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Review

Gene therapy clinical trials, where do we go? An overview

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ABSTRACT

There have been many ups and downs since the introduction of gene therapy as a therapeutic modality for diseases. However, the journey of gene therapy has reached a fundamental milestone, as evidenced by the increasing number of gene therapy products on the market. Looking at the currently approved and under-approval products, as well as the numerous clinical trials in this field, gene therapy has a promising future. Trend of changes in gene therapy strategies, vectors, and targets could be insightful for pharmaceutical companies, policymakers, and researchers. In this paper, following a brief history of gene therapy, we reviewed current gene therapy products as well as gene therapies that may be approved in the near future. We also looked at ten-year changes in gene therapy clinical trials strategies, such as the use of vectors, target cells, transferred genes, and *ex-vivo/in-vivo* methods, as well as the major fields that gene therapy has entered. Although gene therapy was initially used to treat genetic diseases, cancer now has the greatest number of gene therapy clinical trials. Changes in gene therapy strategies, particularly in pioneering countries in this field, may point to the direction of future clinical products.

1. Introduction

Recent advances in cell and gene therapies have enabled the treatment of a wide range of conditions, from congenital disorders to solid cancers. Since the first gene therapy in 1990 [1], numerous efforts have been made, which have culminated in the approval of several gene therapy products in recent years, ushering humanity into a new era of gene therapy. Several milestones along the path of gene therapy research paved the way for science to be translated into products from the bench to the bedside. Examining the trends of successful innovative strategies in this field provides valuable information for future research, improved study designs, and realistic policy-making for gene therapy research centers.

The goal of this overview was to provide an overview of gene therapy clinical trials and to analyze recent trends, with a focus on gene therapy strategies. In this study, we reviewed clinical trials information from Clinicaltrials.gov from 2010 to October 2020. The website was searched using the following keywords: "Gene", "Gene Therapy", "CAR T cell", "Chimeric antigen receptor", "CRISPR", "DC", "Engineered", "Lenti", "Retro", "Adeno", "AAV", "HSV", "Micro RNA", "miR", "miRNA", "siRNA", "T cell", "TCR", "TALEN", "Zinc finger". After duplicate removal, search results were screened based on titles. Data was extracted from the remaining studies. The clinical trial inclusion flowchart is depicted in Fig. 1. The extracted data for final analysis included publication information (start year, country, study start and end dates), type of used vector, type of engineered cells or targeted tissues, transferred genes, *ex-vivo* or *in-vivo* method of gene therapy, clinical trial phase, disease group (Cancer, Genetic disease, infection, and others) and its subsequent subcategories, and the specific condition for which gene therapy is used. It should be noted that if any of the assessed variables had been undetermined or unreachable, they were excluded from the analysis. After extracting trial status, terminated, suspended, or withdrawn clinical trials were excluded. Data were analyzed using Excel 2019 (Microsoft™, Redmond, WA, US).

Abbreviations: AAV, Adeno-associated virus; ADA, Adenosine Deaminase; AIDS, Acquired immunodeficiency syndrome; BCG, Bacillus Calmette–Guérin; CAR, Chimeric antigen receptor; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; DC, Dendritic cell; DMD, Deschene muscular dystrophy; EMA, European Medicines Agency; FDA, United States Food and Drug Administration; HPV, Humanpapillomavirus; HSV, Herpes simplex virus; LCA, Leber congenital amaurosis; scFv, single-chain variable fragment; SCID, Severe Combined Immunoeficiency; SFDA, Chinese State Food and Drug Administration; SMA, Spinal muscular atrophy; SMN, Survival Motor Neuron; VSV, Vesicular stomatitis virus.

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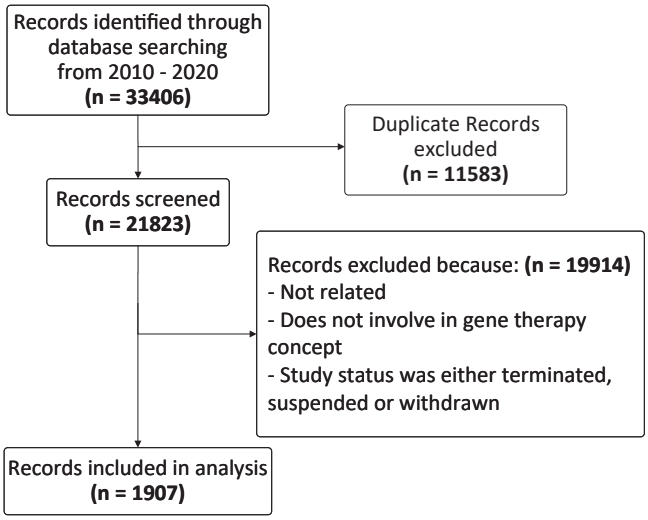

Fig. 1. The flowchart diagram of the inclusion of clinical trials in the study. As described above, there were several clinical trials (n = 171) that were terminated, suspended, or withdrawn. 142 (77%) of them were in the cancer group, while 15 (8%) and 14 (7%) of them were in the genetic diseases and infection groups, respectively. The higher relative number of excluded studies in the cancer group is explained in part by the higher number of clinical trials in this group.

2. A glance at the journey of gene therapy

From the authors' point of view, the history of gene therapy can be divided into four phases, as shown in Fig. 2.

2.1. From the "gene" to development of gene therapy as a tool; basic research phase (1909–1973)

The gene therapy journey began after Wilhelm Johannsen coined the term "gene". [2,3] Francis Crick and James Watson discovered the double-helix structure of DNA after about a half-century [4]. The term "genetic engineering" was first used in the 1930s [5]. The basic principles of gene transfer in bacteria were discovered in the 1960s, which were then tailored into the development of eukaryote transfection techniques [5]. The restriction and ligation enzymes, first described in the 1970s, form the foundation of gene manipulation [6,7]. Recombinant DNA techniques enabled researchers to introduce selected therapeutic gene(s) into engineered vectors [8,9]. With the discovery of viruses' ability to transfer genetic material, viral vectors have emerged as a promising and effective tool for gene transfer [9,10]. These technological advancements enabled scientists to create gene therapy vectors capable of transferring specific genetic materials into target mammalian cells [5].

2.2. Early age of the gene therapy practice with sweet and sour (beginning of clinical trials) (1989–2003)

It took a century full of ups and down until the first clinical trial started. First of all, in 1989, a retrovirus was used to express a neomycin-resistance marker on tumor-infiltrating lymphocytes, which was used in tracking infiltrated lymphocytes in the immunotherapy of melanoma [11]. The next year (1990), scientists at the University of Pennsylvania initiated the first successful gene therapy clinical trial on a four-year-old girl, named Ashanti Desilva who was diagnosed with severe combined immunodeficiency (SCID). A retroviral vector was used to transfer a normal copy of adenosine deaminase *(ADA)* into her T cells [12]. Although she did not become completely needless to recombinant ADA (PEG-ADA), currently she is experiencing normal life [13]. However, the basic strategies of gene therapy were identified earlier, the use of viral vectors accompanied by some adverse events like insertional mutagenesis and immune reactions, which slowed down the progression of clinical gene therapies. In 2000, European researchers in Paris reported a successful clinical trial of X-linked SCID gene therapy aimed to replace the cytokine receptor (*IL2RG*) mutated gene [14], however, 5 of 20 treated children developed leukemia in later stages, as a result of activating an oncogene following the introduction of the transgene by the vector [15]. At the same time in London, Thrasher and his colleagues in a similar gene therapy trial reported another case of leukemia after retroviral-mediated gene therapy for the most common form of SCID

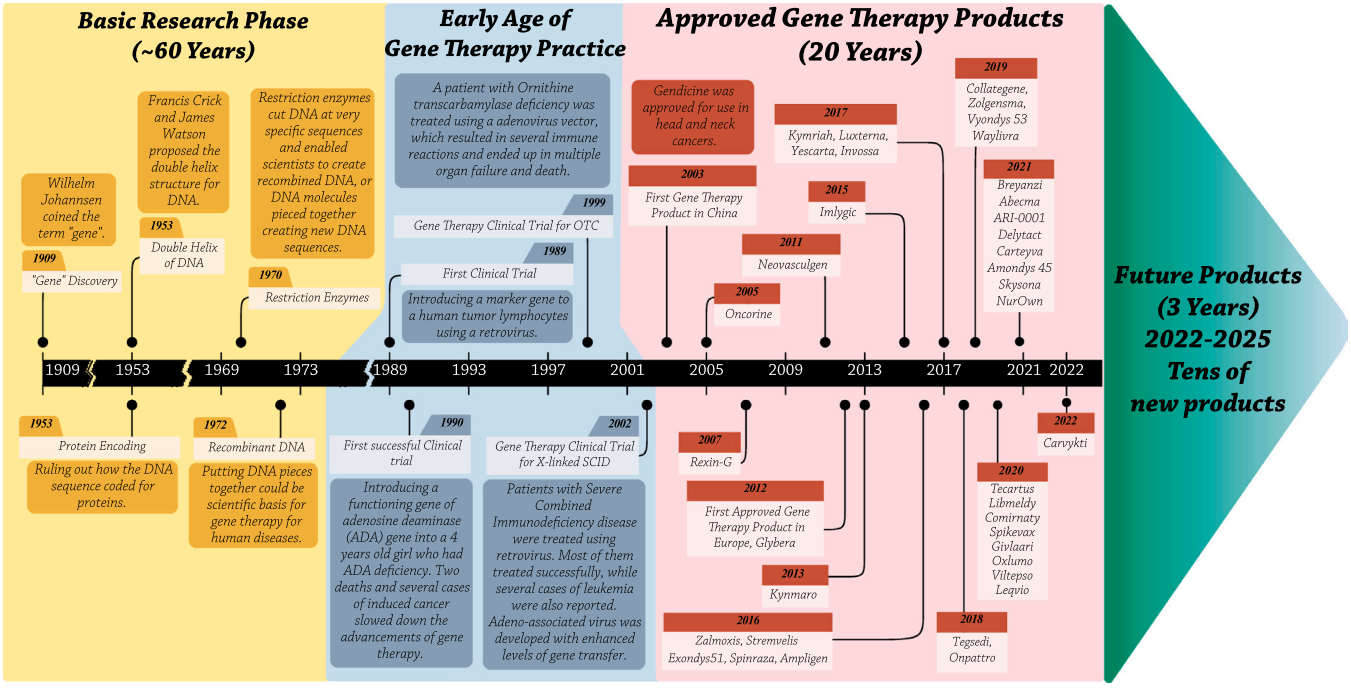

Fig. 2. Historical timeline of gene therapy.

(SCID-X1) [16]. Meanwhile, in 1999, Jesse Gelsinger, an 18 years old boy diagnosed with a rare metabolic disease, who was volunteered to be the 18th person receiving an adenoviral vector encoding a normal copy of ornithine transcarbamylase gene died due to massive coagulation disorder and subsequent multi-organ failure, to be the first dead person of gene therapy [16]. Although these setbacks slowed down the gene therapy progress, they also demonstrated the gene therapy potential and its bright future.

2.3. A phase from bust to boom (starting the approval of gene therapy products) (2003–2022)

In 2003, after approximately 686 clinical trials, the Chinese State Food and Drug Administration (SFDA) approved Gendicine, the first gene therapy product indicated for head and neck cancer [17]. Two years later, the SFDA approved Oncorine, the first oncolytic virus indicated for nasopharyngeal carcinoma [18]. A decade later, in 2012, the number of clinical trials became nearly doubled when European Medicines Agency (EMA) approved the first European gene therapy product, Glybera, indicated for Lipoprotein lipase deficiency [19]. The first *ex-vivo* gene therapy product, Strimvelis, was approved by EMA in 2016. The United States Food and Drug Administration (FDA) approval of two chimeric antigen receptor (CAR) products in 2017, Kymriah and Yescarta, acts as a key milestone that pave the way for future products [20]. Luxturna, the first FDA-approved *in-vivo* AAV (Adeno-associated virus) gene therapy product for Leber congenital amaurosis (LCA), was also approved in 2017 [21]. In 2019, the FDA approved the most expensive drug to date, Zolgensma, an AAV vector indicated for pediatric spinal muscular atrophy [22]. The number of approved gene therapy products increases every year.

Given the new genetic technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR) and zinc fingers, the production of gene-based therapeutics is anticipated to accelerate significantly. CRISPR technology has recently been used ex-vivo to treat sickle cell disease and beta-thalassemia [23], as well as *in-vivo* to treat Transthyretin (ATTR) amyloidosis [24]. Current gene therapy products are generally approved by FDA, EMA, and SFDA [25,26] (Table 1).

2.4. Future products (2022–2025)

Based on previous clinical trials and currently approved products, it appears that this promising field of medicine is advancing faster than ever before, with tens of new approved products expected in the near future. Several products are currently awaiting approval from regulatory agencies. With their approval, we are gradually incorporating gene therapy into the treatment of a broader range of diseases. Table 2 lists these products, indications, and the regulatory agencies that first assess and approved them (Table 2).

3. Gene therapy strategies

Typically, therapeutic gene therapies involve the transfer of genetic material into cells to reverse an abnormal condition or induce a new trait. Depending on the underlying genetic problem, various strategies such as addition, edition (repair), and deletion/knockout (inactivation) could be used [27]. Sometimes gene therapy is used to add a normal and functional copy of an allele to increase gene expression, such as adding a normal human clotting factor IX allele to produce enough factor IX in hemophilia type B [28]. Gene therapy is sometimes used to introduce a modified allele into cells to give them new characteristics, such as CAR (Chimeric antigen receptor) structures in CAR-T cells [29] or suicide genes (e.g. Thymidine Kinase) into cancer cells [30]. Some gene therapies are intended for vaccination, primarily by introducing a specific antigen to stimulate the immune system. This strategy has gained attention, particularly with the development of the COVID-19 vaccine [31,32]. Gene therapy is sometimes used to repair or edit a mutation or defective allele, such as the correction of the survival motor neuron 2 (SMN2) gene transcript with an antisense oligonucleotide in spinal muscular atrophy [33]. CRISPR is a valuable tool in this mechanism [34]. Sometimes gene therapy is used for inactivating the abnormal or defective gene, for example, using siRNA (anti-sense oligonucleotides) or CRISPR to degrade TTR mRNA and reduce TTR protein production in the treatment of hereditary transthyretin-mediated amyloidosis. [24,35,36].

4. The most transferred genes

Investigating the functional type of transferred genetic material (categorized based on prior classification by Ginn et al. [37]) revealed that genes encoding surface antigens were the most common type of gene (more than half of the clinical trials). Among them, different CAR genes (e.g., CD19, CD22, BCMA, etc.) were the most frequently incorporated genes as a surface marker (663 out of the total 948). The next most frequently transferred genes were those encoding cytokines and those encoding enzymes. Suicide genes, structural protein-encoding genes, and genes involved in the secretion of blood coagulation factors were among the other types of transferred genes.

5. Fields of gene therapy

Gene therapy is making inroads into a variety of fields. Starting with genetic diseases, gene therapy is now playing a game-changing role in a variety of other fields [38]. After reviewing 1908 gene therapy clinical trials from 2010 to October 2020, it was concluded that they could be classified into three major fields: cancers, monogenic and polygenic disorders (genetic disorders), infections, and other studies.

5.1. Gene therapy, an emerging treatment for cancers

Although gene therapy is considered a treatment for some genetic disorders, cancer is the most common disease for which gene therapy is used. From 2010 to 2020, cancer-related clinical trials comprised the majority of all studies. Hematological cancers were the most investigated cancers, partly due to the development of a great tool for targeting tumoral cells known as CAR T cells, as well as their suitable nature with excellent accessibility and broad surface interactions [39]. The next most commonly addressed cancers in gene therapy trials were gastrointestinal and nervous system cancers (Fig. 3). Leukemia, lymphoma, melanoma, and multiple myeloma were the most common cancers for which gene therapies were developed (Table 3).

CAR T cells are one of the most well-known and successful gene therapy applications in immunotherapy [40,41]. T cells were engineered to express chimeric receptors on their surface in order to generate CAR T cells. These constructs use the single-chain variable fragment (scFv) of a corresponding antibody as their warhead, allowing them to react against specific molecules, particularly tumor-associated antigens [42]. In addition, along with the promotion of CAR T cells across its generations, they were equipped with a few intracellular co-stimulatory domains responsible for the complete activation of T cells upon contact with the target antigens [43]. The combination of T cell activation and expansion ability with scFv specificity makes CAR T cells one of the most promising cancer-fighting tools [44]. TCR-modified T cells operate in a similar manner, with the exception that they require simultaneous activation of other cellular elements such as HLA to be fully activated [45]. Dendritic cell (DC) vaccines involve ex-vivo antigen-loaded special leukocytes capable of introducing antigens to the immune system and have shown promising results in several advanced-phase (phase 3) clinical trials for the treatment of lymphoma and melanoma [46].

looking at current trials addressing cancer diseases, the majority of them were in phase 1. Although the number of phase 2 trials was high, it appears that only a small number of them were followed by a phase 3

Table 1
Approved gene therapy products.

Year of Approval	Trade name (General name)	Manufacturer	Vector	Transferred gene/ genetic modification	Indication	*Ex-vivo/in-vivo* (target cell)	Approving country/Agency	Details
1998	Vitravene (Fomivirsen) [64]	Isis Pharmaceuticals	RNA	Antisense oligonucleotide against *UL123* gene of CMV	Local treatment of cytomegalovirus retinitis in immunocompromised patients	*In-vivo*	FDA/ EMA	First approved gene therapy drug, later withdrawn from the market in 2002
2003	Gendicine (rAd-p53) [65,66]	Shenzhen SiBiono GeneTech	Adenovirus	*P53*	Head and neck cancer	*In-vivo*	SFDA	First commercial gene therapy drug
2005	Oncorine (H101) [18]	Shanghai Sunway Biotech	Adenovirus	*E1B*-deleted adenovirus	Nasopharyngeal carcinoma	*In-vivo*	SFDA	First oncolytic virus product
2007	Rexin-G (Mx-dnG1/ DeltaRex-G) [67]	Epeius Biotechnologies	Retrovirus	Mutant form of the cyclin G1	Soft tissue sarcoma and osteosarcoma	*In-vivo*	Philippines	
2011	Neovasculgen (Cambiogenplasmid/ PI-VEGF165) [68]	Human Stem Cells Institute	Plasmid	VEGF	Peripheral vascular disease and limb ischemia	*In-vivo*	Russian Ministry of Healthcare, Ukraine	First plasmid-based medicine
2012	Glybera (alipogene tiparvovec) [69]	UniQure	AAV1	lipoprotein lipase (*LPL S447X*)	Familial lipoprotein lipase deficiency	*In-vivo*	EMA	First gene therapy product in the European Union, later withdrawn from the market in 2017
2013	Kynamro (Mipomersen) [64]	Ionis Pharmaceuticals	RNA	Antisense oligonucleotide against *ApoB100*	Homozygous familial hypercholesterolemia	*In-vivo*	FDA, Mexico, Argentina, South Korea	Later withdrawn in 2019 by FDA
2015	Imlygic (Talimogene Laherparevec)[70,71]	Amgen	HSV1	Addition of GM-CSF, deletion of ICP47 and ICP34.5	Melanoma	*In-vivo*	FDA, EMA, UK, Australia	Oncolytic virus product
2016	Zalmoxis [72]	MolMed	Retrovirus	ΔLNGFR and HSV-TK Mut2	Restoring the immune system of the patient after hematopoietic stem cell transplantation	*Ex-vivo* (T cell)	EMA	Genetically modified allogeneic T cell, later withdrawn in 2019 in Germany
2016	Strimvelis (GSK2696273) [73–76]	Orchard Therapeutics	Retrovirus	Adenosine deaminase (*ADA*)	Severe combined immunodeficiency (SCID) due to ADA deficiency	*Ex-vivo* (CD34 + cell)	EMA, UK	First corrective *ex-vivo* stem cell (autologous CD34 + cells) gene therapy in the world
2016	Exondys 51 (Eteplirsen) [77,78]	Sarepta Therapeutics	RNA	Antisense oligonucleotide for *dystrophin*	Duchenne Muscular Dystrophy (DMD)	*In-vivo*	FDA	
2016	Spinraza (Nusinersen) [77,79]	Ionis Pharmaceuticals	RNA	Antisense oligonucleotide for *SMN2*	Spinal Muscular Atrophy	*In-vivo*	FDA, EMA, UK, Canada, Japan, Brazil, Switzerland, Australia, South Korea, SFDA, Argentina, Colombia, Taiwan, Turkey	
2016	Ampligen (Rintatolimod/ Poly (C12U)) [80]	AIM ImmunoTech	RNA	Antisense double stranded RNA oligonucleotide as a TLR3 agonist	Chronic fatigue syndrome/ myalgic encephalomyelitis	*In-vivo*	Argentina, FDA (Compassionate use)	
2017	Kymriah (Tisagenlecleucel) [81]	Novartis	Lentivirus	*CD19* CAR	Relapsed B cell acute lymphoblastic leukemia	*Ex-vivo* (T cell)	FDA, EMA, UK, Japan, Australia, Canada, South Korea	First CAR T cell using lentivirus
2017	Luxturna (Voretigene Neparvovec-rzyl) [82]	Spark Therapeutics (Roche)	AAV2	*RPE65*	RPE65 mutation-associated retinal dystrophy	*In-vivo*	FDA, EMA, UK, Australia, Canada, South Korea	First FDA-approved *In-vivo* AAV gene

(*continued on next page*)

Table 1 (*continued*)

Year of Approval	Trade name (General name)	Manufacturer	Vector	Transferred gene/ genetic modification	Indication	*Ex-vivo/in-vivo* (target cell)	Approving country/Agency	Details
								therapy product
2017	Yescarta (Axicabtagene Ciloleucel) [83]	Kite Pharma (Gilead)	Retrovirus	*CD19* CAR	Relapsed or Refractory large B cell lymphoma	*Ex-vivo* (T cell)	FDA, EMA, UK, Japan, Canada, SFDA	First CAR T cell using retrovirus
2017	Invossa (chondrocytes transduced with TGF-ß1) [84,85]	Kolon TissueGene	Retrovirus	TGF-β1	Moderate Knee Arthritis	*Ex-vivo* (chondrocyte)	Korea	First gene therapy product in Korea
2018	Tegsedi (Inotersen) [86]	Ionis Pharmaceuticals	RNA	Antisense oligonucleotide against transthyretin mRNA	Hereditary Transthyretin-related Amyloidosis	*In-vivo*	EMA, UK, Canada, FDA, Brazil	
2018	Onpattro (Patisiran) [36]	Alnylam	RNA	double-stranded siRNA against transthyretin mRNA	Hereditary Transthyretin-related Amyloidosis	*In-vivo*	FDA, EMA, UK, Japan, Canada, Switzerland, Brazil, Taiwan, Israel, Turkey	
2019	Collategene (Beperminogene perplasmid) [87]	AnGes	Plasmid	Human hepatocyte growth factor (*HGF*)	Critical Limb Ischemia	*In-vivo*	Japan	First Gene therapy product in Japan
2019	Zolgensma (Onasemnogene Abeparvovec-xioi) [88]	Novartis	AAV9	*SMN1*	Pediatric Spinal Muscular Atrophy	*In-vivo*	FDA, EMA, UK, Japan, Australia, Canada, Brazil, Israel, Taiwan, South Korea	Most expensive drug worldwide
2019	Zynteglo (Betibeglogene autotemcel) [89]	Bluebird Bio	lentivirus	βA-T87Q-globin (modified *β-globin* gene)	Adult transfusion-dependent ß-thalassemia	*Ex-vivo* (CD34 + cell)	EMA, UK	Later withdrawn from the market in 2022
2019	Vyondys 53 (Golodirsen) [90]	Sarepta Therapeutics	RNA	Antisense oligonucleotide against *dystrophin*	Duchenne Muscular Dystrophy	*In-vivo*	FDA	
2019	Waylivra (Volanesorsen) [91]	Ionis Pharmaceuticals	RNA	Antisense oligonucleotide against *ApoC3*	Adult Familial Chylomicronemia syndrome	*In-vivo*	EMA, UK, Brazil	
2020	Tecartus (brexucabtagene autoleucel/ KTE-X19) [92]	Kite Pharma (Gilead)	Retrovirus	*CD19* CAR	Relapsed/refractory mantle cell lymphoma	*Ex-vivo* (T cell)	FDA, EMA, UK	
2020	Libmeldy (Atidarsagene autotemcel) [93]	Orchard Therapeutics	Lentivirus	*ARSA* (arylsulfatase A) gene	Metachromatic Leukodystrophy	*Ex-vivo* (CD34 + cell)	EMA, UK	Autologous CD34 + cells encoding ARSA gene
2020	Comirnaty (Tozinameran) [94]	BioNTech	mRNA	lipid nanoparticle-formulated, nucleoside-modified mRNA encoding the SARS-CoV-2 spike (S) protein	COVID-19 vaccination	*In-vivo*	UK, Bahrain, Israel, Canada, FDA, Rwanda, Serbia, United Arab Emirates, Macao, Mexico, Kuwait, Singapore, Saudi Arabia, Chile, Switzerland, EMA, Colombia, Philippines, Australia, Hong Kong, Peru, South Korea, New Zealand, Japan, Brazil, Sri Lanka, Vietnam, South Africa, Thailand, Oman, Egypt, Malaysia	Pfizer-BioNTech COVID-19 mRNA Vaccine
2020	Spikevax (Moderna COVID-19 vaccine/ mRNA-1273, elasomeran) [95]	Moderna Therapeutics	mRNA	mRNA for pre-fusion stabilized Spike glycoprotein of SARS-CoV-2 virus	COVID-19 vaccination	*In-vivo*	FDA, Canada, Israel, EMA, Switzerland, Singapore, Qatar, Vietnam, UK, Philippines,	Moderna COVID- 19 vaccine

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Table 1 (*continued*)

Year of Approval	Trade name (General name)	Manufacturer	Vector	Transferred gene/ genetic modification	Indication	*Ex-vivo/in-vivo* (target cell)	Approving country/Agency	Details
							Thailand, Japan, South Korea, Brunei, Paraguay, Taiwan, Botswana, India, Indonesia, Saudi Arabia, Mexico, Australia, Nigeria, Colombia	
2020	Givlaari (givosiran) [96]	Alnylam	RNA	siRNA aginst aminolevulinate synthase 1 (ALAS1) mRNA	Porphyria	*In-vivo*	FDA, EMA, UK, Canada, Switzerland, Brazil, Israel, Japan	
2020	Oxlumo (lumasiran) [97]	Alnylam	RNA	siRNA against hydroxyacid oxidase 1 (HAO1)	Primary hyperoxaluria type 1	*In-vivo*	EMA, UK, FDA, Brazil	
2020	Viltepso (viltolarsen) [98]	NS Pharma	DNA	Anti-sense oligonucleotide against exon 53 of dystrophin pre-mRNA	Duchenne Muscular Dystrophy	*In-vivo*	FDA, Japan	First DNA-based approved gene therapy product
2020	Leqvio (inclisiran/ ALN-PCSsc, ALN-60212) [99]	Alnylam	RNA	Anti-sense oligonucleotide (siRNA) against proprotein convertase subtilisin Kexin type 9 (PCSK9)	Primary hypercholesterolemia	*In-vivo*	EMA, UK, Australia, Canada, Israel, FDA	
2021	Breyanzi (lisocabtagene maraleucel) [100]	Celgene (Bristol Myers Squibb)	Retrovirus	CD19 CAR	Relapsed or refractory diffuse large B cell lymphoma; follicular lymphoma	*Ex-vivo* (T cell)	FDA, Japan	
2021	Abecma (Idecabtagene vicleuel) [101]	bluebird bio	Lentivirus	BCMA CAR	Multiple myeloma	*Ex-vivo* (T cell)	FDA, Canada, EMA, UK, Japan	
2021	ARI-0001 [102]	Hospital Clínic	Lentivirus	CD19 CAR	Adult relapsed/ refractory acute lymphoblastic leukemia	*Ex-vivo* (T cell)	Spain	
2021	Delytact (teserpaturev) (G47Δ) [103]	Daiichi Sankyo	HSV-1	Triple-mutated, replication-conditional oncolytic virus	Malignant Glioma	*In-vivo*	Japan	The first oncolytic virus for brain cancer
2021	Carteyva (Relma-cel/ relmacabtagene autoleucel) [104]	JW Therapeutics	Lentivirus	CD19 CAR	Relapsed or refractory diffuse large B cell lymphoma	*Ex-vivo* (T cell)	SFDA	
2021	Amondys 45 (casimersen/ SRP-4045) [105]	Sarepta Therapeutics	RNA	Antisense oligonucleotide against exon 45 of dystrophin gene	Duchenne Muscular Dystrophy	*In-vivo*	FDA	
2021	Skysona (elivaldogene autotemcel/ Lenti-D) [106]	bluebird bio	Lentivirus	*ABCD1* gene	Juvenile Cerebral Adrenoleukodystrophy	*Ex-vivo* (CD34 + cell)	EMA	Later withdrawn in 2022
2022	Carvykti (ciltacabtagene autoleucel) [107]	Legend Biotech	lentivirus	BCMA CAR	Relapsed or refractory multiple myeloma	*Ex-vivo* (T cell)	FDA	

FDA: Food and Drug Administration; EMA: European Medicines Agency; SFDA: State Food and Drug Administration of China; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; siRNA: small interfering RNA; BCMA: B-cell maturation antigen; CAR: Chimeric antigen receptor; MSC: mesenchymal stem cells; HSV: Herpes simplex virus.

trial. There was currently one phase IV clinical trial for lymphoma using *CD19* CAR-T cells. Although most cancer clinical trials use *ex-vivo* engineered and expanded cells, *in-vivo* trials are also progressing to advanced stages, most notably using human herpes simplex virus (HSV) as an oncolytic virus. Imlygic (Talimogene Laherparepvec) is an engineered HSV that was approved by the FDA in 2015 as the first FDA-approved oncolytic virus for the treatment of advanced melanoma [47].

Several gene therapy products have been approved for the treatment of cancer to date, including gendicine, oncorine, rexin-G, imlygic, delytact and eight CAR T cells, including kymriah, yescarta, tecartus, breyanzi, abecma, ARI-0001, carteyva and carvykti (Table 1).

5.2. Gene therapy for genetic disorders

The idea of transferring genetic materials to treat diseases (gene therapy) was first applied to genetic disorders. [26] The National Human Genome Research Institute defines genetic disorders as "a disease caused in whole or in part by a change in the DNA sequence away from the normal sequence" [48]. Unlike cancer, most of these disorders, such as thalassemia, Deschene muscular dystrophy (DMD), and cystic

Table 2
Gene therapy products currently waiting for approval.

NO	Agency	Drug name	Indications	Details
1	EMA	A004	Treatment of X linked Retinitis Pigmentosa owing to defects in Retinitis Pigmentosa GTPase Regulator	Adenovirus associated viral vector serotype 5 containing the human RPGR gene
2	EMA	AAV2/8-hCARp. hCNGB3	Treatment of achromatopsia associated with defects in CNGB3	Adenovirus associated viral vector serotype 8 containing the human CNGB3 gene
3	EMA	AMT-060, AMT-061	Treatment of severe hemophilia B	Adeno-associated viral vector serotype 5 containing human factor IX gene or variant
4	EMA	AT132	Treatment of X-linked Myotubular Myopathy	Adeno-associated viral vector serotype 8 containing the human MTM1 gene
5	EMA	BAY2599023	Treatment of hemophilia A	Recombinant adeno-associated virus vector based on the AAV serotype hu37 containing a single-stranded DNA genome encoding a form of human FVIII
6	EMA	CT053	Treatment of patients with relapsed and/or refractory multiple myeloma (MM) whose prior regimens included a proteasome inhibitor, an immunomodulatory agent and an anti-CD38 monoclonal antibody	Fully human anti-BCMA autologous CAR T Cell
7	EMA	FLT180a	Treatment of hemophilia B	Recombinant adeno-associated viral vector serotype S3 containing codon-optimized expression cassette encoding human coagulation factor IX variant
8	EMA	JCAR125	Treatment of relapsed / refractory multiple myeloma whose prior therapies included autologous stem cell transplant if they were eligible, a proteasome inhibitor, an immunomodulatory agent and an anti-CD38 antibody	Autologous CD4 + and CD8 + T-cell populations transduced with a genetically-engineered replication-incompetent, self-inactivating lentiviral vector to express a BCMA-specific CAR
9	EMA	JNJ-68284528	Treatment of adult patients with relapsed or refractory multiple myeloma, whose prior regimens included a proteasome inhibitor, an immunomodulatory agent and an anti-CD38 antibody and who had disease progression on the last regimen	Autologous human T cells genetically modified *ex-vivo* with a lentiviral vector encoding a chimeric antigen receptor (CAR) for B-cell maturation antigen (BCMA)
10	EMA	KB103	Treatment of Dystrophic Epidermolysis Bullosa	Genetically modified replication-incompetent herpes simplex virus-1 expressing collagen VII
11	EMA	MB-CART2019.1	Treatment of patients with relapsed and refractory diffuse large B-cell lymphoma (DLBCL) after frontline therapy and who are ineligible for autologous stem cell transplantation	Autologous anti-CD19/CD20 CAR T transduced cells
12	EMA	NY-ESO-1c259T	Treatment of HLA-A* 0201, HLA-A* 0205, or HLA-A* 0206 allele positive patients with inoperable or metastatic synovial sarcoma who have received prior chemotherapy and whose tumor expresses the NY-ESO-1 tumor antigen	Autologous CD4 and CD8 T cells transduced with lentiviral vector containing an affinity-enhanced T cell receptor to target the cancer-testis tumor antigen NY-ESO-1
13	EMA	OTL-300	Treatment of transfusion-dependent β-thalassemia	Autologous CD34 + cells transduced with lentiviral vector encoding the human beta-globin gene
14	EMA	PF-06838435/SPK-9001	Treatment of hemophilia B	Adeno-associated viral vector containing factor IX gene variant
15	EMA	Rebisufligene etisparvovec	Treatment of Mucopolysaccharidosis Type IIIA, MPS IIIA (Sanfilippo A Syndrome)	A non-replicating, recombinant, self-complementary adeno-associated virus serotype 9 (scAAV9) vector, expressing the human N-sulfoglucosamine sulfohydrolase (hSGSH) cDNA, under the control of a murine small nuclear RNA promoter U1a.
16	EMA	RP-L102	Treatment of Fanconi anemia Type A	Autologous CD34 + enriched cells transduced ex vivo with lentiviral vector carrying the FANCA gene, PGK-FANCA-WPRE
17	EMA	Tasadenoturev (DNX-2401)	Treatment of recurrent glioblastoma in patients for which a gross total resection is not possible or advisable, or for those who refuse further surgery	Adenovirus serotype 5 containing partial E1A deletion and an integrin-binding domain
18	FDA	PR001	Parkinson's disease associated with GBA1 mutations, and for Gaucher disease	Adeno-associated virus 9 (AAV9) to deliver a functional copy of the GBA1 gene
19	FDA/ EMA	Valoctocogene Roxaparvovec (Roctavian)	Hemophilia A	AAV5 that carries a healthy copy of the factor VIII gene under the control of a liver-specific promoter
20	EMA/ FDA	ADP-A2M4 (transduced CD4 + and CD8 + cells)	Treatment of HLA-A* 02 positive patients with inoperable or metastatic synovial sarcoma who have received prior chemotherapy and whose tumor expresses the MAGE-A4 tumor antigen	Transduced CD4 + and CD8 + cells for the treatment of soft tissue sarcomas.
21	EMA	AAV2/8-hCARp. hCNGB3	Treatment of achromatopsia associated with defects in CNGB3	Adenovirus associated viral vector serotype 8 containing the human CNGB3 gene
22	EMA	ARU-1801	Treatment of Sickle Cell Disease	Modified γ-globin lentiviral vector to produce HbFG16D within autologous CD34 + HSCs
23	EMA/ FDA	AT-GTX-501	Slowing disease progression in pediatric patients with variant late infantile neuronal ceroid lipofuscinosis 6 (vLINCL6)	Adeno-associated viral vector, serotype 9, containing the human CLN6 gene
24	EMA	CD30. CAR-T	Treatment of classical Hodgkin lymphoma	CD30-directed genetically modified autologous T cells
25	EMA/ FDA	CT041 (CAR-CLDN 18.2 T cells)	Treatment of patients with advanced gastric cancer who have failed at least 2 prior lines of systemic therapy	Claudin18.2 CAR T Cells
26	EMA/ FDA	CTX001	Treatment of Sickle Cell Disease/ Treatment of transfusion-dependent β-thalassemia	Autologous CD34 + hematopoietic stem cells with a CRISPR-edited erythroid enhancer region of the BCL11A gene
27	EMA/ FDA	MB-107	Treatment of X-linked severe combined immunodeficiency (XSCID) in newly diagnosed infants	*Ex-vivo* lentiviral gene therapy
28	EMA	Obecabtagene autoleucel/ AUTO1	Treatment of relapsed or refractory B cell acute lymphoblastic leukemia	CD19 chimeric antigen receptor (CAR) T-cell therapy
29	EMA	OTL-203	Treatment of Mucopolysaccharidosis type I (MPS-1)	

(*continued on next page*)

Table 2 (*continued*)

NO	Agency	Drug name	Indications	Details
				Autologous CD34 + hematopoietic stem and progenitor cells genetically modified with the lentiviral vector (IDUA LV) encoding for the alpha-L-iduronidase gene
30	EMA	RP-L201	Treatment of Leukocyte Adhesion Deficiency-I	Autologous CD34 + enriched cells transduced with the therapeutic lentiviral vector
31	EMA/ FDA	ABO-102	Mucopolysaccharidosis (MPS) IIIA	Adeno-associated viral (AAV9)-based gene therapy for the treatment of Sanfilippo syndrome type A
32	FDA	ABO-202	CLN1 Disease	Modified viral vector called AAV9 to deliver a correct copy of the PPT1 gene
33	FDA	EB-101	Epidermolysis bullosa (EB)	Gene-corrected cell therapy/ gene transfer to deliver the COL7A1 gene
34	FDA	ADP-A2M4	Synovial sarcoma	ADP-A2M4 involves T-cells with an engineered T-cell receptor (TCR) that targets the cancer-associated protein MAGE-A4
35	EMA/ FDA	NY-ESO-1	Metastatic synovial sarcoma	Cancer vaccine consisting of an immunogenic peptide derived from the cancer-testis antigen (NY-ESO-1), an antigen found in normal testis and various tumors.
36	FDA	Generx	Heart failure	An angiogenic gene therapy which triggers the production of a protein that stimulates new blood vessel growth
37	EMA/ FDA	AT132	X-linked myotubular myopathy (XLMM)	An AAV8 vector containing a functional copy of the human MTM1 (hMTM1) gene
38	FDA	BC-819	Bladder cancer	A recombinant DNA plasmid that directs the expression of a potent toxin specifically in malignant cells
39	FDA	JCAR015	Hematological malignancies - refractory B-cell acute lymphoblastic leukemia (ALL)	CD19-targeting chimeric antigen receptor (CAR) T-cell therapy
40	FDA	CMD-003	Hematological malignancies - relapsed or refractory lymphoma and post-transplant lymphoproliferative disease associated with Epstein Barr virus (EBV)	Autologous cytotoxic T cells, which have been programmed to target EBV antigens
41	FDA	DTX101	Hemophilia B	A non-replicating recombinant adeno-associated virus (AAV) serotype rh10 (AAVrh10) vector that contains a codon-optimized hFIX gene
42	FDA	FCX-007	Recessive dystrophic epidermolysis bullosa (RDEB)	A genetically modified cell product obtained from the subject's own skin cells (Autologous fibroblasts). The cells are expanded and genetically modified to produce functional COL7
43	FDA	Oncoprex	Non-small cell lung cancer (NSCLC)	Consists of the TUSC2 (Tumor Suppressor Candidate 2) gene, the active agent in Oncoprex, complexed with a lipid nanovesicle
44	FDA	TNFerade	Pancreatic cancer	A replication-deficient adenoviral vector expressing human tumor necrosis factor alpha (TNF-α) under the control of the chemoradiation-inducible EGR-1 promoter
45	FDA	HMI-102	Phenylketonuria (PKU)	An investigational gene therapy in clinical development for the treatment of phenylketonuria (PKU) in adults
46	FDA	JVS-100	Ischemic chronic heart failure	A non-viral gene therapy that expresses stromal cell-derived factor-1 (SDF-1), a naturally occurring signaling protein that activates the endogenous tissue repair pathways
47	FDA	LYS-SAF302	Mucopolysaccharidosis (MPS) IIIA	Delivers a functional copy of the human SGSH gene directly to brain cells using the adeno-associated virus carrier, AAVrh.10
48	FDA/ EMA	AAV-CNGB3	Achromatopsia	Adenovirus associated viral vector serotype 8 containing the human CNGB3 gene
49	FDA/ EMA	AAV-RPGR	X-linked retinitis pigmentosa	AAV5-RPGR vector for patients with X-linked retinitis pigmentosa
50	FDA	NSR-REP1	Choroideremia	An AAV vector administered by subretinal injection which provides a functioning CHM gene and expression of the REP-1
51	FDA	OTL-103	Wiskott-Aldrich Syndrome (WAS)	An autologous cell therapy that uses the patient's own CD34 + cells transfected with a lentiviral vector that encodes for a functional Wiskott–Aldrich syndrome protein
52	FDA	P-BCMA-101	Hematological malignancies - multiple myeloma	An autologous chimeric antigen receptor-T cell (CAR-T) therapeutic targeting BCMA
53	FDA	PR0001	Parkinson's disease	Adeno-associated virus 9 (AAV9) to deliver a functional copy of the GBA1 gene to the brain
54	FDA	RGX-111	Mucopolysaccharidosis (MPS) I	AAV9 vector to deliver the human α-I-iduronidase (IDUA) gene to the central nervous system (CNS)
55	FDA	RGX-121	Mucopolysaccharidosis (MPS) II	AAV9 vector to deliver the human iduronate-2-sulfatase (IDS) gene to the central nervous system (CNS)
56	FDA	RT-100	Heart failure	RT-100 AC6 gene transfer involves infusing an inactivated adenovirus vector encoding human adenylyl cyclase type 6 (Ad5. hAC6)
57	FDA	SB-318	Mucopolysaccharidosis (MPS) I	An intravenously delivered Zinc Finger Nuclease (ZFN) Therapeutic for genome editing
58	FDA	SB-525	Hemophilia A	Recombinant adeno-associated virus 2/6 (AAV2/6) vector encoding the cDNA for the B-domain deleted human F8
59	FDA	SB-913	Mucopolysaccharidosis (MPS) II	A zinc finger nuclease (ZFN) in vivo genome editing product candidate for the treatment of patients with Mucopolysaccharidosis Type II
60	FDA	SPK-8001	Hemophilia A	Novel bio-engineered adeno-associated viral (AAV) vector utilizing the AAV-LK03 capsid
61	FDA/ EMA	TOCA 511	Glioblastoma	Gene encoding cytosine deaminase (CD) in a replicating, non-lytic retroviral vector

(*continued on next page*)

Table 2 (*continued*)

NO	Agency	Drug name	Indications	Details
62	FDA	TAC01-CD19	Hematological malignancies - relapsed or refractory diffuse large B-cell lymphoma (DLBCL)	T cell antigen coupler (TAC) therapy
63	FDA	DTX401	Glycogen storage disease type Ia	AAV8 gene therapy designed to deliver stable expression and activity of G6Pase-α
64	FDA	AMT-130	Huntington's disease	An AAV5 vector carrying an artificial micro-RNA specifically tailored to silence the huntingtin gene
65	FDA	VB-111	Glioblastoma multiforme	A non-replicating adenovirus 5 (Ad-5, E1-deleted) carrying a pro-apoptotic human Fas-chimera transgene
66	FDA	VM202	Painful diabetic peripheral neuropathy	A plasmid DNA encoding two isoforms of hepatocyte growth factor (HGF)
67	FDA	VY-AADC	Parkinson's disease	AAV2 capsid as a vector encoding AADC
68	FDA	XC001	Chronic angina	A novel adenoviral vector expressing multiple isoforms of vascular endothelial growth factor (VEGF) promoting an enhanced local angiogenic effect
69	FDA	Ad-RTS-hIL-12	Recurrent glioblastoma	A novel gene therapy expressing IL-12 under the control of an oral activator ligand

*Results were based on searching the Alliance for Regenerative Medicine (https://alliancerm.org/expedited-approval-designations/), EMA Prime list, FDA Regenerative Medicine Advanced Therapy Designation, FDA orphan drug designation list, FDA priority review list, FDA fast track, and breakthrough therapies, FDA accelerated approval, Japan SAKIGAKE Designation, Drugs.com, Evaluate.com, and WHO International Nonproprietary Names. SFDA website was not accessible, therefore not included.

fibrosis, are inherited. More than half of the clinical trials in this group focused on metabolic disorders, eye disorders, and blood coagulation disorders (Fig. 3). Looking more closely, the genetic disorders with the most clinical trials were SMA, DMD, hemophilia B, ADA-SCID, and Beta Thalassemia (Table 3).

The majority of these clinical trials are *in-vivo*, with AAV being the most commonly used vector. When compared to cancers, the proportion of phase 3 clinical trials for genetic diseases was higher (Fig. 4). LCA, SMA, ATTR amyloidosis, primary hyperoxaluria, amyloid neuropathy, DMD, lipoprotein lipase deficiency, familial chylomicronemia syndrome, and hypertriglyceridemia are among the genetic diseases being studied in phase 3 trials. There are also two Phase 4 clinical trials addressing the genetic disease, including the treatment of SMA with Spinraza and Zolgensma, two FDA and EMA-approved gene therapy products. Even though the total number of trials on genetic diseases was less than that of cancer trials, the number of current approved products for genetic disorders is higher (twenty vs thirteen). To this date, there are approved products for ADA-SCID, DMD, SMA, LCA, B-Thalassemia, and a number of metabolic disorders (familial lipoprotein lipase deficiency, primary and familial hypercholesterolemia, hereditary transthyretin amyloidosis, adult familial chylomicronemia syndrome, metachromatic leukodystrophy and cerebral adrenoleukodystrophy) (Table 1).

5.3. Treating infectious disease using gene therapy, a fiction or reality?

There has always been a struggle to find a way to treat some of the most serious infectious diseases, such as acquired immunodeficiency syndrome (AIDS) or malaria. Gene therapy was no exception. Although they constituted a minor portion of gene therapy clinical trials from 2010 to 2020, the emergence of the coronavirus disease (COVID-19) pandemic has resulted in the initiation of 29 trials addressing COVID-19 since December 2019. AIDS, COVID-19, malaria, Ebola, hepatitis C, human papillomavirus infection, and hepatitis B were the infectious diseases with the greatest number of clinical trials (Table 3). The majority of the clinical trials used vaccination strategy to induce immunity against the corresponding diseases. There were only a few gene therapies in advanced stages, i.e. gene therapies using DNA as a vector for hepatitis B. Looking at approved products, it appears that gene therapy strategies have a long way to go before they can effectively address infectious diseases.

5.4. Gene therapy has penetrated everywhere

We see some exotic gene therapy applications on the list of clinical trials from 2010 to 2020. At first glance, gene therapy appears to have few applications for disorders other than cancer, genetic diseases, and infection. However, Gene therapy has been used for peripheral vascular disease, osteoarthritis, diabetic retinopathy, macular degeneration, coronary artery disease, myocardial ischemia and infarction, diabetic neuropathy, allergic rhinitis, heart failure, and hypertrophic scar (Table 3). In addition, there are several approved gene therapy products in this category, including neovasculgen for peripheral vascular disease and limb ischemia, ampligen for chronic fatigue syndrome, invossa for knee arthritis, and nollategene for Critical Limb Ischemia. None of these products have been approved by the EMA, FDA, or SFDA for wide use. Neovasculgen and Collategene are currently being evaluated in phase 4 clinical trials (Table 1).

6. How long does a gene therapy clinical trial last?

It is worth noting that clinical trials for cancer generally last longer than trials for genetic diseases and infectious diseases. Based on the extracted data, a gene therapy clinical trial for cancer or genetic disease would last an average of 5 and 3.5 years from start to finish, respectively. This could be because cancer trials have a higher number of participants than trials for rare genetic disorders. Meanwhile, trials for infectious diseases are typically shorter in duration (one-third of a year). Given the generally long duration and consequently the high cost of clinical trials, gene therapy clinical trials have been confined to well-organized, well-funded, and experienced research institutes.

7. Vectors; how genes are transferred?

Ideal gene therapy vectors should have high transduction efficiency, the ability to induce long-term and stable expression, enough capacity to transport large transgenes, be specific for target cells, be able to transduce both quiescent and proliferating cells, and avoid random insertion and mutagenesis [49,50]. Different vectors with different characteristics have been used for various applications. The most common type of vectors is viral vectors, which can effectively transfer their gene into their host cells [51]. The advantages of viral vectors including higher transduction efficiency, greater ability to be engineered, and highly specific gene delivery, have resulted in a wider range of applications when compared to non-viral vectors [52]. Genes involved in virus replication, assembly, or infection are replaced with therapeutic genes

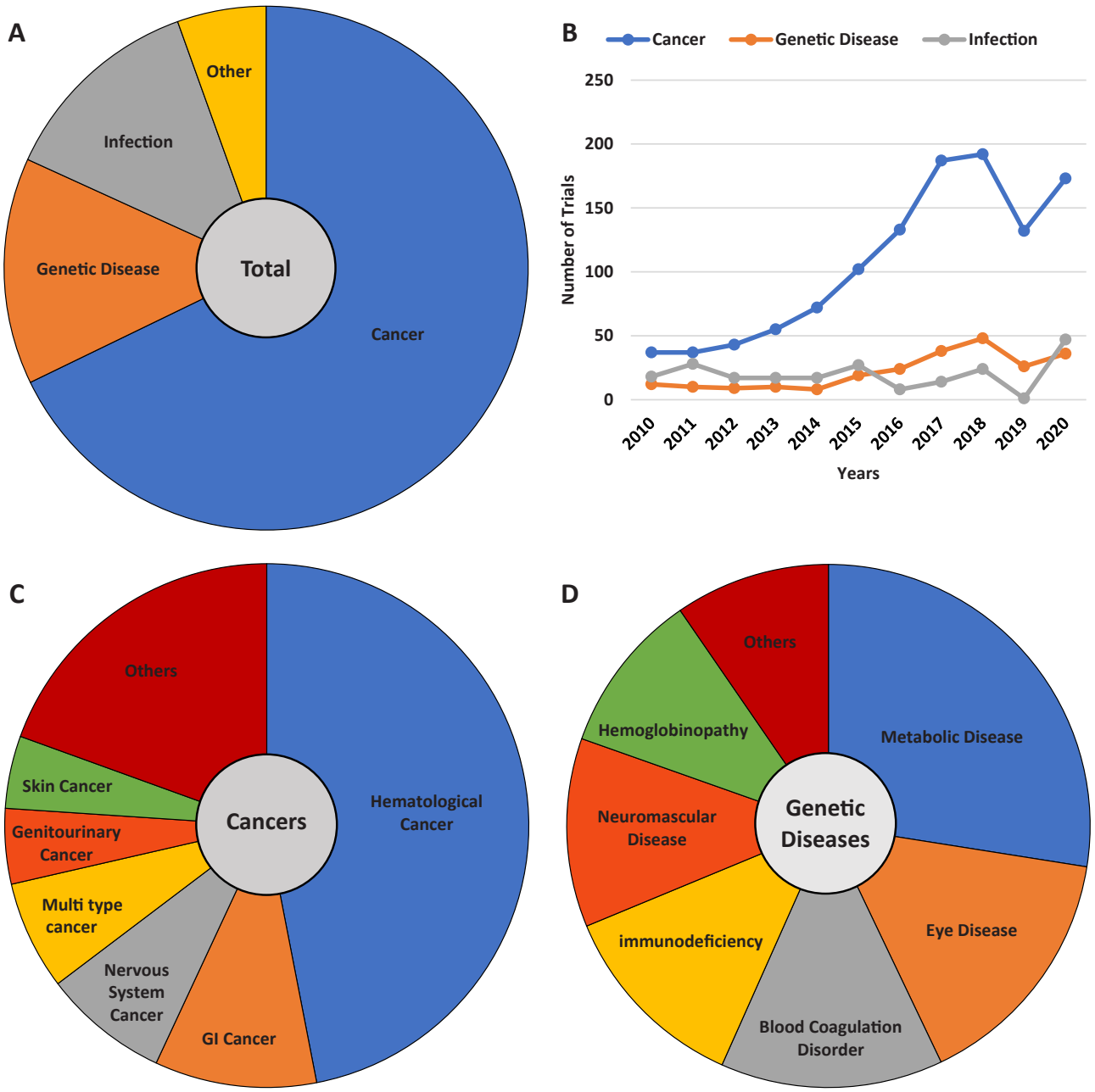

Fig. 3. Gene therapy trials for common disease groups and different disorders. (A) The most common disease groups, their subsequent categories, and disorders targeted by clinical trials from 2010 to 2020. The most common type of diseases on which gene therapy clinical trials were focused were cancers, genetic disorders, and infectious diseases. (B) Number of clinical trials divided by disease groups on which trials are focused. The distribution of the most common type of disease is approximately similar across these years, in which cancers ranked higher in comparison to two other groups. There is a decrease in the number of trials in 2019 in all three groups. (Number of clinical trials in each category were as follows: 2010 (78), 2011 (80), 2012 (80), 2013 (87), 2014 (99), 2015 (149), 2016 (165), 2017 (235), 2018 (270), 2019 (1186), 10-month of 2020 (289), Cancer (1087), Genetic diseases (283) and Infection (244)). (C) Hematological cancers account for about a half of the cancers; and (D) metabolic disease, eye disease, and coagulation disorders account for half of the genetic disorders, on which gene therapy trials were focused.

to create replication-deficient, specific, and safe therapeutic gene therapy vectors. [50] Retroviridae family (including gamma-retrovirus and lentivirus) and adeno-(associated)-viruses were the most common viral vectors. Retroviral and adeno-(associated)-viral vectors enable stable long-term transgene expression [53]. Although retroviral vectors can only infect dividing cells, lentiviral vectors, a more complex genus of retroviridae family, can infect non-dividing cells as well [54]. Lentiviral and retroviral vectors were mainly used in the manufacturing of CAR-T cells and TCR-modified T cells [55]. Adenoviral vectors are well-known for their use as oncolytic viruses and viral vaccines due to their high transgene expression and ability to infect a wide range of quiescent to proliferating cells [56,57]. AAVs are among the most promising candidates for *in-vivo* applications. They can infect both dividing and non-dividing cells, resulting in long-term transgene expression. Furthermore, they do not cause any known illness in humans, and the risk of insertional mutagenesis is low due to their extremely low integration frequency and specific integration site [27,53,58]. Other viral vectors with limited use include HSV, vaccinia, vesicular stomatitis virus (VSV), poxvirus, measles virus, Sendai Virus, oncolytic MG1 Virus, Coxsackievirus, alfa virus, λ-bacteriophage, Newcastle disease virus, and HPV (Fig. 5).

Non-viral vectors, on the other hand, are less popular, owing to their

Table 3
Most common disorders on which gene therapies were focused; Leukemia & lymphoma, multiple myeloma, Spinal muscular dystrophia, Duchenne muscular dystrophy, hemophilia, HIV, COVID-19, peripheral vascular disease, and osteoarthritis were among the most common disorder, gene therapy ever entered.

Disorders addressed by gene therapy with their count of clinical trials (#)					
Cancers	#	Achromatopsia	6	RSV	7
Leukemia	268	Granulomatous disease	6	CMV	5
Lymphoma	232	Parkinson	6	Anthrax	3
Melanoma	84	Mucopolysaccharidosis type 3	6	HSV	3
Multiple Myeloma	84	Adrenoleukodystrophy	6	Zika virus	3
Lung cancer	65	Type 1 diabetes mellitus	5	Hantaan virus	2
Pancreatic Cancer	58	Fabry disease	5	Leishmania	2
Ovarian cancer	56	Lysosomal Storage Disease	5	Mers virus	2
Breast cancer	55	Pompe disease	5	Neisseria	2
Glioblastoma	53	Wiskott-Aldrich Syndrome	5	Dengue virus	1
Hepatocellular carcinoma	51	Fanconi anemia	4	HDV	1
Colorectal Cancer	42	Mucopolysaccharidosis type I	4	Marburg Virus	1
Sarcoma	39	Aromatic L-amino acid decarboxylase deficiency	3	Others	#
Prostate cancer	36	Alpha 1-Antitrypsin Deficiency	3	Peripheral vascular disease	13
Glioma	35	Amyloidosis	3	Osteoarthritis	11
Bladder cancer	28	Mucopolysaccharidosis type 2	3	Diabetic retinopathy	8
Gastric cancer	26	Atherosclerosis	3	Macular degeneration	8
Head and Neck cancer	24	Batten disease	2	Coronary artery disease	7
Mesothelioma	22	Crigler-Najjar	2	Myocardial ischemia and infarction	6
Renal cell carcinoma	21	Familial Chylomicronemia Syndrome	2	Diabetic neuropathy	5
non-small cell lung carcinoma	20	Hypercholesterolemia	2	Allergic Rhinitis	4
Astrocytoma	18	Hypertriglyceridemia	2	Heart failure	4
Squamous cell carcinoma	16	Limb-Girdle Muscular Dystrophy	2	Hypertrophic Scar	4
Cervical cancer	13	Metachromatic Leukodystrophy	2	Bone disorder	2
Neuroblastoma	13	Phenylketonuria	2	Cardiomyopathy	2
Esophageal cancer	11	Retinoschisis	2	Dry eye syndrome	2
HPV	5	Alport Syndrome	1	Neuromyelitis Optica	2
Urinary cancers	4	Amyotrophic lateral sclerosis	1	Ocular hypertension	2
Mantle cell lymphoma	3	Becker Muscular dystrophy	1	Peanut Allergy	2
Merkel cell carcinoma	3	Charcot-Marie-Tooth	1	Rheumatoid arthritis	2
Myelodysplastic syndrome	3	Cystic Fibrosis	1	Uveitis	2
Uterine cancer	3	Danon disease	1	Alzheimer Disease	1
Cervical intraepithelial neoplasia	2	Gaucher disease	1	Dementia	1
Small cell lung carcinoma	2	LPL Deficiency	1	Diarrhea	1
Thyroid cancers	2	Mucopolysaccharidosis type 6	1	Erectile Dysfunction	1
Retinoblastoma	1	OTC Deficiency	1	Hepatic fibrosis	1
Genetic disorders	#	Pemphigus Vulgaris	1	Lipoproteinemia	1
Spinal muscular atrophy	23	Pyruvate Kinase Deficiency	1	Myopic Choroidal Neovascular Membrane	1
Duchenne Muscular Dystrophy	18	Type 2 diabetes mellitus	1	Neuropathic Pain	1
Hemophilia B	17	Infections	#	Osteopetrosis	1
ADA-SCID	16	HIV	62	Overactive Bladder	1
Beta Thalassemia	16	COVID19	29	Peripheral Nerve Injury	1
Leber congenital amaurosis	15	Malaria	20	POMES Syndrome	1
Hemophilia A	14	Ebola	19	Raynaud's Phenomenon	1
Retinitis Pigmentosa	10	HCV	13	Retinal Vein Occlusion	1
Choroideremia	9	HPV	12	Scleroderma	1
Sickle cell anemia	9	HBV	10	Severe to Profound Hearing Loss	1
Primary Hyperoxaluria	9	Influenza	9	Systemic lupus erythematosus	1
Epidermolysis bullosa	9	M.Tuberculosis	9		
Transthyretin Amyloidosis	8	Respiratory Tract Diseases	7		

lower efficiency and duration of gene expression [59]. Plasmids, DNA, and RNAs (siRNA and miRNA) are the most common types of non-viral vectors, with the first two primarily used for "gain of function" (replacing or substituting a specific genetic function in a target cell) and the latter for "loss of function" (reducing the expression of endogenous genes in a sequence-specific manner) purposes [59]. Plasmids have recently entered the market as a vector in the form of Neovasculgen for peripheral vascular disease. They have also been used in the treatment of cancer. Oligonucleotides such as Spinraza, Exondys 51, and Vyondys 53 have also entered the market. Other non-viral vectors with fewer applications include transposons, CRISPRs, ZNFs, Salmonella, yeast, Saccharomyces cerevisiae, Neisseria, Listeria monocytogenes, Bacillus Calmette–Guérin (BCG), Bifidobacterium longum, Escherichia coli, Mycobacterium tuberculosis, gold and lipid nanoparticles (Fig. 5).

From 1989 to 2001, scientists struggled to improve the safety of vectors, which was a major concern about them, in order to prevent known adverse events. [60] Looking at the trend of using different vectors in Fig. 5A, plasmids and RNAs were the most common non-viral vectors with a roughly constant level of use between 2010 and 2020, while DNA and mRNA remained in low use during these years. Lentivirus, adenovirus, and adeno-associated viruses were the most widely used viral vectors between 2013 and 2016, and they are now the leading gene therapy vectors. Retrovirus and HSV, two less common viral vectors, have seen a slight increase in usage since 2017–2018 (Fig. 5).

8. Two methods of gene therapy; engineering cells/tissues inside or outside of the body?

There are two methods for introducing genetic material into cells. *Ex-vivo* gene therapy involves laboratory-based transduction of cells. In this method after harvesting specific types of cells either from the patient (autologous) or a donor (allogeneic), a vector is used to carry the therapeutic gene into the cell. After possible activation or expansion, the transduced cells are returned to the patient. *In-vivo* gene therapy, on the other hand, entails delivering a vector into a patient's body without the use of a cellular vehicle, allowing the gene transfer process to take place

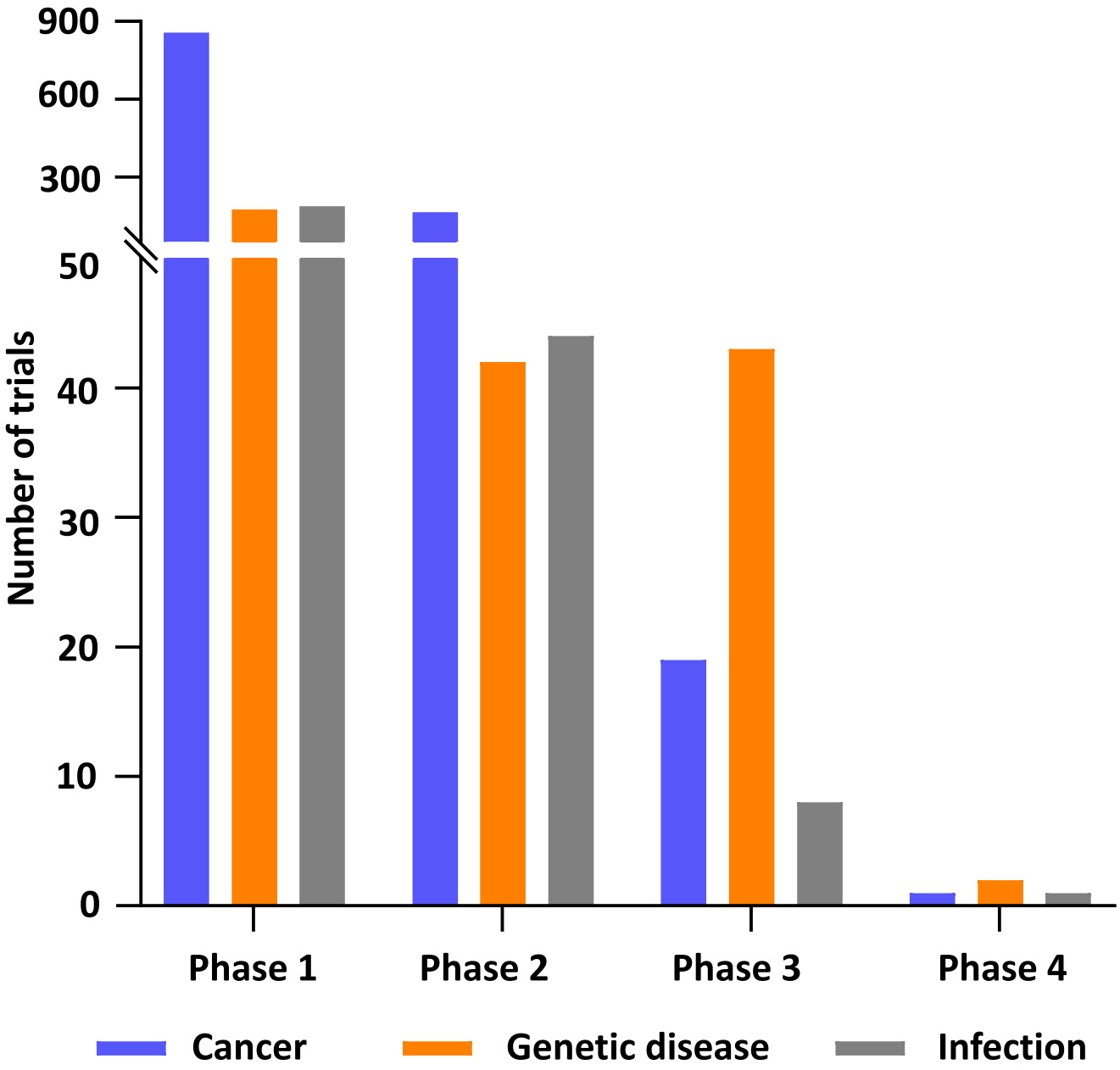

Fig. 4. Number of clinical trials from 2010 to 2020 by their current phase of study and disease group. The majority of cancer clinical trials were in phase 1. Although the number of phase 2 trials was also high, it seems that they did not easily proceed to phases 3 and 4. On the other hand, the high contribution rate of phase 3 clinical trials in genetic diseases, may show higher efficacy and feasibility of gene therapy in genetic disorders. It is noteworthy that the number of commercial gene therapy products for genetic diseases is more than the cancer group.

entirely within the body. [53] Both methods have advantages and disadvantages. We could specifically target the proposed collected cells by ex-vivo gene transfer; additionally, the ability to select and refine the cells of interest prior to transplantation allows us to ensure the desired characteristics of the cells and have a wide range of available vector choices. While ex-vivo genetic manipulation of cells demands a high-tech laboratory, extensive research, and advanced equipment, the *in-vivo* method requires less laboratory work. *in-vivo* gene manipulation is the key method when the cells cannot be separated from the body or not be able to live *ex-vivo* for a long time. Furthermore, the *in-vivo* method is best suited to easily accessible tissues. However, non-specific contact of the vector with cells and a higher risk of immune reactions have limited its use. [61–63].

From 2010 to 2020, there were 1035 (54%) ex-vivo and 872 (45%) *in-vivo* gene therapy clinical trials. As illustrated in Fig. 6, the majority of ex-vivo clinical trials used engineered T cells, followed by hematopoietic stem cells, DC, and NK cells. In contrast, common tissues targeted by *in-vivo* clinical trials include the tumor, bone marrow, eye, central nervous system, heart, and liver. The *in-vivo* method is used in the majority of metabolic disorder trials. Spinraza, Zolgensma, and Luxturna used *in-vivo* methods, while others used ex-vivo methods (Fig. 6).

9. Pioneer countries in gene therapy

In the early 1990s, the National Institute of Health in the United States conducted the first successful clinical trial in gene therapy. [12] Even though the United States has had more gene therapy clinical trials than any other country since that time, the Chinese took the lead in approving the first commercially available gene therapy product (Gendicine). In the late 1990s, several countries in Europe were among those

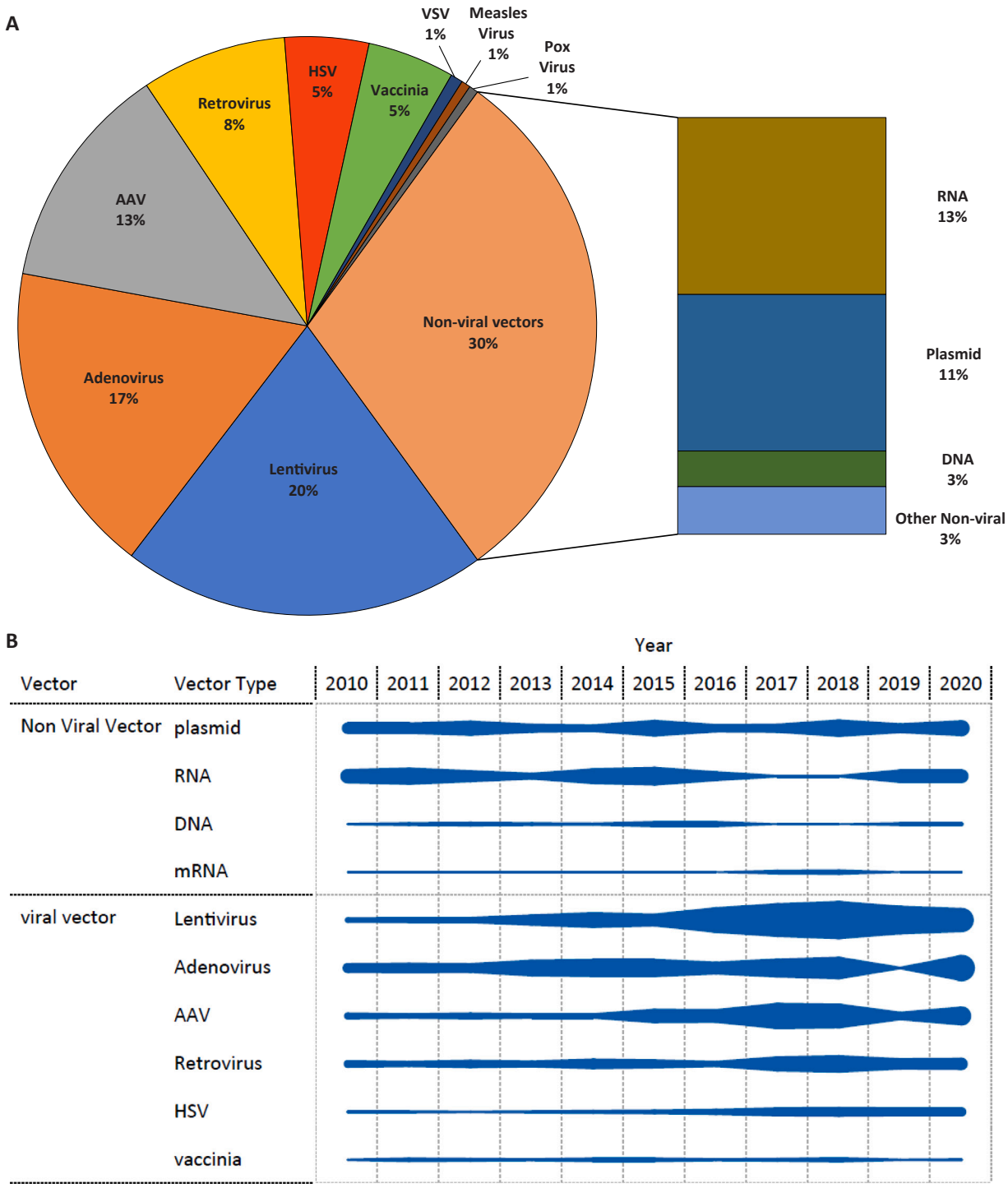

Fig. 5. Trends of using different vectors in gene therapy trials. (A) The contribution part of viral and non-viral vectors and their types in clinical trials from 2010 to 2020. Viral vectors played the main part in transferring the genetic materials, however, the characteristics of each vector made them ideal candidates for specific usages. (e.g., lentivirus & retrovirus for *ex-vivo* gene therapy, AAV for *in-vivo* gene therapy, or RNAs for loss-of-function changes.) RNAs and plasmids were the most common non-viral vectors. (B) The time trend of gene therapy clinical trials using different vectors.

that were the first to implement gene therapy. Currently, the United States and China dominate gene therapy clinical trials, accounting for roughly 80% of all trials. Several other countries, including the United Kingdom, France, Spain, and Germany, have excelled in this promising field in recent years. It should be noted that there is an increasing number of clinical trials being conducted with the collaboration of institutes or universities from multiple countries. The number of gene therapy clinical trials with an international contribution doubled from 2010 to 2020 (Fig. 7).

10. Limitations

Based on the searches conducted on the clinicaltrials.gov website and associated publications, some of the assessed variables were undetermined or inaccessible. This excluded proportion of undetermined values was considerable for some variables, particularly for uncommon

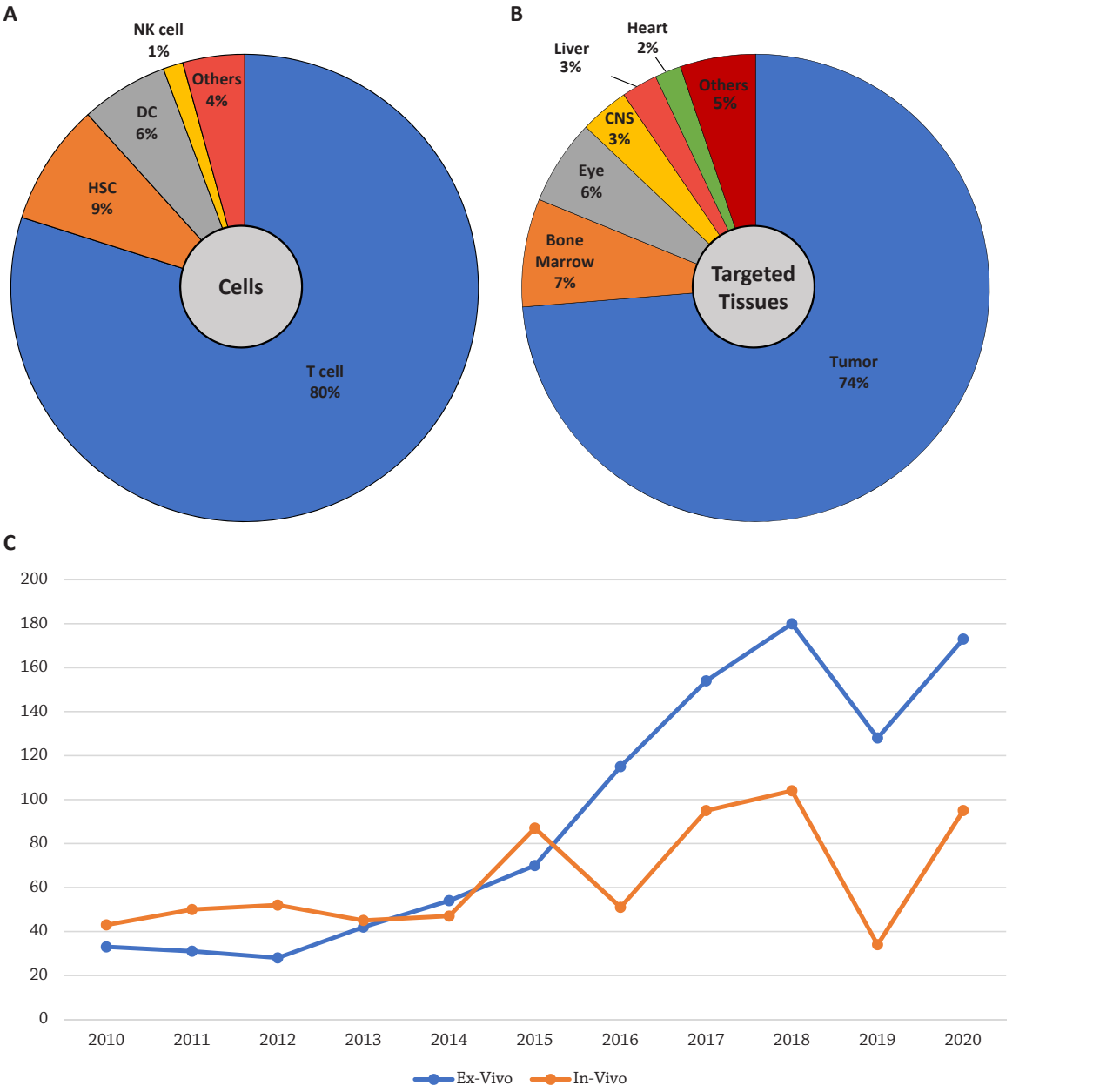

Fig. 6. Trends of *in-vivo* and in-vitro gene therapies and their engineered targets (A & B) The Distribution of each type of cells engineered in *ex-vivo* trials and each type of organ targeted in *in-vivo* Trials from 2010 to 2020. (C) The number of clinical trials using *ex-vivo* and *in-vivo* methods of gene transfer from 2010 to 2020. However, *ex-vivo* gene therapy is the major method used in the gene therapy trials, other diseases would benefit from the *in-vivo* method of gene delivery, either systemic or localized. It should be noted that a great number of viral vectors used for *in-vivo* gene therapy trials were AAVs.

classifications (e.g., *ex-vivo/in-vivo*), which, despite preserving the general view of trends, could treat the comprehensiveness of findings.

11. Conclusions

It has been about three decades since gene therapy was proposed as a method of treating diseases that were thought to be difficult to cure or incurable. Looking at the trend of gene therapy progressions over the last century, it appears that we have entered a new era, as evidenced by the increasing number of approved gene therapy products in the last decade. This overview covered the major aspects of clinical trial strategies used over the last ten years.

Gene therapy strategies have evolved over time. Genetic advances and biotechnology breakthroughs, such as CRISPR-CAS gene editing, have given gene therapy a new role in treating a broader range of diseases. Gene therapy employs a variety of strategies for disease treatment based on the underlying pathology, such as knocking out/deleting/adding a defective allele or adding a correct copy/modified copy of an allele. Vaccination has been accomplished through the addition of new genes coding for key structural subunits of infectious particles. Although gene therapy has primarily been used in cancer and genetic disorders, it has also been studied in advanced phase clinical trials for infectious diseases such as AIDS, malaria, and HPV, as well as acquired diseases such as peripheral vascular disease, osteoarthritis, retinopathies, CAD and heart failure, allergic rhinitis, Alzheimer's disease, etc (Table 3).

A ten-year trend analysis of data on strategies used in gene therapy clinical trials revealed that the use of viral vectors, particularly lentivirus, adenovirus, and AAV, has become more appealing for clinical studies over time. It should be noted that the widespread use of lentiviral

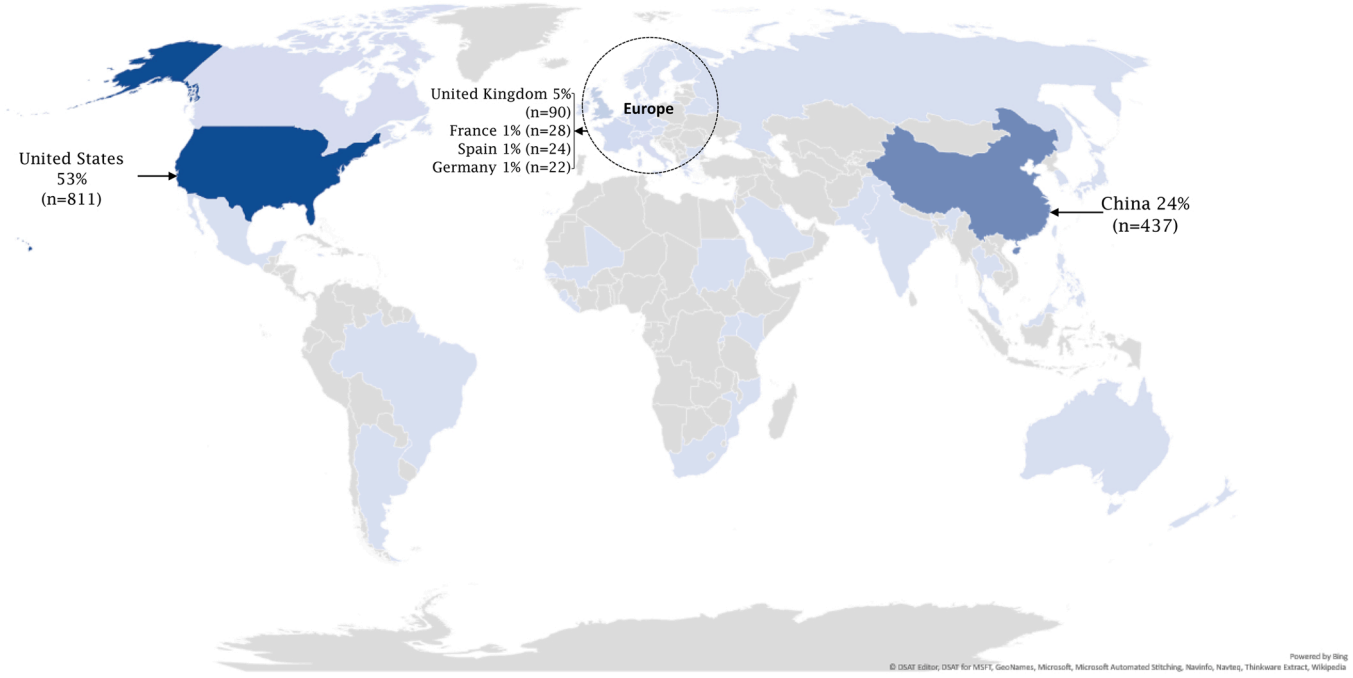

Fig. 7. Heat map of the distribution of clinical trials across the world from 2010 to 2020. The contribution part of the countries with the highest number of gene therapy trials is presented in the lower left of the map. The data were shown as country and percentage of the grand total.

vectors is due to their use in ex-vivo gene therapy of cancer using CAR T cells or TCR modified T cells, whereas AAV is primarily used as an *in-vivo* vector for the treatment of genetic diseases. Among non-viral vectors, RNAs (siRNA, miRNA, shRNA) and plasmids were the most commonly used. Furthermore, our data show that ex-vivo gene therapies have been more common than *in-vivo* gene therapies in the last decade. While ex-vivo gene therapy is the most commonly used strategy for cancer, in vivo gene therapy is more commonly used for genetic diseases and viral therapies.

Examining the trends of strategies employed in successful clinical trials provides valuable information for future investigations, improved study designs, and practical policy-making by pharmaceutical companies and gene therapy research institutes.

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CRediT authorship contribution statement

FA and NA contributed to the design of the study, FA and VM contributed to data gathering and data visualization, VM and FA drafted the manuscript, NA supervised the study, and VM critically revised the manuscript.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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