conditioning required for successful MUD HSCT in ADA deficiency is not known. Because of the relatively high risk posed by myeloablative conditioning for ADA-SCID HSCT [44], reduced intensity conditioning regimens should be considered, although the efficacy with specific agents and dosages needs to be established. Adult bone marrow or peripheral blood stem cells are preferred over umbilical cord blood, as the results with the latter have been relatively poor, based on limited numbers [44].

The data on haplo-HSCT for ADA-deficient patients are relatively limited, as in recent years some centers have abandoned altogether the use of such donors [H.B.G., unpublished data, 27 June 2018]. A large multi-center study previously reported 43% survival among 30 patients who underwent haplo-HSCT, although the data stretched back to the middle of the 1980s, when techniques for T cell depletion were less rigorous and supportive care less advanced than currently [44]. Among these patients, myeloablative conditioning was used in 23 patients, 6 were not conditioned and 1 received reduced intensity conditioning [44]. The use of unconditioned T cell depleted haplo-identical transplants has also been considered by some centers. However, in the largest such series reported, only 7 of 19 patients (33%) demonstrated effective T cell engraftment [91].

It is also expected that the outcome of HSCT using alternative donors will continue to improve in the upcoming years as allogeneic HSCT technologies continue to advance.

Sequence-based HLA typing has been shown to improve outcomes over the earlier era when the less discriminatory serological-based typing methods were used [92]. Improved methods for graft engineering such as TCR alpha-beta+/CD19+ or CD45RA+ (naïve) T cell immunomagnetic bead depletion are showing excellent results in many settings [92]. Haplo-identical HSCT with post-transplant cyclophosphamide for in vivo depletion of allo-reactive donor T cells has also been shown to be effective with low rates of GVHD [94-96]. Thus, these novel techniques may change the approach to ADA-SCID patients needing HSCT and should be implemented in the context of clinical trials to obtain maximal information.

Several options are available for ADA-deficient patients for whom the first definitive therapy failed to restore immunity. Many centers re-institute ERT while a second definitive therapy is planned [Figure 6]. If the first treatment was an allogeneic HSCT, this may be repeated, possibly with a different graft source or more intense conditioning. Second allogeneic HSCTs carry increased risks of complications from added conditioning, infections and GVHD. HSC-GT after an unsuccessful conditioned HSCT is should be carefully considered, as the effects of the conditioning regimen on the bone marrow may compromise its usefulness as a source for the hematopoietic stem cells needed for GT. If HSC-GT as first treatment was not successful, it may be repeated, depending on interval since initial HSC-GT, as exemplified by 2 ADA-deficient patients who failed gamma-retrovirus HSC-GT and then underwent successful lentivirus HSC-GT with reduced intensity conditioning [Gaspar – personal communication]. Alternatively, if HSC-GT is unsuccessful, an allogeneic HSCT may be pursued. Indeed 6 patients, in whom HSC-GT failed, underwent an allo-HSCT, with successful outcomes in 5 patients and chronic GvHD leading to death in the other [A.A., D.B.K and H.B.G., unpublished data, 27 June 2018].

Discussion and recommendation

The information detailed in the Management of adenosine deaminase section led the meeting's participants to the development of a consensus algorithm for the management of ADA-SCID (Figure 6). The authors recognize that management choices depend on experience and knowledge of health care providers, the patient's and family's preferences, institutional policies, access and availability of treatments, national health systems and insurers decisions, new information in the rapidly developing field of ERT, HSCT and HSC-GT. Therefore, the proposed algorithm should serve as a guideline, rather than a mandated structured treatment map.

449 Given the number of important issues concerning optimal treatments and the long-term
450 outcomes of immune as well as neurological, developmental, hearing, fertility, endocrine and
451 other complications, it is vital to establish an unbiased independent registry to encompass all
452 ADA-deficient patients. It is only by collecting these data longitudinally that optimal therapies
453 can be designed for future patients.

454

Figure legends

Figure 1: Timeline for the institution of treatments for adenosine deaminase deficiency

Since the identification of adenosine deaminase defects as a cause for severe combined immune deficiency in 1972, there have been 3 main treatment approaches. Allogeneic hematopoietic stem cells with HLA matched sibling donors (MSD) or matched family donors (MFD) are most commonly used followed by HLA haploidentical and HLA matched unrelated donors (MUD). The stem cells have been obtained from bone marrow, peripheral blood mononuclear cells or umbilical cord blood. Enzyme replacement therapy relied initially on transfusions of red blood cells from healthy donors and subsequently on frequent injections of polyethylene glycol coupled to bovine ADA (PEG-ADA). Autologous ex-vivo corrected hematopoietic stem cell gene therapy used initially gamma-retroviruses to transduce the gene of interest into stem cells, while in recent years the ability of lentivirus is being studied. The addition of reduced intensity conditioning prior to gene therapy is now recognized as critical for the success of the procedure. In the last year, benefits from cryopreservation of the lentiviral vector transduced hematopoietic stem cells are being explored.

Figure 2: Scheme of gamma-retrovirus and lentivirus based gene therapy with busulfan and discontinuation of enzyme replacement therapy.

After obtaining consent of patients/guardians for autologous hematopoietic stem cell (HSC) gene therapy, patients are screened and admitted for bone marrow (BM) harvest and conditioning with low dose busulfan, with adjustment in accordance to pharmacokinetics (pK) predetermined targets. CD34+ HSC are isolated, transduced with gamma retroviral vector (A) or lentiviral vector (B) containing the ADA gene, and reinfused through a central venous catheter (CVC). (A) For gamma-retrovirus, enzyme replacement therapy (ERT) is usually discontinued 14-21 days before gene therapy and patients are treated for 2 days with busulfan. (B) For Lentivirus based HSC-GT, patients are treated for 1 day with busulfan, and ERT is continued during gene therapy until 30 days after infusion.

Figure 3: Effects of busulfan and continued enzyme replacement therapy for 30 days following lentiviral vector gene therapy for ADA deficiency on ADA and dAXP in patients' red blood cells and Immune reconstitution.

Adenosine deaminase (ADA) activity (A), expressed as units of activity and deoxyadenosine phosphates (dAXP) percentage in red blood cells (B), as well as the number of CD3+ T cells (C) and CD19+ B cells (D) in the peripheral blood of patients with ADA deficiency 0-24 months after hematopoietic stem cell gene therapy. Results are the mean and standard deviation from interim analysis of 20 patients treated at the UCLA Mattel Children's Hospital, Los Angeles, Cal, through their most recent study time-point. Normal ranges are ADA activity (A) are 63 ±41 nmol/h/mg, %dAXP (B) <0.2%. Normal ranges (10^{th}-90^{th}%ile) at 1-2 years of age for CD3+ and CD19+ cells are 2.10-6.20 cells/μl × 10^{-3} and 0.72-2.60 cells/μl × 10^{-3}; respectively.

Figure 4: Timeline for the development of adenosine deaminase deficiency hematopoietic stem cell gene therapy

After the identification and cloning of the cDNA for ADA, retrovirus vectors were developed to efficiently deliver ADA. In 1990 the first gene therapy trial was initiated at the National Institute of Health (NIH), using patient's peripheral blood lymphocytes (PBL) followed by the use of CD34+ hematopoietic stem cells. Since 2000, the use of busulfan has been gradually incorporated into HSCT-GT, including lentivirus based trials. In 2016, Strimvelis was approved for clinical use in the EU. Currently the effect of cryopreservation of transduced cells is being explored.

Figure 5: Scheme of cryopreserved lentivirus based gene therapy with pK-adjusted busulfan and continued enzyme replacement therapy.

After obtaining consent of patients/guardians for autologous hematopoietic stem cell gene therapy patients are screened and admitted for bone marrow (BM) harvest. CD34+ cells are isolated from the bone marrow, transduced by lentivirus containing the ADA gene, and cryopreserved. Approximately 30 days later, the patient is admitted again, central venous catheter (CVC) is inserted and the patient is treated with busulfan at dosages that are adjusted in accordance to predetermined pharmacokinetics (pK) targets. Enzyme replacement therapy is discontinued 30 days after the hematopoietic stem cell-gene therapy (HSC-GT).

Figure 6: Consensus algorithm for the management of infants diagnosed with ADA-SCID

Following the diagnosis of severe combined immune deficiency (SCID) caused by inherited defects in adenosine deaminase (ADA) deficiency is established, all patients should receive enzyme replacement therapy, while monitoring for efficacy. Human Leukocyte Antigens (HLA) typing of the patient and close family members should be completed. Infections prophylaxis should be provided in accordance to the guidelines for SCID. Two equal first line treatment options should then be considered. Patients should proceed to receive allogeneic hematopoietic stem cell transplantation (HSCT) from HLA matched sibling donor (MSD) or HLA matched family donor (MFD) donor, if available. Alternatively, eligible patients should proceed to receive autologous hematopoietic stem cell gene therapy (HSC-GT), if available. If HSC-GT or HSCT are not available or are unsuccessful, patients should continue ERT while considering HSCT using alternative sources such as HLA matched unrelated donor (MUD) or HLA haplo-identical family members. Following treatment, patients should be monitored for abnormalities associated with ADA deficiency and for maintained immune reconstitution. Should treatment fail, patients should be re-considered for the different management options.

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A.B.: No conflicts to declare.

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H.B.G.: Chief Scientific Officer at Orchard Therapeutics, who are the owners of Strimvelis in the European Union and who are the license holder for the lentiviral vector gene therapy studies. HBG is co-founder, employee and equity holder in the company.

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863

864 **Table 1. Abnormalities associated with adenosine deaminase deficiency**

Affected cells/tissues	Mechanism	Clinical significance	References
Lymphoid cells	Depressed numbers and function of T, B and NK lymphocytes	Increased susceptibility to infections and autoimmunity, Omenn's syndrome,	3-10
Myeloid cells	Neutropenia and myeloid dysplasia	Not known	11-13
Lungs	Alveolar proteinosis, increased airway resistance	Respiratory distress, bronchiectasis	14-17
Brain	Not known	Neuro-development, cognitive, behavior seizures, hearing defects	18-23
Skeletal	Osteoblast insufficiency	Skeletal dysplasia	24-26
Liver	Not known	Hepatic dysfunction	27-29
Renal	Not known	Atypical hemolytic uremic syndrome	30-32
Tumors	Impaired DNA repair, defective immune surveillance	Dermatofibrosarcoma Protuberans, Lymphoma, Liver cancer	33-37

Dermatofibrosarcoma Protuberans

Table 2. Experience with autologous hematopoietic stem cell gene therapy for ADA deficiency since 2000

	Site, period	Vector Type	Cryo-preserved	Busulfan	ERT* after HSC-GT†	Number of patients	Treatment failure number (%)‡	Reference
1	SR-TIGET§, 2000-2016	Gamma‖	No	Yes	No	22	5 (23%)	83; A.A., unpublished data, 27 June 2018
2	SR-TIGET§, (Strimvelis) 2017	Gamma‖	No	Yes	No	5	0 (%)	A.A., unpublished data, 27 June 2018
3	LA¶/NIH#, 2001-09	Gamma‖	No Yes	No	No	4 6	4 (100%) 3 (50%)	79
4	GOS, 2003-13	Gamma‖	No	Yes	No	8	4 (50%)	81; H.B.G., unpublished data, 27 June 2018
5	LA¶/NIH#,, 2009-12	Gamma‖	No	Yes	No	10	1 (10%)	84
6	LA¶/NIH#,, 2013-16, GOS**, 2012-16	Lenti	No	Yes	Yes – 1 month	40	1 (2%)	D.B.K and H.B.G., unpublished data, 27 June 2018
7	¶LA, 2017	Lenti	Yes	Yes	Yes – 1 month	13	1 (8%)	D.B.K and H.B.G., unpublished data,

								27 June 2018
Total						108	19 (18%)	

‡Need to restart ERT or need for HSCT/HSC-GT

*ERT- enzyme replacement therapy; ||Gamma- Gamma-retrovirus; **GOS- Great Ormond Street, London, UK; †HSC-GT- gene therapy; ¶LA- Los Angeles, California, US; #NIH- National Institute of Health, Bethesda, US;. §SR-TIGET- San Raffaele Telethon Institute for Gene Therapy, Milan, Italy.

Table 3. Comparison of matched sibling donor hematopoietic stem cell transplantation with autologous HSC-gene therapy for ADA deficiency*

	MSD#* HSCT†	HSC-GT‡
Minimum time to procedure (months)	0.5-1	3-6
Performed at close specialized HSCT center†	Yes	Not currently§
Donor availability	<20%	100%
Cost of procedure	<120,000 dollars||	594,000 Euro¶
Chemotherapy conditioning	No	Yes
ERT prior to procedure	Usually not given	Usually given
Bone marrow/PBSC harvest from patient	No	Yes
Bone marrow/PBSC harvest from donor	Yes	No
Years of successful experience	>40	6**-17***
Procedure failure frequency (%)	10-20%	5-20%
Potential for graft versus host disease	Yes	No
Graft versus host prophylaxis	No	No
Procedure related mortality	5.6%	0%
Time to immune reconstitution	3-6 months	6-24 months
Immunoglobulin replacement need	5%	7-10%

*- The data provided in the MSD HSCT and the HSC-GT columns represent compiled results from multiple studies performed at different conditions. Accordingly, the two treatment modalities are not directly comparable.

#MSD- matched sibling donor

†HSCT- hematopoietic stem cell transplantation

‡HSC-GT- hematopoietic stem cell transplantation with autologous gene therapy

§- Cryopreservation might allow in the future for the procedure to be done at a closer HSCT center

||- Does not include added clinical costs, travel and accommodation at HSCT site.

¶- Cost of Strimvelis. Does not include added clinical costs, travel and accommodation at HSC-GT site.

∗- Years of experience with lentivirus vectors

∗∗∗- Years of experience with gamma-retrovirus vectors

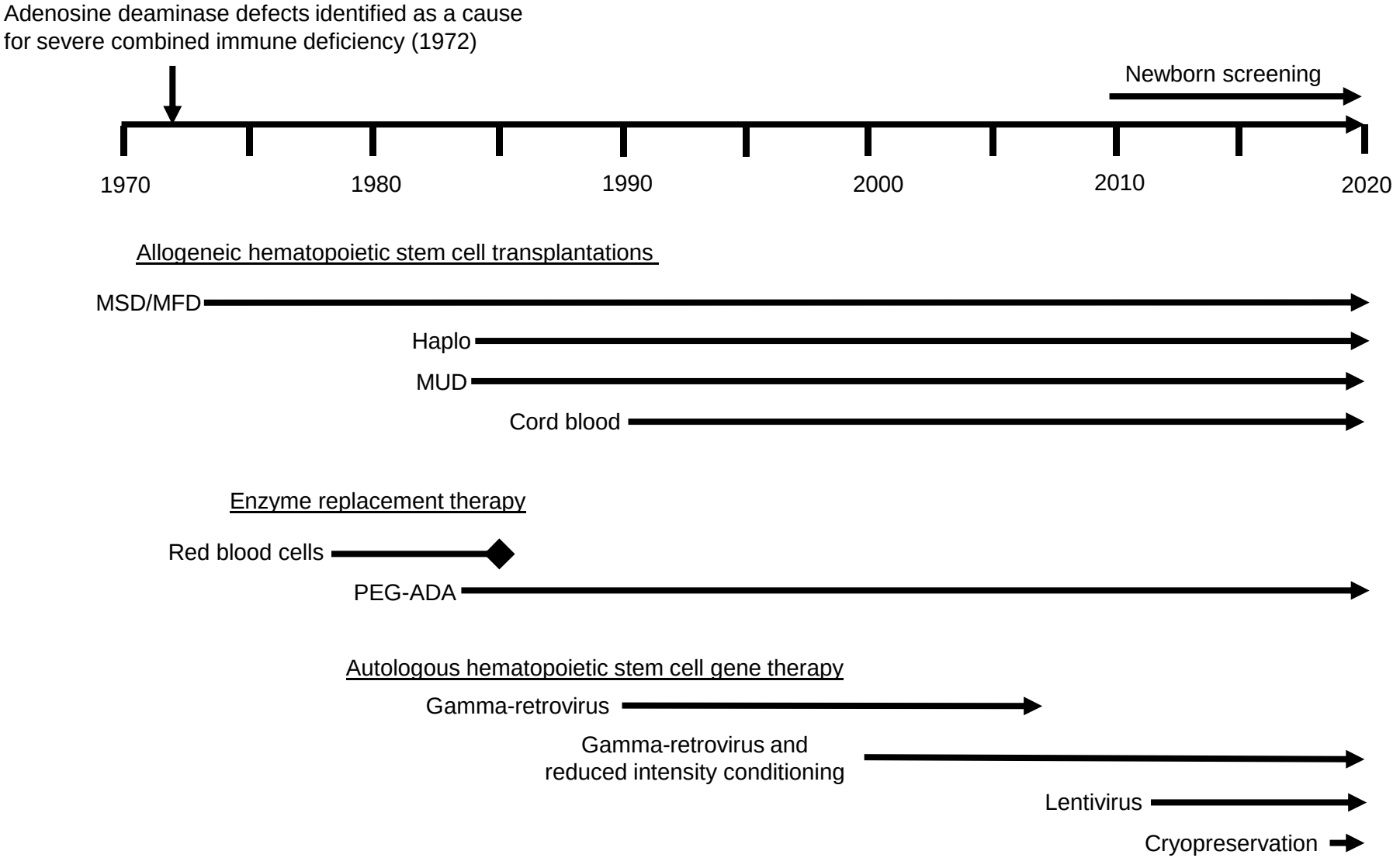
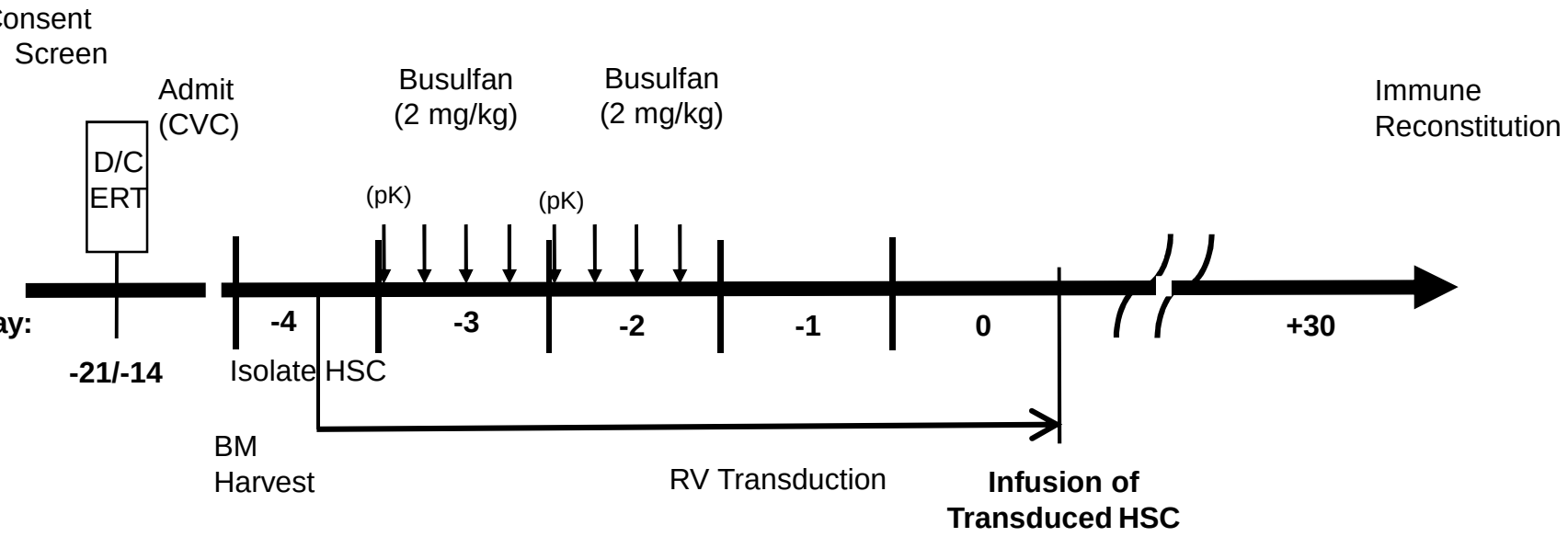


Figure 2: Scheme of gamma- and lenti- virus based gene therapy with busulfan and continued enzyme replacement therapy.



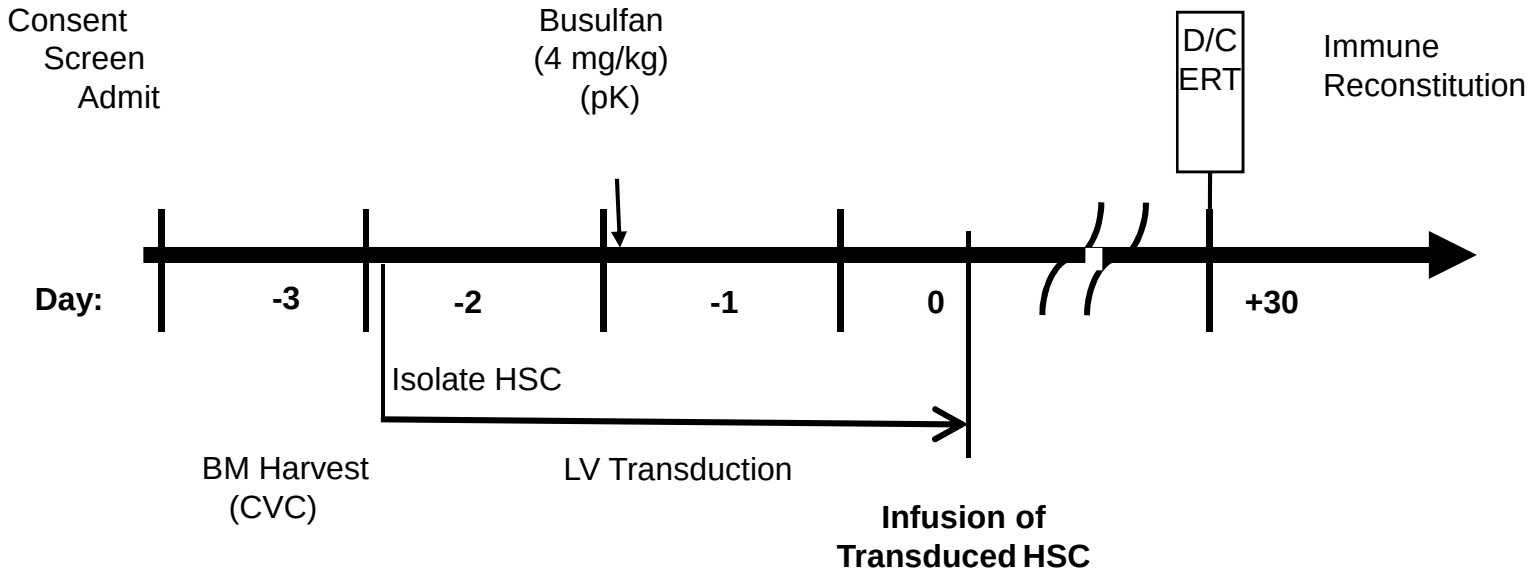


Figure 3: Effects of busulfan and continued enzyme replacement therapy for 30 days following lentiviral vector gene therapy for ADA deficiency on ADA and dAXP in patients' red blood cells

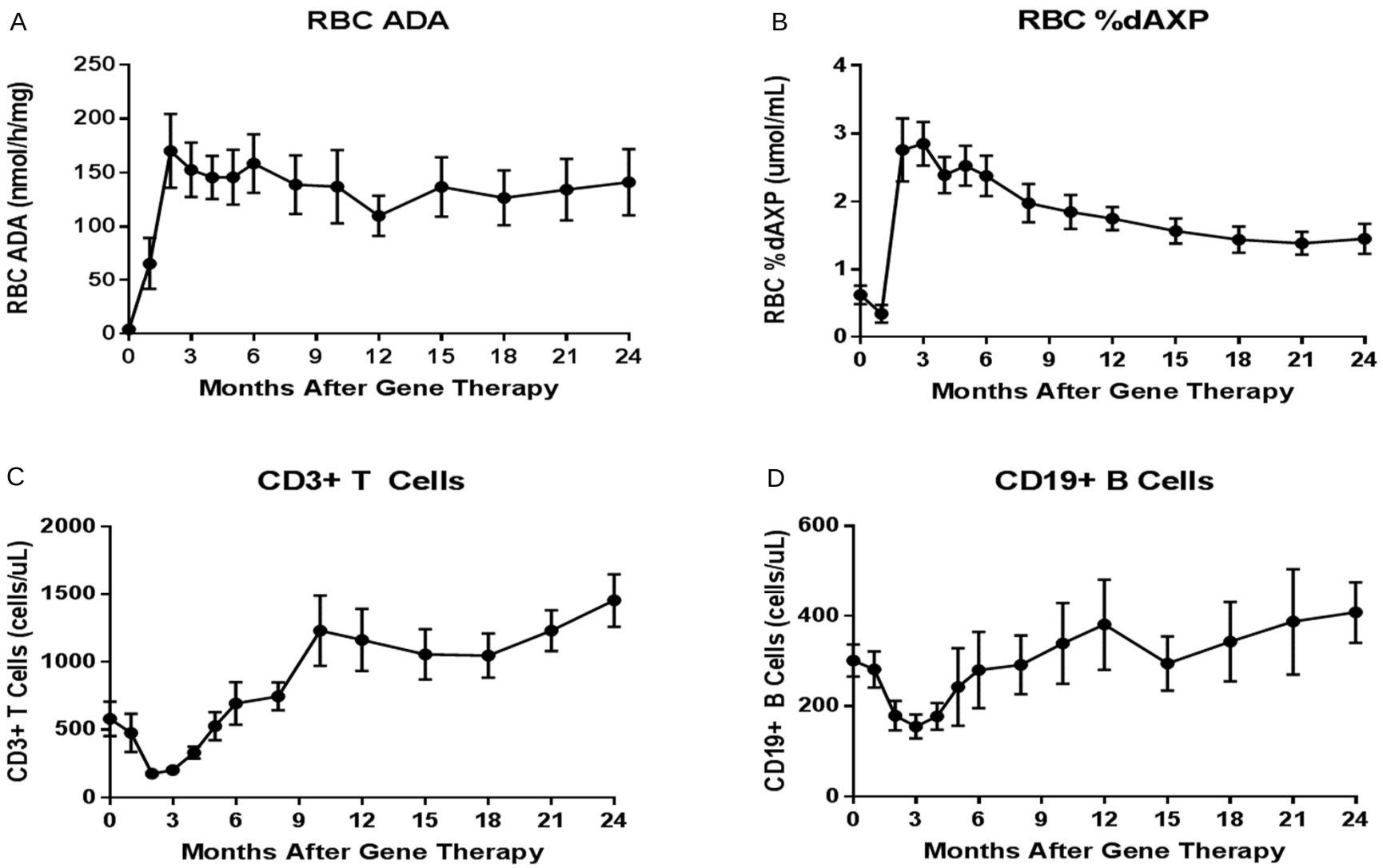


Figure 4: Timeline for the development of adenosine deaminase deficiency hematopoietic stem cell gene therapy

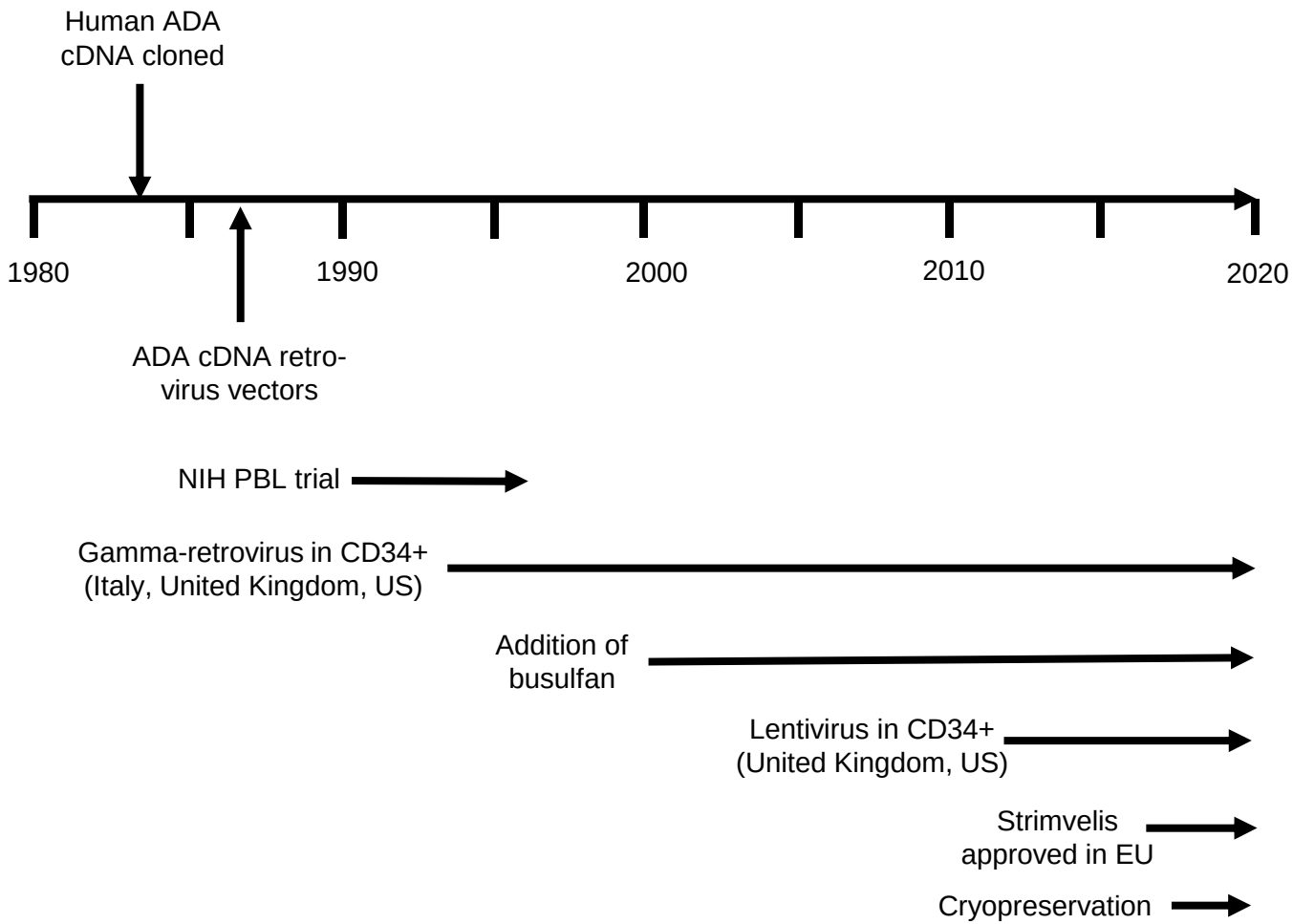
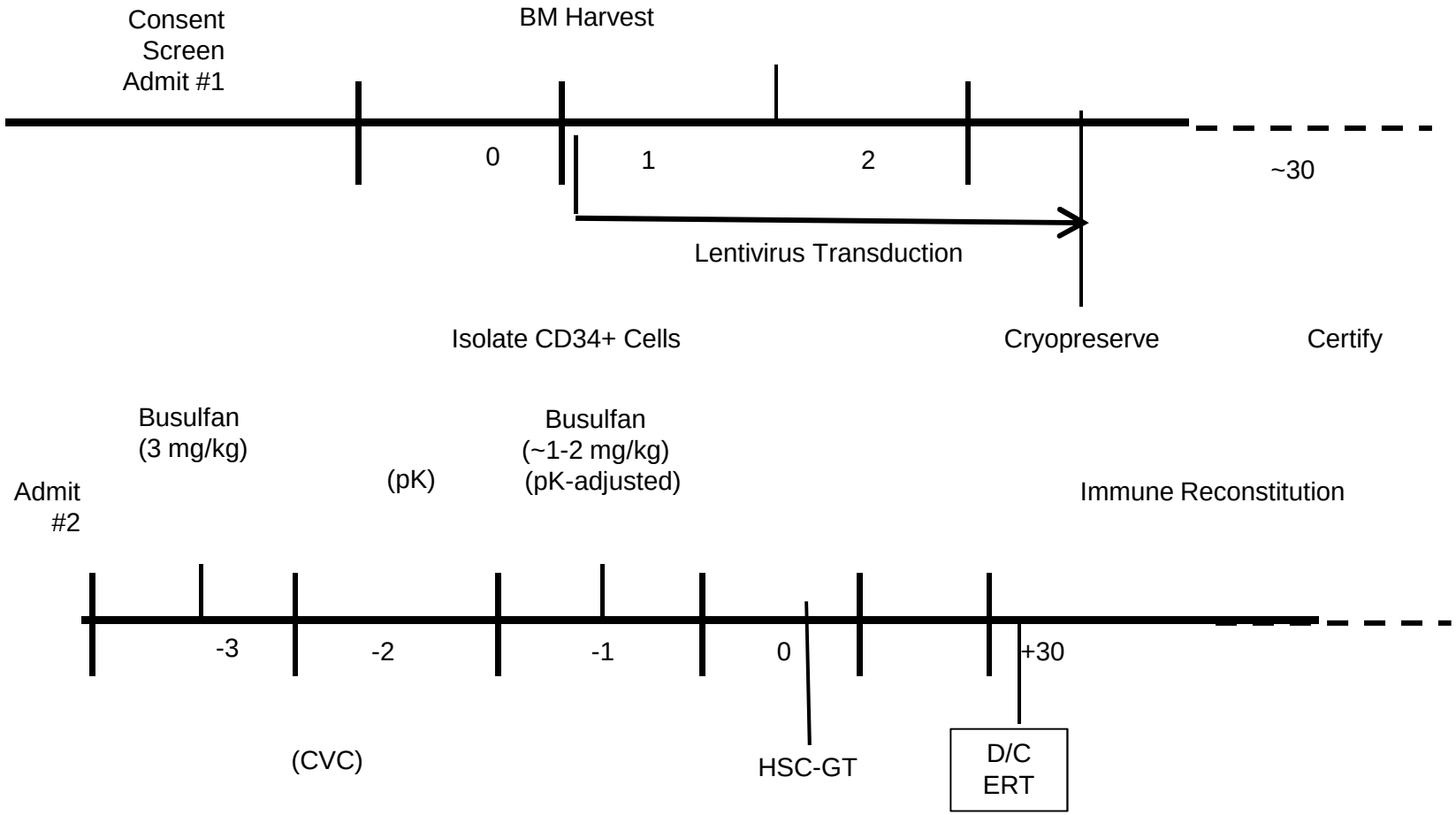


Figure 5: Scheme of cryopreserved lentivirus based gene therapy with pK-adjusted busulfan and continued enzyme replacement therapy.



6: Consensus algorithm for the management of infants diagnosed with ADA-SCID