Current status of ex vivo gene therapy for hematological disorders: a review of clinical trials in Japan around the world

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Abstract Gene therapies are classified into two major categories, namely, in vivo and ex vivo. Clinical trials of human gene therapy began with the ex vivo techniques. Based on the initial successes of gene-therapy clinical trials, these approaches have spread worldwide. The number of gene therapy trials approved worldwide increased gradually starting in 1989, reaching 116 protocols per year in 1999, and a total of 2210 protocols had been approved by 2015. Accumulating clinical evidence has demonstrated the safety and benefits of several types of gene therapy, with the exception of serious adverse events in several clinical trials. These painful experiences were translated backward to basic science, resulting in the development of several new technologies that have influenced the recent development of ex vivo gene therapy in this field. To date, six gene therapies have been approved in a limited number of countries worldwide. In Japan, clinical trials of gene therapy have developed under the strong influence of trials in the US and Europe. Since the initial stages, 50 clinical trials have been approved by the Japanese government. In this review, the history and current status of clinical trials of ex vivo gene therapy for hematological disorders are introduced and discussed.

Keywords Severe combined immune deficiency · Adenosine deaminase deficiency · Chronic granulomatous disease · Wiskott-Aldrich syndrome · Leukemia cell vaccine

Introduction

Clinical trials of human gene therapy initially began with ex vivo techniques. The earliest efforts included a gene-marking study of transfusion of autologous tumor-infiltrating lymphocytes, modified by retroviral transduction of a neomycin resistance gene, in advanced melanoma patients, starting in 1989, and a clinical trial of transfusion of peripheral autologous lymphocytes modified by retroviral transduction of the adenosine deaminase (ADA) cDNA, for treatment of patients with severe combined immunodeficiency (SCID) with ADA deficiency, starting in 1990 [1, 2]. The results of these studies demonstrated the feasibility and safety of retroviral transduction of target genes into peripheral T cells. In addition, the second study showed that transfusion of ADA gene-transduced T cells could confer clinical benefits and save the lives of ADA-SCID patients, who are at high risk of infectious disease.

Based on the initial successes of gene-therapy clinical trials, these new therapeutic approaches have spread worldwide. The number of gene therapy trials approved worldwide increased gradually starting in 1989, reaching 116 protocols per year in 1999, and, essentially, maintaining this rate thereafter. By 2015, a total of 2210 protocols had been approved. Accumulating clinical evidence has demonstrated the safety and benefits of several types of gene therapy. Currently, 78.1 % of 2210 protocols are at phase I or I/II (<http://www.wiley.com/legacy/wileychi/genmed/clinical/>), but six gene therapy drugs have been approved in a limited number of countries [3–7].

However, progress has decelerated due to the occurrence of serious adverse events in several clinical trials, including a fatal systemic inflammatory response syndrome in an ornithine transcarbamylase-deficient patient following intrahepatic arterial injection of adenoviral vector; acute

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lymphoid malignancy in four patients with X-linked severe combined immunodeficiency; and myelodysplasia with monosomy 7 in 2 patients with X-linked chronic granulomatous disease following peripheral-blood transplantation of retrovirally transduced autologous peripheral stem cells [8–10]. These painful experiences were translated backward to basic science. With the goal of preventing similar outcomes in the future, subsequent trials adopted new gene-transfer technologies, selection of appropriate patients, and close monitoring of patients after gene therapy [11–14].

In Japan, clinical trials of gene therapy have developed under the strong influence of trials in the US and Europe. Fifty clinical trials have been approved by the Japanese government, since the initial stages: gene therapy for an ADA deficiency patient was approved in 1995, and cancer gene therapies using GM-CSF and p53 genes were approved in 1998 [15–17]. There have been no remarkable adverse events reported in Japan. Of the 58 trials to date, 37 were for malignancies and 21 were for congenital or acquired non-malignant diseases (<http://www.nihs.go.jp/mtgt/section-1/prtcl/prtcl-jn.html>). These clinical trials have also been strongly supported by the Japan Society of Cell and Gene Therapy, which was established in 1995 (<http://jsct.jp/ABOUT-JSGT/about-jsct1.html>).

Gene therapies can be classified into two major categories, namely, in vivo and ex vivo [18]. Historically, ex vivo gene therapy has been the most widely adopted, because investigators can preliminarily check the safety and predict the efficacy of the gene-transduced target cells in vitro before administration of the therapeutic agents to patients. However, because serious adverse events have been reported, the long-term in vivo toxicity of ex vivo gene therapy must also be carefully considered [9, 10]. Ex vivo gene therapy can successfully treat many hematological disorders, including hematological malignancies. Therefore, in this review, we summarize the current status of ex vivo gene therapy for hematological disorders in Japan and around the world.

Ex vivo gene therapy clinical trials in Japan and around the world

Essentially, all congenital monogenic hematological and immunological disorders can be targeted by gene therapy. Historically, however, several hematological disorders have received the closest attention as target diseases for gene therapy clinical trials, largely because we have a great deal of biochemical information about their pathogenesis. In this review, clinical trials of gene therapy for congenital hematological and immunological disorders are introduced and discussed. Data from “Gene Therapy Clinical Trials

Worldwide,” provided by the *Journal of Gene Medicine* (<http://www.wiley.com/legacy/wileychi/genmed/clinical/>), were used to generate Tables 1 and 2. Data from “List of Gene Therapy Clinical Study Protocols Approved in Japan” provided by the National Institute of Health Sciences, Japan (<http://www.nihs.go.jp/mtgt/section-1/english/prtcl-e/prtcl-e.html>), <http://www.nihs.go.jp/mtgt/section-1/prtcl/prtcl-jn.html> were used to generate Table 3.

Ex vivo gene therapy clinical trials for congenital hematological disorders in Japan and around the world

Since the first gene therapy of a monogenic disease, severe combined immunodeficiency due to ADA deficiency (ADA-SCID) was conducted safely and with clinical benefits [2]; 209 clinical protocols for monogenic diseases have been approved as of July 2015. Almost half of the approved trials involve ex vivo gene therapy. The target diseases include ADA-SCID, X-linked severe combined immunodeficiency (SCID-X1), X-linked chronic granulomatous disease (X-CGD), hemophilia, Wiskott–Aldrich syndrome (WAS), mucopolysaccharidosis, cerebral adrenoleukodystrophy, sickle cell disease, thalassemia, metachromatic leukodystrophy, familial hypercholesterolemia, Gaucher disease, Fanconi anemia, purine nucleoside phosphorylase deficiency, leukocyte adherence deficiency, gyrate atrophy, JAK3 deficiency, and epidermolysis bullosa. In Table 1, the outlines of clinical trials of primary immunodeficiencies (PIDs) and hemoglobinopathies are presented.

PIDs are a heterogeneous group of monogenic conditions caused by altered innate and/or adaptive immune responses. More than 260 disorders involving more than 300 gene mutations have been reported, and these numbers continue to increase due to improved diagnostic modalities for immune system, the introduction of genome-wide association studies, and the next-generation sequencing technologies. Patients’ phenotypes range from asymptomatic to manifestation of life-threatening conditions, including various forms of severe combined immunodeficiency (SCID) [19, 20]. Below, the results of clinical trials for ADA-SCID, SCID-X1, CGD, and Wiskott–Aldrich syndrome are introduced and discussed.

Severe combined immunodeficiency due to ADA deficiency (ADA-SCID)

Inherited ADA deficiency causes a range of phenotypes, the most severe of which is SCID presenting in infancy, usually resulting in early death. Ten-to-fifteen percent of patients have a delayed clinical onset starting at 6–24 months of age, and a smaller percentage of patients have later onset, diagnosed between the age of 4 years and adulthood. The latter patients exhibit less severe infections and

Table 1 Ex vivo gene therapy for hematological congenital diseases

Disease	Trial ID	Country	Clinical phase	Approved year	Genes	Vector	Target cells/route	Principle investigator
ADA-SCID	FR-0006	France	I/II		Adenosine deaminase (ADA)	Retrovirus	CD34+ bone marrow cell/bone marrow transplantation	Alain Fischer
	IL-0004	Israel	I	2006	Adenosine deaminase (ADA)			Shimon Slavin
	IT-0001	Italy	I/II	1992	ADA	Retrovirus	Autologous PBL, BMC/intravenous	Claudio Bordignon
	IT-0008	Italy	I/II	2000	ADA	Retrovirus	Autologous CD34+ bone marrow cells / intravenous after Busulfan	A. Aiuti
	IT-0016	Italy	I/II	2002	ADA	Retrovirus	Autologous CD34+ cells/intravenous	Maria Grazia Roncarolo
	JP-0002	Japan	I/II	1995/2/1	ADA	Retrovirus	Autologous PBL/intravenous	Yukio Sakiyama
	JP-0012	Japan	I	2002/6/17	ADA	Retrovirus	Autologous CD34+ cells/intravenous	Tadashi Ariga
	NL-0001	The Netherlands	I/II		Multi-drug resistance-1	Retrovirus	Autologous CD34+BMC/BMT	Pieter Hoogerbrugge
	NL-0006	The Netherlands	I/II		ADA	Retrovirus	Autologous CD34+BMC/BMT	Dinko Valerio
	UK-0012	UK	I/II	1993/1	ADA	Retrovirus	CD34+ bone marrow cell/bone marrow transplantation	Roland Levinsky
	UK-0199	UK	I/II	2011/11	ADA	Lentivirus	CD34+ bone marrow cell/bone marrow transplantation	Bobby Gasper
	US-0002	USA	I	1990/9/6	ADA neomycin resistance	Retrovirus	PBC, CD34+ PBC, cord blood, placenta cells	Michael Blaese
X1-SCID	US-0337	USA	I	1999	ADA	Retrovirus	CD34+ Cells from Cord Blood or Bone Marrow intravenous	Donald B. Kohn
	US-1006	USA	I/II	2009/10	ADA	EFS lentivirus	CD34+ bone marrow cell/intravenous	Donald B. Kohn
	FR-0014	France	I	2002	Gamma c common chain receptor	Retrovirus	Autologous CD34+ cells/intravenous	Alain Fischer
	FR-0046	France	I/II	2010/12	Gamma c common chain receptor	Retrovirus	CD34+ hematopoietic stem cells	Alain Fischer
	IT-0024	Italy	I		IL-2 receptor gamma	Genome editing	Hematopoietic stem cells	Pietro gienovese

Table 1 continued

Disease	Trial ID	Country	Clinical phase	Approved year	Genes	Vector	Target cells/route	Principle investigator
Chronic granulomatous disease	UK-0061	UK	I	2001/1	Gamma c common chain receptor	Retrovirus		Adrian Thrasher
	UK-0101	UK	I	2003/2	Gamma c common chain receptor	Retrovirus		Inst.Child Health London
	UK-0154	UK	I	2007	Gamma c common chain receptor	Retrovirus		Great Ormond Street Hospital London
	US-0152	USA	I	1996	Gamma c common chain receptor	Retrovirus	CD34+ BMC, CD34+ umbilical cord blood cells/intravenous	Kenneth I. Weinberg
	US-0494	USA	I	2001	Gamma c common chain receptor	Retrovirus	CD34+ cord blood or BMC	Kenneth I. Weinberg
	US-0516	USA	I/II	2002	Gamma c common chain receptor	Retrovirus	CD34+ cells from peripheral blood/intravenous	Harry L. Malech
	US-0950	USA	I	2008/10	Gamma c common chain receptor	Retrovirus	CD34+ cells from BM/intravenous	Luigi Notarangelo
	US-0963	USA	I	2009/1	Gamma c common chain receptor	Lenti (SIN)	Autologous CD34+ cells/intravenous	Brian P. Sorrentino
	US-0964	USA	I	2009/1	Gamma c common chain receptor	Lenti	Autologous CD34+ cells/intravenous	Suk See DeRavin
	CH-0041	Switzerland	I	2005/4	gp91phox	Retrovirus		Reinhard Segar
	CH-0048	Switzerland	I/II	2009/6	SF71-gp91phox	Retrovirus		Reinhard Segar
	DE-0035	Germany	I	2002/3	gp91phox1_NGFR	Retrovirus	Autologous peripheral CD34+/intravenous	Dueter Hoelzer
	DE-0085	Germany	I/II	2013/1	gp91phox	Retrovirus	Autologous peripheral CD34+/intravenous	
	FR-0067	Multi-country; France, UK, Germany	I/II	2015/4	gp91 phox	Lentivirus	Autologous peripheral CD34+/intravenous	
	KR-0009	South Korea	I/II	2007/1/2	gp91(MT91)	Retrovirus	Autologous hematopoietic stem cells	
	UK-0062	UK	I	2000/12	gp91phox	Retrovirus		Adrian Thrasher
	UK-0198	UK	I/II	2011/11	gp91phox	Lentivirus	CD34+ cells	Adrian Thrasher
	US-0104	USA	I/II	1995/4/15	p47phox	Retrovirus	CD34+ PBC/intravenous	Harry L. Malech

Table 1 continued

Disease	Trial ID	Country	Clinical phase	Approved year	Genes	Vector	Target cells/route	Principle investigator
Homophilia	US-0196	USA	I	1997	gp91phox	Retrovirus	CD34+ PBC/intravenous	Franklin O. Smith
	US-0231	USA	I/II	1998	p47phox gp91phox	Retrovirus	Autologous CD34+ PBC/intravenous	Harry L. Malech
	US-1223	USA	I/II	2013/4	gp91phox	Lentivirus	Autologous CD34+ BMC/intravenous	Donald Kohn
	XX-0025	Multi-country: UK, Switzerland, Germany, France	I/II	2013/2	gp91phox	Lentivirus	Autologous CD34+ cells/intravenous	Adrian Thrasher
	XX-0029	Multi-country: UK, Germany, France	I/II	2013/10	gp91 phox (codon optimized)	G1XCGD lentivirus	Autologous CD34+ BMC/intravenous	
	CN-0001	China	I/II	1995/6	Factor IX	Retrovirus	Autologous skin fibroblasts/subcutaneous	J. L. Hsueh
	FR-0038	France	I	2002	Factor VIII	Adenovirus (MaxAdFVIII Vector)		Genstar/France
	US-0247	USA	I	1998	Factor VIII	Naked/plasmid DNA	Autologous fibroblasts/intraperitoneal	David A. Roth
	US-1112	USA	I	2011/6	Factor VIII	Lentivirus	Autologous CD34+ cells/intravenous	Christopher Doering
	DE-0053	Germany	I/II	2006/3	Wiskott Aldrich protein	Retrovirus	Autologous hematopoietic stem cells	Christophe Klein
Wiskott–Aldrich Syndrome	FR-0047	Multi-Country: France, UK	I/II	2011/5	Wiskott Aldrich protein	Lentivirus	Hematopoietic stem cells	Alain Fischer
	FR-0062	Multi-Country: France, UK	I/II	2014/10	Wiskott Aldrich protein	Lentivirus	Autologous CD34+ cells	Marina Cavazzana
	IT-0020	Italy	I/II	2010/5	Wiskott Aldrich protein	Lentivirus	Hematopoietic stem cells	Alessandro Aiuti
	UK-0168	UK	I/II	2008/2	Wiskott Aldrich protein	Lentivirus	Hematopoietic stem cells	Great Ormond Street Hospital London
Mucopolysaccharidosis type I (Hurlers syndrome)	US-1052	USA	I	2010/7	Wiskott Aldrich protein	Lentivirus	CD34+ cells from BM/intravenous	Sung-Yun Pai
	FR-0005	France	I		Alpha-1-iduronidase (IDUA)	Retrovirus	Autologous fibroblast/intraperitoneal	Alain Fischer
Type I (Hurlers syndrome)	UK-0011	UK	I/II	1997/8/1	IDUA	Retrovirus	Autologous BMC/BMT	L. S. Lashford

Table 1 continued

Disease	Trial ID	Country	Clinical phase	Approved year	Genes	Vector	Target cells/route	Principle investigator
Type I (Hurlers syndrome)	UK-0043	UK	I	1997/5	pLX	Retrovirus	Autologous BMC/BMT	Galpin
Type II (Hunter disease)	US-0087	USA	I	1995/8/20	Iduronate-2-Sulfatase (IDS)	Retrovirus	Autologous lymphocytes	Chester B. Whitley
Type VII	US-0758	USA	I	2006/2	Beta-glucuronidase	Lentivirus	Autologous CD34+ cells/intravenous	Mark S. Sands
Cerebral adrenoleukodystrophy	FR-0028	France	I/II	2005	ALD	Lentivirus	Autologous CD34+ cells/intravenous	Cartier N.
	FR-0042	France	III	2009/11	ALD (ABCD-1 gene)	Lentivirus	Hematopoietic stem cells	P. Aubourg
	FR-0066	Multi-Country: France, UK	II/III	2013/9	ALD (ABCD-1 gene)	Lentivirus	Autologous CD34+ cells/intravenous	
Childhood	US-1073	USA	II/III	2010/10	ALD (ABCD-1 gene)	Lentivirus	Autologous CD34+ cells/intravenous	David Williams
Sickle cell anaemia, thalassemia	FR-0029	France	I/II	2006	Human globin (A-T87Q-globin (LentiGlobin)) vec	Lentivirus	Autologous CD34+ cells/intravenous	Genetix-France
Hemoglobinopathies (Sickle Cell Anemia and Thalassemia Major)	FR-0055	France	I/II	2013/1	A-T87Q Globin	Lentivirus	Autologous CD34+ stem cells	
Beta thalassemia	IT-0026	Italy	I/II	2015/5	Beta globin	Lentivirus	Autologous hematopoietic stem cells	Alessandro Aiuti
	US-0852	USA	I	2007/4	Human globin	Lentivirus	Autologous CD34+ cells/intravenous	Farid Boulad
	US-1067	USA	I	2010/10	gamma globin	Lentivirus(SIN)	Autologous CD34+ cells/intravenous	Derek Persons
Beta thalassemia major	US-1164	USA	I	2012/4	A-T87Q-Globin	Lentivirus	Autologous CD34+ cells/intravenous	Donald Kohn
Thalassemia	US-1304	USA	I	2014/4	mRNA or engineered zinc finger nucleases targeting human BCL11A		Autologous CD34+ cells/intravenous	Mark Walters
Sickle cell disease	US-1023	USA	I/II	2010/1	Beta globin with gamma globin exons gene	Lentivirus	Autologous CD34+ cells/intravenous	Malik Punam
	US-1187	USA	I/II	2012/10	Beta globin	AS3-FB Lentivirus	Autologous CD34+ cells/intravenous	Garry Schiller
	US-1254	USA	I	2013/10	Beta globin	BB305 lentivirus	Autologous CD34+ cells/intravenous	John F. Tisdale

Table 1 continued

Disease	Trial ID	Country	Clinical phase	Approved year	Genes	Vector	Target cells/route	Principle investigator
Metachromatic Leukodystrophy	IT-0019	Italy	I/II	2010/5	Arylsulfatase A	Lentivirus	Hematopoietic stem cells/intravenous	Alessandra Biffi
Familial hypercholesterolemia	US-0012	USA	I	1991/11	LDLR	Retrovirus	Autologous hepatocyte/intrahepatic	James M. Wilson
Gaucher disease	US-0046	USA	I	1993/9/3	Glucocerebrosidase	Retrovirus	Autologous CD34+ PBC/BMT	John A. Barranger
	US-0047	USA	I/II	1993/9/3	Glucocerebrosidase	Retrovirus	Autologous CD34+ PBC/BMT	Stefan Karlsson
	US-0061	USA	I	1994/11/15	Glucocerebrosidase	Retrovirus	Autologous G-CSF mobilized CD34+ PBC/intravenous	Friedrich G., Schuening
Fanconi anemia	US-0078	USA	I/II	1995/2/12	Fanconi Anemia Complementation Group C	Retrovirus	Autologous hematopoietic progenitor/intravenous	Johnson M.Liu
	US-0291	USA	I/II	1999	Complementation Group A	Retrovirus	Autologous hematopoietic progenitor/intravenous	Christopher E. Walsh
	US-0370	USA	I	2000	Complementation Group A& C	Retrovirus	Autologous CD34+ PBC/intravenous	James M. Croop
	US-0520	USA	I	2002	herpes simplex virus thymidine kinase	Retrovirus	Allogeneic T lymphocytes/intravenous	Paul J. Orchard
	US-0895	USA	I	2008/1	Complementation Group A	Lentivirus	Autologous CD34+ PBC/intravenous	Pamela S. Becker
	US-1105	USA	I	2011/4	Complementation Group A	Lentivirus	Autologous CD34+ cells/intravenous	W.Scott Goebel
Purine nucleoside phosphorylase deficiency	XX-0020	Multi-country: Spain, France, UK	I/II	2012/10/8	FANCA gene	Lentivirus	Filgrastime and Mozobil	
	US-0111	USA	I/II	1995/7	Purine Nucleoside Phosphorylase	Retrovirus	Autologous PBC/intravenous	R. Scott McIvor
Leukocyte adherence deficiency	US-0204	USA	I	1997	CD18(beta 2 integrin)	Retrovirus		
Gyrate atrophy	US-0295	USA	I	1999	Ornithine aminotransferase	Retrovirus	Autologous keratinocytes/skin patch administration	Robert B. Nussenblatt

Table 1 continued

Disease	Trial ID	Country	Clinical phase	Approved year	Genes	Vector	Target cells/route	Principle investigator
JAK3 Deficiency	US-0446	USA	I	2001	JAK-3	Retrovirus	Autologous CD34+ hematopoietic stem cells	Rebecca H. Buckley
Inherited autosomal recessive/junctional epidermolysis bullosa	US-0423	USA	I/II	2000	Laminin 5-beta3	Retrovirus	Skin patch administration	Alexa Kimball
Recessive dystrophic epidermolysis bullosa (RDEB)	US-0827	USA	I/II	2007/1	Human collagen type 7 A1	Retrovirus	Autologous keratinocytes/skin patch administration	Albert T Lane
	US-1370	USA	I/II	2015/1	Human collagen type 7 A1	Lentivirus	Autologous dermal fibroblast/intradermal	M. Peter Marinkovich

BMC bone marrow cells, *BMT* bone marrow transplantation, *PBC* peripheral blood cells, *ADA* adenosine deaminase, *IL* interleukin, *EFS* short form elongation factor-1 α promoter

more gradual immunologic deterioration. ADA deficiency accounts for approximately 15 % of all SCID cases and one-third of cases of autosomal recessive SCID. ADA is an enzyme that catalyzes the irreversible deamination of adenosine and deoxyadenosine in the purine catabolic pathway (<http://www.omim.org/>) [20]. Enzyme activity is highest in lymphoid tissues, particularly the thymus. The lack of ADA results in accumulation of toxic purine metabolites, causing lymphocytes to undergo apoptosis during differentiation and selection. Enzyme replacement therapy (ERT) with polyethylene glycol-modified bovine ADA is clinically useful but non-curative. To achieve a definitive cure, hematopoietic stem cell transplantation (HSCT) from an unaffected, matched sibling donor is the treatment of choice. For those without well-matched donors, ex vivo gene therapy with autologous peripheral T cells is considered to be the treatment of choice. The first clinical trial of gene therapy for ADA-SCID, involving two children, started in 1990. Infusions of retrovirally ADA gene-transduced T cells were achieved safely, and normalized blood T cells (including many cellular and humoral immune responses) and integrated vector and ADA gene expression in T cells persisted after the discontinuation of therapy. The usefulness of T-cell-directed gene transfer in the treatment of ADA-SCID was also demonstrated by another clinical trial; however, the effects required multiple rounds of T-cell infusions and were not sufficient to alleviate the requirement for ERT, although the dose could be reduced [2, 22]. To develop a longer lasting source of ADA-expressing T cells, the ADA gene was retrovirally transduced into hematopoietic stem cells, including bone marrow cells, BM CD34+ cells, and umbilical cord blood CD34+ cells, and infused into patients. These results demonstrated the feasibility of hematopoietic cell gene therapy using a gamma retrovirus vector. The level of ADA expression, however, was not sufficient to confer significant clinical benefits [21–24]. Several strategies were adopted to overcome these problems: optimization of gene transduction methods, non-myeloablative preconditioning with busulfan or melphalan to create space in the bone marrow, and discontinuation of ERT around the time of reinfusion of gene-modified stem cells [19, 21]. Nine out of ten ADA-SCID patients treated with gene therapy after non-myeloablative preconditioning with busulfan maintained lasting immune reconstitution, and eight and five of the ten were able to discontinue ERT or intravenous immune globulin, respectively, emphasizing the importance of non-myeloablative preconditioning for discontinuation of ERT [25–27].

More than 40 ADA-SCID patients have been with gamma-retroviral ADA gene therapy in the US and Europe, and 100 % of these patients survived. Furthermore, unlike other PIDs, such as X-SCID, X-CGD, and WAS, no severe adverse events related to insertional mutagenesis have

Table 2 Ex vivo gene therapy for hematological malignancies

Category	Disease	Trial ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
Leukemia	Acute leukemia	AU-0006	Australia	I/II	1997/3	Interleukin-2 PG1-B7-1	Adenovirus + retrovirus	Autologous leukemia cells	
	Acute leukemia, myelodysplastic syndrome or chronic myeloid leukemia	AU-0032	Australia	I	2014/5	icasp-9	Retrovirus	T cells/intravenous	Siok Tey
	Acute lymphoblastic leukemia	CN-0037	China	I	2014/10	CD28 CAR CD137 CAR	Lentivirus	T cells/intravenous	Hongkui Deng
	Acute leukemia, Chronic myelogenous leukemia	DE-0018	Germany	I	2002/6	Herpes simplex virus thymidine kinase (HSV-TK) del-taLNGFR	Retrovirus	Allogeneic lymphocytes/intravenous	Bernd Hertenstein
	Acute and chronic leukaemia, plasmacytoma	DE-0034	Germany	I/II	2001/9	Herpes simplex virus thymidine kinase (HSV-TK)	Retrovirus	Allogeneic T cells/intravenous	Axel Rolf Zander
	Acute leukemia	DE-0084	Germany	III	2012/11	Herpes simplex virus thymidine kinase (HSV-TK)del-taLNGFR	Retrovirus	Allogeneic lymphocytes/intravenous	
	Leukemia, lymphomia (GVHD risk after allogeneic BMT)	FR-0011	France	I/II		Herpes simplex virus thymidine kinase (HSV-TK)	Retrovirus	Allogeneic CD3+ PBC/intravenous	Pierre Tiberghien
	Hematological malignancies	FR-0043	France	I/II	2010/3	Herpes simplex virus thymidine kinase	Retrovirus		Sebastien Maury
	Malignant hemopathies	IL-0001	Israel	I	1997/10	Herpes simplex virus thymidine kinase	Retrovirus	Allogeneic PBL, T cells/intravenous	Shimon Slavin
	Hematological malignancies	IT-0017	Multi-country:Italy, Germany	I/II	2002	Herpes simplex virus thymidine kinase	Retrovirus	Allogeneic PBL, T cells/intravenous	Fabio Ciceri
	Leukemia	IT-0018	Italy	III	2008	Herpes simplex virus thymidine kinase	Retrovirus	Haploidentical donor PBL/intravenous	
	Acute leukemia	IT-0021	Italy	III	2009/6	Herpes simplex virus thymidine kinase	Retrovirus	Haploidentical donor PBL/intravenous	Fabio Ciceri
	Leukemia	IT-0023	Italy	I/II	2014/4	Inducible Caspase 9 Suicide Gene/AP1903	Retrovirus	/Intravenous	Franco Locatelli
	Leukemia	JP-0015	Japan	I/II		Herpes simplex virus thymidine kinase	Naked/plasmid DNA	Allogeneic donor PBL/intravenous	Masafumi Onodera
	Leukemia	JP-0018	Japan	I	2008	Herpes simplex virus thymidine kinase	Retrovirus	Allogeneic donor PBL/intravenous	National Cancer Center

Table 2 continued

Category	Disease	Trial ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
	Hematological malignancies	JP-0020	Japan	I	2008/10	Herpes simplex virus thymidine kinase	Retrovirus	Alloneic donor PBL/intravenous	
	Acute myelogenous leukemia and myelodysplastic syndrome	JP-0027	Japan	I	2013/8	MS3-WT1-siT cell receptor	Retrovirus	Autologous T cells/intravenous	Shinichi Kageyama
	Refractory or relapsed hematological malignancies	NL-0028	The Netherlands	I	2011/7	T Cell Receptor alpha and beta Chain	Retrovirus		
	Chronic myelogenous leukemia	UK-0060	UK	I	2000/10	Herpes simplex virus thymidine kinase	Retrovirus	Allogeneic donor PBL/intravenous	
	Acute myelogenous leukemia	UK-0119	UK	I	2004	Interleukin-2, B7.1 (CD80)	Lentivirus	Allogeneic donor PBL/intravenous	
	CD 19+ B-lymphoid malignancies	UK-0126	UK	I	2005/6	CD19-specific chimeric T cell receptor	Retrovirus	Autologous T cells/intravenous	Christie Research centre Manchester
	Leukemia	UK-0150	UK	I	2006/12	T cell receptor specific for Wilms' tumour antigen 1	Retrovirus	Autologous T cells/intravenous	Royal Free Hospital
	Acute lymphoblastic leukemia	UK-0169	UK	I	2008/2	CD19-specific T cell receptor	Retrovirus	Allogeneic T cells/intravenous	Great Ormond Street Hospital
	Leukemia, lymphoma, multiple myeloma	US-0146	USA	I	1996	Herpes simplex virus thymidine kinase	Retrovirus	Allogeneic PBC/intravenous	Charles L. Link
	Chronic myelogenous leukemia	US-0188	USA	I	1997	Antisense BCR/ABL Dihydrofolate reductase	Retrovirus	Autologous CD34+ PBC/BMT	Catherine Verfaillie
	Chronic myelogenous leukemia	US-0206	USA	I	1997	Herpes simplex virus thymidine kinase Hygromycin phosphotransferase	Retrovirus	Allogeneic CD4+ T cells, CD8+ T cells	Mary E. D. Flowers
	Chronic myelogenous leukemia (CML), multiple myeloma, non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL)	US-0241	USA	I/II	1998	Herpes simplex virus thymidine kinase	Retrovirus	Alloneic donor PBL/intravenous	William I. Bensinger
	Chronic lymphocytic leukaemia	US-0242	USA	I	1998	CD154 (CD40-ligand)	Adenovirus	Leukemic cells/intravenous	Thomas J. Kipps

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
	Leukemia	US-0308	USA	I	1999	Herpes simplex virus thymidine kinase	Retrovirus	Allogeneic CD8+ cells/ intravenous	Edus Warren
	Acute leukemia	US-0319	USA	I	1999	Interleukin-2 (IL-2) CD154 (CD40Higand)	Adenovirus	Autologous fibroblasts/subcutaneous	Malcolm K. Brenner
	Myelodysplasia or acute myelogenous leukemia	US-0369	USA	I	2000	Granulocyte-macrophage colony stimulating factor	Adenovirus	Autologous leukemia cells/ intradermal + subcutaneous	Daniel J. DeAngelo
	Chronic lymphocytic leukaemia	US-0401	USA	II	2000	CD154 (CD40-ligand)	Adenovirus	Autologous leukemia cells/ intravenous	William G. Wierda
	Acute or chronic myelogenous leukemia	US-0433	USA	I	2000	Neomycin resistance(neoR)	Retrovirus	Allogeneic bone marrow cells/BMT	Malcolm K. Brenner
	Leukemia, Multiple Myeloma	US-0435	USA	I/II	2000	Granulocyte-macrophage colony stimulating factor(GM-CSF)	Naked/plasmid DNA	Autologous tumor cells with an allogeneic GM-CSF producing bystander cell line/intradermal	Ivan Borrello
	Chronic lymphocytic leukaemia	US-0460	USA	I	2001	Interleukin-2 (IL-2) CD154 (CD40-ligand)	Adenovirus	Autologous leukemia cells/ subcutaneous	Malcolm K. Brenner
	Acute myelogenous leukemia	US-0479	USA	I/II	2001	Granulocyte-macrophage colony stimulating factor(GM-CSF)	Naked/plasmid DNA	Autologous tumor cells with an allogeneic GM-CSF producing bystander cell line/intradermal	Ivan Borrello
	Acute lymphoblastic leukemia	US-0535	USA	I	2002	CD19-specific chimeric T cell receptor Hygromycin phosphotransferase Herpes simplex virus thymidine kinase	Naked/plasmid DNA	Autologous cytolytic T cell clone/intravenous	Laurence J. N. Cooper
	Chronic lymphocytic leukaemia	US-0553	USA	I	2002	Interleukin-2 (IL-2) CD154 (CD40-ligand)	Naked/plasmid DNA	Autologous leukemia cells/ subcutaneous	Malcolm K. Brenner
	Chronic myelogenous leukemia	US-0602	USA	I	2003	Granulocyte-macrophage colony stimulating factor(GM-CSF)	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	Hyam I. Levitsky

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
	Chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), myelodysplasia, Graft-vs-Host Disease (GVHD)	US-0605	USA	I	2003	Herpes simplex virus thymidine kinase (HSV-TK)	Retrovirus	Allogeneic T cells/intravenous	Steven Kornblau
	Myelodysplasia (MDS) or refractory acute myeloid leukemia(AML)	US-0621	USA	I	2004	Granulocyte-macrophage colony stimulating factor (GM-CSF)	Adenovirus	Autologous leukemia cells/subcutaneous	Vincent T. Ho
	Chronic myelogenous leukemia (CML)	US-0670	USA	I	2004	Granulocyte-macrophage colony stimulating factor (GM-CSF)	Lipofection	Allogeneic K562 cells/intradermal+subcutaneous	Catherine Wu
	Hematological malignancies	US-0712	USA	I	2005/5	Human telomerase reverse transcriptase (hTERT)	RNA transfer	Autologous dendritic cells/intradermal	David Rizzieri
	Chronic lymphocytic leukaemia	US-0717	USA	I	2005/7	Interleukin-2 (IL-2) CD154 (CD40-ligand)	Adenovirus	Autologous leukemia cells/subcutaneous	George Carrum
	Chronic lymphocytic leukaemia (CLL)	US-0721	USA	I	2005/7	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cell/intravenous	Renier Brentjens
	Chronic lymphocytic leukaemia	US-0744	USA	I	2005/11	Granulocyte-macrophage colony stimulating factor (GM-CSF)	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	Ian W. Flinn
	Chronic lymphocytic leukaemia	US-0757	USA	I	2006/1	CD154 (CD40-ligand)	Adenovirus	Autologous leukemia cells/intravenous	William Wierda
	Chronic myelogenous leukemia	US-0761	USA	I	2006/2	Granulocyte-macrophage colony stimulating factor	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	B. Douglas Smith
	Chronic myelogenous leukemia	US-0763	USA	I	2006/3	Granulocyte-macrophage colony stimulating factor	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	B. Douglas Smith
	Hematological malignancies	US-0766	USA	I/II	2006/4	Herpes simplex virus thymidine kinase (HSV-TK)	Retrovirus	Allogeneic T cells/intravenous	Steven M. Kornblau
	EBV+ Hodgkin's or non-Hodgkin's lymphoma	US-0771	USA	I	2006/4	LMP2A and L MP1	Adenovirus	Autologous cytolytic T cells/intravenous	Stephen Gottschalk

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
	Acute myelogenous leukemia	US-0789	USA	I/II	2006/7	Human telomerase reverse transcriptase	RNA transfer	Autologous dendritic cells/intradermal	John DiPersio
	B-cell chronic lymphocytic leukemia	US-0800	USA	I	2006/7	Interleukin-2 (IL-2) CD154 (CD40-ligand)	Adenovirus	Autologous leukemia cells/subcutaneous	Malcolm K. Brenner
	Hematological malignancies	US-0803	USA	I	2006/9	Granulocyte-macrophage colony stimulating factor	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	Carol Ann Huff
	B-cell acute lymphocytic leukemia	US-0813	USA	I	2006/10	Anti-CD 19-41 BB-Cdzeta	Retrovirus	Haploidentical natural killer cell/intravenous	Dario Campana
	Chronic lymphocytic leukaemia	US-0817	USA	I	2006/10	Granulocyte-macrophage colony stimulating factor	Lipofection	Allogeneic K562 cells/intradermal+subcutaneous	Catherine J. Wu
	Myelodysplastic Syndrome (MDS)	US-0907	USA	I	2008/3	Granulocyte-macrophage colony stimulating factor CD154 (CD40-ligand)	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	Javier Pinilla
	CD 19+ B-lymphoid malignancies	US-0922	USA	I	2008/4	CD19 antigen specific-zeta T cell receptor	Sleeping Beauty transposon	Autologous T cells/intravenous	Partow Kebriaei
	MDS, AML	US-0937	USA	I	2008/8	Granulocyte-macrophage colony stimulating factor	Lipofection	Allogeneic K562 cells/intradermal+subcutaneous	Vincent T. Ho
	B-cell malignancies	US-0940	USA	I	2008/9	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Steven A. Rosenberg
	Chronic lymphocytic leukaemia (CLL), B-Cell lymphoma	US-0941	USA	I	2008/9	Chimeric antigen receptor-Kappa-CD28 endodomain	Retrovirus	Autologous T cells/intravenous	Malcolm K. Brenner
	Acute lymphoblastic leukemia (ALL)	US-0945	USA	I	2008/10	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Robert Krance
	Chronic lymphocytic leukaemia (CLL)	US-0952	USA	I	2008/10	CD154 (CD40-ligand)	Adenovirus	Autologous leukemia cells/intravenous	Januario E. Castro
	Acute lymphoblastic leukemia (ALL)	US-0985	USA	I	2009/7	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	RenierBrentjens
	B-cell malignancies	US-0998	USA	I	2009/9	CD19 antigen specific-zeta T cell receptor	Retrovirus	Allogeneic T cells/intravenous	Michael Bishop
	B-cell malignancies	US-1003	USA	I	2009/10	CD19 antigen specific-zeta T cell receptor	Sleeping Beauty transposon	Allogeneic HLA matched T cells/intravenous	Laurence J. N. Cooper

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
	B-cell malignancies	US-1022	USA	I	2010/1	CD19 antigen specific-zeta T cell receptor	Sleeping Beauty transposon	Allogeneic umbilical cord blood derived lymphocytes/intravenous	Laurence Cooper
	CD 19+ B-lymphoid malignancies	US-1025	USA	I	2010/1	CD19 antigen specific-zeta T cell receptor	Lentivirus	Autologous CD3+ T cells/intravenous	David Porter
	Leukemia, elimination of graft vs. host disease	US-1054	USA	I	2010	Herpes simplex virus thymidine kinase (HSV-TK) Nerve growth factor receptor	Retrovirus	Allogeneic T cells/intravenous	Jayesh Mehta
	Unmutated IgVH chronic lymphocytic leukemia	US-1059	USA	I	2010/7	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous CD3+ T cells/intravenous	Renier Brentjens
	Acute lymphoblastic leukemia (ALL)	US-1091	USA	I	2011/2	CD19 antigen specific-zeta T cell receptor	Retrovirus	Allogeneic Epstein Bar virus-specific T cells/intravenous	Nancy A. Korman
	CD 19+ B-lymphoid malignancies	US-1161	USA	I	2012/4	CD19 antigen specific-zeta T cell receptor	Lentivirus	Autologous T cells/intravenous	Rebecca Gardner
	Acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and chronic myeloid leukemia (CML)	US-1169	USA	I/II	2012/6	Alpha and beta chains of Wilms' tumor antigen 1 (WT1) T cell receptor	Lentivirus	Allogeneic T cells/intravenous	Merav Bar
	MDS/AML	US-1185	USA	II	2012/9	Granulocyte-macrophage colony stimulating factor	Adenovirus	Autologous leukemia cells/Intradermal + subcutaneous	Glenn Dranoff
	B-cell chronic lymphocytic leukemia	US-1192	USA	I	2012/10	CD19 antigen specific-zeta T cell receptor	Sleeping Beauty transposon	Autologous CD4+ and CD8+ T cells/intravenous	William Wierda
	B-cell non-Hodgkin lymphoma	US-1196	USA	I	2012/10	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Craig Sauter
	CD19+ leukemia and lymphoma	US-1197	USA	II	2012/12	CD19 antigen specific-zeta T cell receptor	Lentivirus	Autologous T cells/intravenous	Noelle Frey
	Acute lymphoblastic leukemia	US-1201	USA	I	2013/1	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Kevin J. Curran
	B-cell chronic lymphocytic leukemia	US-1203	USA	I	2013/1	CD19 antigen specific chimeric antigen receptor (CAR) CD3 zeta and CD137 signaling	Sleeping beauty transposon	Autologous CD4+ and CD8+ T cells/intravenous	Chitra Hosing

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
	Advanced CD19+ B cell malignancies	US-1213	USA	I/II	2013/3	CD19 antigen specific chimeric antigen receptor (CAR) epidermal growth factor receptor	Lentivirus	Autologous central memory T cells/intravenous	Cameron Turtle
	B-cell chronic lymphocytic leukemia	US-1225	USA	I	2013/4	CD19 antigen specific-zeta T cell receptor	Sleeping beauty transposon	Autologous CD4+ and CD8+ T cells/intravenous	Laurence J. N. Cooper
	Pediatric acute lymphoblastic leukemia	US-1233	USA	I/II	2013/6	CD19-specific chimeric antigen receptor truncated tpidermal growth factor receptor (EGFRt)	lentivirus	Autologous T cells/intravenous	Rebecca Gardner
	B-lineage malignancies	US-1236	USA	I	2013/7	CD19 antigen specific-zeta T cell receptor	Sleeping beauty transposon	Allogeneic umbilical cord blood derived lymphocytes/intravenous	Partow Kebriaei
	Chemotherapy resistant or refractory acute lymphoblastic leukemia	US-1259	USA	II	2013/10	CD19 antigen specific-zeta T cell receptor/TCR and 4-1BB signaling domains	Lentivirus	Autologous T cells/intravenous	Noelle Frey
	Relapsed or refractory acute myeloid leukemia	US-1287	USA	I	2014/1	CD133 specific chimeric antigen receptor (CAR) epidermal growth factor receptor	Lentivirus	Autologous T cells/intravenous	L. Elizabeth Budde
	CD 19+ leukemia or lymphoma	US-1291	USA	I	2014/1	Humanized CD19 antigen specific-zeta T cell receptor/TCR and 4-1BB signaling domains	Lentivirus	Autologous T cells/intravenous	Shannon L. Maude
	CD19 B cell lymphoproliferative neoplasms	US-1292	USA	I	2014/2	CD19 antigen specific chimeric antigen receptor (CAR) epidermal growth factor receptor	Lentivirus	Autologous central memory T lymphocytes (Tcm)/intravenous	Tanya Siddiqui
	Relapsed/refractory ALL, relapsed ALL post allogeneic SCT	US-1299	USA	II	2014	CD19 chimeric antigen receptors Expressing tandem TCR and 4-1BB costimulatory domains	Lentivirus	Autologous T cells/intravenous	Noelle Frey

Table 2 continued

Category	Disease	Trial ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
Myeloma	Relapsed/refractory ALL, relapsed ALL post allogeneic SCT	US-1300	USA	II	2014/4	CD19 chimeric antigen receptors Expressing tandem TCR and 4-1BB costimulatory domains	Lentivirus	Autologous T cells/intravenous	Stephen Grupp
	Acute myeloid leukemia, advanced myelodysplastic syndrome and multiple myeloma	US-1319	USA	I	2014/6	NKG2DL chimeric antigen receptor (CAR)/CD3/DAP10/human NKG2D cDNA	Retrovirus	Autologous T cells/intravenous	Susan Baumeister
	B cell malignancies: ALL or lymphoma	US-1320	USA	I	2014/6	CD22 chimeric antigen receptors	Lentivirus	Autologous T cells expressing CD22 chimeric antigen receptors/intravenous	Terry J. Fry
	Elimination of graft versus host disease	US-1325	USA	I/II	2014/7	Inducible caspase 9 suicide gene/AP1903	Retrovirus	Allogeneic T cells/intravenous	Helen Heslop
	Relapsed-refractory ALL, relapsed ALL post allogeneic SCT	US-1351	USA	II	2014/10	CD19 chimeric antigen receptors expressing tandem TCR and 4-1BB costimulatory domains	Lentivirus	Autologous T cells/intravenous	Paul L. Martin
	B-lineage malignancies	US-1353	USA	I	2014/10	CD19 antigen specific-zeta T cell receptor interleukin-15 (IL-15)	Sleeping beauty transposon	Autologous primary CD3+ T cells/intravenous	Partow Kebriaei
	Acute myelogenous leukemia	XX-0012	Multi-country: Italy, UK, Israel	I/II	2001/8	Herpes simplex virus thymidine kinase (HSV-TK)	Retrovirus	Allogeneic lymphocytes/intravenous	Fabio Ciceri
	Multiple myeloma	CA-0011	Canada	I		Interleukin-12, B7.1	Adenovirus	Autologous myeloma cells, BMT	A. K. Stewart
	Multiple myeloma	CA-0012	Canada	I		Interleukin-2	Adenovirus	Autologous myeloma cells	A. K. Stewart
	Recurrent or persistent multiple myeloma	US-0107	USA	I	1995/7	Herpes simplex virus thymidine kinase	Retrovirus	Allogeneic T cells/intravenous	Nikhil C. Munshi

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
Hodgkin Disease	Multiple myeloma	US-0997	USA	II	2009/8	Granulocyte-macrophage colony stimulating factor (GM-CSF)		Allogeneic K562 cells/intravenous	Ivan Borrello
	Multiple myeloma	US-1056	USA	I	2010/7	High affinity T cell receptor specific for MAGE-A3 or NY-ESO-1	Lentivirus	Autologous T cells/intravenous	Aaron Rapoport
	Multiple Myeloma	US-1208	USA	I	2013/1	Chimeric antigen receptors Expressing tandem TCR and 4-1BB costimulatory domains		Autologous T cells expressing CD19/intravenous	Edward Stadtmauer
	Multiple myeloma	US-1303	USA	I	2014/4	BCMA (B cell maturation antigen) chimeric antigen receptors expressing tandem TCR and CD28 costimulatory domains		Autologous T cells/intravenous	James N. Kochenderfer
	EBV + Hodgkin disease	US-0473	USA	I	2001	LMP2A neomycin resistance (NeoR)	Adenovirus + retrovirus	Cytotoxic T lymphocytes/intravenous	Benedikt Gahn
	EBV+ Hodgkin's or non-Hodgkin's lymphoma	US-0608	USA	I	2003	Latent membrane protein 2A (LMP2A)	Adenovirus	LMP2A specific cytotoxic T cells/intravenous	
	Hodgkin's Lymphoma	US-0698	USA	I	2005	Granulocyte-macrophage colony stimulating factor (GM-CSF)	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	Richard Ambinder
	Hodgkin's Lymphoma	US-0823	USA	I	2007/1	Granulocyte-macrophage colony stimulating factor (GM-CSF)	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	Yvette Leslie Kasamon
	Hodgkin's lymphoma/non-Hodgkin's lymphoma	US-1034	USA	I	2010/4	CD30 antigen specific-zeta T cell receptor	Retrovirus	Autologous Epstein Barr Virus cytotoxic T Lymphocytes/intravenous	Helen Heslop
	Hodgkin's lymphoma/non-Hodgkin's lymphoma	US-1066	USA	I	2010/9	CD30 antigen specific-zeta T cell receptor	Retrovirus	Autologous or syngeneic activated T cells/intravenous	Helen Heslop
	Refractory or relapsed Hodgkin lymphoma	US-1346	USA	I	2014/8	Anti-CD 19 TCR 4-1BB	mRNA electroporation	Autologous T cells/intravenous	Susan R. Rheingold

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
Lymphoma	Refractory or relapsed Hodgkin lymphoma	US-1349	USA	I	2014/9	Anti-CD 19 TCR 4-1BB	mRNA electroporation	Autologous T cells/intravenous	Jakub Svoboda
	Lymphoma	CN-0042	China	I/II	2014/10	Anti-CD30 CAR	Lentivirus	Autologous T cells/intravenous	Jun Zhu
	B cell lymphoma	CN-0043	China	I/II	2014/9	Anti-CD19 CAR	Lentivirus	Autologous T cells/intravenous	Jun Zhu
	Relapse or refractory CD30 positive lymphoma	CN-0048	China	I/II	2014/10	Anti-CD30 CAR (4th generation)	Lentivirus	Autologous T cells/intravenous	Jun Zhu
	B cell lymphoma	CN-0049	China	I/II	2014/9	Anti-CD19 CAR (4th generation)	Lentivirus	Autologous T cells/intravenous	Jun Zhu
	Lymphoma, malignant melanoma, renal, colon cancer	DE-0007	Germany	I		Interleukin-7, interleukin-2	Naked/plasmid DNA	Autologous tumor cells/subcutaneous	Ingo Schmidt-Wolf
	AIDS related lymphoma	DE-0078	Germany	I/II	2008/10	Membrane anchored C46	Retrovirus	Gene-modified stem cells	Axel R. Zander
	B-cell non-Hodgkin lymphoma	JP-0030	Japan	I/II	2014/5	Anti-CD19 CAR	Retrovirus	Autologous T cells/intravenous	Keiya Ozawa
	Refractory B cell malignancy	SE-0011	Sweden	I/II	2014/4	Anti-CD19 CAR	Retrovirus	Autologous T cells/intravenous	Gumilla Enblad
	Lymphoma	UK-0002	UK	I/II	1994/12	Multi-drug resistance-I	Retrovirus	Autologous CD34+ PBC/BMT	Devereux
CD20+ B-lymphoid malignancies	Cutaneous T-cell lymphoma, malignant melanoma, breast, head and neck cancer	US-0199	USA	I	1997	Interleukin-12	Retrovirus	Autologous fibroblasts/intra-tumoral	Chan H. Park
	CD20+ B-lymphoid malignancies	US-0330	USA	I	1990	scFvFc-zeta T cell receptor against CD30	Naked/plasmid DNA	Autologous CD8+ T cells/intravenous	Michael Jensen
	Non-Hodgkin B-cell lymphoma	US-0376	USA	I	2000	Dihydrofolate reductase (DHFR) cytidine deaminase	Retrovirus	Autologous CD34+ PBC/intravenous	Joseph Bertino
	Lymphoma	US-0400	USA	I	2000	Multi-drug resistance-I	Retrovirus	Autologous CD34+ PBC/intravenous	Pamela S. Becker
	Follicular non-Hodgkin's lymphoma	US-0491	USA	I	2001	CE7R-specific scFvFc-zeta T cell receptor	Naked/plasmid DNA	Autologous CD8+ T cells/intravenous	Oliver W. Press

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
Lymphoma	Epstein–Barr virus lymphoma, elimination of graft versus host disease	US-0627	USA	I/II	2004	Herpes simplex virus thymidine kinase (HSV-TK) ^r	Dicistronic retroviral vector, termed NIT, encoding a mutant human nerve growth factor receptor (mLNGFR) and herpes simplex virus thymidine kinase (HSV-TK)	Allogeneic T cells/intravenous	Richard J. O'Reilly
		US-0713	USA	I	2005/6	Granulocyte-macrophage colony stimulating factor (GM-CSF) humanized <i>Escherichia coli</i> beta-galactosidase	Vaccinia virus	Cytokine-induced killer (CIK) Cells/intravenous	Robert S. Negrin
		US-0724	USA	I	2005/7	LMP2A dominant negative TGF- β receptor II (DNRII)	Adenovirus +retrovirus	Autologous dendritic cells/intravenous	Catherine Bollard
		US-0776	USA	I	2006/4	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Rammurti T. Kamble
		US-0793	USA	I	2006/7	CD19 antigen specific-zeta T cell receptor	Lentivirus	Autologous T cells/intravenous	David L. Porter
		US-0832	USA	I	2007/2	Granulocyte-macrophage colony stimulating factor	Naked/plasmid DNA	Allogeneic K563 cells/intradermal+subcutaneous	Eric D. Jacobson
		US-0863	USA	I	2007/7	CD20-specific scFvFc-zeta T cell receptor	Naked/plasmid DNA	Autologous T cells/intravenous	Oliver W. Press
		US-0892	USA	I	2008/1	CD19 antigen specific chimeric antigen receptor	Lentivirus	Autologous T cells/intravenous	Leslie Popplewell
		US-0915	USA	I	2008/4	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Caros A. Ramos
		US-1062	USA	I/II	2010/8	CD19 antigen specific chimeric antigen receptor	Lentivirus	Autologous T cells/intravenous	Leslie Popplewell

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
	Non-Hodgkin's lymphoma	US-1183	USA	I	2012/9	CD19 antigen specific chimeric antigen receptor	Lentivirus	Autologous central memory T cells/intravenous	Leslie Popplewell
	Non-Hodgkin's lymphoma, chronic lymphocytic leukemia	US-1191	USA	I	2012/10	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Carlos A. Ramos
	Non-Hodgkin's lymphoma	US-1250	USA	I	2013/8	CD19 antigen specific chimeric antigen receptor epidermal growth factor receptor	Lentivirus	Autologous central memory T cells/intravenous	Leslie Popplewell
	CD19+ oymphoma	US-1258	USA	II	2013/10	CD19 antigen specific-zeta T cell receptor/TCR and $\pm$ IBB signaling domains	Lentivirus	Autologous T cells/intravenous	Stephen Schuster
	Non-Hodgkin's lymphoma, chronic lymphocytic leukemia	US-1276	USA	I	2013/11	CD19 antigen specific zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Malcolm K. Brenner
	Lymphoma	US-1279	USA	I	2013/11	Epidermal growth factor receptor	Lentivirus	Autologous central memory T lymphocytes (Tcm)/intravenous	Samer K. Khaled
	Non-Hodgkin lymphoma	US-1339	USA	I/II	2014/7	CD19 antigen specific-zeta T cell receptor KTE-C19)	Retrovirus	Autologous T cells/intravenous	Steven Rosenberg
	Mantle cell lymphoma	US-1376	USA	II	2015/1	CD19 antigen specific-zeta T cell receptor	Retrovirus	Autologous T cells/intravenous	Michael Wang
	Mantle cell lymphoma	CN-0036	China	I/III	2014/3	CD19CAR	retrovirus	Autologous T cells/intravenous	Vu Zhao
	Mantle cell lymphoma	US-0654	USA	II	2004	Granulocyte-macrophage colony stimulating factor (GM-CSF) CD15+ (CD+0-ligand)	Naked/plasmid DNA	Allogeneic K562 cells/intradermal	Sophie Dessureault
	Hematological malignancies	DE-0079	Multi-country: Germany, Italy	I/II	2002	Herpes simplex virus thymidine kinase (HSV-TK)			B. Hertenstein

Table 2 continued

Category	Disease	Trail ID	Country	Clinical phase	Approved Year	Genes	Vector	Target cells/route	Principle investigator
Graft-versus-host disease	Graft-versus-host disease	US-0849	USA	I	2007/4	Inducible caspase 9 suicide gene	Retrovirus	Allogeneic T cells/intravenous	Malcolm K. Brenner
	Graft-versus-host disease	US-1092	USA	I	2011/3	Inducible caspase 9 suicide gene	Retrovirus	Haploidentical donor T cells/intravenous	Malcolm K. Brenner
	Graft-versus-host disease	US-1158	USA	I/II	2012/4	Inducible caspase 9 suicide gene/API903	Retrovirus	Allogeneic (partially mismatched, related donor) T cells/intravenous	Hillard Lazarus
	Graft-versus-host disease	US-1159	USA	I/II	2012/4	Inducible caspase 9 suicide gene/API903	Retrovirus	Allogeneic T cells/intravenous	Richard E. Champlin

CAR chimeric antigen receptor, *PBL* peripheral blood lymphocytes, *PBC* peripheral blood cells, *BMT* bone marrow transplantation

been reported in any of these patients, despite equivalent vector design and integration profiles similar to those seen in other trials [21, 28]. Clonal analysis of long-term repopulating cell progeny in vivo revealed highly polyclonal T-cell populations and shared retroviral integration sites among multiple lineages, demonstrating the engraftment of multipotent HSCs [29]. ADA enzyme production may cross-rescue ADA-deficient lymphocytes, thus placing less replicative stress on the gene-corrected cells [21]. To achieve safer gene therapy for ADA⁺SCID patients, current trials have adopted self-inactivating HIV-1-based lentiviral vectors. Lentiviral vectors are pseudotyped with the vesicular stomatitis virus glycoprotein, which uses the broadly expressed low-density lipoprotein receptor for cell entry and thus achieves broad-cell tropism. At least 30 ADA⁺SCID patients have been treated with lentiviral vectors in the US and the UK. Excellent immune reconstitution has been achieved in these patients, with no vector-related complications [21].

In Japan, the first gene therapy for an ADA⁺SCID patient was initiated in 1995. The patient, a 5-year-old Japanese male, received periodic infusions of retrovirally ADA gene-transduced autologous T cells. The percentage of peripheral-blood lymphocytes carrying the transduced ADA gene remained stable at 10–20 % during the 12 months since the fourth infusion. ADA enzyme activity in the patient's circulating T cells, which was barely detectable before gene transfer, increased to levels comparable to those of a heterozygous carrier individual, and was associated with increased T-cell counts and improvement in the patient's immune function. In 2003–2004, two ADA⁺SCID patients, including one who received T-cell gene therapy, safely received retrovirally ADA gene-transduced autologous bone marrow CD34⁺ cells without any cytoreductive conditioning after discontinuation of ERT. Partial recovery of immunity due to moderate systemic detoxification was achieved, allowing temporary discontinuation of ERT for 6 and 10 years in patients 1 and 2, respectively. Vector integration-site analyses confirmed that hematopoiesis was reconstituted by a limited number of clones, some of which had myelo-lymphoid potential. These results emphasize that cytoreductive conditioning is important for achieving optimal benefits from SCGT [30] (Table 3).

X-linked severe combined immunodeficiency (SCID-X1)

X-linked severe combined immunodeficiency (SCID-X1) is caused by mutation in the gene encoding the gamma subunit of the interleukin-2 receptor (γ_c), a key subunit of the cytokine receptor complex for interleukin (IL)2, IL4, IL7, IL9, IL15, and IL21. Common γ_c deficiency is the most frequent genetic form of SCID, accounting for 50–60 % of cases, and is typically characterized by an absence of

Table 3 Ex vivo gene therapy for hematological disorders in Japan

Approved year	Affiliation	Disease	Gene	Vector	Status (cases)
(1) 1995	Hokkaido Univ.	ADA def	ADA	Retro	Finished (1)
(2) 2002	Cancer Inst.	Breast Ca	MDR1	Retro	Finished (3)
(3) 2002	Tsukuba Univ.	Leukemia (relapse)	HSV-TKΔLNGFR	Retro	Under way (10)
(4) 2002	Hokkaido Univ.	ADA def	ADA	Retro	Under way (2/4)
(5) 2002	Tohoku Univ.	X-SCID	Common γ -chain	Retro	Not started
(6) 2007	Takara Bio	Leukemia (relapse)	HSV-TKΔLNGFR	Retro	Under way (9)
(7) 2009	N.C.C.	Leukemia (relapse)	HSV-TKΔLNGFR	Retro	Under way
(8) 2012	N.C.C.H.D	X-CGD	hCYBB	Retro	Under way
(9) 2013	Multiple Univ.	Leukemia and MDS	WT-1 TCR $\alpha\beta$	Retro	Under way (9)
(10) 2014	Jichi Univ.	B cell lymphoma	CD19 CAR	Retro	Under way

NCC National Cancer Center, *NCCHD* National Center for Child Health and Development, *ADA def* adenosine deaminase deficiency, *retro* retroviral vector, *ex vivo* gene therapy, *Ca* cancer, *X-SCID* X linked severe combined immune deficiency, *X-CGD* X-linked chronic granulomatous disease, *MDS* myelodysplastic syndrome, *MDR1* multidrug resistance protein 1, *HSV* herpes simplex virus, *TCR* T cell receptor, *CAR* chimeric antigen receptor

mature T cells and natural killer (NK) lymphocytes due to a complete blockade in their development [31]. SCID-X1 is a lethal condition that can be cured by allogeneic stem cell transplantation. Between 1999 and 2002, the first ex vivo gene therapy was conducted in France, using retrovirally common γ c gene-transduced CD34+ bone marrow cells. The patients in this trial were 10 SCID-X1 boys without HLA-identical stem cell donors. Immune functions, including normalization of the numbers and phenotypes of T cells, the repertoire of T-cell receptors, and the in vitro proliferative responses of T cells to several antigens after immunization, persisted for up to 2 years after treatment [32]. Subsequently, a similar study was initiated in the UK. A total of 20 patients were enrolled and successfully treated with ex vivo-transduced CD34+ cells without any conditioning regime [33]. In the US, five older patients were treated with a similar protocol. Functional reconstitution, however, was not achieved in those patients, probably due to loss of thymic function by the time gene therapy was initiated. The promising results in France and the UK were subsequently hampered by the occurrence of lymphoid malignancies. Five patients developed acute T-cell leukemia; four entered remission after standard chemotherapy, but one died. All of the leukemias were associated with viral integration in oncogenes; in four of the cases, the integration occurred in *LMO2*. The enhancer activity of the viral LTR was the most likely cause of the initial aberrant expression of these oncogenes and dysregulation of the cell cycle; subsequent accumulation of additional genetic abnormalities, such as associated rearrangements in *BM11*, *CCND2*, and *NOTCH1*, led to frank leukemic transformation [34]. In the French cases, transduced T cells were detected for up to 10.7 years after gene therapy. Seven patients, including three of the leukemia survivors, exhibited sustained immune reconstitution, whereas the

three others required immunoglobulin-replacement therapy. Sustained thymopoiesis was established by the persistent presence of naive T cells, even after chemotherapy, in three patients. The T-cell receptor repertoire was diverse in all patients. Transduced B cells were not detected. Correction of immunodeficiency improved the patients' health. After nearly 10 years of follow-up, gene therapy had corrected the immunodeficiency associated with SCID-X1.

Gene therapy is considered to be an option for patients who do not have an HLA-identical donor for HSCT and for whom the risks are deemed acceptable; the treatment is associated with a risk of acute leukemia [35, 36]. Patients who underwent gene therapy also demonstrated a clear advantage in terms of T-cell development relative to patients who underwent HSCT with a mismatched donor; moreover, patients treated with gene therapy exhibited faster T-cell reconstitution and better long-term thymic output. Interestingly, this advantage of gene therapy relative to haploidentical HSCT seemed to be independent of the presence of clinical graft-versus-host disease in the latter condition [37]. Parallel trials in Europe and the US evaluated treatment with a self-inactivating (SIN) γ -retrovirus vector containing deletions in viral enhancer sequences expressing γ c (SIN- γ c). All of the patients, nine boys with SCID-X1, received bone marrow-derived CD34+ cells transduced with the SIN- γ c vector, without cytoreductive conditioning. After 12.1–38.7 months of follow-up, seven of the boys exhibited recovery of peripheral-blood T cells that were functional and led to resolution of infections. The patients remained healthy thereafter. The kinetics of CD3+ T-cell recovery was not significantly different from those observed in previous trials. Assessment of insertion sites in peripheral blood from patients in the current trial revealed significantly less clustering of insertion sites within *LMO2*, *MECOM*, and other lymphoid proto-oncogenes than in the

previous trials. Thus, the modified γ -retrovirus vector is a safer method for treatment of SCID-X1 than the first-generation Moloney murine leukemia virus vector expressing γc [13].

The gamma chain of the human IL-2 receptor was originally cloned in Japan in 1992 [38]. Gene therapy using a γ -retrovirus vector expressing the gamma chain of the human IL-2 receptor was approved in Japan in 2002. The clinical trial was not started, however, because lymphoid malignancy arose in French patients in 2002.

X-linked chronic granulomatous disease (X-CGD)

Chronic granulomatous disease is a genetically heterogeneous immunodeficiency disorder resulting from an inability of phagocytes to kill microbes that they have ingested. This impairment in killing is caused by any of several defects in the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (phox) complex, which generates the microbicidal ‘respiratory burst’, patients carrying mutations in subunits of NADPH oxidase suffer from defects in neutrophil function (<http://www.omim.org/>). The fully assembled NADPH oxidase, which consists of the cytosolic phox proteins (p47^{phox}, p67^{phox}, and p40^{phox}, respectively, encoded by *NCF1*, *NCF2*, and *NCF4*), translocated to the membrane-bound flavocytochrome (gp91^{phox} and p22^{phox}, respectively, encoded by *CYBB* and *CYBA*), leads through a series of reactions to the production of reactive oxygen species (ROS), which are essential for phagocytic killing of invading microbes. Mutations in *CYBB*, which encodes gp91^{phox}, lead to the X-linked form of this disease; these mutations account for 60–80 % of all CGD patients in most European countries, North America and Japan. The next most common form, representing about one-third of cases, is an autosomal recessive disease resulting from mutations in *NCF1* (encoding p47^{phox}) on chromosome 7. CGD patients are susceptible to recurrent and severe bacterial and fungal infections, notably *Staphylococcus* and *Aspergillus*, and are also affected by inflammatory complications in the lungs and gastrointestinal and genitourinary tracts. Conventional therapy involves a broad regimen of antifungals, antibiotics, anti-inflammatory medications, and HSCT, but its effectiveness is limited [34, 39, 40].

Initial trials of ex vivo gene therapy for X-CGD were conducted in the US in 1995 and 1998, targeting p47^{phox} and gp91^{phox} deficiency, respectively, using gamma-retroviral vectors to transduce granulocyte colony stimulating factor (G-CSF)-mobilized CD34⁺ HSCs without any cytoreductive conditioning. A total of 10 patients were involved. In both trials, only transient production of ROS-producing neutrophils was detected, and no remarkable long-term clinical benefits accrued [34, 39–41].

Subsequent clinical trials in Germany used gamma-retroviral vectors to transduce granulocyte colony stimulating factor (G-CSF)-mobilized CD34⁺ HSCs after a non-myeloablative conditioning regimen. Two adult patients exhibited substantial gene transfer to neutrophils, leading to a large number of functionally corrected phagocytes and notable clinical improvement. Large-scale analysis of the distribution of retroviral integration sites in both individuals revealed activating insertions in *MDS1-EVII*, *PRDM16*, or *SETBP1* that influenced regulation of long-term hematopoiesis by enhancing gene-corrected myelopoiesis 3–4-fold. Thus, gene therapy in combination with bone marrow conditioning was successfully used to treat CGD, an inherited disease affecting the myeloid compartment [42]. Subsequently, a total of 13 patients were treated in similar manner, and transient clinical benefits due to gene transfer were observed [10, 34, 43–47]. Three patients, including two adults, exhibited a temporary increase in gene-marked neutrophils followed by the clearance of infections. However, insertional mutagenesis occurred due to integration into the *MECOM* (*MDS/EVII* complex) and *PRDM16* oncogene loci, and transactivation by the viral SFFV LTR was similar to that observed in SCID-X1 patients. Two adults with fatal outcomes exhibited silencing of transgene expression due to methylation of the viral promoter, and myelodysplasia with monosomy 7 due to insertional activation of ectopic viral integration site 1 (*EVII*). Forced overexpression of *EVII* in human cells disrupts normal centrosome duplication, linking *EVII* activation to the development of genomic instability, monosomy 7, and clonal progression toward myelodysplasia [34, 46]. The frequency of these adverse events highlighted the fact that only gp91^{phox}-transduced cells with gain-of-function events could persist in patients who underwent ex vivo gene therapy using LTR-driven retrovirus vectors [18]. Moreover, ectopic expression of the gp91^{phox} transgene and the highly activated bone marrow environment caused by X-CGD patients’ chronic infections was thought to promote the loss of gene-corrected cell engraftment. To increase the safety and efficacy of the gene therapy for X-CGD patients, subsequent trials adopted self-inactivating gamma retrovirus (SIN- γ RV) and lentiviral vectors (SIN-LVs) with an internal promoter and a fully myeloablative conditioning regimen (12–16 mg/kg busulfan), prior to gene therapy. The vector currently under evaluation in multicenter trials is an SIN-LV configuration with a chimeric promoter consisting of myeloid-specific Cathepsin G and c-Fes regulatory elements; this promoter element allows preferential expression in myeloid cells and differentiated granulocytes [28, 48].

In Japan, a clinical trial for gene therapy to treat X-CGD was first performed in 2014. This trial, in a 27-year-old man, used a gp91^{phox} gamma-retroviral vector in autologous G-CSF-mobilized CD34⁺ HSCs after busulfan

conditioning. The patient received 3.9×10^8 CD34+ cells, including 76.8 % of gp91phox-expressing cells. Although clinical symptoms of lymphadenitis and CGD-colitis improved until day 95 of transplantation and no genotoxicity was observed, the number of gene-transduced cells was decreased to about 0.1 % of neutrophils at day 182 probably due to the reduction of gene-modified CD34+ stem cells. At present, gene therapy was considered to be useful as a short-term treatment for CGD patients who had no HLA-matched donor for HSCT [49].

Other ex vivo gene therapies for hematological and non-hematological congenital disorders

Wiskott–Aldrich syndrome (WAS) is a rare, complex, X-linked recessive PID disorder caused by mutations in the WAS gene that encodes the WAS protein (WASp). WAS is characterized by recurrent infections, microthrombocytopenia, eczema, and elevated risk of autoimmune manifestations and tumors. WASp is a key regulator of actin polymerization in hematopoietic cells, and contains domains involved in signaling, cell locomotion, and immunologic-synapse formation. The complex biological features of this disease result from multiple dysfunctions in different subgroups of leukocytes, including functional defects in T and B cells, disturbed formation of the NK-cell immunologic synapse, and impaired migratory responses in all leukocyte subgroups. Severe WAS leads to early death from infection or bleeding. Currently, the only curative therapy involves allogeneic HSCT, which is itself associated with considerable risk of death or complications related to transplantation. Therapy with WAS gene-corrected autologous HSCs, thus, represents a valid alternative approach for patients lacking a suitable donor or those who are more than 5 years old [18, 50].

The first gene therapy for WAS was conducted in Germany in 2006; the trial involved two patients. Sustained expression of WASp in HSCs, lymphoid and myeloid cells, and platelets after ex vivo gene therapy was achieved using autologous CD34+ HSCs transduced with gamma retroviral vector after non-myeloablative preconditioning. T and B cells, natural killer (NK) cells, and monocytes were functionally corrected. After treatment, the patients' clinical condition markedly improved, with resolution of hemorrhagic diathesis, eczema, autoimmunity, and predisposition to severe infection. Comprehensive insertion-site analysis revealed vector integration that targeted multiple genes controlling growth and immunologic responses in a persistently polyclonal hematopoiesis [50]. Subsequently, 10 patients with severe WAS were treated using the HSC gene therapy. Nine of the ten patients exhibited sustained engraftment and correction of WASp expression in lymphoid and myeloid cells and platelets. Gene therapy

resulted in partial or complete resolution of immunodeficiency, autoimmunity, and bleeding diathesis. Analysis of retroviral insertion sites revealed >140,000 unambiguous integration sites and a polyclonal pattern of hematopoiesis in all patients soon after gene therapy. Seven patients developed acute leukemia [one with acute myeloid leukemia (AML), four with T-cell acute lymphoblastic leukemia (T-ALL), and two with primary T-ALL with secondary AML associated with a dominant clone; vector integration occurred at *LMO2* (six T-ALL), *MDS1* (two AML), or *MNI* (one AML) locus]. Thus, *LMO2*-driven leukemogenesis is not specific for X-SCID gene therapy, but is also seen in WAS gene therapy. Cytogenetic analysis revealed additional genetic alterations, including chromosomal translocations. This study showed that hematopoietic stem cell gene therapy for WAS is feasible and effective; however, the use of γ -retroviral vectors was associated with a substantial risk of leukemogenesis [51].

In 2010, three WAS patients in Italy received bone marrow-derived CD34+ cells genetically modified with an SIN lentiviral vector encoding functional WASp after a reduced-intensity conditioning regimen. All three patients exhibited stable engraftment of WASp-expressing cells and improvements in platelet counts, immune functions, and clinical scores. Vector integration analyses revealed highly polyclonal and multilineage hematopoiesis resulting from the gene-corrected HSCs. Lentiviral gene therapy did not induce selection of integrations near oncogenes, and no aberrant clonal expansion was observed after 20–32 months [52]. Between 2010 and 2014, seven WAS patients in France and England received a single infusion of CD34+ cells genetically modified with an SIN lentiviral vector. Six of the seven patients were alive at the time of last follow-up and exhibited sustained clinical benefit. One patient died 7 months after treatment due to a preexisting drug-resistant herpes virus infection. Eczema and susceptibility to infections resolved in all six patients. Autoimmunity improved in five patients. No severe bleeding episodes were recorded after treatment, and at last follow-up, all six surviving patients were free of blood product support and thrombopoietic agonists. Hospitalization days were reduced from a median of 25 days during the 2 years before treatment to a median of 0 days during the 2 years after treatment. All six surviving patients exhibited high-level, stable engraftment of functionally corrected lymphoid cells. The degree of myeloid cell engraftment and of platelet reconstitution correlated with the dose of gene-corrected cells administered [53]. No evidence of vector-related toxicity was observed either clinically or by molecular analysis in either of these clinical trials using SIN lentiviral vectors, although long-term follow-up is still required [52, 53].

Ex vivo gene therapy for congenital diseases other than PIDs has also been developed. Sickle cell disease (SCD)

and β -thalassemia major (β -TM), the latter defined clinically as transfusion-dependent cases regardless of the underlying genotype, are the most common monogenic disorders worldwide, with approximately 400,000-affected conceptions or births each year. Over the past 8 years, clinical trials have evaluated gene therapy by ex vivo lentiviral transfer of a therapeutic β -globin gene derivative (β (AT87Q)-globin) into hematopoietic stem cells, driven by cis-regulatory elements that confer high, erythroid-specific expression. β (AT87Q)-globin is used as both a strong inhibitor of HbS polymerization and a biomarker. While long-term studies are underway in multiple centers in Europe and in the US, proof-of-principle of efficacy and safety has already been obtained in multiple patients with β -TM and SCD [54].

LG001 and HGB-205 were the first gene therapy studies worldwide to treat β -TM and SCD subjects, respectively, achieving the first conversion to long-term transfusion independence of a β -TM patients and the first evidence of clinical benefit in SCD [54, 55]. An SIN lentiviral vector expressing HPV569 β (AT87Q)-globin was used in the LG001 trial. Four β -TM patients were enrolled, 3 of whom were treated between 2006 and 2011. Transduced cells successfully engrafted in two of them. The HPV drug product was well tolerated, with no non-hematologic or drug product-related serious adverse events. One of the two treated patients receiving no backup cells exhibited clinical benefits as evidenced by transfusion independence for approximately 8 years. Integration-site analysis revealed the relative dominance of a clone bearing the vector in intron 3 of the *HMGA2* gene, which reached a maximum representation of 30 % of transduced myeloid cells 15 months after gene therapy. The BB305 SIN lentiviral vector was the second generation of the HPV569, lacking the *cHS4* insulator, and exhibited elevated vector titers and transduction efficiency. Several clinical trials using BB305 had been initiated in France, two separate international, and USA for β -TM and SCD patients [54].

The following two ex vivo gene therapies are for non-hematological diseases, but HSCs were used as therapeutic vehicle. First, X-linked adrenoleukodystrophy (ALD) is a severe brain demyelinating disease in boys that is caused by a deficiency in the ALD protein, an adenosine triphosphate-binding cassette transporter encoded by the *ABCD1* gene. ALD progression can be halted by allogeneic hematopoietic cell transplantation (HCT). An ex vivo gene therapy trial for two ALD patients without any matched donors was initiated in 2005. Lentivirally *ABCD1* gene-transduced autologous CD34+ cells were reinfused into the patients after fully myeloablative preconditioning. Over a span of 24–30 months of follow-up, polyclonal reconstitution was observed, suggesting successful gene transduction into HSCs. Beginning 14–16 months after infusion of the

genetically corrected cells, progressive cerebral demyelination in the two patients stopped, a clinical outcome comparable to that achieved by allogeneic HCT. Thus, lentiviral-mediated gene therapy of HSCs provided clinical benefits in ALD [56].

Second, metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. Patients with MLD exhibit progressive motor and cognitive impairment and die within a few years of symptom onset. Three presymptomatic patients who exhibited genetic, biochemical, and neurophysiological evidence for late infantile MLD received lentivirally ARSA gene-transduced HSCs. After receiving the gene-corrected HSCs, the patients exhibited extensive and stable ARSA gene replacement, which led to high enzyme expression throughout hematopoietic lineages and in cerebrospinal fluid. Analyses of vector integrations revealed no evidence of aberrant clonal behavior. The disease did not manifest or progress in the three patients for 7–21 months beyond the predicted age of symptom onset. These findings suggested the clinical usefulness of lentiviral gene therapy for MLD patients [57].

Ex vivo gene therapy clinical trials for hematological malignancies in Japan and around the world

Ex vivo gene therapy clinical trials for hematological malignancies have been primarily performed to enhance host immune function in patients who could not receive stem-cell transplantation due to a lack of appropriate donors, who relapsed, or who were refractory to standard chemotherapies (Table 2). As recent advances in gene therapy using the receptor gene-modified T-cell therapy are excellently reviewed by Davila ML and Sadelain M in this special issue, in the following, we will discuss immune therapy using suicide gene-transduced donor lymphocytes and gene-modified tumor vaccines.

Suicide gene therapy

The most effective and established cell therapy approach is allogeneic HSCT, which is currently the only cure for patients with several high-risk hematological malignancies. Alloreactive T cells induce potent graft-versus-tumor effect (GvT) and also trigger detrimental graft-versus-host disease (GvHD). Gene therapy technology allows T cells to exert the GvT effect, while avoiding or controlling GvHD.

Herpes simplex virus thymidine kinase (HSV-TK) is the suicide gene most extensively tested in humans. Expression of HSV-TK in donor lymphocytes confers sensitivity to the anti-herpes drug ganciclovir (GCV). Progressive improvements in suicide genes, vector technology, and transduction protocols have ameliorated the toxicity of GvHD, while

preserving the antitumor efficacy of allogeneic HSCT. Several clinical trials in the last 20 years documented the safety and the efficacy of the HSV-TK approach and established its important role in cellular therapy against cancer. Recently developed gene therapies have improved immune effector cell survival, homing, function, safety, and specificity using high-avidity tumor-reactive T-cell receptors (TCRs) or chimeric antigen receptors, and suicide gene therapy has been reappraised with the goal of avoiding or controlling the toxic effects induced by these innovative therapies [58].

After the successful administration and long-term detection of retrovirally gene-modified Epstein–Barr virus (EBV)-specific T cells in the US [59], eight Italian patients who relapsed or developed EBV-induced lymphoma after T-cell-depleted BMT were treated with donor lymphocytes retrovirally transduced with the HSV-TK suicide gene. The transduced lymphocytes survived for up to 12 months, and exerted antitumor activity in five patients. Three patients developed GvHD, which was effectively controlled by ganciclovir-induced elimination of the transduced cells. These data suggested that genetic manipulation of donor lymphocytes increased the efficacy and safety of allo-BMT and expanded its application to a larger number of patients [60]. Based on these initial clinical trials, several groups around the world have exploited suicide gene therapy to control T-cell reactivity in the context of allogeneic HSCT. The major advantage of this approach is that the donor T cells permanently acquire sensitivity to the prodrug GCV, allowing donor T-cell alloreactivity to be selectively eliminated without the administration of immunosuppressive drugs that might interfere with the natural process of post-transplant immune reconstitution. In the TK suicide gene/prodrug system, only alloreactive gene-modified T cells that proliferate actively during GVHD are killed by GCV, whereas resting transduced T cells and untransduced cells are spared. TK gene therapy has proven to be safe, with no documented adverse events related to the gene-transfer procedure, including appearance of replication-competent retroviruses or genotoxic effects of vector integration [61–71].

Based on these promising results, the safety and efficacy of suicide gene-modified donor T cells were tested in the more challenging condition of haploidentical HSCT. In this setting, the risk of GvHD is particularly high due to the immunological disparity between donor and host [58, 61]. In a phase I/II, multicenter, non-randomized trial of haploidentical stem-cell transplantation, and retrovirally HSV-TK-transduced donor lymphocytes (TK cells) were administered after transplantation. The primary study endpoint was immune reconstitution, defined as circulating CD3⁺ count ≥ 100 cells per μL in two consecutive observations. Fifty patients received haploidentical stem-cell transplants for high-risk leukemia. Immune reconstitution

was not recorded before infusion of TK cells. Starting 28 days after transplantation, 28 patients received TK cells; and 22 patients achieved immune reconstitution (median, 75 days from transplantation and 23 days from infusion). Ten patients developed acute GVHD and one developed chronic GVHD, which were controlled by the induction of the suicide gene. Overall survival at 3 years was 49 % for 19 patients who were in remission from primary leukemia at the time of stem-cell transplantation. After TK-cell infusion, the last death due to infection was at 166 days, and this was the only infectious death after day 100. No acute or chronic adverse events related to the gene-transfer procedure were observed [72].

In candidates for haploidentical stem-cell transplantation, infusion of TK cells was considered to be effective in accelerating immune reconstitution, while controlling GVHD and protecting patients from late mortality [72]. TK cells played an active role in supporting a thymic-dependent pathway of immune reconstitution, which resulted in maturation and differentiation of donor hematopoietic precursors in the recipient thymus. This process was especially remarkable, because the cohort consisted of adults with a median age of 51, a stage of life usually characterized by low thymic output. Thus, infusion of genetically modified donor T cells after HSCT can drive recovery of thymic activity in adults, leading to immune reconstitution [61, 72, 73]. The HSV-TK strategy is currently under evaluation in a phase III clinical trial in patients undergoing haploidentical HSCT for high-risk acute leukemia. Preliminary results of this ongoing trial confirmed the potential clinical benefit of the T-cell gene-transfer technology integrated with T-cell depletion haploidentical HSCT, and highlighted the role of early immune reconstitution as a surrogate endpoint for survival outcomes and the dose-related antileukemic effects of TK [58].

An inducible T-cell safety switch based on the fusion of human caspase 9 to a modified human FK-binding protein, allowing conditional dimerization, was recently developed. When exposed to a chemical inducer of dimerization (CID), the inducible caspase 9 is activated, leading to the rapid death of cells expressing this construct [74]. Alloplete haploidentical T cells expressing the inducible caspase 9 suicide gene could reconstitute immunity post-transplant, and administration of CID could eliminate these cells from both peripheral blood and the central nervous system (CNS), leading to rapid resolution of GVHD and GVHD-associated cytokine release syndrome [75]. The safety and efficiency of repeated CID treatments for persistent or recurring toxicity from T-cell therapies have also been demonstrated [76]. This approach was considered to be useful for the rapid and effective treatment of toxicities associated with infusion of engineered T lymphocytes [75, 76].

In Japan, clinical trials designed to examine the feasibility, safety, and efficacy of donor lymphocyte infusion (DLI) of TK cells were approved in 2002 and 2009. DLI of TK cells was safely performed in a total of eight patients. However, these TK cells disappeared rapidly, probably due to their insufficient *in vivo* expansion in these patients [77, 78]. A comparison of onset with remission of acute GVHD confirmed that TK cells were predominantly eliminated and that proliferative CD8(+) non-TK cells were also depleted in response to ganciclovir administration. The TCR V β -chain repertoire of both TK cells and non-TK cells changed markedly after administration of ganciclovir. In addition, whereas the TCR repertoire of non-TK cells returned to a normal spectratype long after transplantation, that of TK cells remained skewed. With the long-term prophylactic administration of acyclovir, TK cells expanded oligoclonally, and the frequency of splice variants of TK cells increased. Known cancer-associated genes were not evident near the oligoclonally expanded HSV-TK insertion sites [78, 79].

Gene-manipulated leukemia cell vaccines

Both autologous and allogeneic whole tumor cells were clinically developed as another form of cellular vaccine for cancer. The advantage of using the whole tumor-cell approach is that the tumor antigens do not have to be prospectively identified, and multiple antigens can be simultaneously targeted. Cytokine-expressing whole tumor cell approaches, including GM-CSF-expressing cells (GVAX), have been extensively clinically investigated in the context of solid tumors [15, 80]. Here, we briefly discuss clinical trials of GVAX for hematological malignancy.

Although allogeneic HSCT affords durable clinical benefits for many patients with hematologic malignancies, mediated via the GvL effect, patients with high-risk acute myeloid leukemia (AML) or advanced myelodysplasia (MDS) often relapse, underscoring the need to intensify tumor immunity within this cohort. In a phase I clinical trial, high-risk AML or MDS patients were immunized with irradiated, autologous, GM-CSF gene-transduced cells early after allogeneic, and non-myeloablative HSCT. Despite the administration of a calcineurin inhibitor as prophylaxis against GVHD, vaccination elicited local and systemic reactions that were qualitatively similar to those previously observed in non-transplanted, immunized solid-tumor patients. While the frequencies of acute and chronic GVHD were not elevated, nine of ten who completed vaccination achieved durable complete remissions, with a median follow-up of 26 months. Six long-term responders exhibited marked reductions in the levels of soluble NKG2D ligands, and three exhibited normalization of cytotoxic lymphocyte NKG2D expression as a

function of treatment. These results established the safety and immunogenicity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic HSCT, and raised the possibility that this combinatorial immunotherapy might potentiate GvL effects in patients [81]. In a phase II study of immune gene therapy, autologous leukemia cells admixed with GM-CSF-secreting K562 cells were administered to adult patients with acute myeloid leukemia. "Primed" lymphocytes were collected after a single pre-transplantation dose of immunotherapy and reinfused with the stem cell graft. Fifty-four subjects were enrolled; 85 % achieved complete remission; and 52 % received pre-transplantation immunotherapy. For all patients who achieved complete remission, the 3-year relapse-free survival (RFS) rate was 47.4 %, and overall survival was 57.4 %. For the 28 immunotherapy-treated patients, the RFS and overall survival rates were 61.8 and 73.4 %, respectively. Post-treatment induction of delayed-type hypersensitivity reactions to autologous leukemia cells was associated with a higher 3-year RFS rate (100 vs 48 %). Minimal residual disease was monitored by the quantitative analysis of Wilms tumor-1 (WT1), a leukemia-associated gene. A decrease in WT1 transcripts in blood was noted in 69 % of patients after the first immunotherapy dose, and was also associated with a higher 3-year RFS (61 vs 0 %). Immunotherapy in combination with primed lymphocytes and autologous stem cell transplantation showed encouraging signs of potential activity in acute myeloid leukemia [82].

A pilot study was performed to determine whether K562/GM-CSF immunotherapy could improve clinical responses to imatinib mesylate (IM) in patients with chronic myeloid leukemia (CML) in cytogenetic but not molecular complete remission. Nineteen patients, with a median duration of previous imatinib mesylate therapy of 37 months, were vaccinated. Mean PCR measurements of BCR-ABL for the group decreased significantly following vaccination. A progressive decline in disease burden was observed in thirteen patients, of whom eight had increasing disease burdens before vaccination. Twelve patients, including seven subjects who became PCR-undetectable, achieved their lowest tumor burden measurements following vaccination. Thus, the K562/GM-CSF vaccine improved molecular responses in patients on imatinib mesylate, including achievement of complete molecular remissions, despite long durations of previous imatinib mesylate therapy [83].

Conclusion

Ex vivo gene therapies have been gradually developing, and seem to lead the gene and cell therapies. The large quantities of information obtained during the translation

of preclinical findings regarding ex vivo gene therapies to clinical trials facilitated the development of new modalities for the treatment of congenital intractable diseases and hematological malignancies. Particularly for congenital diseases, newly developed gene-editing technology, excellently reviewed by Voytas and Tolar in this special issue, will unquestionably change current gene therapy approaches using autologous HSCs [84]. In the case of malignancies, the recent development of gene-modified T-cell technologies, including chimeric antigen receptors and immune checkpoint inhibitors, will change the gene therapy modality for treatment of solid tumors as well as hematological malignancies [85, 86].

In Japan, expedited approval system consisting of conditional and time-limited authorization based on two laws known as the “The Act on the Safety of Regenerative Medicine” and the “Pharmaceuticals, Medical Devices and Other Therapeutic Products Act” has been introduced to approve new regenerative medical products, including ex vivo gene therapy since 2014. Under the traditional approval process, long-term data collection and evaluation in clinical trials for regenerative medical products were needed. New separate approval process, however, will make it possible for the regenerative, including ex vivo gene therapy medical product obtain conditional, time-limited approval, if an exploratory clinical trial predicts likely clinical benefit [87]. This new act would accelerate the development of ex vivo gene therapy in Japan.

Needless to say, further clarifications of the pathogenesis and molecular mechanisms of intractable congenital disorders and hematological malignancies, as well as further development of gene-transfer technologies, are required to achieve our goal of ideal gene therapy.

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