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REVIEW ARTICLE

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Gene therapy clinical trials worldwide to 2023—an update

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Abstract

To date, 3,900 gene therapy clinical trials have been completed, are ongoing or have been approved worldwide. Our database brings together global information on gene therapy clinical activity from trial databases, official agency sources, published literature, conference presentations and posters kindly provided to us by individual investigators or trial sponsors. This review presents our analysis of clinical trials that, to the best of our knowledge, have been or are being performed worldwide. As of our March 2023 update, we have entries on 3,900 trials undertaken in 46 countries. We have analyzed the geographical distribution of trials, the disease indications (or other reasons) for trials, the proportions to which different vector types are used, and which genes have been transferred. Details of the analyses presented, and our searchable database are on *The Journal of Gene Medicine* Gene Therapy Clinical Trials Worldwide website at https://a873679.fmphost.com/fmi/webd/GTCT. We also provide an overview of the progress being made around the world, and discuss key trends since the previous review, namely the unprecedented increase in gene therapy clinical trial activity, including the implementation of genome editing technology with the potential to transform the field moving forward.

KEYWORDS
clinical trials, database, gene therapy, *The Journal of Gene Medicine*, worldwide

1 | INTRODUCTION

The gene therapy landscape has changed dramatically since our previous review, which was published soon after approval of the first adeno-associated virus (AAV)-based gene therapy by the US Federal Food and Drug Administration (FDA), Luxterna™, in December 2017.[1] Since then, the field has seen the approval of over 20 new gene therapy products (see Tables 1–3) with notable examples including Zolgensma®, Hemgenix® and Onpattro™, the latter being the first siRNA therapy to reach the market. Improvements in gene transfer efficiency and rapid implementation of genome editing technologies together with significant commercial investment has brought the clinical horizon increasingly close, and with it, new challenges as the field advances. This review will provide a snapshot of the current status of the field at the time of this publication and highlight some of these key challenges including a renewed focus on factors that influence patient safety. Other resources that provide contemporary information are also available, with reports from the American Society of Gene and Cell Therapy (ASGCT) and the Alliance of Regenerative Medicine notable examples.

2 | ACCELERATING CLINICAL SUCCESS AND TRANSLATION-RELATED CHALLENGES

Gene therapy clinical trials have so far predominantly provided important proof-of-concept data, demonstrating that gene therapy is generally well tolerated with relatively few serious adverse events observed. Although clinical success has been infrequent as the field has matured, importantly, it has uncovered risks such as insertional mutagenesis and immune responses to the gene delivery system or transgene product that were not initially predicted by the preclinical

TABLE 1 Approved viral vector-based gene therapy products[†].

Brand name	Generic name	Date of approval	Original approving agency	Indication	Delivery system; administration	Manufacturer
Gendicine	N/A	October 2003	State Food and Drug Administration of China (SFDA)	Head and neck squamous cell carcinoma	Adenoviral; *intratumoral*	Shenzhen SiBiono GeneTech
Oncorine	H101	November 2005	SFDA	Refractory nasopharyngeal cancer	Adenoviral; *intratumoral*	Shanghai Sunway Biotech
Rexin-G	Mx-dnG1	December 2007	Food and Drug Administration Philippines	Solid tumors, metastatic pancreatic cancer	γ-Retroviral; *in vivo*	Epeius Biotechnologies
Glybera®	Alipogene tiparvovec	November 2012	European Medicines Agency (EMA)	Lipoprotein lipase deficiency	AAV, *in vivo*	uniQure (withdrawn in 2017)
Imlygic® (T-VEC)	Talimogene Laherparepvec	October 2015	US Food and Drug Administration (FDA)	Melanoma	Herpes simplex virus-1; *intratumoral*	Amgen
Strimvelis™	N/A[‡]	June 2016	EMA	Adenosine deaminase deficiency	γ-Retroviral; *ex vivo*	GlaxoSmithKline
Luxterna™	Voretigene neparvovec	December 2017	FDA	Retinal dystrophy (biallelic *RPE65* mutation)	AAV; *in vivo*	Spark Therapeutics, Inc
Zolgensma®	Onasemnogene abeparovec	May 2019	FDA	SMA	AAV; *in vivo*	Avexis
Zynteglo®	Betibeglogene autotemcel	May 2019	EMA	β-Thalassemia	Lentiviral; *ex vivo*	Bluebird Bio
Libmeldy™	Gentherapeutikum	December 2020	EMA	Metachromatic leukodystrophy (ARSA deficiency)	Lentiviral; *ex vivo*	Orchard Therapeutics
Delytact	Teserpaturev/G47Δ	June 2021	Pharmaceuticals and Medical Devices Agency, Japan	Delytact	Herpes simplex virus-1; *intratumoral*	Daiichi Sankyo Company, Limited
Skysona™	Elivaldogene autotemcel	July 2021	EMA	Cerebral adrenoleukodystrophy	Lentiviral; *ex vivo*	Bluebird Bio
Upstaza™	Eladocagene exuparvovec	July 2022	EMA	Aromatic L-amino acid deficiency	Lentiviral; *ex vivo*	PTC Therapeutics International Limited, Irland
Roctavian®	Valoctocogene roxaparvovec	August 2022	EMA	Hemophilia A	AAV; *in vivo*	BioMarin Pharmaceutical
Hemgenix®	Etranacogenedezaparvovec	November 2022	FDA	Hemophilia B	AAV; *in vivo*	CSL Behring LLC; Unicure
Adstiladrin®	Nadofaragene firadenovec-vncg	December 2022	FDA	Bladder cancer	Adenoviral; intravesical instillation	Ferring Pharmaceuticals
Vyjuvek	Beremagene geperpavec-svdt	May 2023	FDA	Dystrophic epidermolysis bullosa (DEB)	Herpes simplex virus-1; topical	Krystal Biotech, Inc
Elevidys	Delandistrogene moxeparvovec	June 2023	FDA	Duchene muscular dystrophy	AAV; *in vivo*	Sarepta Therapeutics
Lyfgenia	Lovotibeglogene autotemcel	December 2023	FDA	Sickle cell disease	Lentiviral; *ex vivo*	Bluebird Bio
Beqvez	Fidanacogene elaparvovec-dzkt	January, 2024	Health Canada	Hemophilia B	AAV; *in vivo*	Pfizer, Inc.

[†]Current at the time of publication.
[‡]N/A, not applicable.

TABLE 2 Approved cloned T-cell receptor cell products[†].

Brand name	Generic name	Date of approval	Original approving agency	Indication	Target antigen	Delivery system	Manufacturer
KYMRIAH®	Tisagenlecleucel	August 2017	US Food and Drug Administration (FDA)	B-cell acute lymphoblastic leukaemia (ALL); B-cell non-Hodgkin lymphoma (NHL)	CD19	Lentiviral vector	Novartis Pharmaceuticals
YESCARTA®	Axicabtagene ciloleucel	October 2017	FDA	B-cell NHL; follicular lymphoma	CD19	Lentiviral vector	Kite Pharma, Incorporated
Breyanzi™	Lisocabtagene maraleucel	February 2021	FDA	B-cell NHL	CD19	Lentiviral vector	Juno Therapeutics
Abecma™	Idecabtagene vicleucel	March 2021	FDA	Multiple myeloma	Anti-B cell maturation antigen (BCMA)	Lentiviral vector	Bristol Myers Squibb
Carteyva®	Relmacabtagene autoleucel (Relma-cel)	July 2021	China National Medical Products Administration (NMPA)	Relapsed or refractory large B-cell lymphoma	CD19	Lentiviral vector	JW Therapeutics
TECARTUS®	Brexucabtagene autoleucel	October 2021	FDA	Mantel cell lymphoma; B-cell ALL	CD19	Lentiviral vector	Kite Pharma, Incorporated
CARVYKTI®	Ciltacabtagene autoleucel	February 2022	FDA	Relapsed and refractory multiple myeloma (RRMM)	BCMA	Lentiviral vector	Janssen Biotech
Fucaso; CT103A	Equecabtagene autoleucel	July 2023	NMPA	RRMM	BCMA	Lentiviral vector	IASO Biotherapeutics
CNCT19	Inaticabtagene autoleucel	November, 2023	NMPA	Relapsed and refractory B-cell acute lymphoblastic leukemia	CD19	Lentiviral vector	Juventas Cell Therapy
Zevor-cel; CT-053	Zevorcabtagene autoleucel	March, 2024	NMPA	RRMM	BCMA	Lentiviral vector	CARsgen Therapeutics

[†]Current at the time of publication.

TABLE 3 Approved non-viral gene therapy products[†].

Brand name	Generic name	Date of approval	Original approving agency	Indication	Therapeutic nucleic acid; administration	Manufacturer
Vitravene	Fomivirsen	August 1998	US Food and Drug Administration (FDA)	Cytomegalovirus infection	Antisense oligomer (ASO); intravitreal injection	Ionis Pharmaceuticals (withdrawn)
Macugen	Pegaptanib	December 2004	FDA	Neovascular (wet) age-related macular degeneration	Polynucleotide aptamer; intravitreal injection	Eyetech Pharmaceuticals
Neovasculgen	Cambiogenplasmid	December 2012	Russian Ministry of Healthcare	Peripheral artery disease, including critical limb ischemia	Plasmid DNA; intraneural injection	Human Stem Cells Institute
Kynamro	Mipomersen	January 2013	FDA	Homozygous familial hyperchlolesterolemia	ASO; subcutaneous injection	Ionis Pharmaceuticals (withdrawn)
Defitelio	Defibrotide	October 2013	European Medicines Agency (EMA)	Veno-occlusive disease/sinusoidal obstructive syndrome	Single-stranded oligonucleotides; intravenous infusion	Jazz Pharmaceuticals

(Continues)

TABLE 3 (Continued)

Brand name	Generic name	Date of approval	Original approving agency	Indication	Therapeutic nucleic acid; administration	Manufacturer
Exondys 51	Eteplirsen	September 2016	FDA	Duchenne muscular dystrophy (DMD)	Phosphorodiamidate morpholino oligomer (PMO); intrathecal injection	Sarepta Therapeutics
Ampligen®	Rintatolimod	August 2016	National Administration of Drugs, Food and Medical Technology, Argentina	Myalgic encephalomyelitis/ chronic fatigue syndrome	Mismatched double-stranded RNA; intravenous infusion	AIM ImmunoTech (formerly Hemispherx Biopharma)
SPINRAZA®	Nusinersen	December 2016	FDA	Spinal muscular atrophy	ASO; intrathecal injection	Ionis Pharmaceuticals
TEGSEDI®	Inotersen	July 2018	EMA	Hereditary transthyretin-mediated amyloidosis (hATTR) polyneuropathy	ASO; subcutaneous injection	Ionis Pharmaceuticals
Onpattro™	Patisiran	August 2018	EMA and FDA	hATTR	LNP containing small interfering RNA (siRNA); intravenous infusion	Alnylam Pharmaceuticals
Collategene®	N/A‡	March 2019	Ministry of Health, Labour and Welfare of Japan	Critical limb ischemia	Plasmid DNA; intramuscular injection	AnGes, Inc.
Waylivra	Volanesorsen	May 2019	EMA	Familial chylomicronaemia syndrome	ASO; subcutaneous injection	Ionis Pharmaceuticals
Givlaari	Givosiran	November 2019	FDA	Acute hepatic porphyria	siRNA; subcutaneous injection	Alnylam Pharmaceuticals
Vyondys 53	Golodirsen	December 2019 (confirmatory trial required)	FDA	DMD	PMO; subcutaneous injection	Sarepta Therapeutics
Viltepso	Viltolarsen	December 2019	FDA	DMD	Morpholino ASO; intravenous infusion	Nippon Shinyaku Pharma, Inc.
Oxlumo	Lumasiran	November 2020	EMA and FDA	Primary hyperoxaluria type 1	siRNA; subcutaneous injection	Alnylam Pharmaceuticals
Leqvio	Inclisiran	December 2020	EMA	Primary hypercholesterolemia	siRNA subcutaneous injection	Novartis/ Alnylam Pharmaceuticals
Amondys 45	Casimersen	February 2021	FDA	DMD	PMO; subcutaneous injection	Sarepta Therapeutics
Amvuttra™	Vutrisiran	June 2022	FDA	hATTR polyneuropathy	siRNA; subcutaneous injection	Alnylam Pharmaceuticals
Qalsody	Tofersen	April 2023	FDA	Amyotrophic lateral sclerosis	ASO; intrathecal injection	Ionis Pharmaceuticals
Rivfloza	Nedosiran	October 2023	FDA	Hyperoxaluria	siRNA; subcutaneous injection	Dicerna Pharmaceuticals

TABLE 3 (Continued)

Brand name	Generic name	Date of approval	Original approving agency	Indication	Therapeutic nucleic acid; administration	Manufacturer
Casgevy	Exagamglogene autotemcel	November 2023	Medicines and Healthcare products Regulatory Agency	Transfusion-dependent β-thalassemia and severe sickle cell disease	Non-viral (Ribonucleoprotein [RNP]); *ex vivo*	Vertex Pharmaceuticals and CRISPR Therapeutics
Wainua	Eplontersen	December 2023	FDA	hATTR polyneuropathy	ASO; subcutaneous injection	Ionis Pharmaceuticals

[†]Current at the time of publication.
[‡]N/A, not applicable.

model system. This has led to further refinements of the delivery systems and treatment protocols used in subsequent trials. The increasing number of trials undertaken with demonstrated benefit to patients, however, has revealed several new challenges for the field to consider as it moves forward. A renewed focus on patient safety is one of these factors as gene therapy expands to include potential therapeutic targets that were previously out of technological reach. Notably, safety considerations relating to the use of recombinant AAV vectors, including toxicity associated with the use of high vector doses, immune responses and vector integration with the potential risk of genotoxicity, have all been a recent focus for the field and highlighted by both the ASGCT[2] and European Society for Gene and Cell Therapy.[3] Other issues that are still to be fully resolved include (i) the challenge of developing therapies for rare and ultra-rare diseases,[4,5] (ii) the ability to manufacture clinical-grade vector at scale to supply current demand,[6–10] (iii) the high cost of gene therapy products, (iv) ensuring equitable access to treatments[11–14] and (v) maintaining a path to the clinic by ensuring freedom to operate.

3 | TECHNOLOGICAL INNOVATION; THE CATALYST OF PROGRESS

3.1 | The rapid uptake of genome editing technology

Over the past few years, there has been a rapid uptake of gene editing technologies over the traditional gene addition approaches because they offer the advantage of precise and permanent changes in the genome of living organisms. Gene addition involves the introduction of a functional copy of a gene into the target cell and is often used to address genetic disorders caused by the absence or malfunction of a specific gene.

One significant milestone since the 2017 review has been the increased interest in genome editing technologies for their ability to introduce precise and permanent genomic modifications. The recent rise in developing programmable nucleases, such as zinc-finger nucleases, transcription activator-like effector nucleases and particularly clustered regularly interspaced short palindromic repeat (CRISPR)–Cas-associated nucleases, has greatly accelerated the path for gene editing to move from concept to clinical practice.[15] One important example is the revolutionary discovery of base-editing in 2016 and its translation into the clinic to treat the first patient with T-cell leukemia in 2022.[16,17] In October 2016, a patient with lung cancer became the first to be treated with CRISPR/Cas9 modified T cells in a Chinese clinical trial (NCT02793856) with 12 additional patients treated to date. In 2020, data from this trial were published in *Nature Medicine* reporting the safety and feasibility of the treatment with minimal side effects.[18] In 2019, the first *ex vivo* CRISPR-based genome editing of blood stems cells was performed and the cells were infused i.v. back into patients with beta thalassemia in Germany in a combined phase I, II and III trial.[19] One of the challenges highlighted from this trial to date is the side effects that result from the chemotherapy that is necessary to eliminate the pre-existing bone marrow cells for the edited cells to engraft.[19] More recently in 2020, for the first time *in vivo*, AAV-based CRISPR/Cas9 was administered to two patients by direct injection into the eye to treat Leber congenital amaurosis (NCT03872479).[20] The trial is reporting data indicative of improvement in some of the diseases parameters; however, no data have been published yet. Recent breakthroughs in the delivery technology of these genome editing tools have offered precise, efficient and permanent ways to modify the DNA of living organisms.

3.2 | Modulating the epigenome

CRISPR-Cas9 has now exceeded its capability as just a gene-editing tool. It has been repurposed to produce a catalytically impaired inactive Cas9 for applications including precise gene regulation, epigenetic editing, chromatin engineering and remodeling.[21] CRISPR activation and repression systems allow for customized control of the cellular phenotype and fate by directing the Cas protein to a specific site by a gRNA and utilizing an effector domain to exert an effect on a specific area in the genome without modifying the underlying DNA sequence.[22] For instance, robust CRISPR/Cas gene activation domains include the VP64-p65-Rta (VPR) system, a tripartite fusion of VP64, p65 and Rta, and the SAM system, consisting of VP64, p65 and HSF1.[23,24] Alternatively, gene repression effector domains have been demonstrated using the fusion of a novel KRAB–MeCP2 bipartite repressor domain to a catalytically inactive Cas9 (dCas9).[25] Multiple

copies of these effector domains can be recruited to the same site using the SunTag system to achieve signal amplification.[26] Modulating the epigenome has long been considered an interesting opportunity for therapeutic intervention in a variety of disease contexts with several approved pharmacological therapies marketed, mainly for cancer.[27] Examples of these drugs include DNA methyltransferase inhibitors, histone deacetylase inhibitors, histone methyltransferase inhibitors and bromodomain inhibitors;[27] however, the main issue with these currently marketed epigenetic drugs is the lack of specificity and the unintended genome-wide off-target effects, hence necessitating precision genomic control utilizing the CRISPR systems. An observational clinical trial was established in 2019 to develop an *ex vivo* system from patient-derived mesenchymal stem cells to develop a CRISPR/Cas9 epigenome editing approach for Kabuki Syndrome (NCT03855631). The field of epigenome editing is still relatively new and there are many questions yet to be addressed, including the durability of the epigenome edits and the toxicity of the effector domains, and the assays utilized to study the epigenome are still under development.[28]

3.3 | Virus-based gene delivery systems

The proportion of approved gene therapy products and those currently in clinical development that utilize viral vectors for gene delivery has been reported to be 89% according to the ASGCT report.[29] The key first step in gene therapy is efficient gene delivery to the target tissues, which is carried out by gene delivery vehicles called vectors.[30] In the past 5 years, the gene therapy field has witnessed a wave of novel viral-based vector products that have gained regulatory approval (Table 3). Viral systems are commonly modified to render them replication incompetent and exploit favorable aspects of virus biology to enter the host cells and deliver the transgene of interest.[31,32] Viral vector-based platforms have been utilized for a spectrum of disorders ranging from cancer therapies to treating monogenic disorders with life-changing outcomes. As more patients have received these therapies, it has become evident that four main challenges still exist that limit the utility of viral vectors. These include the need to increase transduction efficiency, lower the dose, evade the host immune system, control gene expression levels and achieve more consistent inter-patient therapeutic effect. Ongoing work to address these challenges is generating technological innovations that have the potential to leapfrog current therapies and unlock the potential of these delivery vehicles.

Current gene therapies fall into two broad categories: *in vivo* and *ex vivo* therapies. In particular, *in vivo* gene therapies require the direct infusion of the gene therapy into the patient's blood stream or target organ to deliver the genetic material into the required cells.[33] Most viral vectors used for *in vivo* delivery in the clinic to date include adenovirus, AAV and herpes simplex virus.[33] γ-Retroviral and, more recently, lentiviral vectors have predominantly been utilized for *ex vivo* applications owing to the integrating nature of the virus and their broad tissue tropism. Retroviral-based vector systems have been used in many clinical trials to introduce genes into mature T cells to generate immunity against cancer through the delivery of chimeric antigen receptors (CARs) or cloned T-cell receptors (CAR-T therapy).[34] Trials targeting primary immunodeficiencies have shown immense therapeutic benefit using *ex vivo* retroviral vector-based approaches,[35–39] although adverse events resulting from insertional mutagenesis were significant drawback to the gene therapy field at the time of their occurrence.[40–43] The risk of insertional mutagenesis has been reduced by using next-generation, self-inactivating, lentiviral vectors, with those developed from non-human lentiviruses hypothesized to be more acceptable for *in vivo* applications owing to their parental viruses being non-infectious to humans.[44] The main application of lentivirus vectors for *in vivo* gene therapies is to treat monogenic diseases and chronic disorders including neurological diseases, ophthalmological diseases and metabolic diseases.[33] In addition to the aforementioned viral vector systems, other vectors also used in clinical trials albeit to a lesser extent include vesicular stomatitis virus, modified vaccinia virus Ankara, arenavirus, Sendai virus and measles virus.[45–48]

3.4 | AAV and capsid technology

The AAV viral capsid forms a fundamental component of viral-vector gene therapy. The AAVs package their genome in a non-enveloped icosahedral capsid of ~3.8 MDa, consisting of 60 subunits of three distinct viral proteins (VPs), all of which perform an essential role in cell-entry and transduction. Adeno-associated viruses have a packaging capacity of 4.7 kb genome and can be pseudo-serotyped whereby different capsid proteins from different serotypes can carry the same genome but can target different tissues, hence different AAV serotypes have different tissue tropism.[49,50] The capsid determines which cell types are targeted, the efficiency of cell entry, transgene expression and the probability that the gene therapy is detected and eliminated by the immune system. An array of naturally occurring AAV serotypes were initially isolated from non-human primate tissues and were aligned to their parental capsids to identify several residues of interest. To address the challenges associated with AAV delivery and pave the path for clinical translation, three main capsid engineering technologies have been devised to genetically modify the AAV capsid including rational design, directed evolution and computationally designed ancestral capsid.[51] For example, AAV1 and AAV7 display high muscle tropism and in aligning these capsids to AAV2, a new variant termed AAV2.5 was generated by the insertion of five conserved residues from AAV1 into the AAV2 capsid. This capsid variant has been used to administer an AAV vector containing a mini dystrophin to patients with Duchene muscular dystrophy with greater transduction efficiency than AAV2.[52,53] The immense success shown using the AAV9 capsid in the treatment of infants with spinal muscular atrophy (SMA) highlights the potential of vectors based on AAV. It also highlights a major obstacle facing the gene therapy field: the pre-existing natural and therapy-induced anti-capsid humoral immunity that precludes some children from receiving the treatment needed. A major

body of work is currently underway in the field to engineer an AAV9 variant that can escape the natural immune response to overcome this hurdle[54] while also engineering novel AAV9 variants that can de-target the liver to avoid most of the AAV9 from accumulating in the liver, hence improving clinical safety and efficacy.[55]

3.5 | The challenge of delivery across the blood–brain barrier

One of the obstacles in using rAAV for delivering genes into the central nervous system (CNS) is the low penetration of vectors across the human blood–brain barrier (BBB). An important example is Zolgensma®, an FDA-approved gene therapy product that uses an rAAV9 vector to deliver the *SMN1* gene into motor neurons for the treatment of patients with SMA.[56] Despite the clinical success of Zolgensma®, rAAV9 has low BBB penetration and CNS transduction in comparison with other tissues. Therefore, high vector doses are required for systemic delivery, causing unwanted gene transfer to other tissues, leading to varying degrees of adverse side effects, including death.[57] This highlights the clinical need to improve AAV transduction efficiency in the brain. Two main approaches have emerged to address this issue, the first being to engineer the AAV capsid to obtain higher CNS transduction.[58–61] For example, using an evolutionary approach Deverman et al. developed an AAV9 capsid mutant, AAV-PHP.B, that exhibits approximately 40-fold higher transduction efficiency in the mouse CNS, while maintaining peripheral organ transduction at similar levels.[62] This variant, however, failed to show a similar transduction profile in all strains of mice and in non-human primates[61,63–66] with transduction efficiency found to be dependent on expression of the Ly6a receptor,[65] highlighting the need for predictive preclinical model systems.

Another approach is to utilize cell-penetrating peptides (CPP) to enhance the internalization of AAV capsids into cells via the process of endocytosis. Several studies have investigated the applications of CPP-derived therapeutics, some of which have entered clinical trials.[67–70] In the past few years, CPPs have been shown to significantly improve the transduction of AAV2 and AAV8 in mouse muscles,[71] and others such as LAH4, LEPTIN30 and APOE, can significantly enhance the *in vitro* transduction efficiency of AAV9. Shuttle peptides to improve delivery across the BBB have also been developed and were shown to enhance AAV8 transduction in the brain and the liver following systemic administration.[72] Of particular interest are AAV.CPP.16 and AAV.CPP.21, two variants that were developed to display cell-penetrating peptides on the viral capsid. These capsids showed improved transduction efficiencies of cells of the CNS upon systemic delivery in both mouse and macaques.[73] It is still unknown whether these variants will be translatable to humans given the interspecies differences in the physiological parameters and molecular compositions of the BBB that could result in differential CNS uptake of rAAVs.[73] Although significant progress has been made, more effort is required to facilitate the translation of these modified AAVs from rodent models to primate species and ultimately to humans.

3.6 | Lipid nanoparticles and other non-viral delivery systems

Transient gene delivery systems using non-viral vector platforms have become an attractive target in clinical settings. In comparison with vector mediated gene delivery, non-viral methods have the advantage of simplicity and reproducibility of production, larger packaging capacity and reduced biosafety concerns.[74] However, their gene delivery potential is relatively less efficient in comparison with recombinant viral vector systems and the stability of the effect can be transient depending on the cargo of interest. The field is currently advancing nucleic acid stability, overcoming extracellular and intracellular barriers and enhancing the potency of the effect.[75] Some strategies to deliver genetic material into the cells using non-viral approaches include lipid nanoparticles (LNP), naked DNA, plasmids, cationic polymers and conjugate complexes.[76] The LNP systems are currently the lead non-viral delivery system for translating clinical potential of genetic drugs. Onpattro™ (Patisiran) is the first LNP/siRNA-gene therapy product that was approved by the FDA to treat hereditary transthyretin amyloidosis (hATTR) by silencing target genes in hepatocytes.[77] Since then, LNP formulations have been devised to harness efficient Apolipoprotein E (ApoE)-mediated hepatocyte transfection resulting in >80% of LNP dose to be accumulated in the liver.[78] Lipid nanoparticles are currently being re-purposed in other applications where transient gene expression is desirable.[79] An extensively studied example is the transient expression of Cas9 and other DNA endonucleases for genome editing to limit the number of off target effects.[80,81] In 2020, a trial sponsored by Intellia with sites in the EU, UK and New Zealand, dosed the first participants with an LNP CRISPR/Cas9 to treat hATTR by a single i.v. injection.[82] Transient gene delivery systems can be desirable to reduce the off-target effect and improve the safety of genome editing. Another interesting challenge in this field is the delivery of DNA cargo, as opposed to RNA cargo, using non-viral delivery systems. One of the hurdles with delivering DNA involves the immune-mediated silencing of the transgene expression and the challenge of crossing the nuclear membrane to reach the nucleus. This is in contrast to mRNA delivery to the cytoplasm, achieved using contemporary LNP formulations which are optimized for endosomal uptake and cytosolic release rather than nuclear delivery.[83] Current work is ongoing to modify the lipid composition of the LNPs to facilitate nuclear transport and aid in transgene expression.[77]

3.7 | Bioinformatics, machine learning and artificial intelligence

Notable challenges in the gene therapy field include the complexities and heterogeneity of the solution realm, obstacles in manufacturing and supply chains in personalized therapies and the difficulty of appropriately matching therapies to the suitable patients. The use of artificial intelligence and machine learning has provided simple ways to decode these functional complexities. A great example of

machine learning utilization is the formation of neural networks based on the human brain and these involve a series of artificial neurons that filter data to produce an output. Each neuron examines the input provided from other neurons within the same network and decides whether to output that information to another neuron based on the filtering equation.[84] Another example that involves the use of artificial intelligence algorithms in cell therapy is by enabling researchers to analyze vast amounts of data and compare it against a patient genetic profile and medical history to allow medical practitioners and researchers to identify the best cells for a particular patient and the likelihood of a successful outcome.[85] Traditional older algorithms are programmed with fixed instructions to predict sites by relying on a predefined set of binding rules. The newer models, however, can be trained on real-world experimental data and can outperform older models through deep learning.[86]

In 2005, the first next generation sequencer (NGS), the Genome Sequencer 20 was launched by Roche.[87] Since then, there have been substantial developments in sequencing technologies to map the genome, transcriptome, epigenome and the of individual cells.[88] The immense power of single cell technology is particularly highlighted in systems with cellular heterogeneity to determine molecular mechanisms of cellular states. For instance, large-scale perturbation screens (Perturb-seq) have been successfully combined with CRISPR technology to explore single cell transcriptional consequences with a respective CRISPR perturbation.[89] Furthermore, sequencing tools to screen the epigenome have hit an inflexion point in the past 5 years. The epigenome performs a critical function in determining cellular identity with different chromatin organization to regulate gene expression. Screening epigenetic marks that target DNA methylation, chromatin accessibility and histone modifications at single cell resolution became within reach through bisulphite sequencing, transposase-accessible chromatin using sequencing and cleavage under targets & release using nuclease to profile antibody-targeted DNA-binding proteins *in situ* with high resolution.[90]

Multiple tools have been developed based on NGS technology in the past 5 years to better understand viral and vector integration events following gene therapy administration. One notable example is the development of Isling, a tool for detecting integration of wild-type viruses and clinical vectors using NGS with less computational timing.[91] The development of these tools and *in silico* modelling will have a significant impact on the outcome of novel therapies in the clinic.

3.8 | The need for predictive preclinical model systems

Despite the accelerated success of the gene therapy field in the past 5 years in clinical settings, the field is still limited by the lack of predictive preclinical model systems that can mimic human disease conditions faithfully at the pre-clinical stage prior to embarking on a clinical trial. In the gene therapy field for instance, the need for predictive pre-clinical models is essential to assess the variables related to the use of viral vectors such as safety, efficacy, dosage and localization of transgene expression.[92] In the context of AAV, there are safety issues related to AAV-associated host immunity and the capacity of AAV vectors to integrate into the host genome, raising concerns of genotoxicity. To better predict these issues and develop methods to overcome them for clinical translation while keeping in mind both efficacy and safety, during clinical trials, preclinical model systems that can accurately recapitulate human biology are required. Pre-clinical models in the gene therapy field strive to achieve homogeneity and translatability into the clinic. The failure of some clinical trials to date can be attributed to inadequacy of available pre-clinical animal models rendering clinical translatability of the gene therapy products highly unpredictable. An interesting example is the discovery of AAV-PHP.B noted above, a variant of AAV9 with enhanced CNS tropism in C57BL6 mice that also extends to rats.[62,93,94] However, the transduction of AAV-PHP.B appeared to be mouse-strain specific, did not translate in B6C3 mice and showed differential levels of transduction in rhesus macaques[59,95] with high-level gene transfer dependent on expression of the Ly6a receptor.[65] Depending on the target organ of interest, newer biologically predictive model systems have emerged including the xenograft FRG mouse for liver targeting therapies and this model is useful for selecting novel liver tropic AAV variants[96] as it shows promise in recapitulating the human liver molecular mechanisms.[97] Other systems currently used in the field include 3D organoids, murine models and microphysiological body-on-a-chip platforms.[98]

4 | PROGRESS AND CURRENT TRENDS IN GENE THERAPY

As defined by the FDA, human gene therapy "seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use"[99] using the transfer of engineered genetic material. So, what was originally envisaged as a treatment for monogenic disease using simple gene addition strategies now extends to infectious disease and cancer, as well as CAR-T cell and RNA-based therapies. While the list of gene therapies currently in use in the clinic is too expansive to summarize here, we have highlighted some key examples that provide important insights for the field.

4.1 | Gene therapy for spinal muscular atrophy

Spinal muscular atrophy is a rare autosomal recessive genetic disorder affecting motor neurons, resulting in progressive muscle atrophy and weakness, and is a leading cause of infant mortality. Spinal muscular atrophy is best considered a single disease caused by mutations in the Survival Motor Neuron 1 (*SMN1*) gene with differing severities caused by *SMN2* copy number variation. Notably, the most common mutation in *SMN1* (reported in up to 95% of cases) is the deletion of exon 7. Symptoms begin at birth or within the first 6 months of life, with children having limited movement, being unable to sit without assistance, having trouble breathing, feeding and swallowing, and

being unlikely to survive past 2 years of age without therapeutic intervention.

Gene therapy to treat SMA is arguably one of the most noteworthy clinical success stories for the field to date, with infants treated prior to the onset of symptoms maintaining or achieving all assessed motor milestones, including independent walking.[100] The first clinical trials to evaluate the safety and efficacy of AVXS-10, a recombinant AAV9 vector containing the human *SMN1* gene under the control of a strong ubiquitous promoter, began recruiting patients in 2014 (NCT02122952, NCT03505099). This treatment, now known as Zolgensma® (onasemnogene abeparvovec), was approved by the FDA on 24 May 2019[101] and is available in over 45 countries.[102]

In addition to remarkable clinical benefit, the success of this gene therapy has highlighted other important considerations for the field as it matures. These include the utility of newborn screening programs to identify eligible patients before the clinical manifestations of the disease, health system readiness and equal access to expensive gene therapy treatments. Another major consideration is immune system-mediated toxicity, particularly when high vector doses are used for disease correction. For example, hepatotoxicity is a known risk associated with the use of Zolgensma,® where most patients display elevated liver function test results (alanine aminotransferase, aspartate amino transferase and/or bilirubin concentrations) which are mitigated using prophylactic prednisolone in the treatment protocol.[103] In rare cases, however, patients have died because of acute liver failure.[104] Toxicity has also been implicated in the death of four patients with pre-existing liver disease in a clinical trial for X-linked myotubular myopathy (NCT03199469), three of whom received the highest vector dose.[105–107] The appearance of previously unseen serious adverse events resulting from thrombotic microangiopathy have also been reported in a small number of patients treated with Zolgensma®.[108] Other toxicities include myocarditis, observed in trials for DMD[109,110] and inflammatory responses noted in a clinical trial for familial amyotrophic lateral sclerosis.[111] The latter does not appear to be related to the dorsal root ganglion pathology reported in non-human primates following AAV9 vector administration, which is probably the result of transgene overexpression.[112,113]

Biodistribution analysis of tissues from SMA patients treated by gene therapy at autopsy also highlights the urgent need to improve the efficiency of the delivery system. While the AAV9 capsid is considered the "gold standard" to cross the blood–brain barrier, it does so inefficiently.[114] Specifically, after systemic delivery of Zolgensma®, although vector DNA and mRNA transcripts were detectable in the CNS, vector genomes were widely distributed throughout the body, with the liver notably having between 300- and 1,000-fold more copies than CNS tissue.[115] This has led to multiple studies that aim to develop AAV capsids capable of crossing the blood–brain barrier with greater efficiency to reduce vector-mediated toxicity, observed particularly when using high doses,[73,116–118] and there is considerable effort to engineer AAV capsid variants that have been de-targeted from the liver.[119–122] Strategies to prevent patients from being excluded from clinical trials because of the presence of pre-existing anti-capsid antibodies[123] are also actively being investigated and include engineering and chemical modification of the capsid and physical association of the vector with microvesicles and exosomes.[124]

4.2 | Gene therapy for transthyretin amyloidosis

Transthyretin amyloidosis, also known as ATTR amyloidosis or hATTR, is a life-threatening condition resulting from progressive accumulation of misfolded transthyretin (TTR) protein, causing the formation of amyloid fibrils (amyloidosis) that are deposited into various tissues, with the heart and nerves predominantly affected. Notably, transthyretin is predominantly produced by the liver, making it the logical target for gene therapies targeting this disease. Gene transfer systems with efficient delivery to the liver, such as LNPs, have been examined extensively. To date, many of these therapies have aimed to treat progressive polyneuropathy by stabilizing circulating transthyretin or reducing its synthesis using synthetic RNA molecules. Specifically, Tegsedi® (inotersen; ASO), Onpattro™ (patisiran; siRNA) and Amvuttra™ (vutrisiran; siRNA) have all been licensed to treat ATTR polyneuropathy.[125] In addition, eplontersen (Ionis Pharmaceuticals; ASO) is currently in a phase III trial to treat both ATTR polyneuropathy and cardiomyopathy (NCT05071300). These treatments, however, require frequent repeat administration (between weekly and once every 3 months depending on the product) owing to the short-lived nature of the RNA molecules delivered.

In contrast, a landmark gene therapy trial showing clear therapeutic benefit uses CRISPR/Cas9-based editing to treat transthyretin amyloidosis without the need for repeat administration (NCT04601051).[82] In this phase I trial, a LNP is used to encapsulate a messenger RNA for *Streptococcus pyogenes* Cas9 protein and a single-guide RNA targeting the transthyretin (*TTR*) gene (NTLA-2001). Following systemic delivery, expected frame-shift mutations introduced into *TTR* in hepatocytes, the consequence of imperfect non-homologous end joining following double strand break repair, has been shown to reduce the concentration of serum transthyretin.[126] Results from the first two patient groups (0.1 and 0.3 mg/kg) demonstrated a durable, dose-dependent reduction of serum transthyretin between 47 and 96% after a single administration.[82] Interim data presented for two higher doses of 0.7 and 1.0 mg/kg of NTLA-2001 showed a >90% reduction in all individuals, which was sustained for the follow-up period.[127] More recently, Intellia has released updated data for over 60 patients, including 29 patients who have reached at least 12 months of follow-up, which confirm durable serum TTR reduction.[128] This demonstrates that genome editing can be used to successfully knock out gene function in the liver using LNP-mediated delivery and will probably supplant other available RNA therapies that require frequent re-administration depending on the target disease and treatment context. In addition, transient delivery of editing reagents offers key safety advantages over more durable viral vector-mediated delivery which include (i) removing the need to include promoter-enhancer elements in the vector with the attendant risk of insertional mutagenesis and (ii) preventing long-term expression of Cas9 which has been shown to induce a p53-mediated DNA damage response.[129–133]

4.3 | Gene therapy for sickle cell anemia and transfusion-dependent β-thalassemia

Hemoglobin is the major component of red blood cells and responsible for oxygen transportation throughout the body. Hemoglobinopathies are, collectively, the most common group of monogenic disorders worldwide,[134] making the development of gene therapies to target these diseases highly attractive. These blood disorders are caused by mutations in the β-globin locus, resulting in defective β-globin production which affects the formation of hemoglobin A, the most common form of hemoglobin in adults.[111] Sickle cell disease and thalassemia are the two most common hemoglobinopathies and gene therapy strategies to cure these disorders all employ *ex vivo* manipulation of hematopoietic stem and progenitor cells and autologous transplantation following myeloablative conditioning. These strategies aim to either restore normal β-globin chain synthesis using lentiviral vector-mediated gene addition or reactivation of the fetal γ-globin chain using genome editing technology.[135]

One gene therapy trial showing impressive results uses CRISPR/Cas9 editing technology to modify patient hematopoietic stem and progenitor cells to treat both β-thalassemia and sickle cell disease. These modified cells, exagamglogene autotemcels, previously known as CTX001, are then infused back into the patient following myeloablative busulfan conditioning (NCT03655678, CLIMB THAL-111; NCT03745287, CLIMB SCD-121). Specifically, the editing event is directed to the erythroid-specific enhancer region of BCL11A, a master regulator of the fetal-to-adult hemoglobin switch. Importantly, this disrupts the binding site of the GATA1 transcription factor located at the site of CRISPR-Cas9 cleavage[19] resulting in a mutation-independent approach with high clinical translatability. The results from the first two patients, one with transfusion-dependent thalassemia and the other with sickle cell disease, demonstrated high levels of editing (between 69 and 83%) in the infused product, with rates maintained in the bone marrow and peripheral blood cells for more than 1 year after treatment. This resulted in increased fetal hemoglobin levels, transfusion independence and for the patient with sickle cell disease, the elimination of hallmark vaso-occlusive episodes.[19] Results from a further 44 patients with transfusion-dependent thalassemia and 31 patients with sickle cell disease have been reported with all patients at greater than 1 year follow-up having stable numbers of edited *BCL11A* alleles in their bone marrow hematopoietic stem and progenitor cells and peripheral blood mononuclear cells and all patients showing clinical improvement.[136,137]

Based on these results, the FDA has recently accepted the Biologics License Application for exagamglogene autotemcel for the treatment of transfusion-dependent β-thalassemia and severe sickle cell disease by trial sponsors Vertex Pharmaceuticals Incorporated in collaboration with CRISPR Therapeutics.[138,139] Notably, exagamglogene autotemcel was the first genome editing-based filing to be accepted for review by the FDA[140] and has now been approved by both the UK's Medicines and Healthcare products Regulatory Agency[141] and the FDA,[142] being sold under the brand name Casgevy. This provides alternatives to Bluebird Bio's lentiviral vector-based betibeglogene autotemcel (Zynteglo®) for β-thalassemia and lovotibeglogene autotemcel for sickle cell disease, with the FDA accepting lovotibeglogene autotemcel for priority review in June 2023.[143] Notably, lovotibeglogene autotemcel was approved by the FDA on 8 December 2023 (sold under the brand name Lyfgenia) on the same day Casgevy.[142] Trials that target the BCL11A enhancer using zinc finger nucleases for both β-thalassemia (ST-400; NCT03432364) and sickle cell disease (BIVV003; NCT03653247) are in development by Sangamo Therapeutics; however, further investment to conduct a phase III trial using BIVV003 is currently on hold, possibly owing to a high level of competition in this space.[138]

5 | SOURCES OF DATA

Recently, there has been stricter enforcement around the necessity to register clinical trials prior to initiation in a recognized registry. Together with increasing register transparency and a move toward open data, the availability of information concerning the initiation of clinical trials has increased since our last review. The World Health Organization International Clinical Trials Registry Platform (ICTRP, https://trialsearch.who.int/) lists 20 registries that are searchable simultaneously according to criteria such as country, date of initiation and keywords and this is our primary source of data. Sources include the Australian New Zealand Clinical Trials Registry, the Chinese Clinical Trial Registry, ClinicalTrials.gov, Clinical Trials Information System, the EU Clinical Trials Register, International Standard Randomised Controlled Trial Number (ISRCTN), The Netherlands National Trial Register, The Brazilian Clinical Trials Registry, the Clinical Trials Registry—India, the Clinical Research Information Service—Republic of Korea, the Cuban Public Registry of Clinical Trials, the German Clinical Trials Register (last data file imported on 4 September 2023), the Iranian Registry of Clinical Trials, the Japan Registry of Clinical Trials, the Pan African Clinical Trial Registry, the Sri Lanka Clinical Trials Registry, the Thai Clinical Trials Registry, the Peruvian Clinical Trials Registry, the Lebanese Clinical Trials Registry and the International Traditional Medicine Clinical Trial Registry. None of these platforms are specific to gene therapy and while the ICTRP platform has attempted to simplify the identification of gene therapy trials by offering a filter that enables users to refine searches using additional key words, such as genome editing, the function relies on investigators flagging their trials as such, which only occurs for a minority of trials. The identification of relevant trials continues to rely on manual, keyword-based searches. Although over the years we have developed a sensitive search strategy, it is possible that relevant trials described using uncommon terms are missed. In addition, while there is a requirement to register all trials, there is no obligation to provide a specific minimum dataset and as such some trials do not initially include basic information such as where the trial is taking place or the nature of the investigational product. Some of the databases included in the ICTRP are also routinely searched individually because they may contain more detailed information or additional fields not included in ICTRP, which has created a common registry that includes

all datasets. In addition, because the search engine algorithm is different, searching individual databases usually yields additional results. For efficiency we focus on databases where the bulk of gene therapy trials are registered, namely clinicaltrials.gov (USA), the European Union Register (https://www.clinicaltrialsregister.eu), The Chinese registry (https://www.chictr.org.cn), the Japanese registry (https://jrct.niph.go.jp/) and the Australia and New Zealand registry (https://www.anzctr.org.au/). It is important to note that national registers may contain trials taking place in other countries. This is particularly the case for clinicaltrials.gov (https://www.clinicaltrials.gov/), which, while hosted by the US government, is a global registry. As such it is the second most important source of data for identifying trials. Following its exit from the European Union, the UK no longer registers its trials in the European Union trial registry and UK trials are registered through ISRCTN (https://www.isrctn.com/), clinicaltrials.gov or both. The existence of meta or global trials databases and the requirement for trial registration has made the need for contact with national health and regulatory agencies to obtain information about trials largely redundant. Once trials are identified, where information is insufficient, additional information is obtained from the peer-reviewed literature searched through PubMed, the websites of gene therapy companies and occasionally press releases. Finally, various entities have begun to develop disease-specific databases to address gaps in availability of contemporary clinical trial results, with the European Association for Haemophilia and Allied Disorders being a notable example (see https://eahadgtd.mdsas. com/).[144]

6 | NUMBER OF TRIALS PER YEAR

Since the 2017 review, we have identified an additional 1,303 gene therapy trials. The number of trials initiated or approved each year is steadily increasing, reaching a peak of 246 trials in 2020 (Figure 1). Notably, the proportion of gene therapy trials from Asia is increasing more rapidly, which is particularly evident since the time of our last review (Figure 2).

7 | COUNTRIES PARTICIPATING IN GENE THERAPY TRIALS

Gene therapy clinical trials have been performed on all five continents (Figure 3) spanning 46 countries (Figure 4). We have located data on trials from eight new countries since our last review,[145] these being

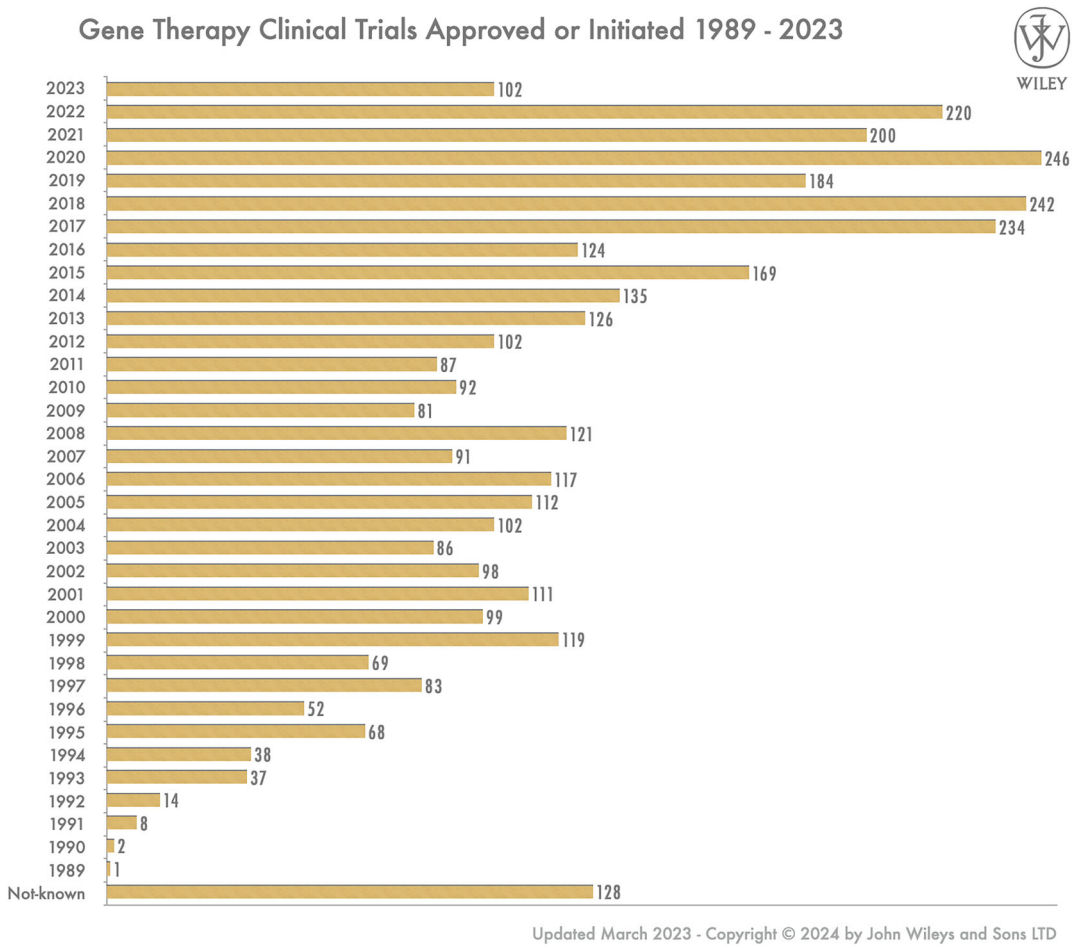

FIGURE 1 Gene therapy clinical trials approved or initiated 1989–2023.

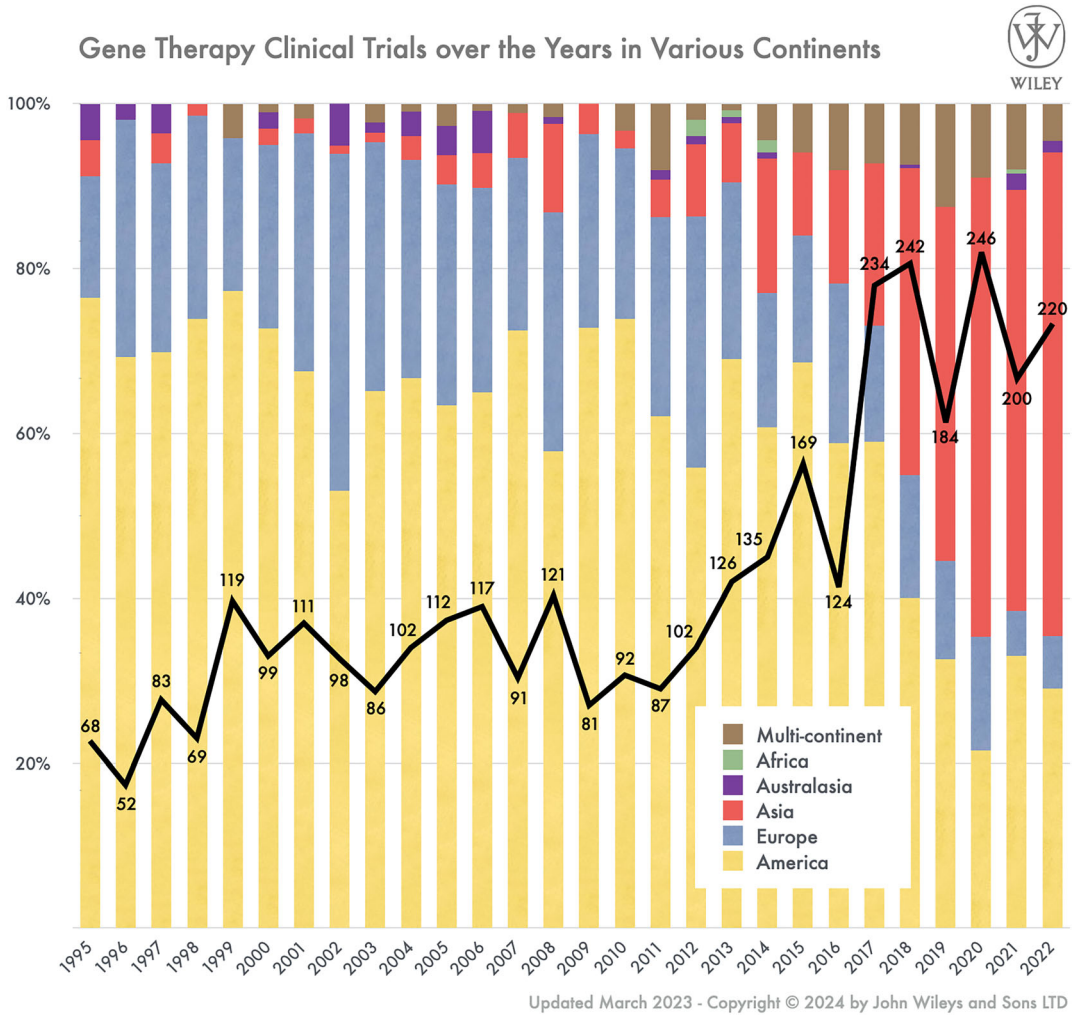

FIGURE 2 Gene therapy clinical trials over the years in various continents. The black line indicates the total number of trials recorded for each year.

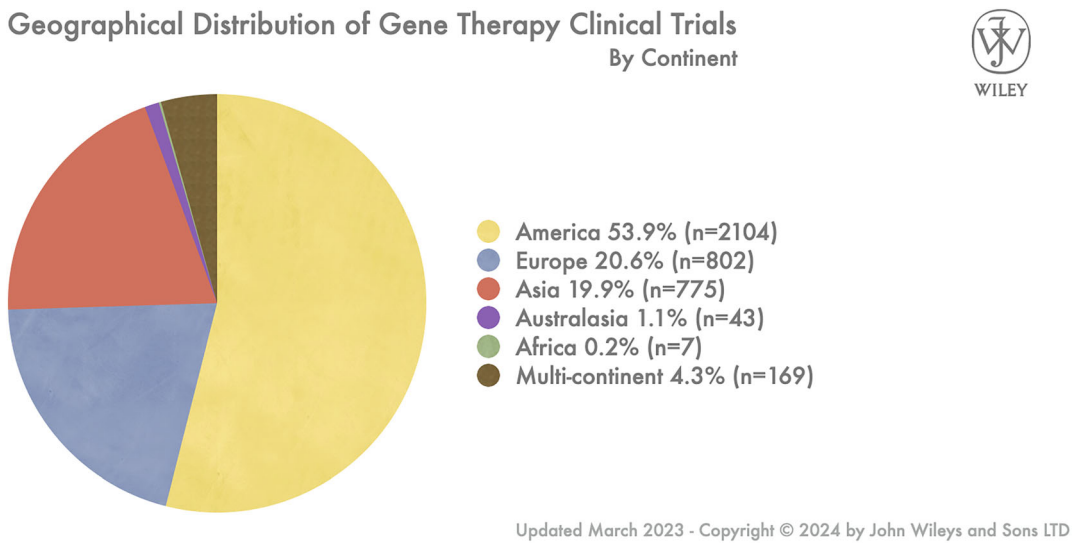

FIGURE 3 Geographical distribution of gene therapy clinical trials (by continent).

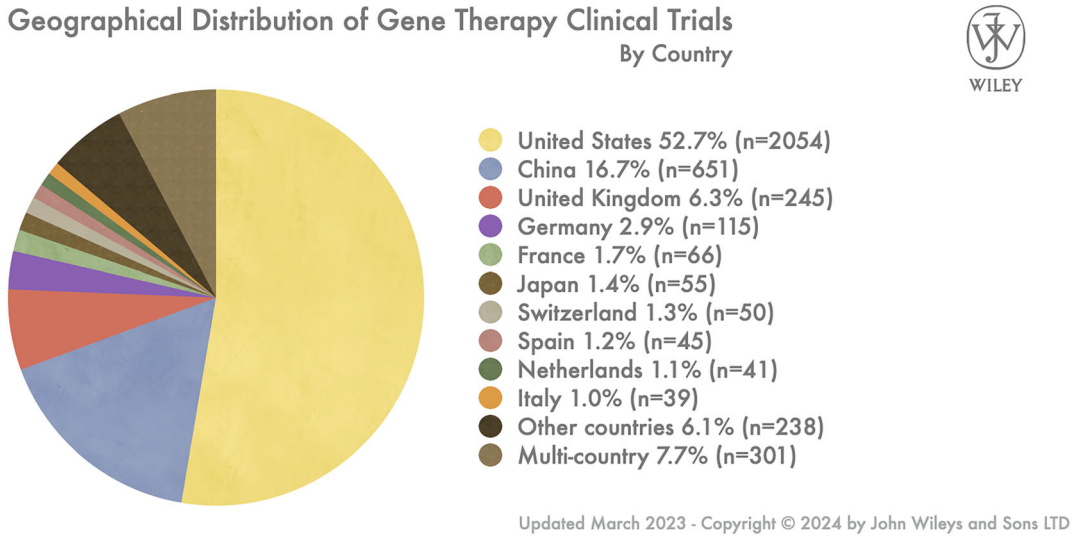

FIGURE 4 Geographical distribution of gene therapy clinical trials (by country).

Belarus, Malaysia, Slovenia and Thailand (all with one trial each), Brazil and Colombia (both with three trials) and India and Iran (both with five trials). While the continental distribution of trials has not considerably changed, and largely reflects research and development expenditure, there has been significant growth in Asia reaching 19.9% of all gene therapy trials, up from 6.5% in 2017 and 3.4% in 2007. This is due to a significant increase in the number of trials reported from China, increasing from 84 trials in 2017 (3.2% of all trials) to 651 trials (16.7% of all trials) at the time of this review, the overwhelming majority of these being CAR-T cell therapies.

The USA continues to undertake the most trials globally (52.7%), with 2054 trials. Other trials in the Americas include 30 from Canada (0.8%), three from Brazil and Colombia, two from Mexico, and one from Argentina. Within Europe, the distribution of trials remains relatively unchanged since the 2017 review: the UK accounts for 6.3% of the world total with 245 trials, Germany 2.9% (115 trials), France 1.7% (66 trials), Switzerland 1.3% (50 trials) and Spain 1.2% (45 trials). From Asia, the most significant increase comes from China, which has jumped from 26 trials in 2012 (1.4% of all trials) and 84 trials in 2017 (3.2% of all trials) to 651 trials (16.7% of all trials), now being the second country behind the USA. Japan has increased from 44 to 55 trials (now 1.4% of all trials) and South Korea has risen from 20 trials in 2017, currently standing at 28 trials (0.7%). Israel comes next with 13 trials, then Singapore and Taiwan with six and India and Iran both with five trials. Kuwait, Malaysia, Thailand and Saudi Arabia have reported a single trial each.

There have been modest increases in trial entries for Australia (from 32 in 2017 to 39 in 2023) and New Zealand (from two in 2017 to four in 2023) with no new trials reported for Sweden (13 trials), Finland (six trials) and Norway (four trials). Most notably, there has been a marked increase in the number of trials reported as multi-country, more than doubling from 130 reported in our 2017 review to 301 trials (7.7%) currently. This probably reflects both increased collaborative interaction between research centres as well as the need to access patient populations from more than one country, particularly in the case of rare and ultrarare diseases.

8 | DISEASES TARGETED BY GENE THERAPY

The overwhelming majority (81.4%) of gene therapy clinical trials to date have addressed cancer (68.3%) and inherited monogenic diseases (13.1%) (Figure 5), with the latter achieving unequivocal success in the clinic for multiple organ systems including the liver, brain and eye.[146–148] Notably, the relative proportion of trials for each broad indication category has remained relatively stable over the years (Figure 6). There has been a reduction in the proportion of trials targeting both infectious and cardiovascular diseases; however, these are still the next most common indications behind cancer and monogenic diseases, at 5.0 and 4.9% of all trials, respectively.

8.1 | Cancer

Thus far, most of the clinical trials in gene therapy have been aimed at the treatment of cancer (2,663 trials in total). Many different cancers have been targeted, including but not limited to lung, gynecological, skin, urological, neurological and gastrointestinal tumors as well as pediatric tumors and hematological malignancies. A range of strategies have been applied to treat cancer, from inserting tumor suppressor genes to immunotherapy, oncolytic virotherapy, gene-directed enzyme pro-drug therapy and CAR-T cell therapy.

The *p53* gene is by far the most transferred tumor suppressor gene, although others such as cytochrome *P450* and *BRCA-1* have been used in cancer trials. There are also some clinical trials that combine p53 gene transfer with chemotherapy or radiotherapy. Immunotherapy to treat cancer aims to intensify the normally weak humoral

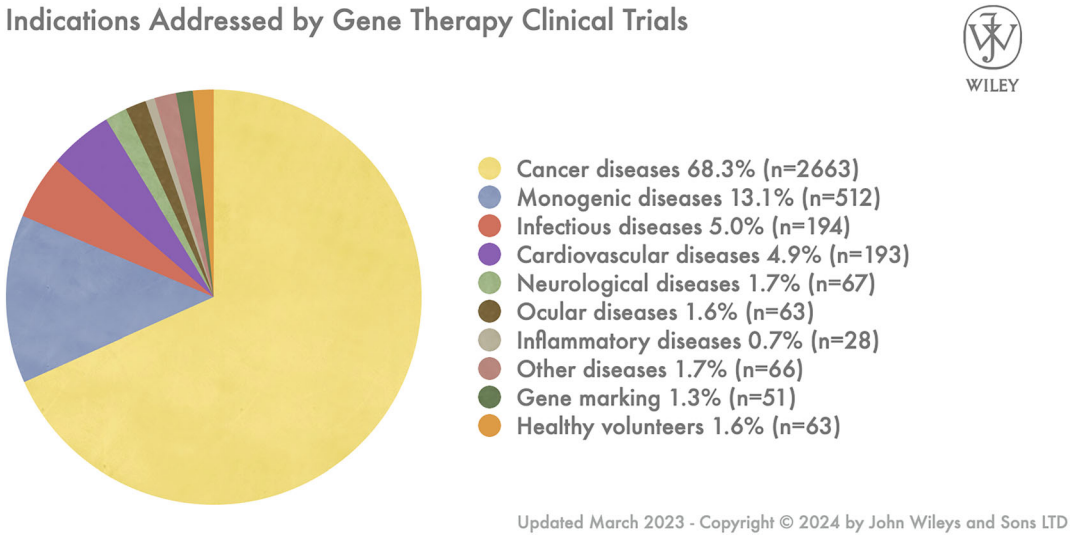

FIGURE 5 Indications addressed by gene therapy clinical trials.

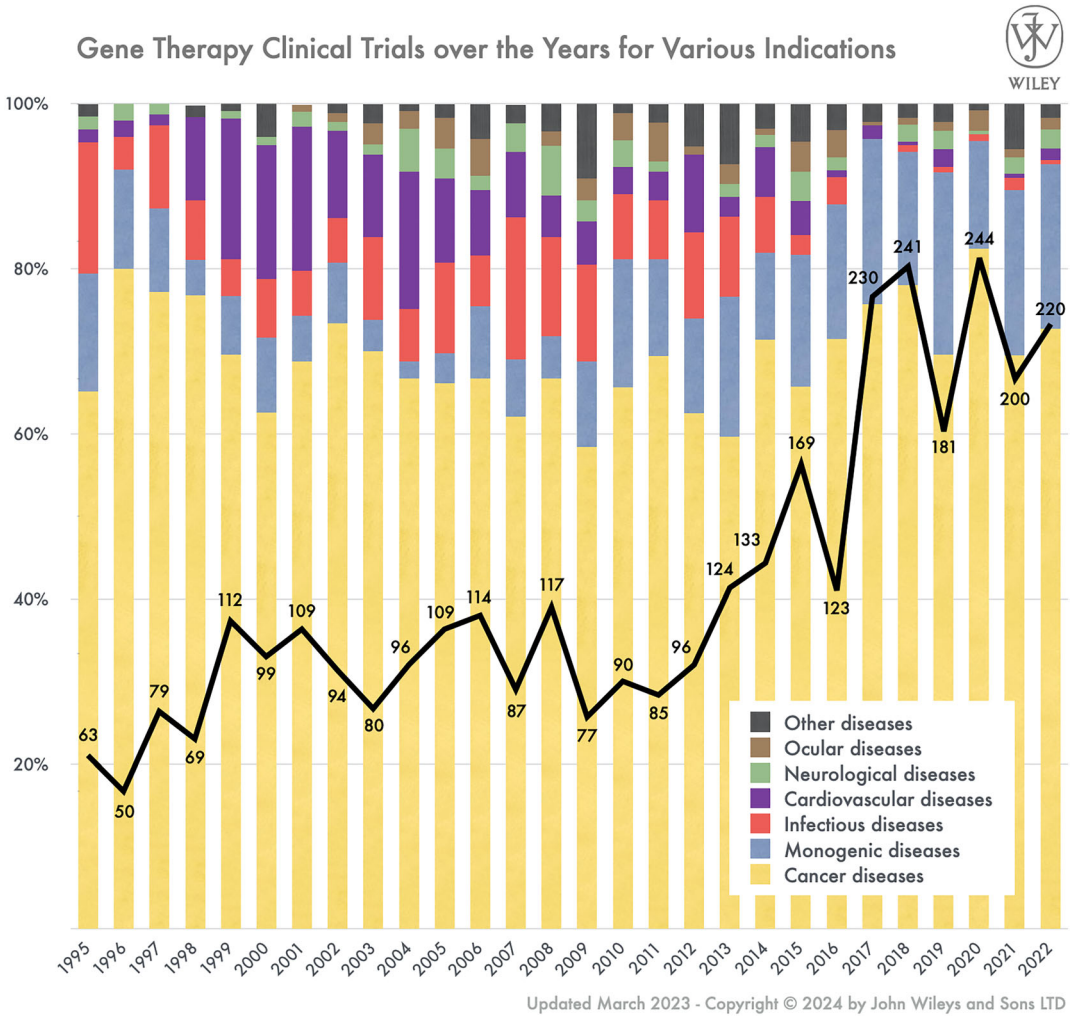

FIGURE 6 Gene therapy clinical trials over the years for various indications. The black line indicates the total number of trials recorded for each year.

and/or cellular reactions to tumor antigens in tumor-bearing hosts. Different strategies have been employed, including vaccination with tumor cells engineered to express immunostimulatory molecules, vaccination with recombinant viral vectors encoding tumor antigens, vaccination with host cells engineered to express tumor antigens or tumor-derived RNA, naked DNA vaccines and intra-tumoral injection of vectors encoding cytokines or major histocompatibility molecules. Notably, on 16 December 2022 the FDA approved Adstiladrin® (Nadofaragene Firadenovec-vncg) for the treatment of adult patients with high-risk non-muscle-invasive bladder cancer.[149] Adstiladrin® is a non-replicating adenoviral vector-based gene therapy that delivers the human interferon α-2b cDNA directly to the bladder epithelium.[150,151] Also in advanced stages are clinical trials for the treatment of cervical dysplasia (high-grade squamous intraepithelial lesions) caused by human papillomavirus, with the DNA vaccine VGX-3100 (phase III, NCT03185013)[152–154] and recombinant Ad-p53 therapy for the treatment of advanced stage oral and maxillofacial malignant tumors (phase IV, NCT00902083) and malignant thyroid tumors (phase IV, NCT00902122).

The use of CAR-T cell therapy, with initial reports published now more than a decade ago,[155,156] has shown impressive clinical responses, particularly for anti-CD19 cell therapy against B cell malignancies.[157] Kymriah became the first CAR-T cell therapy approved by the FDA for acute lymphoblastic leukemia which, on 30 August 2022, celebrated the 5 year anniversary of this landmark approval. Notably, in the time since the publication of the 2017 review, there has been a clear emergence of CAR-T cell therapies. These now make up a substantial proportion of the cancer trials reported; however, they hardly existed when the last review was published. Although trials targeting solid tumours are predominantly in early clinical phases,[158,159] encouraging outcomes have been reported[160] and the FDA has recently approved the first tumor-derived autologous T cell immunotherapy, Amtagvi (lifileucel), to treat melanoma in adult patients.[161] Targeting solid tumors has a unique set of challenges that include the identification of an appropriate target antigen and the ability of CAR-T cells to traffic to and infiltrate solid tumors, which unlike B cell leukemias, are physically limited by barriers such as the tumor stroma.

8.2 | Monogenic diseases

Although the scope of gene therapy is continually expanding, its predominant target has been cancer, with monogenic disease being the next most targeted group of indications (Table 4). We have identified 512 trials for inherited monogenic disorders, 13.5% of which target immunodeficiency disorders, with adenosine deaminase deficiency (ADA-SCID) and SCID-X1 the most common targets. Immunodeficiency disorders are a group of diseases in which gene therapy has shown lasting and clinically meaningful therapeutic benefit[162] since the landmark trial was published in *Science* now more than two decades ago.[36] There has been an increase in the proportion of trials treating hemophilia, which is now the second most common disease indication (11.4%) behind immunodeficiency disorders when combining those targeting both hemophilia A and hemophilia B (56 trials in

TABLE 4 Conditions for which human gene transfer trials have been approved.

Monogenic disorders
Achromatopsia
Acute intermittent porphyria
Adrenoleukodystrophy
Adenosine deaminase deficiency
Adrenomyeloneuropathy
Alpha-1 antitrypsin deficiency
Angelman syndrome
Aromatic L-amino acid deficiency
Artemis deficient severe combined immunodeficiency
Batten disease
Becker muscular dystrophy
Beta thalassemia
Bietti crystalline dystrophy
Canavan disease
Chronic granulomatous disease
Congenital adrenal hyperplasia
Crigler–Najjar Syndrome
Cystic fibrosis
Duchenne muscular dystrophy
Fabry disease
Familial adenomatous polyposis
Familial hypercholesterolaemia
Familial lecithin–cholesterol acyltransferase deficiency
Fanconi anaemia
Friedrich ataxia
Galactosialidosis (neuraminidase deficiency with β-galactosidase deficiency)
Gaucher disease
Gyrate atrophy
GM1 gangliosidosis (β-galactosidase-1 deficiency)
Haemophilia A and B
Hereditary angioedema
Hereditary inclusion body myopathy-2
Hereditary transthyretin amyloidosis
Hunter syndrome (mucopolysaccharidosis type II)
Hurler syndrome (mucopolysaccharidosis type I)
Janus Kinase 3 (JAK3) Deficient Severe Combined Immunodeficiency (SCID)
IPEX syndrome
Krabbe disease (globoid cell leukodystrophy)
Late infantile neuronal ceroid lipofuscinosis
Lecithin–cholesterol acyltransferase deficiency
Leukocyte adherence deficiency
Limb girdle muscular dystrophy (β-sarcoglycan deficiency)
Lipoprotein lipase deficiency
Metachromatic leukodystrophy

(Continues)

TABLE 4 (Continued)

Monogenic disorders
Menkes syndrome
Netherton syndrome
Ornithine transcarbamylase deficiency
Phenylketonuria
Pompe disease
Primary hyperoxaluria type 1
Purine nucleoside phosphorylase deficiency
Pyruvate kinase deficiency
RAG1 deficiency (RAG1-SCID)
Recessive dystrophic epidermolysis bullosa (junctional, recessive dystrophic)
Sandhoff disease
Sanfilippo A (mucopolysaccharidosis type IIIA)
Sanfilippo B (mucopolysaccharidosis type IIIB)
Sickle cell disease
Sly syndrome (mucopolysaccharidosis type VII)
Spinal muscular atrophy type 1
Spinal muscular atrophy type 2
Spinal muscular atrophy type 3
Tay Sachs disease
Usher syndrome
Wilson disease
Wiskott–Aldrich syndrome
von Gierke disease (glycogen storage disease type Ia)
X-linked myotubular myopathy
X-linked severe combined immunodeficiency (SCID-X1)
Cardiovascular disease
Anaemia of end stage renal disease
Angina pectoris (stable, unstable, refractory)
Congestive heart failure
Coronary artery disease
Coronary artery stenosis
Critical limb ischaemia
Intermittent claudication
Myocardial ischaemia
Myocardial infarction
Peripheral vascular disease
Pulmonary hypertension
Venous ulcers
Infectious disease
Adenovirus infection
COVID-19
Cytomegalovirus infection
Epstein–Barr virus
Hepatitis B and C
Human immunodeficiency virus/AIDS

(Continues)

TABLE 4 (Continued)

Infectious disease
Influenza
Japanese encephalitis
Malaria
Paediatric respiratory disease
Respiratory syncytial virus
Tetanus
Tuberculosis
Cancer
Gynaecological—breast, ovary, cervix, vulva
Nervous system—glioblastoma, leptomeningeal carcinomatosis, glioma, astrocytoma, neuroblastoma, retinoblastoma
Gastrointestinal—abdominal, colon, colorectal (bowel), liver metastases, post-hepatitis liver cancer, pancreas, gall bladder, hepatocellular carcinoma
Genitourinary—prostate, renal, bladder, ano-genital neoplasia
Skin—melanoma (malignant/metastatic)
Head and neck—nasopharyngeal carcinoma, squamous cell carcinoma, oesophaegeal cancer
Lung—adenocarcinoma, small cell/non-small cell, mesothelioma
Haematological—leukaemia, lymphoma, multiple myeloma
Sarcoma
Germ cell
Li–Fraumeni syndrome
Thyroid
Neurological diseases
Alzheimer disease
Amyotrophic lateral sclerosis
Carpal tunnel syndrome
Charcot–Marie–Tooth neuropathy type 1A
Chronic traumatic brain injury
Cubital tunnel syndrome
Diabetic neuropathy
Epilepsy
Frontotemporal dementia
Huntington disease
Giant axonal neuropathy
Late infantile neuronal ceroid lipofuscinosis
Multiple sclerosis
Multiple system atrophy
Myasthenia gravis
Neuromyelitis optica spectrum disorders
Pain
Parkinson disease
Peripheral neuropathy
Spastic paraplegia
Traumatic brain injury

(Continues)

TABLE 4 (Continued)

Ocular diseases
Achromatopsia
Age-related macular degeneration
Choroideraemia
Diabetic macular edema
Diabetic retinopathy
Glaucoma
Leber congenital amaurosis
Leber hereditary optic neuropathy
Macular telangiectasia type 2
Retinitis pigmentosa
Stargardt disease
Superficial corneal opacity
X-linked retinoschisis
Inflammatory diseases
Arthritis (rheumatoid, inflammatory, degenerative)
Degenerative joint disease
Immune nephritis
Osteoarthritis
Severe inflammatory disease of the rectum
Systemic lupus erythematosus
Ulcerative colitis
Other indications
Bone regeneration
Chronic renal disease
Cocaine dependence
Diabetic ulcer/foot ulcer
Detrusor overactivity
Erectile dysfunction
Fractures
Hearing loss
Hereditary inclusion body myopathy
Hypertrophic Scarring
Graft vs. host disease/transplant patients
Kidney transplantation/rejection
Oral mucositis
Parotid salivary hypofunction
Systemic scleoderma
Type 1 diabetes
Wound healing

total). Hemophilia serves as a paradigm for non-cell autonomous disease targets, with gene addition strategies already showing success in the clinic. This disease, however, has a low therapeutic threshold because an increase in plasma clotting factors of as little as 1–2% of wild-type levels can confer significant phenotypic improvement by converting severe- to moderate disease and above 6%, a mild phenotype.[163] Trials targeting cystic fibrosis are now the third most frequent indication (7.7%) with very few clinical trials initiated since the 2017 review (NCT05248230) despite it being the most common inherited life-shortening genetic disease in Europe and the USA.

8.3 | Other indications

There have been 194 trials (5.0% of the total) aiming to treat infectious diseases, with most approaches using the delivery system to express a therapeutic molecule or antigen. Human immunodeficiency virus infection is the major target in this category (70.3%) with 135 trials. Clinical trial activity for other infectious diseases has been relatively stable since the 2017 review. Trials aiming to treat hepatitis account for 10.3% of trials for infectious disease. Malaria is the next most common target at 6.7% and a small number of trials for Epstein–Barr virus, cytomegalovirus diseases, pediatric respiratory infection, tuberculosis, respiratory syncytial virus, influenza, adenovirus infection and *Clostridium tetani* infection have also been conducted. Unsurprisingly, since our 2017 review, clinical trials to treat SARS-Cov-2 infection have been initiated to address COVID-19, which was declared a pandemic by the World Health Organization on 11 March 2020 and no longer a public health emergency on 5 May 2023.

Cardiovascular gene therapy is the fourth most common application for gene therapy at 4.9% of trials (193 in total; down from 6.9% in 2017). The expectation is that gene therapy will provide alternative treatments such as therapeutic angiogenesis for myocardial and lower limb ischemia, regeneration and repair, and modulation of cardiac function in heart failure.[164] An example of the latter includes enhancing action potential conduction and electrical excitability in the heart to treat cardiac arrhythmia.[165] Although clinical success has yet to be reported, studies aimed at improving gene delivery to cardiac tissue are ongoing.[166,167] The number of trials aimed at treating ocular disease has increased from 34 trials in 2017 to 63 trials at the time of this review. This continued interest reflects the fact that the eye remains an attractive target for *in vivo* gene therapy applications, being a discrete organ with a relatively small target cell population and the possibility of reduced inflammation after therapy because the eye is a relatively immune-privileged organ. Approval of the first AAV gene therapy product (Luxturna, Spark Therapeutics) by the FDA in December 2017 has also generated commercial interest for developing treatments for blinding eye disease. Clinical trials for choroideremia (NCT03496012, NCT03584165, NCT04483440), X-linked RPGR gene-related retinitis pigmentosa (NCT04794101, NCT04671433, NCT04850118) and achromatopsia (NCT03758404) are recent examples, all using AAV-based gene delivery.

9 | GENES TRANSFERRED

There have been a vast number of gene types used in human gene therapy trials (Figure 7), making it impossible to discuss each one here. As would be expected, the gene types transferred most frequently are those primarily used to combat cancer, the disease most treated by gene therapy. These categories (antigens, cytokines, tumor

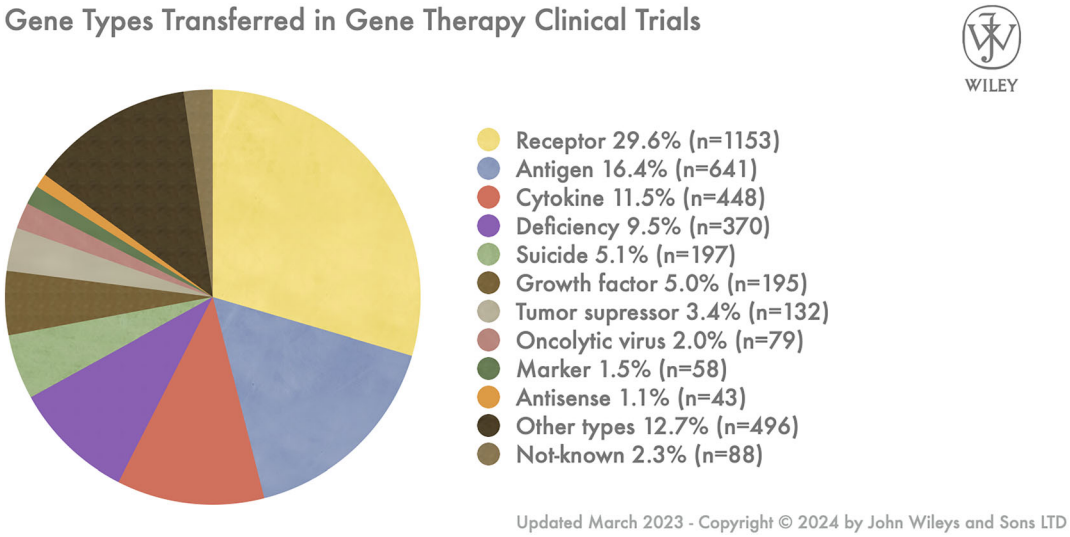

FIGURE 7 Gene types transferred in gene therapy clinical trials.

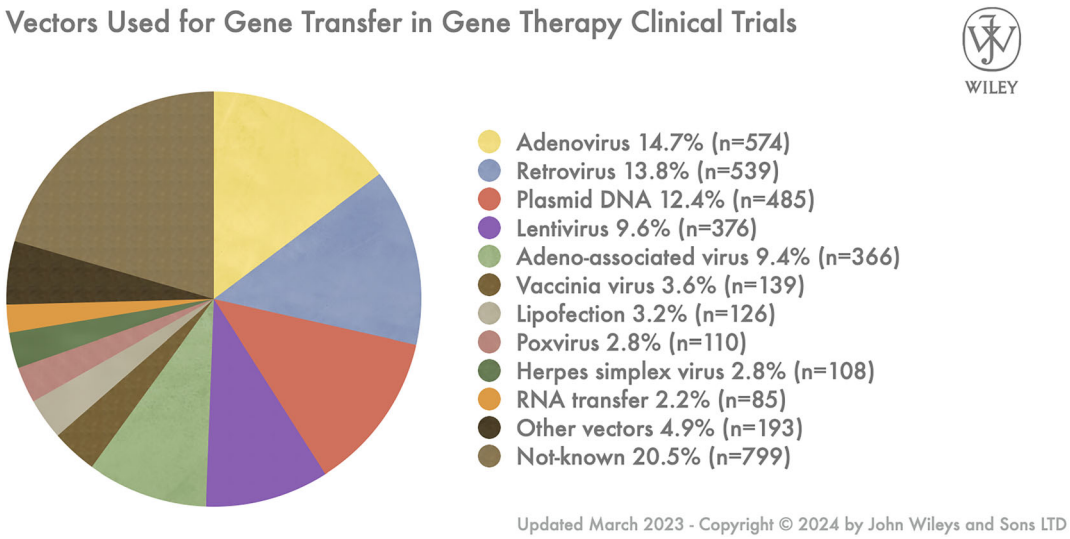

FIGURE 8 Vectors used for gene transfer in gene therapy clinical trials.

suppressors and suicide enzymes) account for 36.4% of trials, although it should be noted that antigens specific to pathogens are also being used in vaccines. In addition, there has been a substantial increase in the use of receptors (most used for cancer gene therapy) at 29.6%, with this category now the most represented gene type transferred. This is an increase from 11.6% at the last review and correlates with an increase in the use of CAR T immunotherapy, particularly from China. Growth factors were transferred in 5.0% of trials, with many of these being aimed at cardiovascular diseases, most commonly genes from the fibroblast growth factor and vascular endothelial growth factor families. Deficiency genes were used in 9.5% of trials, and in 2.0% of trials, oncolytic viruses were transferred (rather than specific therapeutic genes) with the aim of destroying cancer cells, and 2.2% of trials involved the transfer of RNA (miRNA, shRNA, shRNA), with the aim of blocking the expression of a target gene.

10 | VECTORS USED IN GENE THERAPY

A variety of different vectors and delivery techniques have been applied in gene therapy trials (Figure 8). Although non-viral approaches are becoming increasingly common, viral vectors remain by far the most popular approach, having been used in almost 60% of the trials performed to date.

10.1 | Viral vectors

In recent years there has been a steady decline in the use of retroviral vectors (currently only 13.8% compared with 17.4% of trials in 2017, 19.7% in 2012, 22.8% in 2007 and 28% in 2004), owing to the move toward lentiviral vectors with a more favorable integration

profile (now at 9.6%, an increase from 7.3% in 2017 and 2.9% in 2012). This proportion is likely to be higher because the gene transfer system for many of the recently initiated CAR-T cell trials from China are not indicated but are likely to use a lentiviral vector delivery system. A similar trend has been observed with the use of adeno-associated virus which has increased from 4.9% in 2012 to 9.4% at the time of this review. Indeed, in the 10-year period from 2013 to the time of this review, there are records for 253 trials using AAV delivery and only 97 trials prior, with 1994 being the earliest year recorded (only one trial each in 1994, 1995, 1997 and 1998). Adenoviruses are still the most used vector (14.7% of all trials), but this has declined further since 2017, when they comprised 20.5% of all trials (23.3% in 2012). They can carry a larger DNA payload than retroviruses (up to 35 kb), but their capacity may still be too small to accommodate certain genes required for clinical applications. The main advantages of adenoviral vectors are their abilities to achieve high efficiency of transduction, high levels of gene expression (though transient) and the transduction of non-dividing cells. Vectors based on other viruses have been used less widely, with the most common of these being vaccinia virus (139 trials; 3.6%), poxvirus (110 trials; 2.8%) and herpes simplex virus (108 trials; 2.8%).

10.2 | Non-viral vectors

Safety concerns and the limited therapeutic payload of viral vectors have prompted the development of synthetic vectors not based on viral systems. The simplest non-viral gene delivery system uses "naked" DNA, plasmid DNA in most cases, which when injected directly into certain tissues, particularly muscle, produces significant levels of gene expression, although lower than those achieved with viral vectors. Importantly, the efficiency of naked DNA delivery can be improved dramatically when combined with *in vivo* electroporation[168] and there have been an additional 41 trials recorded since the last review. While most of these trials target cancer, phase III trials for critical limb ischemia are recruiting patients (NCT04275323, NCT04274049). The proportion of all trials using naked (plasmid) DNA has reduced since the time of the 2017 review (12.4% of trials compared with 16.5% in 2017, 18.3% in 2012, 18% in 2007), and it is the most popular non-viral system used in clinical trials. This is followed by lipofection, which involves cationic lipid/DNA complexes (used in 3.2% of all trials). Notably, trials using LNPs to deliver genome editing machinery to knock out unwanted gene function have entered the clinic and are showing promising results (NCT04601051, NCT05697861 and NCT05120830).

11 | CLINICAL TRIAL PHASES

As was the case in our last review, almost 80% of gene therapy clinical trials performed to date are phase I or I/II (Figure 9); the two categories combined represent 79.9% of all gene therapy trials. Phase II trials make up 14.7% of the total and phase II/III and III represent only 5.1% of all trials. The proportion of trials in phase II, II/III and III has been relatively stable over time (19.8% compared with 21.9% in 2017, 21.2% in 2012, 19.1% in 2007 and 15% in 2004, respectively), indicating that while progress has been made in bringing gene therapy closer to routine clinical application, the overwhelming majority of trials are still in early-stage clinical development. Finally, there has been an increase in the number of single subject ($N = 1$) clinical trials, from six trials reported in 2017 to 11 at the time of this review. This number is expected to increase as the regulatory framework for trials involving single participants is established, resulting from an increasing need to develop therapies for patients with ultrarare disease being identified through improved diagnostics.

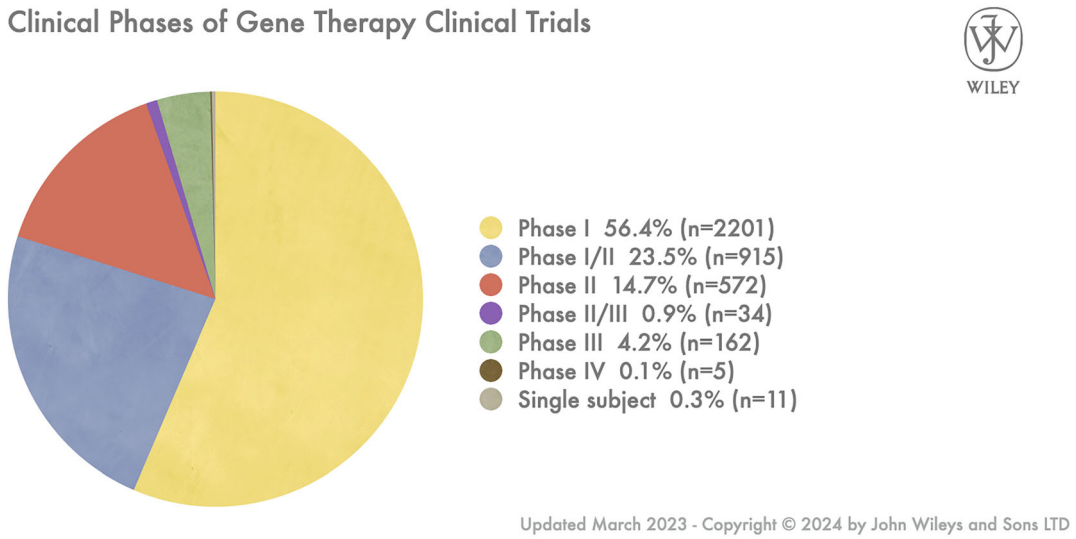

FIGURE 9 Phases of gene therapy clinical trials.

12 | CONCLUDING REMARKS

It is approaching 35 years since the first authorized gene transfer study took place at the National Institutes of Health in 1989, providing the first direct evidence that human cells could be genetically modified and safely returned to a person.[169] Since then, although trials demonstrating therapeutic benefit have been reported relatively infrequently, there has been a wave of recent approvals and many more products at an advanced stage of development. With therapeutic success becoming increasingly common, patient safety has never been more paramount, particularly with the advent of gene editing technologies that introduce permanent changes into the genome. Notably, clinical outcomes are driving new avenues of research to improve the specificity and efficiency of the gene transfer technology while minimising immunogenicity and undesired off-target effects. Continued progress in these areas will ultimately be required for gene therapy to realize its full potential, particularly to bring more challenging disease targets within therapeutic reach.

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CONFLICT OF INTEREST STATEMENT

ME and MRA receive payment from John Wiley and Sons Ltd for their work on *The Journal of Gene Medicine* Gene Therapy Clinical Trials Worldwide database. SLG, MM and IEA declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used in this review is available at *The Journal of Gene Medicine*'s searchable Gene Therapy Clinical Trials Worldwide database at https://a873679.fmphost.com/fmi/webd/GTCT.

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