HGT

Open camera or QR reader and scan code to access this article and other resources online.

Advances and Challenges in the Development of Gene Therapy Medicinal Products for Rare Diseases

Juan A. Bueren[1–3,†,*] and Alberto Auricchio[4,5,†]

[1]Biomedical Innovation Unit, Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain.
[2]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.
[3]Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Madrid, Spain.
[4]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy.
[5]Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy.
[†]These authors currently serve as President and Vice-President of the European Society for Gene and Cell Therapy, respectively.

The development of viral vectors and recombinant DNA technology since the 1960s has enabled gene therapy to become a real therapeutic option for several inherited and acquired diseases. After several ups and downs in the gene therapy field, we are currently living a new era in the history of medicine in which several *ex vivo* and *in vivo* gene therapies have reached maturity. This is testified by the recent marketing authorization of several gene therapy medicinal products. In addition, many others are currently under evaluation after exhaustive investigation in human clinical trials. In this review, we summarize some of the most significant milestones in the development of gene therapy medicinal products that have already facilitated the treatment of a significant number of rare diseases. Despite progresses in the gene therapy field, the transfer of these innovative therapies to clinical practice is also finding important restrictions. Advances and also challenges in the progress of gene therapy for rare diseases are discussed in this opening review of a *Human Gene Therapy* issue dedicated to the 30th annual Congress of the European Society for Gene and Cell Therapy.

Keywords: ATMPs, gene therapy, rare diseases

INTRODUCTION

In 1972, Friedmann and Roblin anticipated that gene therapy might ameliorate human genetic disorders.[1] Since then, several integrative and nonintegrative vectors have been generated. In parallel with this, hundreds of clinical trials have been initiated using *ex vivo* and *in vivo* gene therapy approaches with the aim of providing curative treatments for inherited and acquired diseases, many of which are life-threatening diseases affecting pediatric patients.[2,3] In addition to approaches based on the use of viral vectors, the generation of new nonviral vectors and gene editing tools has facilitated the design of gene targeting approaches not only in preclinical models but also in human clinical trials.[4,5]

In this study, we present a historic view in the development of *ex vivo* and *in vivo* gene therapy approaches that have resulted in the approval of innovative gene therapy medicinal products.

PROGRESS OF *EX VIVO* GENE THERAPIES FOR MONOGENIC DISORDERS

Ex vivo gene therapy approaches are based on the collection of target cells from the body of affected patients, followed by their *ex vivo* genetic correction and reinfusion in the patient. *Ex vivo* gene therapy has been mainly used for the treatment of diseases that had been successfully treated by allogeneic cell transplantation.

*Correspondence: Dr. Juan A. Bueren, Biomedical Innovation Unit. Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT), Avenida Complutense 40. 28040, Madrid, Spain. E-mail: juan.bueren@ciemat.es

DOI: 10.1089/hum.2023.152

This is the case of diseases treated by hematopoietic stem cell transplantation (HSCT), such as primary immunodeficiencies, red blood cell diseases and other blood-cell related disorders. Additionally, several neurometabolic conditions have been treated by HSC gene therapy based on the potential for genetically corrected hematopoietic cells to migrate and release the therapeutic transgenic protein to cells of the central nervous system (CNS).

In all these HSC gene therapies, autologous bone marrow (BM) or mobilized peripheral blood (PB) $CD34^+$ cells are purified, transduced *ex vivo*, and thereafter infused into the patient (Fig. 1), in many instances after conditioning regimens capable of depleting endogenous HSCs.[6]

In addition to HSC gene therapy, *ex vivo* gene therapy has been also used for the treatment of different genodermatoses, a group of genetic diseases frequently associated with severe skin lesions. Similar to HSC gene therapy, autologous skin biopsies from affected patients are obtained to establish keratinocyte cultures, which are then genetically modified *ex vivo* and used to cover denuded areas of the skin (Fig. 1).[7]

PIONEERING PRECLINICAL STUDIES OF HSC GENE THERAPY

The first preclinical studies demonstrating the insertion of genes into the genome of mouse HSCs were published in the years 1984–1986, and were based on the efficacy of gamma-retroviral vectors (RVs) to stably transfer marker genes into mouse BM cells.[8–10] These experimental studies not only demonstrated the stable gene marking of self-renewing HSCs but also showed the possibility of tracking genetically marked HSCs characterized by different repopulations and differentiation properties.

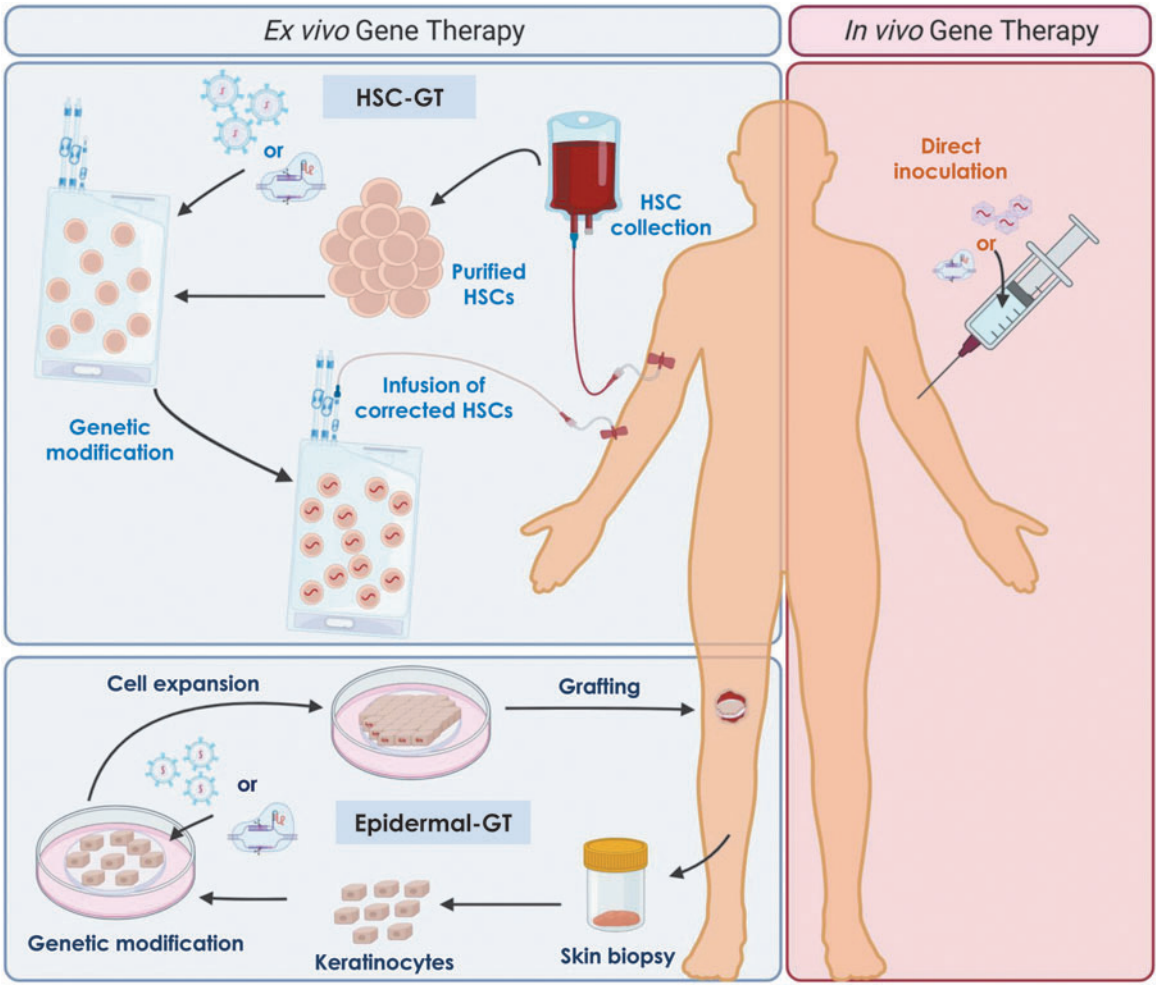

Figure 1. Schematic representation of *ex vivo* and *in vivo* gene therapies. The figure represents common procedures used for *ex vivo* and *in vivo* gene therapy. In the case of *ex vivo* gene therapies, autologous HSCs or keratinocytes are genetically modified *ex vivo* either with integration-competent vectors or with gene editing systems, and thereafter reinfused in the patient. In contrast to *ex vivo* gene therapy, *in vivo* gene therapy is based on the direct inoculation of vectors, in most instances AAVs, harboring the therapeutic sequences. Alternatively, nanoparticles carrying gene editing molecules are directly administered to the patient. AAV, adeno-associated viral; HSC, hematopoietic stem cell.

Since RVs could only stably transduce dividing cells, and given that under physiological conditions most HSCs are quiescent, a new HIV-derived self-inactivating lentiviral vector (SIN-LV) system capable of transducing quiescent cells was developed by L. Naldini et al. in 1996.[11] These SIN-LVs carry a large deletion in the U3 region of the 3′ LTR that completely inactivates its promoter/enhancer activity and in turn contain an internal promoter driving the transcription of the transgene. The relevance of these new vectors in HSC gene therapy became even more evident after the discovery of the genotoxic potential of RVs. Experimental studies conducted in 2002 at the Hannover Medical School showed for the first time the generation of leukemias in mice that had been infused with RV-transduced HSCs. Interestingly, these leukemias were associated with proviral insertions within the *Evi1* (ecotropic viral integration site-1) proto-oncogene, whose transactivation by the viral LTR promoter was considered the primary leukemogenic event.[12]

Subsequent studies related to these observations demonstrated that RVs had a preferential integration near the start of the transcriptional units, whereas LVs showed a preferential integration anywhere in the transcriptional unit.[13] Due to the different integration patterns of RVs and LVs and also because of the specific design of SIN-LVs, these vectors were shown to be much safer vectors compared with conventional RVs.[14] Similar to SIN-LVs, a second generation of RVs—SIN-RVs—was generated by C. Baum's team, which was also characterized by improved safety properties compared with the first generation RVs.[15–17]

INITIAL HSC GENE THERAPIES IN PATIENTS WITH PRIMARY IMMUNODEFICIENCIES

In parallel with advances in the design and use of RVs and LVs in disease animal models, several HSC gene therapy trials were initiated in the 1990s. The first clinical trial showing at least a minimal therapeutic benefit was conducted at the NIH in two children with adenosine deaminase severe combined immunodeficiency (SCID-ADA). In this trial, autologous T cells were transduced with an RV that carried a wild-type copy of the *ADA* gene. In contrast to current HSC gene therapy protocols based on a single infusion of transduced HSCs, up to 12 infusions of transduced T cells were administered to these patients, who remained dependent on enzyme replacement therapy.[18]

The efficacy of this gene therapy approach was improved by the combined administration of PB T cells with T cell-depleted BM cells, each transduced with a different ADA-RV. The cellular and humoral immunity of these patients was improved, and the discontinuation of the ADA replacement treatment resulted in progressive increases of corrected BM-derived PB T cells.[19] A further improvement of this protocol was based on the use of purified BM $CD34^+$ cells and the administration of a cytotoxic nonmyeloablative conditioning to facilitate the engraftment of corrected HSCs. In addition, in contrast to previous trials, these patients had not been pretreated by any ADA-enzyme replacement therapy, thus enhancing the proliferation advantage of corrected T cells. A sustained hematopoietic repopulation with gene-corrected myeloid and lymphoid cells was observed in these patients, who also showed marked improvements in their immune system.[20,21]

Subsequent data from SCID-ADA trials demonstrated that RV-mediated gene therapy had a favorable safety profile and was effective in restoring the immune function of these patients in the long term.[22,23] The clinical studies carried out by A. Aiuti et al. facilitated that in 2016 the San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) and GlaxoSmithKline (TIGET/Telethon-GSK) obtained the European marketing authorization for *Strimvelis*, an advanced therapy medicinal product (ATMP) that consisted of SCID-ADA $CD34^+$ cells transduced with the ADA-RV. These cells became the first genetically modified cell product approved as a commercial ATMP, which is currently licensed to Orchard Therapeutics (see Ref.[24] and Table 1).

The results of a new clinical trial in two patients with a different primary immunodeficiency, SCID-X1, were reported by Cavazzana and Fischer in the year 2000 at the Necker Children's Hospital. BM $CD34^+$ cells from these patients were transduced with an RV that carried the common *γ-chain cytokine receptor* (*γc*) gene and then infused into nonconditioned patients, as initially conducted in the first SCID-ADA trials. Normalized numbers of T, B, and NK cells with improved function were observed in most of these patients.[25] Similar results were observed in a clinical trial conducted by A. Thrasher's team at Great Ormon Street Hospital (GOSH) in four patients, all of whom showed substantial immunological and clinical improvements.[26]

Strikingly, 3 years after the initiation of the SCID-X1 trial in France, two patients showed deregulated premalignant cell proliferation due to the RV enhancer transactivation of the *LMO2* gene oncogene.[27,28] Similarly, clonal T cell acute lymphoblastic leukemias were observed in 2 of the 10 SCID-X1 patients treated in the United Kingdom,[29] revealing for the first time the leukemogenic potential of RVs in humans.

During those years, another RV-mediated gene therapy trial was conducted by M. Grez in Frankfurt. In this case, two adults with X-linked chronic granulomatous disease (X-CGD) were infused with $CD34^+$ cells that had been transduced with an RV carrying the *gp91phox* gene after conditioning with a nonmyeloablative treatment. In both patients, a high proportion of functionally corrected phagocytes were observed, although this process was associated with the expansion of gene-corrected myeloid cells due to insertional mutagenesis.[30] Unfortunately, both subjects developed myelodysplasia and showed silencing of transgene expression due to methylation of the viral promoter.[31]

Table 1. *List of gene therapy medicinal products with marketing authorization in Europe and the United States*

Modality	*Vector*	*Target*	*Via*	*Indication*	*Gene*	*Trade Name*	*Approval EU*	*Approval USA*	*Company*
Ex vivo	gRV	HSCs	IV	ADA-SCID	*ADA*	STRIMVELIS	May 2016	—	Orchard Ther
	SIN-LV	HSCs	IV	Transfusion-dependent β-Thalassemia	β^{A-T87Q} *globin*	ZYNTEGLO	June 2019 withdrawn March 2022	August 2022	bluebird bio
	SIN-LV	HSCs	IV	Metachromatic Leukodystrophy	*ARSA*	LIBMELDY	December 2020	—	Orchard Ther
	SIN-LV	HSCs	IV	Cerebral adrenoleukodystrophy	*ABCD1*	SKYSONA	July 2021 withdrawn November 2021	September 2022	bluebird bio
In Vivo (Viral)	AAV1	Muscles	IM	Lipoprotein lipase deficiency	*LPL*	GLYBERA	October 2012 Expired Oct 2017	—	Uniqure Biopharma/ Chiesi Farmaceutici
	AAV2	Retina	Sub-retinal	Leber congenital amaurosis type 2 Retinosis pigmentaria type 20	*RPE65v2*	LUXTURNA	Nov 2018	Dec 2017	Spark Ther/Novartis Europharm
	scAAV9	Motor neurons	IV	Spinal muscular atrophy	*SMN1*	ZOLGENSMA	May 2020	May 2019	Novartis
	AAV2	Brain	Intra-putamen	AADC deficiency	*DDC*	UPSTAZA	July 2022	—	PTC Therapeutics,
	AAV5	Liver	IV	Haemophilia A	*BDD-Factor VIII*	ROCTAVIAN	Aug 2022	June 2023	Biomarin International
	AAV5	Liver	IV	Haemophilia B	*Padua FactorIX*	HEMGENIX	Febr 2023	Nov 2022	CSL Behring Gmbh
	AAVrh74	Muscles	IV	Duchenne muscular dystrophy	*Micro-dystrophin*	ELEVIDYS	—	June 2023	Sarepta Therapeutics
	HSV1	Skin	Topic	Dystrophic epidermolysis bullosa	*Col7A1*	VYJUVEK	—	May 2023	Krystal Biotech
In Vivo (Non viral)	Antisense oligonucl.	Motor neurons	IT	Spinal muscular atrophy	*SMN2-ASO*	SPINRAZA	May 2017	Dec 2016	Biogen
	siRNA-liposome	PNs, Heart, Other	IV	Hereditary transthyretin amyloidosis	*Transtirethrin siRNA*	ONPATTRO	Aug 2018	Aug 2018	Alnylam Pharma
	siRNA	Liver	SC	Acute hepatic porphyria	*Aminolevulinate synthase 1-siRNA*	GIVOSIRAN	March 2020	Nov 2019	Alnylam Pharma
	siRNA	Liver/Kidney	SC	Primary hyperoxaluria type 1	*glycolate oxidase-siRNA*	OXLUMO	Nov 2020	Nov 2020	Alnylam Pharma

AADC, aromatic L-amino acid decarboxylase; AAV, adeno-associated viral; *ARSA*, arylsulfatase A; EU, European Union; gRV, gammaretroviral vector; HSCs, hematopoietic stem cells; HSV, herpes simplex virus; IM, intramuscular; IV, intravenous; IT, intrathecal; PN, peripheral nerves; SIN-LV, self-inactivated lentiviral vector.

Finally, another RV-mediated gene therapy trial was carried out at Muenchen University in patients with the Wiskott-Aldrich syndrome (WAS) who received busulfan conditioning before cell infusion. While 9 of 10 patients showed sustained engraftment and partial or complete resolution of the clinical signs of the disease (immunodeficiency, autoimmunity, and bleeding diathesis), 7 patients developed acute leukemia associated with RV genotoxicity.[32,33]

Risks associated with the use of RVs became evident in most HSC gene therapy trials,[3] which implied the necessity of improving the safety of therapeutic vectors in the clinic. In the particular case of SCID-ADA, an extremely low probability of insertional oncogenesis by the therapeutic RV has been observed,[3] probably due to the metabolic nature of the disease. Therefore, this is the only RV-mediated HSC gene therapy that continues under approved clinical practice.

SAFE AND EFFICIENT HSC THERAPIES WITH SELF-INACTIVATED RV AND LV IN MONOGENIC BLOOD CELL DISORDERS AND NEUROMETABOLIC DISEASES

The low genotoxic potential of SIN vectors was first demonstrated in preclinical studies,[14,34,35] and thereafter in numerous clinical trials in patients with hematopoietic and neurometabolic diseases (see review in Ref.[3]).

Primary immunodeficiencies

Due to the high frequency of leukemias observed in the RV-mediated WAS trial, a new trial was developed at the HSR-TIGET using an SIN-LV carrying the *WAS* gene under the control of its own promoter, which is currently licensed to Orchard. After infusion of transduced cells in three preconditioned WAS patients, stable long-term polyclonal hematopoietic reconstitution was seen in PB cells. In addition, increased numbers of T cell and platelets and protection from bleeding and severe infections were observed in most patients.[36,37] Another clinical trial in patients with WAS was carried out in France and in the United Kingdom using a similar SIN-LV (LV-w1.6 WASp). Also these patients showed sustained clinical benefit, including resolution to infections, eczema, and autoimmunity, and no signs of genotoxicity.[38]

Very recently, data from a new clinical trial in five patients with WAS who had been treated with the LV-w1.6 WASp have been reported. Clinical improvement of eczema, infections, and bleeding diathesis was observed in all these patients. In addition, although levels of the transgenic WAS protein were subphysiological, the immune function of these patients was also consistently improved.[39] These clinical studies reveal the efficacy and safety of LV-mediated gene therapy in a monogenic disease previously characterized with a high risk of RV-mediated leukemogenesis.

In the case of SCID-X1, a multinational gene therapy trial was conducted using an SIN-RV that carried the γc cDNA under the control of the human *elongation factor-1α* regulatory element (*hEF1α*). As in previous SCID-X1 trials with RVs, transduced $CD34^+$ cells were infused into patients who had not received any preparative conditioning. Seven of the eight evaluable patients showed recovery of functional PB T cells, as well as infection resolution. The kinetics of T cell recovery was similar to the one observed in trials with RVs, while no insertional oncogenesis events were evidenced in these patients.[40] A new clinical trial in patients with SCID-X1 was developed at the NIH using an *EF1α-γc* SIN-LV. In this case, five SCID-X1 patients who had been previously treated with haploidentical HSCT but with persistent immune dysfunction were infused with transduced $CD34^+$ cells after nonmyeloablative busulfan conditioning.

A significant expansion of corrected T, NK, and B cells, and a sustained restoration of humoral responses and clinical improvement were observed in these patients.[41] One additional trial in eight infants with SCID-X1 who received low-dose busulfan conditioning was conducted at the St. Jude Children's Research Hospital. Multilineage engraftment of transduced cells and a significant reconstitution of functional T cells, B cells, and NK cells were seen in most patients, who again showed polyclonal reconstitution without evidence of clonal dominance.[42]

Although the genotoxicity of RVs in SCID-ADA patients was shown to be extremely low,[3] a U.S./U.K. clinical trial was conducted with an SIN-LV in which the *ADA* gene was driven by the elongation factor short (*EFS*) promoter. A total of 50 patients were treated, resulting in more than 95% event-free survival and robust immune reconstitution, without evidence of leukemogenesis events in any of these patients.[43]

The outcome of patients treated in another SCID trial, Artemis-SCID, has been recently reported by UCSF Benioff Children's Hospital. In this case, $CD34^+$ cells were transduced with an SIN-LV carrying the human *Artemis* cDNA under the transcriptional regulation of its endogenous promoter, and then infused in busulfan-conditioned patients. Most of the treated patients showed T cell immune reconstitution, as well as sufficient B cell numbers to permit discontinuation of immunoglobulin G (IgG) infusions, without evidence of clonal expansions.[44]

A more recent trial was initiated at the Leiden University Medical Centre in patients with SCID-RAG1. In this trial, conditioned patients were infused with an SIN-LV in which the *RAG1* cDNA was driven by the myeloproliferative sarcoma virus regulatory sequences (*MND* promoter). As in the other SCID trials, the two patients so far included in this trial had shown improved immune function, with no insertional events predictive of genotoxic events.

As happened with other HSC trials, X-CGD gene therapy has progressed toward the initiation of new clinical trials by an international consortium that included UCLA, GOSH, and Net4GCD, among other partners. In this case, an SIN-LV carrying the codon-optimized *CYBBa* cDNA under the regulation of a chimeric promoter with regulatory elements of the *cathepsin G* and *Cfes* genes was constructed.[45] At 1 year post-treatment, most of the evaluable patients showed stable insertion of the therapeutic provirus in hematopoietic cells, as well as persistence of oxidase-positive neutrophils in PB, and no evidence of clonal dysregulation or transgene silencing. In addition, most of these patients were able to discontinue the antibiotic prophylaxis required for X-CGD patients.[46]

Another primary immunodeficiency associated with defects in the expression of β2-integrins, leukocyte adhesion immunodeficiency type I (LAD-I), has been recently addressed by LV-mediated gene therapy. The severe form of LAD-I remains a life-threatening condition with a very limited 2-year survival in the absence of HSCT.[47] An SIN-LV carrying the *ITGB2* gene driven by the *cathepsin G/Cfes* chimeric promoter was generated at CIEMAT.[48] Nine patients were conditioned with busulfan and then infused with transduced $CD34^+$ cells in a global phase I/II clinical trial sponsored by Rocket Pharmaceuticals, Inc. (Rocket Pharma). All treated patients have shown sustained gene marking and restored membrane expression of CD18, CD11a, and CD11b in PB leukocytes, as well as a marked reduction of infection-related hospitalizations.

As in the previous LV-mediated gene therapy trials, a highly polyclonal integration pattern has been observed in these patients,[49] revealing the favorable efficacy and safety profile of this new therapy for patients with severe LAD-I.

Red blood cell diseases

The first clinical evidence showing significant clinical improvement in a β-thalassemic patient treated by gene therapy was reported by Cavazzana-Calvo et al.[50] A busulfan-conditioned β^E/β^0 thalassemic patient was infused with $CD34^+$ cells transduced with an SIN-LV carrying the antisickling $\beta^{A\text{-}T87Q}$ gene that harbors a critical amino acid from the γ-globin chain that prevents HbS polymerization, and which is driven by regulatory elements of the β-globin locus. Net increases in red blood cells and transfusion independence were achieved in this patient. Although a myeloid-biased cell clone predominated in this patient for some time, this effect was shown to be transient and did not imply safety concerns.[50]

A subsequent trial used a similar LV (LentiGlobin BB305) in which insulator elements were removed to improve the vector titer. Most of the β^E/β^0 patients became transfusion independent, and β^0/β^0 patients either became transfusion independent or showed a significant reduction in the transfusion requirements.[51] In 2019, bluebird bio obtained the marketing authorization for *Zynteglo* ($CD34^+$ cells transduced with LentiGlobin BB305) from the European Commission, and then in 2022 from the U.S. FDA (see Table 1).

Additional clinical trials have also shown the efficacy of gene therapy in β-thalassemic patients. This was the case of

the clinical trial developed at HSR-TIGET in patients treated by the intrabone administration of $CD34^+$ cells transduced with the GLOBE LV. Transfusion requirements were reduced in the adults, and three of the four evaluable pediatric patients became transfusion independent.[52]

In the case of sickle cell disease (SCD), the first study showing clinical improvement was published in 2017 in a patient treated with the LentiGlobin BB305 LV.[53] The clinical improvement associated with this treatment has been recently confirmed in a cohort of 23 patients. In this trial funded by bluebird bio, almost 50% of the hemoglobin values corresponded to the antisickling globin, which led to reduced hemolysis and complete resolution of severe vaso-occlusive events.[54]

Because the expression of fetal hemoglobin (HbF) ameliorates the disease of patients with β-thalassemia and SCD, different approaches have been developed based on the major role of the zinc-finger transcription factor BCL11A in the silencing of γ-globin expression in adult human cells. These novel therapeutic strategies aim to interfere *BCL11A* expression, or alternatively to prevent the interaction of this repressor with γ-globin regulatory sequences. A LV-mediated gene therapy approach was conducted at Boston Children's Hospital and sponsored by bluebird bio for patients with SCD. In this trial, $CD34^+$ cells were transduced with an SIN-LV encoding a short-hairpin micro-RNA (*shmiR*) targeting the *BCL11A* mRNA. High levels of HbF and improved clinical status were observed in these patients.[55]

A different clinical approach sponsored by CRISPR Therapeutics and Vertex Pharmaceuticals aimed at the targeting of the *BCL11A* erythroid-specific enhancer with the CRISPR-Cas9 system. More than a year after the infusion of edited $CD34^+$ cells, marked increases in HbF were observed, concomitant with the achievement of transfusion independence.[56]

Additional gene therapy and editing trials have been conducted for hemoglobinopathies, which have been extensively reviewed by Ferrari et al. in this *Human Gene Therapy* issue.

A non-hemoglobinopathy RBC monogenic syndrome that has been recently treated by gene therapy is pyruvate kinase deficiency (PKD). This is a rare inherited hemolytic anemia caused by mutations in the *PKLR* gene. An SIN-LV carrying the *PKLR* gene under the regulation of the PGK promoter was generated at CIEMAT, which showed evident efficacy to correct the disease in PKD mouse models.[57] A global phase I clinical trial is currently ongoing under the sponsorship of Rocket Pharma in splenectomized and transfusion-dependent patients with severe PKD. Currently, three patients have been treated using myeloablative busulfan conditioning and infused with transduced $CD34^+$ cells. The two adult patients with longer follow-up have shown polyclonal HSC reconstitution and extensive improvement in their anemia to normal or near-normal levels, such that neither patient has required RBC transfusions postengraftment.[58]

BM failure syndromes

To date, Fanconi anemia (FA) is the only bone marrow failure (BMF) syndrome that has entered into gene therapy trials. Since FA cells are characterized by DNA repair defects and given that FA patients are prone to develop myelodysplasia, acute myeloid leukemia, and also solid tumors—even more frequently after allogeneic HSCT—none of the clinical trials conducted so far has used genotoxic conditioning before infusion of transduced cells.

In contrast to many other disorders, the efficacy of FA gene therapy has remained elusive for a long time.[59–62] Aiming at developing efficient gene therapy in FA patients, a new vector—PGK.FANCA.Wpre* LV—was generated at CIEMAT for FA patients of the FA-A complementation group. Using optimized transduction conditions, engraftment and a strong *in vivo* proliferation advantage of corrected FA-A HSCs were first demonstrated in transplanted immunodeficient mice.[63]

To improve the collection of HSCs from FA patients, an academic trial sponsored by Hospital Vall d'Hebron was developed. This trial showed the efficacy and safety of filgrastim and plerixafor to mobilize $CD34^+$ cells in patients with this BMF syndrome.[64] A subsequent gene therapy trial was then initiated in 2016 under the sponsorship of Hospital Infantil Universitario Niño Jesús. Based on the natural reversion of BMF observed in FA mosaic patients,[65] and given the experimental evidence showing *in vivo* proliferation advantage of transduced FA HSCs,[63] transduced FA $CD34^+$ cells were infused into patients in the absence of any conditioning regimen to minimize cancer risks. Remarkably, data reported during the short-term follow-up of the first treated patients showed progressive engraftment of multipotent HSCs that harbored the *FANCA* gene.[66]

More recent data corresponding to the follow-up of these patients for up to 7 years postinfusion have also revealed BMF reversion in patients with higher levels of engraftment.[67] In addition, comparative single-cell RNA sequencing analyses from corrected and uncorrected $CD34^+$ cells have also demonstrated the efficacy of gene therapy to correct the altered transcriptional program characteristic of FA HSCs.[68] These results constituted the basis for a global phase II trial in FA-A patients sponsored by Rocket Pharma. Current data of this trial are confirming the safety and also the efficacy of gene therapy in FA patients treated in the early stages of BMF; 7 of 12 patients treated in the absence of conditioning demonstrated evidence of progressively increasing BM and PB genetic correction, progressively increasing resistance of BM progenitor cells to DNA damage (mitomycin C resistance), and concomitant hematologic stability.[69]

Neurometabolic diseases

The first clinical trial conducted with an SIN-LV was initiated almost 20 years ago in two children with a severe

brain demyelinating disease, X-linked adrenoleukodystrophy (X-ALD). Autologous $CD34^+$ cells were transduced with an SIN-LV carrying the *ABCD1* cDNA under the control of the *MND* promoter. Transduced cells were then infused into patients who received myeloablative conditioning. All hematopoietic lineages showed ABCD1 expression. In addition, a halt in the progression of cerebral demyelination was observed, providing evidence of the clinical efficacy associated with the migration of gene-corrected hematopoietic cells of the CNS.[70] Additional gene therapy trials have been conducted in patients with ALD and other neurodegenerative monogenic disorders, in most instances using myeloablative conditioning regimens to facilitate the engraftment of transduced HSCs.

Seventeen patients were first treated at Harvard Medical School in a cerebral ALD (CALD) trial sponsored by bluebird bio. $CD34^+$ cells transduced with the Lenti-D vector (similar to the one used in the French trial) were infused. Most of these patients showed sustained engraftment of corrected cells and were free from major functional disability.[71] Recent studies have shown that 2 out of the 55 treated patients developed myelodysplastic syndrome, likely mediated by the provirus insertion and dysregulation of specific proto-oncogenes. This constituted the first genotoxic effect reported in an LV-mediated gene therapy trial, although in this case a viral promoter—the *MND U3* viral promoter—was used to drive therapeutic ABCD1 levels in cells of the CNS.

Despite these genotoxic events, the positive benefit/risk ratio of this therapy warranted the continuation of this trial in patients with such a severe disease. The ATMP consisting of autologous $CD34^+$ cells transduced with the ABCD1-LV (*Skysona*) received European marketing approval in 2021, as well as FDA-accelerated approval in 2022.

The first clinical trial in patients with metachromatic leukodystrophy (MLD) was carried out at HSR-TIGET, a lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. Patients with early-onset MLD were treated with $CD34^+$ cells that had been transduced with the *hPGK.ARSA* LV. A preliminary efficacy and safety trial showed high levels of enzyme expression not only in hematopoietic cells but also in the cerebrospinal fluid, halting the progression of the disease.[72] These results were confirmed thereafter in a total of 29 patients, most of whom showed preserved cognitive function and motor development.[73] No evidence of genotoxic insertions were observed in any of the treated patients, consistent with the safety of SIN-LVs harboring eukaryotic promoters. This gene therapy ATMP was licensed to Orchard Therapeutics, which obtained in December 2020 the European marketing authorization for *Libmeldy*.

Excellent outcomes have been also recently reported in a trial conducted at HSR-TIGET in patients with Hurler syndrome (mucopolysaccharidosis type I), also licensed to Orchard Therapeutics. Affected children were infused with autologous $CD34^+$ cells transduced with an SIN-LV carrying the α-L-iduronidase (*IDUA*) driven by the *hPGK* promoter. All the eight treated patients showed IDUA activity in their cerebrospinal fluid, and stable cognitive performance and motor skills.[74]

Preliminary results from a clinical trial conducted by a Canada/U.S. consortium have been reported in patients with type 1 Fabry disease who were infused with $CD34^+$ cells transduced with an SIN-LV carrying the alpha-galactosidase A gene (*EF1α-αGal A* LV). Three of the five treated patients could discontinue the enzyme replacement therapy or up to 3 years of follow-up, and neither in this case serious adverse events associated with the investigational product were observed.[75]

Taken together, the results of clinical trials in patients with specific neurometabolic diseases indicate that HSC gene therapy can be efficiently and safely used to deliver therapeutic proteins to cells of the CNS, halting the progression of these neurodegenerative diseases in most patients.

ADVANCES OF *EX VIVO* GENE THERAPY FOR RARE SKIN DISEASES

Following similar processes such as those developed for HSC gene therapy, *ex vivo* gene therapy has been also used for the treatment of patients with severe genetic skin lesions known as genodermatoses (Fig. 1).

The first *ex vivo* clinical gene therapy study in a patient affected by LAM5-β3-deficient junctional epidermolysis bullosa (JEB) was conducted by Mavilio et al. at the University of Modena and Reggio Emilia in 2006. Keratinocyte cultures from an adult JEB patient were transduced with an RV carrying the *LAMB3* cDNA. Corrected epidermal grafts were then transplanted onto surgically prepared regions of the patient. A firmly adherent epidermis in the absence of blisters was observed in this patient during the 1-year follow up of this study. Analyses of the provirus integration sites showed that regenerated epidermis was maintained by a defined repertoire of transduced stem cells and did not evidence genotoxicity risks such as the ones reported in RV-mediated HSC gene therapy.[76]

In 2016, the results of a clinical trial in patients with recessive dystrophic epidermolysis bullosa, another devastating skin disease generated by mutations in *collagen type VII alpha 1 chain* (*COL7A1*), were reported by Siprashvili et al. from Stanford University. Autologous keratinocytes from four patients were transduced with an RV carrying full-length *hCOL7A1* cDNA, and then transplanted onto each of the patients. This trial showed expression of type VII collagen at the dermal–epidermal junction. Although wound healing was noted in some of

the corrected grafts, variable responses that generally declined over time were observed.[77]

In 2017, a compassionate life-saving gene therapy treatment was initiated at Ruhr University Hospital and the University of Modena for a patient with a devastating life-threatening form of JEB generated by a splice site mutation in *LAMB3*. A skin biopsy from a nonblistering area was used to establish keratinocyte cultures, which were then transduced with an RV carrying the *LAMB3* cDNA. Transduced epidermal grafts were applied on prepared dermal wound beds. The results of this clinical study showed that transduced keratinocytes regenerated a fully functional epidermis on the patient. Insertion site and clonal tracing studies revealed that human epidermis was sustained by a limited number of long-lived stem cells that could extensively self-renew and repopulate terminally differentiated keratinocytes.[78]

A more recent trial was performed at the GOS Institute of Child Health in six patients with Netherton syndrome (NS) caused by mutations in *SPINK5*. In this trial, keratinocytes were transduced with an SIN-LV carrying the *SPINK5* under the control of the human involucrin promoter. Genetically modified epithelial sheets were successfully generated in three subjects, although only transient functional correction was observed.[79]

The advantages and also limitations currently associated with the *ex vivo* gene therapy of patients with severe skin diseases have been recently described.[7] Alternative approaches based on *in vivo* gene therapy have been developed for the treatment of patients with dystrophic epidermolysis bullosa (DEB), in this case using a herpes simplex virus type I (HSV-I), which has very recently obtained marketing authorization in the United States (see Table 1).

THE (R)EVOLUTION OF *IN VIVO* GENE THERAPY

Efforts to deliver genes *in vivo* for the therapy of human diseases, whether inherited or acquired, began at the beginning of the 1990s around the same time *ex vivo* gene therapy was developed. In those times, the most efficient vector system that emerged for transducing several tissues and organs to a high level, particularly the liver, was based on a replication-defective adenovirus serotype 5.[80] However, its short-lived transgene expression due to cell-mediated immunity against transduced cells that expressed residual adenoviral proteins made it clear that a ''naked'' vector was needed *in vivo*.[80] In addition, acute toxicity was observed following systemic administration of Ad vectors which caused the first gene therapy death in a clinical trial.[80] While ''gutted'' helper-dependent adenoviral vectors were generated that were devoid of any adenoviral coding sequences and resulted in long-term transgene expression, their systemic administration was still associated with acute toxicity. Therefore, a less immunogenic vector system than the one based on adenovirus was needed for *in vivo* gene therapy of human diseases that require long-lasting transgene expression. Among the other viruses that were explored as vectors for *in vivo* gene therapy, one emerged as the most promising for its unique combination of low immunogenicity and high transduction levels in nonreplicating tissues.

The first recombinant adeno-associated viral (AAV) vectors were generated by the group of Muczyczka and Samulski at the University of Florida[81] based on the rationale that parental AAV vectors are naturally replication-deficient in the absence of helper viruses and that no human diseases were associated with AAV infection. AAV is a small nonenveloped virus that contains a short single-stranded DNA genome of about 5 kb.[82] The recombinant vector only contains the inverted terminal repeats and no viral coding sequences.[82] The strengths and weaknesses of this Trojan horse reside in its small size.

On one hand, it crosses multiple barriers, including endothelial cells that line vessels[83] and the blood–brain barrier.[84] This unique ability has been instrumental to generate effective therapies for untreatable deadly diseases, such as spinal muscular atrophy or Duchenne muscular dystrophy (DMD) where affected tissues are hard to transduce due to the presence of physical barriers and/or require body-wide targeting that can be achieved following a simple intravenous administration. On the other hand, AAV does not package genomes larger than 5 kb efficiently, which prevents its use in gene therapy for conditions that require the delivery of large coding sequences.

Over the last 20 years, a number of improvements to the original AAV serotype 2 (AAV2) delivery system have been brought forward, including among others, (1) the discovery of dozens of natural AAV serotypes and the development of engineered capsid variants through a rational design or directed evolution that provide a unique portfolio of vectors with different tropisms and transduction characteristics,[85] (2) self-complementary AAV vectors that contain a double-stranded, shorter genome, which overcomes the second-strand synthesis limitation step for transduction,[86] and (3) dual AAV vectors that deliver larger genes split into two halves each independently packaged in AAV that are then reconstituted in a single larger gene[87] or larger protein[87] in target cells upon coinfection by both dual vectors, which doubles AAV transfer capacity.

The first *in vivo* gene therapy product approved in the Western world was *Glybera* in 2012, an AAV1 delivered intramuscularly in patients with a monogenic form of hyperlipidemia[88] (Table 1). The combination of the high cost of treatment and the rarity of the condition led to Glybera market withdrawal after only one patient received the approved drug.[89] This was the first alarm pointing to the complex challenge of the sustainability of gene therapies.

It took a few more years to get to the first AAV-based ATMP approved both by EMA and FDA. This was the

case of *Luxturna*, an AAV2 vector delivered intraocularly for the treatment of an inherited severe form of childhood blindness (Table 1).[90] This success was built on a multi-year effort from the University of Pennsylvania/Children's Hospital of Philadelphia at the beginning of 2000s with a milestone proof-of-concept in a dog model of Leber congenital amaurosis type 2 (LCA2).[91] This led to a successful multicenter phase I/II clinical trial[92] followed by a phase III trial, which unequivocally showed the efficacy of the treatment in addition to its safety.[92]

Another major breakthrough was the accelerated approval of *Zolgensma*, a life-saving gene therapy based on intravenous delivery of high doses of self-complementary AAV9 for treatment of type 1 spinal muscular atrophy (SMA1; Table 1). In the phase I/II study that led to approval, all SMA1 patients survived beyond 2 years of age at which patients with their genotype typically die, and some of them reached and maintained critical psychomotor milestones, which have never been achieved before, with most improvement observed in patients treated in the first few weeks/months after birth.[93] This highlighted the need for newborn screening to allow early intervention in those lethal and progressive conditions for which a therapy becomes available.

In the last 2–3 years, several AAV-based products have been additionally approved, including one for a monogenic form of Parkinson's disease based on local intracerebral delivery of the therapeutic vector, *Upstaza* (Table 1),[94] and two AAVs for hemophilia A (*Roctavian*) and hemophilia B (*Hemgenix*). These are provided as a single intravenous administration of the vector that predominantly targets the liver, which is thus converted in a factory for systemic long-term secretion of the clotting factor, therefore eliminating the need for prophylactic or therapeutic infusions of the protein[95,96] (see review from T. Vandendriessche in this issue of *Human Gene Therapy* and Table 1).

Another AAV vector that is given as a single intravenous injection at high doses to target muscle in patients with DMD has been approved in June 2023, *Elevidys* (Table 1).[97] It has taken decades to reach this landmark approval given the challenges posed by the large size of the dystrophin coding sequence, which had to be reduced to fit in a single AAV vector, and the need to target muscles body-wide for the therapy to be effective.

Very recently, a herpes simplex virus type 1 (HSV-1) vector has received approval for commercialization in US for the treatment of both the dominant and recessive forms of dystrophic epidermolysis bullosa (DEB) with mutations in the *collagen type VII alpha 1 chain (COL7A1)* gene. *Vyjuvek* is a topically applied HSV-1 vector generated by Krystal Biotech that can transduce keratinocytes and fibroblasts, thus restoring the expression of collagen type VII in patients with DEB[98] (Table I).

In addition to viral-based ATMPs that are currently on the market, several non-viral gene therapy ATMPs were approved since 2016 for the treatment of rare genetic diseases (Table 1). *Spinraza* is an antisense oligonucleotide that is administered intrathecally in patients with spinal muscular atrophy, enabling the *SMN2* gene to produce the full-length protein, thus replacing the missing SMN protein. A number of small-interfering RNA products have also received commercial authorization (Table I). These include, *Onpattro*, an siRNA liposome that down-regulates the expression of defective transthyretin, thereby reducing the formation of amyloids and relieving the symptoms of hereditary transthyretin (hATTR) amyloidosis, and two others *Givosiran* and *Oxlumo* for acute hepatic porphyria and primary hyperoxaluria type I, respectively.

As happened in the field of *ex vivo* gene therapy, new viral and nonviral vectors are now in advanced clinical trials for the *in vivo* treatment of genetic and acquired diseases. For those that require systemic administrations of high doses of AAV vectors such as SMA1 or DMD, rare acute severe adverse events, including thrombotic microangiopathy (TMA) and deaths have been reported that are due to the immunogenicity of high AAV capsid loads.[99] These events were observed more consistently in a clinical gene therapy trial for X-linked myotubular myopathy where an underlying liver disease was present.[100]

In vivo genome editing is currently being explored for therapeutic purposes, and the retina has been at the forefront of this development given its favorable risk/benefit. The first-in-human of CRISPR-Cas9 has occurred in patients with a rare form of childhood blindness, Leber congenital amaurosis type 10.[101] Despite promising clinical safety data, the sponsor has discontinued investments in this and other ocular indications. In the case of liver diseases, the efficient targeting of hepatocytes following a single systemic administration of CRISPR-Cas9-lipid nanoparticles has resulted in the significant phenotype improvement of patients with transthyretin amyloidosis,[102] suggesting that the time is ripe for further implementation of liver genome editing with nonviral delivery methods.

CONCLUDING REMARKS

Advances in the generation of integration competent vectors have markedly improved the efficacy and also the safety associated with *ex vivo* HSC gene therapies during the last decade. Consequently, several gene therapy HSC-based ATMPs have obtained marketing authorization in Europe and in the United States (Table 1). Following the first European marketing authorization for *Strimvelis* (HSCs transduced with ADA-RVs; Orchard therapeutics, EU-2016), additional ATMPs consisting of LV-transduced HSCs have also obtained marketing approval in different countries. This is the case for *Zynteglo* (HSCs transduced with the LentiGlobin BB305; bluebird bio, EU-2019 and US-2022) for the treatment of β-thalassemia, and also for *Libmeldy* (HSCs transduced with the ARSA-

LV for MLD; Orchard Therapeutics, EU-2020) and for *Skysona* (HSCs transduced with the ABCD1-LV for CALD; bluebird bio, EU-2021 and US-2022).

Unfortunately, some of these ATMPs (*Zynteglo* and *Skysona*) have been recently discontinued in Europe because of commercial disagreements. Also, the SCID-ADA and WAS gene therapy programs will probably be discontinued due to commercial issues. The complexity and thus the cost of these *ex vivo* gene therapies have raised unexpected alarms in their application to diseases with unmet clinical needs.

In addition to *ex vivo* gene therapy, *in vivo* gene therapy has shown to be a safe and an effective therapy in humans. However, also some challenges remain to make this transformative therapeutic approach widely available. One is the high prevalence of preexisting anti-AAV immunity in humans who are natural hosts for this virus.[103] This is being approached by a variety of strategies, including pretreatment with IgG-cleaving enzymes that transiently deplete neutralizing antibodies that would otherwise block AAV infection following their systemic administration.[104] Another is the human-specific cell-mediated immune response to AAV vector-transduced cells, which results in their elimination. This can be resolved in several cases by administration of a short regimen of oral corticosteroids.[103]

History has taught us that it takes decades to characterize the safety and efficacy of a gene delivery platform such as AAV before it can be used reliably in patients. Although the accelerated path of anti-COVID-19 vaccines has shown that this time can be significantly shortened, only time will tell if there will be a paradigm shift in *in vivo* gene therapy from AAV to nonviral vectors.

As it is the case with *ex vivo* gene therapies, the complexity and high costs associated with the large-scale manufacturing of viral vectors used for *in vivo* gene therapy currently pose challenges to the sustainability of this therapeutic modality. The development of easy-to-manufacture nonviral vectors such as lipid and nonlipid nanoparticles similar to the ones used for the COVID-19 vaccination, should indeed help in the development of more accessible gene therapy ATMPs. Additionally, the combination of these new delivery systems together with the revolution of versatile and precise approaches of genome editing should further enhance the implantation of personalized gene therapy in the clinic.

Common efforts from pharmaceutical companies, scientists, clinicians, and regulators are required to find imaginative approaches that would improve the accessibility of affordable gene therapy medicinal products for the treatment of a much higher proportion of patients with rare diseases.[105,106]

AUTHOR DISCLOSURE

J.A.B. is coinventor on patents licensed to Rocket Pharmaceuticals, Inc., related to the use of LVs for gene therapy and is a consultant and shareholder from the same company. A.A. is coinventor of patents related to the use of AAV vectors for gene therapy. A.A. is cofounder, shareholder, and consultant of InnovaVector srl and AA-VantardeBio srl.

FUNDING INFORMATION

J.A.B. acknowledges the following Institutions for support of gene therapy programs at CIEMAT: ''Ministerio de Ciencia e Innovación y Competitividad y Fondo Europeo de Desarrollo Regional (FEDER)'' (SAF2015-68073-R, SAF2015-64152-R, and RTI2018-097125-B-I00); Next Generation EU; Plan de Recuperación Transformación y Resilencia (Instituto de Salud Carlos III; TERAV) (RD12/0019/0023); Programs of the European Commission (HEALTHF5-2012-305421 and EUROFANCOLEN); the ''Ministerio de Sanidad, Servicios Sociales e Igualdad'' (EC11/060 and EC11/550). A.A. acknowledges funding from the European Union's EIC Pathfinder program under grant agreement No. 101071041 ''AAVolution'', the European Union's Horizon 2020 Research and Innovation Program under Grant Agreement No 825825 ''Upgrade'', the Foundation Fighting Blindness Project Grant No. TA-NMT-0621-0815-TIGEM ''RetMir,'' and the EU- NextGenerationEU grant CN00000041 ''National Center for Gene Therapy and Drugs based on RNA Technology'' (concession number 1035 of June 17, 2022-PNRR MUR-M4C2—Investment 1.4 Call ''National Centers''), code project (CUP): E63C22000940007.

REFERENCES

1. Friedmann T, Roblin R. Gene therapy for human genetic disease? Science 1972;175(4025):949–955; doi: 10.1126/SCIENCE.175.4025.949

2. Dunbar CE, High KA, Joung JK, et al. Gene therapy comes of age. Science 2018;359(6372):eaan4672; doi: 10.1126/SCIENCE.AAN4672

3. Tucci F, Galimberti S, Naldini L, et al. A systematic review and meta-analysis of gene therapy with hematopoietic stem and progenitor cells for monogenic disorders. Nat Commun 2022;13(1):1315; doi: 10.1038/s41467-022-28762-2

4. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012;337(6096):816–821; doi: 10.1126/SCIENCE.1225829

5. Raguram A, Banskota S, Liu DR. Therapeutic in vivo delivery of gene editing agents. Cell 2022;185(15):2806–2827; doi: 10.1016/J.CELL.2022.03.045

6. Ferrari G, Thrasher AJ, Aiuti A. Gene therapy using haematopoietic stem and progenitor cells. Nat Rev Genet 2021;22(4):216–234; doi: 10.1038/S41576-020-00298-5

7. Jayarajan V, Kounatidou E, Qasim W, et al. Ex vivo gene modification therapy for genetic skin diseases-recent advances in gene modification technologies and delivery. Exp Dermatol 2021;30(7):887–896; doi: 10.1111/EXD.14314

8. Williams DA, Lemischka IR, Nathan DG, et al. Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse. Nature 1984;310(5977):476–480; doi: 10.1038/310476A0

9. Dick JE, Magli MC, Huszar D, et al. Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/Wv mice. Cell 1985; 42(1):71–79; doi: 10.1016/S0092-8674(85)80102-1

10. Lemischka IR, Raulet DH, Mulligan RC. Developmental potential and dynamic behavior of hematopoietic stem cells. Cell 1986;45(6):917–927; doi: 10.1016/0092-8674(86)90566-0

11. Naldini L, Blömer U, Gallay P, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 1996; 272(5259):263–267; doi: 10.1126/SCIENCE.272.5259.263

12. Li Z, Düllmann J, Schiedlmeier B, et al. Murine leukemia induced by retroviral gene marking. Science 2002;296(5567):497; doi: 10.1126/SCIENCE.1068893

13. Wu X, Li Y, Crise B, et al. Transcription start regions in the human genome are favored targets for MLV integration. Science 2003; 300(5626):1749–1751; doi: 10.1126/SCIENCE.1083413

14. Montini E, Cesana D, Schmidt M, et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 2006;24(6):687–696; doi: 10.1038/NBT1216

15. Kraunus J, Schaumann DHS, Meyer J, et al. Self-inactivating retroviral vectors with improved RNA processing. Gene Ther 2004;11(21):1568–1578; doi: 10.1038/SJ.GT.3302309

16. Zychlinski D, Schambach A, Modlich U, et al. Physiological promoters reduce the genotoxic risk of integrating gene vectors. Mol Ther 2008; 16(4):718–725; doi: 10.1038/MT.2008.5

17. Modlich U, Navarro S, Zychlinski D, et al. Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. Mol Ther 2009;17(11):1919–1928; doi: 10.1038/MT.2009.179

18. Blaese RM, Culver KW, Miller AD, et al. T lymphocyte-directed gene therapy for ADA-SCID: Initial trial results after 4 years. Science 1995;270(5235):475–480; doi: 10.1126/SCIENCE.270.5235.475

19. Bordignon C, Notarangelo LD, Nobili N, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. Science 1995;270(5235):470–475; doi: 10.1126/SCIENCE.270.5235.470

20. Aiuti A, Slavin S, Aker M, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science 2002;296(5577):2410–2413; doi: 10.1126/SCIENCE.1070104

21. Aiuti A, Cattaneo F, Galimberti S, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 2009; 360(5):447–458; doi: 10.1056/NEJMOA0805817

22. Ferrua F, Aiuti A. Twenty-five years of gene therapy for ADA-SCID: From bubble babies to an approved drug. Hum Gene Ther 2017;28(11):972–981; doi: 10.1089/HUM.2017.175

23. Shaw KL, Garabedian E, Mishra S, et al. Clinical efficacy of gene-modified stem cells in adenosine deaminase-deficient immunodeficiency. J Clin Invest 2017;127(5):1689–1699; doi: 10.1172/JCI90367

24. Aiuti A, Roncarolo MG, Naldini L. Gene therapy for ADA-SCID, the first marketing approval of an ex vivo gene therapy in Europe: Paving the road for the next generation of advanced therapy medicinal products. EMBO Mol Med 2017;9(6): 737–740; doi: 10.15252/EMMM.201707573

25. Cavazzana-Calvo M, Hacein-Bey S, De Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 2000;288(5466):669–672; doi: 10.1126/SCIENCE.288.5466.669

26. Gaspar HB, Parsley KL, Howe S, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet 2004;364(9452):2181–2187; doi: 10.1016/S0140-6736(04)17590-9

27. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 2003;302(5644):415–419; doi: 10.1126/SCIENCE.1088547

28. Hacein-Bey-Abina S, Garrigue A, Wang GP, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008;118(9):3132–3142; doi: 10.1172/JCI35700

29. Howe SJ, Mansour MR, Schwarzwaelder K, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 2008;118(9):3143–3150; doi: 10.1172/JCI35798

30. Ott MG, Schmidt M, Schwarzwaelder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 2006;12(4):401–409; doi: 10.1038/NM1393

31. Stein S, Ott MG, Schultze-Strasser S, et al. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med 2010;16(2):198–204; doi: 10.1038/NM.2088

32. Boztug K, Schmidt M, Schwarzer A, et al. Stem-cell gene therapy for the Wiskott-Aldrich syndrome. N Engl J Med 2010;363(20):1918–1927; doi: 10.1056/NEJMOA1003548

33. Braun CJ, Boztug K, Paruzynski A, et al. Gene therapy for Wiskott-Aldrich syndrome—Long-term efficacy and genotoxicity. Sci Transl Med 2014;6(227):227ra33; doi: 10.1126/SCITRANSLMED.3007280

34. Modlich U, Bohne J, Schmidt M, et al. Cell-culture assays reveal the importance of retroviral vector design for insertional genotoxicity. Blood 2006;108(8):2545–2553; doi: 10.1182/blood-2005-08-024976

35. Schwarzer A, Talbot SR, Selich A, et al. Predicting genotoxicity of viral vectors for stem cell gene therapy using gene expression-based machine learning. Mol Ther 2021;29(12):3383–3397; doi: 10.1016/j.ymthe.2021.06.017

36. Aiuti A, Biasco L, Scaramuzza S, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science 2013; 341(6148); doi: 10.1126/SCIENCE.1233151

37. Ferrua F, Cicalese MP, Galimberti S, et al. Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: Interim results of a non-randomised, open-label, phase 1/2 clinical study. Lancet Haematol 2019;6(5):e239–e253; doi: 10.1016/S2352-3026(19)30021-3

38. Hacein-Bey Abina S, Gaspar HB, Blondeau J, et al. Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. JAMA 2015;313(15):1550–1563; doi: 10.1001/JAMA.2015.3253

39. Labrosse R, Chu J, Armant M, et al. Outcomes of hematopoietic stem cell gene therapy for Wiskott-Aldrich Syndrome. Blood 2023; In Press; doi: 10.1182/BLOOD.2022019117

40. Hacein-Bey-Abina S, Pai S-Y, Gaspar HB, et al. A modified γ-retrovirus vector for X-linked severe combined immunodeficiency. N Engl J Med 2014;371(15):1407–1417; doi: 10.1056/NEJMOA1404588

41. De Ravin SS, Wu X, Moir S, et al. Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 2016;8(335):335ra57; doi: 10.1126/SCITRANSLMED.AAD8856

42. Mamcarz E, Zhou S, Lockey T, et al. Lentiviral gene therapy combined with low-dose busulfan in infants with SCID-X1. N Engl J Med 2019;380(16): 1525–1534; doi: 10.1056/NEJMOA1815408

43. Kohn DB, Booth C, Shaw KL, et al. Autologous ex vivo lentiviral gene therapy for Adenosine Deaminase Deficiency. N Engl J Med 2021;384(21): 2002–2013; doi: 10.1056/NEJMOA2027675

44. Cowan MJ, Yu J, Facchino J, et al. Lentiviral gene therapy for artemis-deficient SCID. N Engl J Med 2022;387(25):2344–2355; doi: 10.1056/NEJMOA2206575

45. Santilli G, Almarza E, Brendel C, et al. Biochemical correction of X-CGD by a novel chimeric promoter regulating high levels of transgene expression in myeloid cells. Mol Ther 2011;19(1): 122–132; doi: 10.1038/MT.2010.226

46. Kohn DB, Booth C, Kang EM, et al. Lentiviral gene therapy for X-linked chronic granulomatous disease. Nat Med 2020;26(2):200–206; doi: 10.1038/S41591-019-0735-5

47. Almarza Novoa E, Kasbekar S, Thrasher AJ, et al. Leukocyte adhesion deficiency-I: A comprehensive review of all published cases. J Allergy Clin Immunol Pract 2018;6(4):1418–1420.e10; doi: 10.1016/J.JAIP.2017.12.008

48. Leon-Rico Di, Aldea M, Sanchez-Baltasar R, et al. Lentiviral vector-mediated correction of a mouse model of leukocyte adhesion deficiency type I. Hum Gene Ther 2016;27(9):668–678; doi: 10.1089/HUM.2016.016

49. Kohn D, Sevilla J, Rao G, et al. Autologous ex-vivo lentiviral gene therapy for pediatric patients with Severe Leukocyte Adhesion Deficiency-I (LAD-I): Interim results from an Ongoing Phase 1/2 Study. Mol Ther 2023;31(4S1):727–728.

50. Cavazzana-Calvo M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature 2010;467(7313):318–322; doi: 10.1038/NATURE09328

51. Thompson AA, Walters MC, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent β-thalassemia. N Engl J Med 2018;378(16):1479–1493; doi: 10.1056/nejmoa1705342

52. Marktel S, Scaramuzza S, Cicalese MP, et al. Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ß-thalassemia. Nat Med 2019;25(2):234–241; doi: 10.1038/S41591-018-0301-6

53. Ribeil J-A, Hacein-Bey-Abina S, Payen E, et al. Gene therapy in a patient with sickle cell disease. N Engl J Med 2017;376(9):848–855; doi: 10.1056/nejmoa1609677

54. Kanter J, Walters MC, Krishnamurti L, et al. Biologic and clinical efficacy of lentiglobin for sickle cell disease. N Engl J Med 2022;386(7):617–628; doi: 10.1056/nejmoa2117175

55. Esrick EB, Lehmann LE, Biffi A, et al. Post-transcriptional genetic silencing of BCL11A to treat sickle cell disease. N Engl J Med 2021;384(3):205–215; doi: 10.1056/NEJMOA2029392

56. Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med 2021;384(3):252–260; doi: 10.1056/NEJMOA2031054

57. Garcia-Gomez M, Calabria A, Garcia-Bravo M, et al. Safe and efficient gene therapy for Pyruvate Kinase Deficiency. Mol Ther 2016;24(7):1187–1198; doi: 10.1038/MT.2016.87

58. Shah AJ, Lopez Lorenzo JL, Sevilla J, et al. Global Phase 1 Study results of lentiviral mediated gene therapy for severe pyruvate kinase deficiency. Mol Ther 2023;31(4S1):118–119.

59. Liu JM, Young NS, Walsh CE, et al. Retroviral mediated gene transfer of the Fanconi anemia complementation group C gene to hematopoietic progenitors of group C patients. Hum Gene Ther 1997;8(14):1715–1730; doi: 10.1089/HUM.1997.8.14-1715

60. Liu JM, Kim S, Read EJ, et al. Engraftment of hematopoietic progenitor cells transduced with the Fanconi Anemia Group C Gene (FANCC). Hum Gene Ther 1999;10(14):2337–2346; doi: 10.1089/10430349950016988

61. Kelly PF, Radtke S, von Kalle C, et al. Stem cell collection and gene transfer in Fanconi anemia. Mol Ther 2007;15(1):211–219; doi: 10.1038/SJ.MT.6300033

62. Adair JE, Chandrasekaran D, Sghia-Hughes G, et al. Novel lineage depletion preserves autologous blood stem cells for gene therapy of Fanconi anemia complementation group A. Haematologica 2018;103(11):1806–1814; doi: 10.3324/HAEMATOL.2018.194571

63. Río P, Navarro S, Guenechea G, et al. Engraftment and in vivo proliferation advantage of gene-corrected mobilized CD34+ cells from Fanconi anemia patients. Blood 2017;130(13):1535–1542; doi: 10.1182/BLOOD-2017-03-774174

64. Sevilla J, Navarro S, Rio P, et al. Improved collection of hematopoietic stem cells and progenitors from Fanconi anemia patients for gene therapy purposes. Mol Ther Methods Clin Dev 2021;22:66–75; doi: 10.1016/J.OMTM.2021.06.001

65. Ramírez MJ, Pujol R, Trujillo-Quintero JP, et al. Natural gene therapy by reverse mosaicism leads to improved hematology in Fanconi anemia patients. Am J Hematol 2021;96(8):989–999; doi: 10.1002/AJH.26234

66. Río P, Navarro S, Wang W, et al. Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia. Nat Med 2019;25(9):1396–1401; doi: 10.1038/S41591-019-0550-Z

67. Rio P, Sevilla J, Navarro S, et al. Long-term phenotypic correction of Fanconi Anemia-A patients treated by gene therapy in early stages of the bone marrow failure. Mol Ther 2022;30(4S1):370.

68. Lasaga M, Río P, Vilas-Zornoza A, et al. Gene therapy restores the transcriptional program of hematopoietic stem cells in Fanconi anemia. Haematologica 2023; In Press; doi: 10.3324/HAEMATOL.2022.282418

69. Czechowicz A, Sevilla J, Booth C, et al. Lentiviral-mediated gene therapy for Fanconi Anemia [Group A]: Results from Global RP-L102 Clinical Trials. Mol Ther 2023;31(4S1):118.

70. Cartier N, Hacein-Bey-Abina S, Bartholomae CC, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science (80-) 2009;326(5954):818–823; doi: 10.1126/science.1171242

71. Eichler F, Duncan C, Musolino PL, et al. Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. N Engl J Med 2017;377(17):1630–1638; doi: 10.1056/nejmoa1700554

72. Biffi A, Montini E, Lorioli L, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science 2013;341(6148):1233158; doi: 10.1126/SCIENCE.1233158

73. Fumagalli F, Calbi V, Natali Sora MG, et al. Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: Long-term results from a non-randomised, open-label, phase 1/2 trial and expanded access. Lancet 2022;399(10322):372–383; doi: 10.1016/S0140-6736(21)02017-1

74. Gentner B, Tucci F, Galimberti S, et al. Hematopoietic stem- and progenitor-cell gene therapy for Hurler Syndrome. N Engl J Med 2021;385(21):1929–1940; doi: 10.1056/NEJMOA2106596

75. Khan A, Barber DL, Huang J, et al. Lentivirus-mediated gene therapy for Fabry disease. Nat Commun 2021;12(1):1178; doi: 10.1038/S41467-021-21371-5

76. Mavilio F, Pellegrini G, Ferrari S, et al. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nat Med 2006;12(12):1397–1402; doi: 10.1038/NM1504

77. Siprashvili Z, Nguyen NT, Gorell ES, et al. Safety and wound outcomes following genetically corrected autologous epidermal grafts in patients with Recessive Dystrophic Epidermolysis Bullosa. JAMA 2016;316(17):1808–1817; doi: 10.1001/JAMA.2016.15588

78. Hirsch T, Rothoeft T, Teig N, et al. Regeneration of the entire human epidermis using transgenic stem cells. Nature 2017;551(7680):327–332; doi: 10.1038/NATURE24487

79. Di WL, Lwin SM, Petrova A, et al. Generation and clinical application of gene-modified autologous epidermal sheets in Netherton Syndrome: Lessons learned from a Phase 1 Trial. Hum Gene Ther 2019;30(9):1067–1078; doi: 10.1089/HUM.2019.049

80. Wilson JM. Adenovirus-mediated gene transfer to liver. Adv Drug Deliv Rev 2001;46(1–3):205–209; doi: 10.1016/S0169-409X(00)00125-3

81. Samulski RJ, Berns KI, Tan M, et al. Cloning of adeno-associated virus into pBR322: Rescue of intact virus from the recombinant plasmid in human cells. Proc Natl Acad Sci 1982;79(6):2077–2081; doi: 10.1073/pnas.79.6.2077

82. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. Nat Rev Genet 2020;21(4):255–272; doi: 10.1038/s41576-019-0205-4

83. Gregorevic P, Blankinship MJ, Allen JM, et al. Systemic delivery of genes to striated muscles using adeno-associated viral vectors. Nat Med 2004;10(8):828–834; doi: 10.1038/nm1085

84. Foust KD, Nurre E, Montgomery CL, et al. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol 2009;27(1):59–65; doi: 10.1038/nbt.1515

85. Pupo A, Fernández A, Low SH, et al. AAV vectors: The Rubik's cube of human gene therapy. Mol Ther 2022;30(12):3515–3541; doi: 10.1016/j.ymthe.2022.09.015

86. McCarty DM. Self-complementary AAV vectors; Advances and applications. Mol Ther 2008; 16(10):1648–1656; doi: 10.1038/mt.2008.171

87. Tornabene P, Trapani I. Can adeno-associated viral vectors deliver effectively large genes? Hum Gene Ther 2020;31(1–2):47–56; doi: 10.1089/hum.2019.220

88. Ylä-Herttuala S. Endgame: Glybera finally recommended for approval as the first gene therapy drug in the European Union. Mol Ther 2012; 20(10):1831–1832; doi: 10.1038/MT.2012.194

89. Senior M. After Glybera's withdrawal, what's next for gene therapy? Nat Biotechnol 2017; 35(6):491–492; doi: 10.1038/NBT0617-491

90. Keeler AM, Flotte TR. Recombinant adeno-associated virus gene therapy in light of Luxturna (and Zolgensma and Glybera): Where are we, and how did we get here? Annu Rev Virol 2019;6(1):601–621; doi: 10.1146/annurev-virology-092818-015530

91. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. Nat Genet 2001;28(1):92–95; doi: 10.1038/ng0501-92

92. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: A randomised, controlled, open-label, phase 3 trial. Lancet 2017; 390(10097):849–860; doi: 10.1016/S0140-6736(17)31868-8

93. Mendell JR, Al-Zaidy S, Shell R, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med 2017;377(18):1713–1722; doi: 10.1056/NEJMoa1706198

94. Hwu W-L, Muramatsu S, Tseng S-H, et al. Gene Therapy for aromatic L -amino acid decarboxylase deficiency. Sci Transl Med 2012;4(134): 134ra61; doi: 10.1126/scitranslmed.3003640

95. Ozelo MC, Mahlangu J, Pasi KJ, et al. Valoctocogene roxaparvovec gene therapy for hemophilia A. N Engl J Med 2022;386(11):1013–1025; doi: 10.1056/NEJMOA2113708

96. Pipe SW, Leebeek FWG, Recht M, et al. Gene therapy with etranacogene dezaparvovec for Hemophilia B. N Engl J Med 2023;388(8):706–718; doi: 10.1056/NEJMoa2211644

97. Mendell JR, Sahenk Z, Lehman K, et al. Assessment of systemic delivery of rAAVrh74.MHCK7. micro-dystrophin in children with Duchenne Muscular Dystrophy. JAMA Neurol 2020;77(9):1122; doi: 10.1001/jamaneurol.2020.1484

98. Dhillon S. Beremagene geperpavec: First approval. Drugs 2023; In Press; doi: 10.1007/S40265-023-01921-5

99. Philippidis A. Novartis confirms deaths of two patients treated with Gene Therapy Zolgensma. Hum Gene Ther 2022;33(17–18):842–844; doi: 10.1089/hum.2022.29216.bfs

100. Philippidis A. Fourth boy dies in Clinical Trial of Astellas' AT132. Hum Gene Ther 2021;32(19–20): 1008–1010; doi: 10.1089/hum.2021.29182.bfs

101. Maeder ML, Stefanidakis M, Wilson CJ, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nat Med 2019;25(2):229–233; doi: 10.1038/s41591-018-0327-9

102. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. N Engl J Med 2021;385(6):493–502; doi: 10.1056/NEJMoa2107454

103. Verdera HC, Kuranda K, Mingozzi F. AAV vector immunogenicity in humans: A long journey to successful gene transfer. Mol Ther 2020;28(3): 723–746; doi: 10.1016/j.ymthe.2019.12.010

104. Leborgne C, Barbon E, Alexander JM, et al. IgG-cleaving endopeptidase enables in vivo gene therapy in the presence of anti-AAV neutralizing antibodies. Nat Med 2020;26(7):1096–1101; doi: 10.1038/s41591-020-0911-7

105. Aiuti A, Pasinelli F, Naldini L. Ensuring a future for gene therapy for rare diseases. Nat Med 2022;28(10):1985–1988; doi: 10.1038/S41591-022-01934-9

106. Fox T, Bueren J, Candotti F, et al. Access to gene therapy for rare diseases when commercialization is not fit for purpose. Nat Med 2023;29(3): 518–519; doi: 10.1038/S41591-023-02208-8

Received for publication August 24, 2023;
accepted after revision September 9, 2023.

Published online: September 10, 2023.