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CONCISE CLINICAL REVIEW

Lentiviral Gene Therapy for Cystic Fibrosis

A Promising Approach and First-in-Human Trial

Jane C. Davies[1,2*], Deepika Polineni[3*], A. Christopher Boyd[2,4], Scott Donaldson[6], Deborah R. Gill[2,7], Uta Griesenbach[1,2], Stephen C. Hyde[2,7], Raksha Jain[8], Gerry McLachlan[2,5], Marcus A. Mall[9,10,11‡], and Eric W. F. W. Alton[1,2‡]

[1]National Heart and Lung Institute, Imperial College London, London, United Kingdom; [2]U.K. Respiratory Gene Therapy Consortium, Oxford, United Kingdom; [3]Division of Allergy and Pulmonary Medicine, Department of Pediatrics, Washington University School of Medicine in St. Louis, St. Louis, Missouri; [4]Centre for Genomic and Experimental Medicine, Institute of Genetics and Cancer, and [5]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom; [6]Division of Pulmonary Diseases and Critical Care Medicine, Department of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [7]Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom; [8]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; [9]Department of Pediatric Respiratory Medicine, Immunology, and Critical Care Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany; [10]German Center for Lung Research (DZL) associated partner site, Berlin, Germany; and [11]German Center for Child and Adolescent Health (DZKJ) partner site, Berlin, Germany

ORCID IDs: 0000-0003-3506-1199 (J.C.D.); 0000-0001-8551-3734 (D.P.); 0000-0003-4802-0388 (S.D.); 0000-0002-5264-054X (D.R.G.); 0000-0002-8254-4540 (U.G.); 0000-0002-8877-4005 (S.C.H.); 0000-0002-2407-7374 (R.J.); 0000-0001-5362-9804 (G.M.); 0000-0002-4057-2199 (M.A.M.); 0000-0003-3852-6764 (E.W.F.W.A).

Abstract

Cystic fibrosis (CF) is a genetic disease caused by mutations in the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene. Although CF is a multiorgan disease, the leading causes of morbidity and mortality are related to progressive lung disease. Current understanding of the effects of the broad spectrum of *CFTR* mutations on CFTR function has allowed for the development of CFTR modulator therapies. Despite the remarkable impact that these therapies have had, there remains a significant proportion of people with CF (estimated at 10–15% of the global CF population) who are genetically ineligible for, or intolerant of, current CFTR-targeting therapies and whose therapeutic needs remain unmet. Inhaled genetic therapies offer the prospect of addressing the unmet pulmonary treatment need in people with CF, with several approaches, including gene addition therapy (the focus of this review), RNA-based therapies, antisense oligonucleotides, and gene editing, being explored. Various nonviral and viral vectors have been investigated for CF gene addition therapy for mutation-agnostic restoration of CFTR function in the lungs. Lentiviral vectors offer the prospect of highly efficient and long-lasting gene expression, and the potential to be safely and, in contrast to other commonly used viral vectors, effectively redosed. A third-generation lentiviral vector pseudotyped with Sendai virus F and HN envelope proteins (rSIV.F/HN) has been developed for the treatment of CF. Promising preclinical results support the progression of this vector carrying a full-length *CFTR* transgene (BI 3720931) into a first-in-human clinical trial expected to begin in 2024.

Keywords: CFTR; integrating vectors; lentivirus; genetic therapy; mutation-agnostic treatment

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*Co–first authors.

‡Co–senior authors.

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Correspondence and requests for reprints should be addressed to Eric Alton, F.R.C.P., F.Med.Sci., National Heart and Lung Institute, Imperial College London, Emmanuel Kaye Building, 1B Manresa Road, London SW3 6LR, UK. E-mail: e.alton@imperial.ac.uk.

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Introduction to Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that is estimated to affect approximately 160,000 individuals across 94 countries (1–3). CF is caused by pathogenic mutations in the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene (1, 2, 4). The *CFTR* gene encodes an anion channel that transports chloride and bicarbonate across the apical surface of multiple epithelial cell types in the lungs and other organs (1, 2, 4) (Figure 1). The CFTR channel also regulates sodium transport via the epithelial sodium channel, ENaC (5, 6). Reduced or absent functioning of the CFTR channel in the airways of people with CF results in a cycle of chronic pulmonary infection, inflammation, and progressive structural lung damage (1, 2, 4). Although yet to be fully elucidated, factors implicated in this include impaired mucus hydration and subsequent mucociliary clearance, as well as reduced pH that potentially leads to impaired bacterial killing (1, 2, 4, 7) (Figure 1). Extrapulmonary clinical manifestations can include chronic sinusitis and nasal polyps; exocrine pancreatic insufficiency leading to malnutrition, pancreatitis, and CF-related diabetes; liver and intestinal disease; bone disease; and reduced fertility in both men and women (1, 2, 4, 8).

Since the identification of the *CFTR* gene in 1989, more than 2,000 different variants have been discovered, of which approximately 700 have been determined to be disease-causing mutations (2, 4, 9–11). Seven classes of *CFTR* mutations have been identified, impacting either the quantity or function of CFTR protein at the cell surface (Figure 2) (7, 12, 13).

An understanding of the effects of mutations on CFTR function has allowed for the development of small molecules that restore defective CFTR function caused by a subset of mutation classes; these treatments are known as CFTR modulators (2, 12).

Current Therapies for CF: CFTR Modulator Therapies

CFTR modulators are systemic therapies, administered orally, that have transformed the landscape of clinical care for people with CF through unprecedented improvements in lung function, pulmonary exacerbations, weight and nutritional status, quality of life, and other clinical outcomes, all of which likely translate into substantially increased life expectancy (11, 14, 15). There are two types of CFTR modulators currently in use: potentiators that enhance channel function and correctors that increase the proportion of mature CFTR protein that is successfully trafficked to the cell surface (7, 14).

To date, four CFTR modulator therapies have been approved for the treatment of CF (11): ivacaftor, a CFTR potentiator; two corrector-potentiator combinations, lumacaftor-ivacaftor and tezacaftor-ivacaftor; and triple combination therapy, elexacaftor-tezacaftor-ivacaftor (ETI). ETI

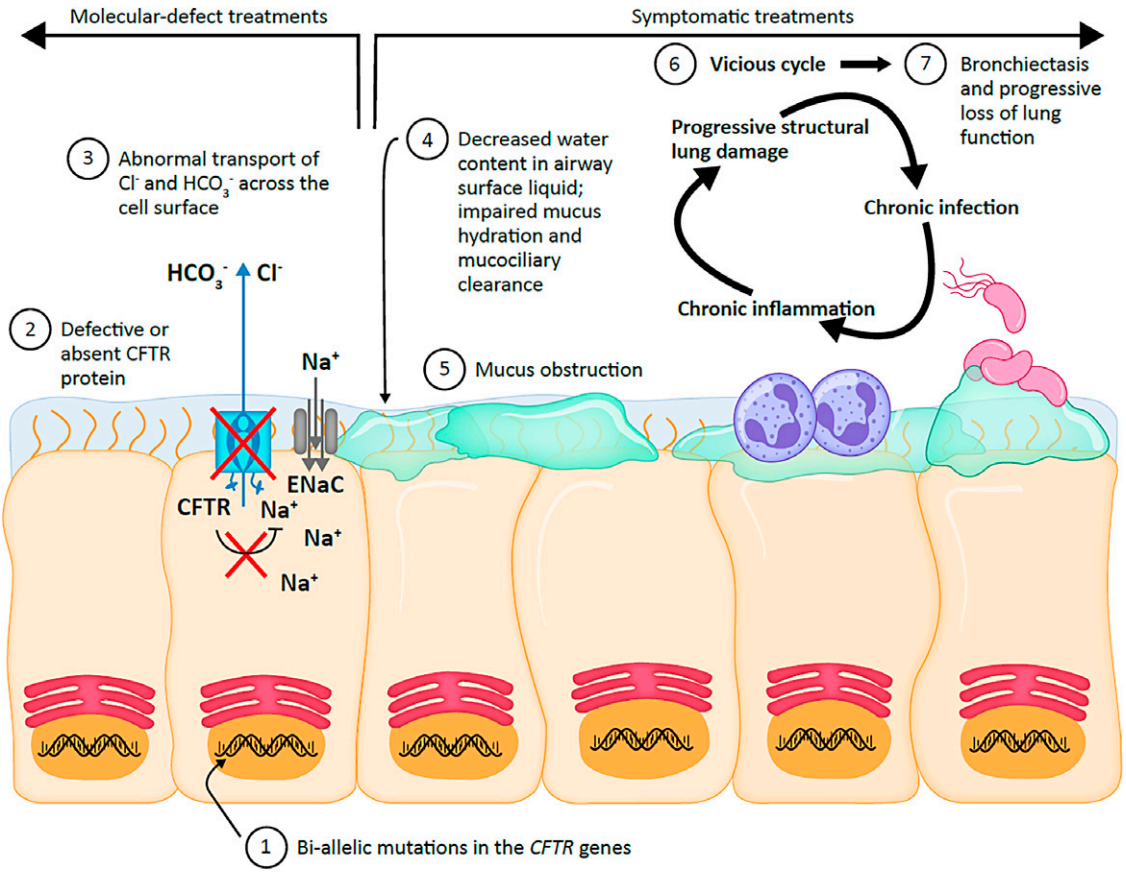

Figure 1. Mechanism of disease. CFTR = cystic fibrosis transmembrane conductance regulator; Cl^- = chloride ion; ENaC = epithelial sodium channel; HCO_3^- = bicarbonate ion; Na^+ = sodium ion.

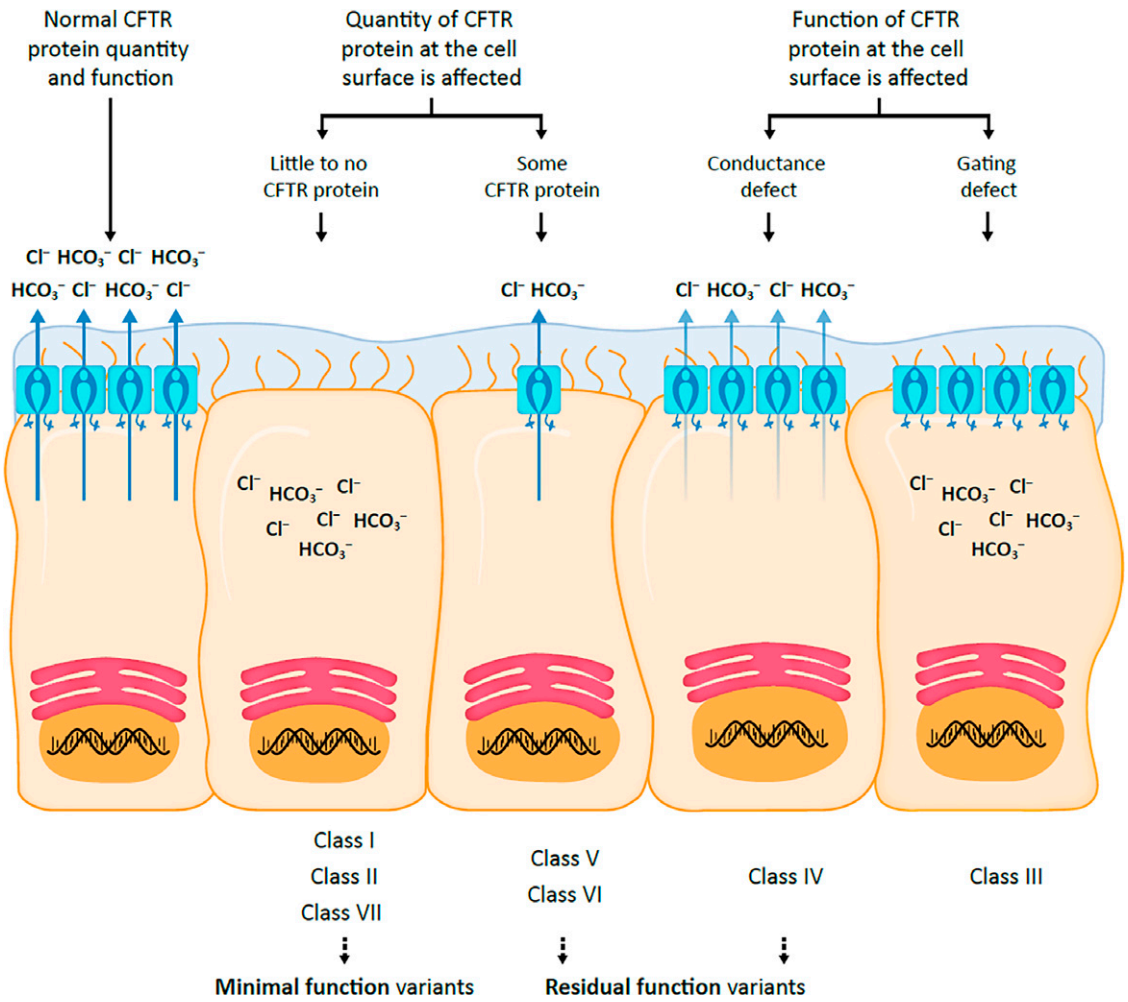

Figure 2. *CFTR* mutation classes. CFTR = cystic fibrosis transmembrane conductance regulator; Cl^- = chloride ion; HCO_3^- = bicarbonate ion.

has resulted in robust improvements in lung function (change in percent predicted FEV_1 [$ppFEV_1$] of up to 13.8%) and other clinical outcomes, such as reduced frequency of pulmonary exacerbations, increased body mass index, and improved patient-reported quality of life (Cystic Fibrosis Questionnaire–Revised, respiratory domain) (3, 7, 11, 12, 16, 17). Recent results from an observational study showed that ETI restores CFTR function in the airway and intestinal epithelia to ∼40–50% of that of healthy people (15).

However, despite the remarkable impact that CFTR modulator therapies have had on CF clinical care, there is still a significant proportion of people with CF who are ineligible for, or intolerant of, CFTR modulator therapies and for whom there remains a high unmet therapeutic need (10–15% of the global CF population, based on current estimates) (11, 18–21).

Unmet Need for New Therapeutic Approaches for CF

Available CFTR modulator therapies are only beneficial when there is sufficient CFTR protein with therapeutic binding sites for them to act on (22). Various CF-causing mutations, including frameshift, nonsense, and splicing mutations, result in no CFTR protein or very low amounts of CFTR protein that is truncated and dysfunctional. As these mutations are largely insensitive to CFTR modulator therapies, people possessing them are ineligible for such therapies (22, 23). The most recent estimates indicate that approximately 5% of people with CF in the United States aged 12 years and older have mutations that are ineligible for CFTR modulator therapy, and these mutations are more common in ethnically and racially minoritized people with CF (24). Thus, there is a higher proportion of minoritized people with CF who are genetically ineligible for CFTR modulator therapy than in the overall CF population, resulting in a CF health disparity (25, 26). In addition, not all people with CF who are genetically eligible for modulator therapies are able to tolerate them because of adverse effects (12, 27). In sum, there is an unmet need for new therapeutic approaches for people with CF who still rely on traditional CF care (symptom-directed treatments), and alternative approaches are needed to restore CFTR function (11, 12). This will help to close the widening health outcome gap between modulator-treated and non–modulator-treated people with CF (11).

In addition, for those who are eligible for and able to tolerate CFTR modulators, lifelong daily oral administration is required, and significant drug–drug interactions have been reported (18, 19). For example, triazoles

(first-line antifungal treatment in people with CF) have been associated with significant, bidirectional drug–drug interactions with CFTR modulators through interference with the cytochrome P450 system (28). Therefore, longer-lasting therapeutic approaches that reduce the need for concomitant therapies could reduce the treatment burden for people with CF, although if administered by inhalation, the systemic benefit of CFTR modulator therapy would be lost. Clearly, even in the CFTR modulator era, there is an ongoing need for new treatment strategies targeting CFTR.

Genetic Therapies in Development for CF

Inhaled genetic therapies offer the prospect of addressing the unmet pulmonary treatment need in people with CF (29, 30). There are several approaches being investigated for CF, including gene addition therapy (the focus of this review) (Figure 3), RNA-based therapies such as mRNAs and transfer RNAs, antisense oligonucleotides (ASOs), and gene editing. Currently, these therapeutic approaches are in clinical use for several indications but are still in preclinical or early clinical trial stages for CF (29).

There are several RNA molecules being assessed for their therapeutic potential in CF (29). A promising mutation-agnostic therapeutic approach for CF is full-length CFTR mRNA addition (30). Three early-phase clinical trials of inhaled mRNA therapy for CF (CFTR mRNA encapsulated in a lipid nanoparticle) are in progress (30–32): RCT2100 in healthy volunteers (33), ARCT-032 in healthy volunteers and adults with CF (34), and VX-522 in adults with CF who are ineligible for CFTR modulator therapy (35).

ASOs are short, synthetic, single-stranded nucleic acids that bind to mutant mRNA transcripts; in doing so, they modulate gene expression either through mRNA repair permitting transcript read-through or by correcting splicing mutations (36, 37). There are several ASOs targeting nonsense and splicing mutations under preclinical and clinical development for treatment of CF (22). In 2023, a phase I study of SPL84, an ASO for the treatment of people with CF with at least one 3849 + 10kbC→T variant, was completed (38). SPL84 was shown to be safe and well tolerated in healthy volunteers who received a single dose (38). A phase II efficacy study is planned for 2024 (38).

Gene editing to correct *CFTR* mutations is still in the early preclinical development

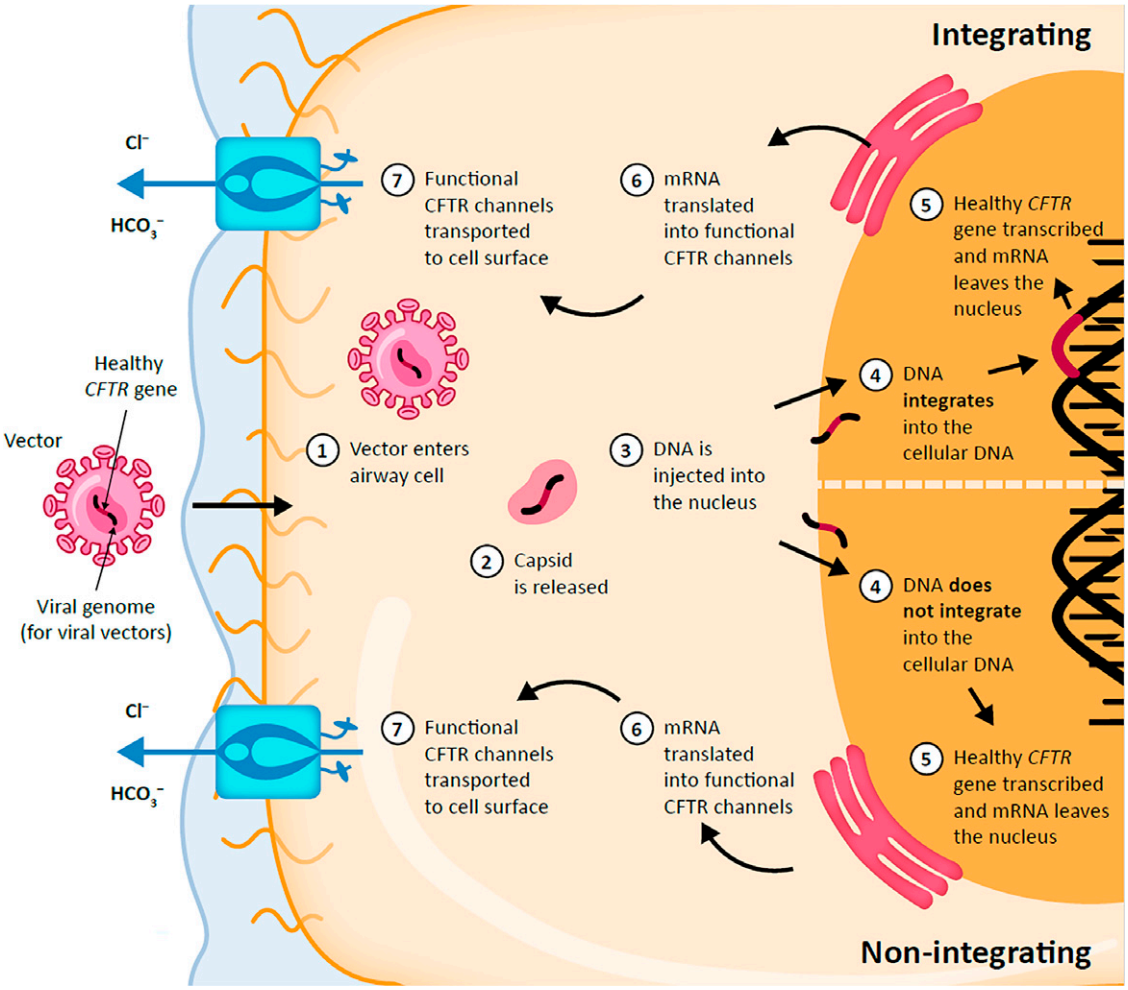

Figure 3. Viral gene therapy for cystic fibrosis. CFTR = cystic fibrosis transmembrane conductance regulator; Cl^- = chloride ion; HCO_3^- = bicarbonate ion.

stage, with both *in vivo* clustered regularly interspaced short palindromic repeats (CRISPR)-based and non–CRISPR-based gene editing approaches assessed in animal models (39).

Gene Addition Therapy

Gene addition therapy is a mutation-agnostic approach that refers to the treatment of a genetic disease through the introduction of the normal gene into cells via a vector (Figure 3). Many gene therapy trials for CF have been performed since the identification of the *CFTR* gene (36 trials involving approximately 600 people with CF); however, for various reasons relating to efficacy and safety, no gene therapy for CF has reached the market to date (18). There are two types of vectors that have been investigated for CF gene therapy, namely nonviral (e.g., liposomes) and viral vectors (e.g., based on adenoviruses [Ads], adeno-associated viruses [AAVs], herpes simplex viruses [HSVs], and lentiviruses). The potential benefits and limitations of these vectors are shown in Table 1.

Nonviral vectors. Nonviral vectors offer many benefits, including an almost unlimited packaging capacity, negligible risk of insertional oncogenesis (as they do not integrate into the DNA of transduced cells), and increased biocompatibility (low cytotoxicity and immunogenicity) compared with viral vectors (36, 41). However, efficient delivery of nonviral vectors to the lung remains a challenge (41). In a phase IIb trial, monthly repeated administration of the nonviral *CFTR* gene liposome complex pGM169/GL67A over a 1-year period was shown to be safe, well tolerated and produced significant lung function benefit compared with placebo (36, 42). In addition, multiple repeat dosing without loss of expression was demonstrated with this vector (42). However, the magnitude of efficacy was modest and insufficient to warrant further development, highlighting the need for more potent vectors (19, 42).

In addition to liposomes, there are several other nonviral vectors in development for the treatment of CF, including lipid nanoparticles, extracellular vesicles, and polymeric nanoparticles (43). Although several nonviral vectors are in clinical (RCT2100, ARCT-032, and VX-522 using lipid nanoparticles) (33–35) and preclinical (Nanite using polymeric nanoparticles) (44) development to deliver CFTR mRNA to people with CF, there are currently no ongoing clinical trials using nonviral vectors for *CFTR* gene addition. There are, however, several nonviral vectors for gene addition therapy in early development (extracellular vesicles [45] and polymeric nanoparticles [46]).

Viral vectors. Viral vectors may offer the advantage of improved efficiency of gene delivery (Figure 3). Ad vectors were one of the first viral vectors investigated for use in gene therapy because of their natural lung tropism and large carrying capacity (40). However, traditional Ad vectors have been considered unsuitable for gene therapy for CF because of their short duration of expression requiring frequent readministration and vector-induced immune responses preventing efficient gene expression after readministration (30, 40). As such, helper-dependent Ad (HD-Ad) vectors have since been developed based on Ad vectors through the removal of viral genes; this makes HD-Ad vectors less immunogenic after repeat administration (47). No clinical trials using HD-Ad vectors for *CFTR* gene addition are currently underway.

Although there is evidence of some degree of integration emerging, AAVs have typically been considered a type of vector that does not integrate into the DNA of transduced cells and therefore offer a very low risk of insertional oncogenesis (31, 36, 48). At low doses, AAVs only elicit modest inflammatory responses, with evidence from clinical trials suggesting that immunogenicity is dose dependent (49, 50). They also have a small packaging capacity and thus cannot carry the full-length *CFTR* coding sequence coupled with typical transcriptional control sequences (30, 31, 36). Furthermore, efficacy of AAVs can be impacted by preexisting immunity as well as adaptive immune responses after vector administration (51, 52). Importantly, efficacious readministration has not been demonstrated with AAVs (30). 4D-710 is an AAV vector–based gene therapy that is currently being assessed in a phase I/II single-dose trial in adults with CF who are ineligible for, or unable to tolerate, CFTR modulator therapy (NCT05248230) (31, 36, 53). To overcome the packaging limitation, the vectors carry *CFTR* minigenes, in which a portion of the coding sequence has been removed (54); alternatively, novel AAV/bocavirus hybrid vectors have been created that sufficiently extend the packaging capacity to allow full-length *CFTR* cDNA to be used (55, 56).

HSV-based vectors are nonintegrating and comparatively transient expression vectors that have a large packaging capacity and are considered noninflammatory (36).

Table 1. Benefits and Limitations of Nonviral and Viral Vectors

		Viral Vectors			
	Nonviral Vectors	Ad Vectors	AAV Vectors	HSV Vectors	Lentiviral Vectors
Large packaging capacity	✓	✓		✓	✓
Almost no risk of insertional oncogenesis	✓	✓	✓	✓	
Noninflammatory	✓		✓	✓	✓
Transduction of terminally differentiated epithelial cells	✓	✓	✓	✓	✓
Integrating vector (gene expression for the lifetime of the transduced cell)*					✓
Successful readministration to the lung without loss of efficacy	✓†				✓

Definition of abbreviations: AAV = adeno-associated virus; Ad = adenovirus; HSV = herpes simplex virus.
*Because of the turnover of transduced airway epithelial cells, a single dose may not be sufficient to achieve lifelong therapeutic benefit in humans. Therefore, the ability to safely and effectively readminister may also be a prerequisite for a successful gene therapy.
†Multiple repeat dosing without loss of expression has been demonstrated with the nonviral *CFTR* (cystic fibrosis transmembrane conductance regulator) gene liposome complex pGM169/GL67A (42). However, it remains unclear whether multiple repeat dosing is possible without loss of efficacy for all nonviral vectors, as there is currently insufficient clinical experience with newer vectors.

Although they are better known for their use in the nervous system and for skin diseases, HSV-1 vectors are also being developed for use in the lung (36). Although efficacy after repeated administration with HSV vectors has been shown to be maintained after cutaneous administration (36), at the time of writing, the authors are not aware of data showing that efficacy is maintained in the lungs. KB407 is a replication-defective HSV-1 vector encoding two copies of the full-length human *CFTR* gene for the treatment of CF (30, 36). Duration of expression is short, and safety of weekly administration is currently being assessed in a phase I/IIa clinical trial in adults with CF, regardless of their CFTR genotype (NCT05504837) (36, 57, 58).

Lentiviral vectors integrate into the DNA of transduced cells, allowing for long-term gene expression throughout the lifetime of the cell after a single dose (18, 31, 36, 40). They have a large packaging capacity and can therefore carry the full-length *CFTR* coding sequence (30, 36). Lentiviral vectors are recognized for their very weak inflammatory properties (59). Moreover, in contrast to Ad, AAV, and HSV, lentiviral vectors have been successfully readministered to the respiratory epithelium without loss of efficacy (30, 60). Lentiviral vectors can be modified with different viral envelope proteins (known as pseudotyping) to improve their tropism for different primary cell types (61).

A highly successful gene therapy for CF would require efficient and long-lasting gene expression. The ability to safely and effectively redose therapy may also be a prerequisite given the turnover of transduced airway epithelial cells. Lentiviral vectors have the potential to meet these needs and are the focus of the remainder of this review.

Gene Therapy with Lentiviral Vectors

There are several lentiviral vector–based gene therapies that have been investigated for the pulmonary treatment of CF. These include lentiviral vectors pseudotyped with VSV-G (62) and GP64 (63) envelope proteins, both of which are in preclinical development. The *CFTR* transgene DNA was detected in five out of six nonhuman primates treated with a lentiviral vector pseudotyped with VSV-G envelope proteins (62). Evidence of functional CFTR was reported in newborn CF pigs that received a lentiviral vector pseudotyped with GP64 envelope proteins (63). In addition, a lentiviral vector pseudotyped with Sendai virus envelope is expected to enter phase I/II testing in 2024 and will be discussed in further detail later in this review.

Expression beyond the lifetime of the surface epithelial cells, which would thereby remove the need for repeated dosing, would require progenitor and/or stem cell transduction. A lentiviral vector has been shown to transduce human airway progenitor cells (primary basal cells) *in vitro* without altering the differentiation potential (64). *In vivo*, such basal cells in the pseudostratified proximal airways are difficult to target; however, progenitor and/or stem cells of the distal lower airways may be more readily transduced related to the simpler, single-cell layer anatomy of this region.

Safety concerns associated with viral vector–based gene therapy, in particular integrating vectors such as lentiviral vectors, include acute inflammation and genotoxic events such as insertional oncogenesis caused by activation of protooncogenes or disruption of tumor suppressor genes (65, 66). Although not new, considerable advances have been made recently to improve the safety of lentiviral vectors.

As lentiviral vectors are integrating vectors, insertional oncogenesis is a safety concern (18, 31, 40, 66). Transcriptionally active long terminal repeats (LTRs) with strong promoter and enhancer sequences have been identified as major drivers of genotoxicity in early generations of lentiviral vectors (first-generation vectors included large portions of the lentiviral genome; second-generation vectors excluded the lentiviral accessory genes *vif*, *vpr*, *vpu*, and *nef*) (66, 67). Subsequently, the safety of lentiviral vectors has been improved through the development of third-generation self-inactivating (SIN) vectors (68, 69), which lack lentiviral *tat* (67) and the transcriptional control elements in the 3′LTR and are therefore much less capable of activating protooncogenes (36, 40, 66, 70) (Figure 4A). Indeed, in a 2022 systematic literature review and meta-analysis that assessed the incidence of genotoxicity in hematopoietic stem and progenitor cells by vector type, no oncogenic events were reported across 34 studies using lentiviral vectors (71). Subsequent to publication of the review, three oncogenic cases associated with vector insertion were reported in trials of the lentiviral vector elivaldogene autotemcel (eli-cel; Skysona™) designed to treat cerebral adrenoleukodystrophy (72). The vector includes a transgene promoter derived from a retroviral LTR element (MNDU3 promoter), which has been implicated as the responsible genotoxic element (72). Overall, excluding eli-cel–treated subjects, SIN lentiviral vectors have demonstrated a reassuring safety profile (Figure 4B). In contrast, in the 2022 systematic literature review and meta-analysis mentioned above, first-generation (non-SIN) gammaretroviral vectors were responsible for 21 oncogenic events across 20 studies ($n = 118$ subjects) (71).

An additional safety feature in the production of third-generation lentiviral vectors involves the splitting of the necessary viral genes onto several different plasmids, thereby reducing the risk of recombination and replication-competent vector development during manufacturing (a hypothetical risk and not seen to date) (73, 74). Lentiviral vectors have been further modified to prevent recombination *in vivo*, such as minimizing sequence similarities between the vector genome and viral *gag/pol* transcripts (75).

Several third-generation lentiviral vector–based gene therapies have been approved for various indications (Table 2). In addition, several clinical trials using lentiviral vectors for the treatment of various indications, including metabolic disorders, cancers, immune disorders, and rare genetic diseases, are ongoing (40).

rSIV.F/HN: A Third-Generation Lentiviral Vector

Historically, no viral vector has met the requirements for clinical use in CF. Major shortcomings included poor gene transfer (in part due to the receptors for viral vectors being predominantly located on the basolateral surface of airway epithelium) and limited capacity for repeat administration (36, 76).

A third-generation lentiviral vector pseudotyped with Sendai virus F and HN envelope proteins (rSIV.F/HN) has been developed as a platform for the treatment of CF (76). The receptors for the F/HN pseudotype are located on the apical surface of airway epithelia, thus enabling cell entry upon inhaled delivery (76, 77). The vector has also been designed to mitigate safety risks, as it is SIN and replication incompetent (76). As mentioned above, concerns regarding

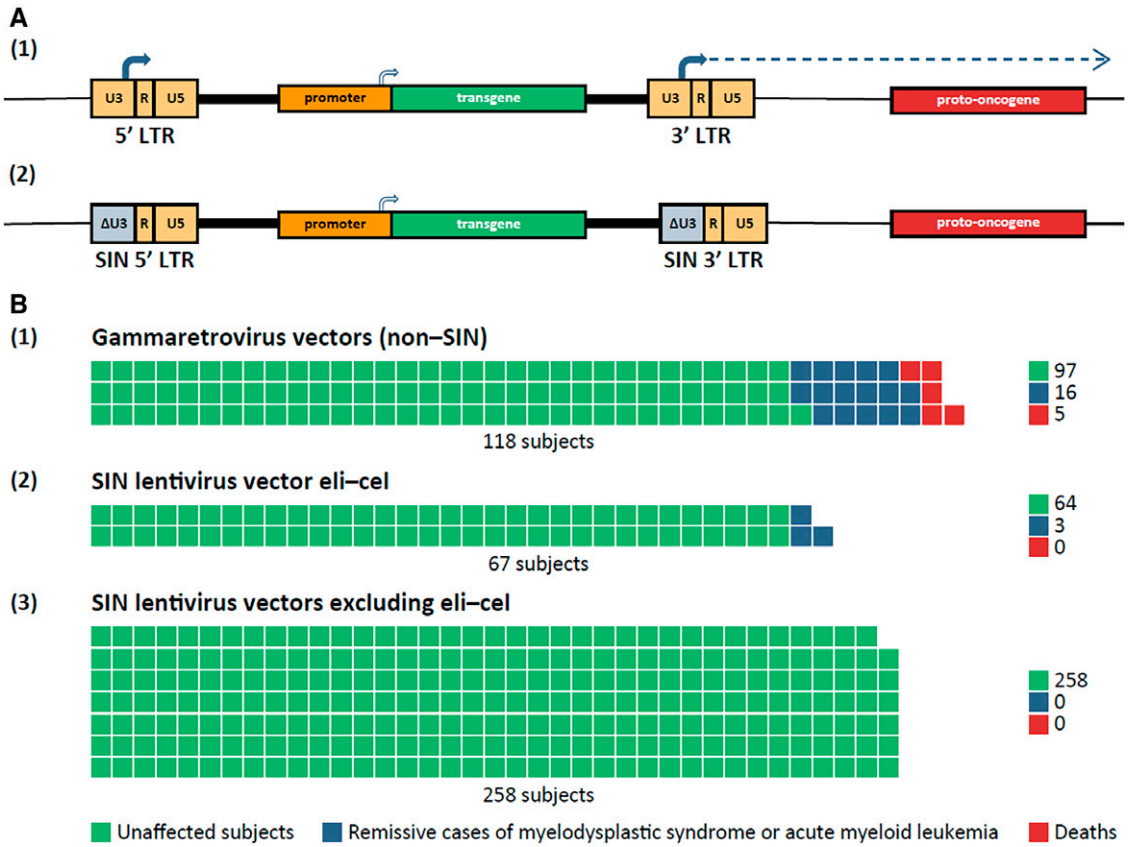

Figure 4. Safety of lentiviral gene therapy. (*A*) Deletion of long terminal repeat (LTR) promoter/enhancer reduces genotoxic risks. (*1*) Integrated second-generation retroviral vector with intact LTRs (non–self-inactivating [non-SIN]). The promoter/enhancer element in the U3 region of the 3′ LTR (solid arrow) has the potential to activate (dotted arrow) a cellular protooncogene. (*2*) Deletion of the U3 element (ΔU3) to ablate the LTR-initiated genotoxic effect is a feature of all modern SIN vectors (third-generation vectors). Host genomic DNA is shown as a thin horizontal black line. (*B*) Waffle charts of frequency of occurrence of oncogenic events after retroviral gene therapy (each square represents an individual subject). (*1*) Subjects treated with early-generation gammaretroviral vectors without SIN deletions. (*2*) Subjects treated with elivaldogene autotemcel (eli-cel), a SIN lentiviral vector (i.e., with ΔU3 deletions in the LTRs) but whose internal transgene promoter is derived from a retroviral LTR. (*3*) Subjects treated with SIN lentiviral vectors lacking any LTR-derived promoter/enhancers. Eli-cell data and inferences from Jun 9–10, 2022 meeting of the U.S. Food and Drug Administration Cellular, Tissue, and Gene Therapies Advisory Committee (72); other data from Reference 71.

the safety of integrating viral vectors were raised after the development of leukemia in people with severe combined immunodeficiency after treatment with a first-generation γ retroviral vector–transduced bone marrow (76, 78). In addition to the improved biosafety of third-generation lentiviral vectors, the risk of insertional oncogenesis is also influenced by the target cell population and is likely to be considerably lower in differentiated nondividing epithelial cells compared with rapidly dividing bone marrow stem cells (76, 78). Indeed, a nonexhaustive insertion site analysis in genomic DNA from nasal and lung airway

Table 2. Approved Lentiviral Vector-based Gene Therapies

Approved Lentiviral Gene Therapy	Indication	Approval Agency (yr)
Zynteglo	β-thalassemia	EMA (2019)* (83); FDA (2022) (84)
Libmeldy or Lenmeldy	Metachromatic leukodystrophy	EMA (2020) (85); FDA (2024) (86)
Skysona	Cerebral adrenoleukodystrophy	EMA (2021)* (87); FDA (2022) (88)
Lyfgenia	Sickle cell disease	FDA (2023) (89)
Kymriah	CAR T therapy for certain cancer types	FDA (2017) (90); EC (2022) (91)
Breyanzi	CAR T therapy for certain cancer types	FDA (2021) (92); EC (2022) (93)

Definition of abbreviations: CAR = chimeric antigen receptor; EC = European Commission; EMA = European Medicines Agency; FDA = U.S. Food and Drug Administration.
*Marketing authorization for Zynteglo (94) and Skysona (95) has subsequently been withdrawn.

epithelial cells extracted from mice transduced *in vivo* by instilled rSIV.F/HN revealed no preference for integration near known oncogenic loci; insertion sites were amplified using linear amplification–mediated PCR adapted for SIV LTRs, and sequence analysis revealed that 73% of insertion sites were located in transcription units, a frequency distribution pattern typical of lentiviruses (70). A comprehensive insertion site analysis performed later in primary human bronchial epithelial cells from people with CF (F508del/F508del) transduced *ex vivo* by rSIV.F/HN also concluded that the insertion site distribution was consistent with the rSIV.F/HN vector being of low genotoxic potential (data not shown).

In mouse models, the rSIV.F/HN vector was shown to transduce the respiratory epithelium with an efficiency of ~15%, a level that is likely to be relevant for clinical benefit in humans (70). Transduction with the vector led to stable gene expression that persisted for at least 2 years after transduction (78). In addition, monthly repeat administration was possible without significant loss of gene expression; the degree of expression was identical after one and three doses and was >4 log orders higher than in nontransduced mice (78). No evidence of chronic toxicity was observed during the 2-year follow-up period (78). Moreover, preexisting and acquired immune responses appear not to interfere with vector readministration efficacy (70).

Furthermore, in nonhuman primates that received a single aerosolized dose of the rSIV.F/HN vector carrying a reporter gene, no evidence of toxicity was reported (79). Transduction levels in airway epithelial cells ranged from 9% to 12%, and high amounts of vector-specific mRNA were detected (79). The rSIV.F/HN vector has also been shown to transduce human lungs maintained in an *ex vivo* lung perfusion system (unpublished data) and achieve persistent gene expression in ovine precision-cut lung slices (78).

The rSIV.F/HN vector carrying a reporter gene has been shown to transduce differentiated human airway epithelium *ex vivo* (78). In addition, persistent gene expression was observed in differentiated human air–liquid interface cultures at amounts far higher than air–liquid interface cultures transduced with the previous nonviral gene transfer agent GL67A (78).

In preparation for a first-in-human clinical trial, the rSIV.F/HN vector carrying a codon-optimized and CpG-depleted CFTR (soCFTR2) cDNA under the control of a CpG-free hybrid promoter (hCEF) (rSIV.F/HN-hCEF-soCFTR2) has been assessed in key translational preclinical studies (70). Codon optimization allows for enhanced translation, thus increasing CFTR transgene expression. CpG motifs within plasmid DNA have been shown to induce acute inflammatory responses when delivered to the lungs of CF animal models and people with CF (80). As such, the use of CpG-depleted cDNA has previously resulted in a reduction in host inflammatory response (80). Preclinical data have shown that codon optimization and CpG depletion of CFTR cDNA does not alter the structure and function of CFTR in cell culture and can lead to persistent CFTR expression *in vivo* in mice (80). In these translational preclinical studies, rSIV.F/HN-hCEF-soCFTR2 led to expression of functional chloride channels *in vitro* as assessed by the iodide efflux assay (70). Furthermore, when transduced into the nasal epithelium of CF knockout mice, significant amounts of vector-specific mRNA were detectable after both 1 and 4 weeks after transduction (70). In addition, rSIV.F/HN-hCEF-soCFTR2 transduction resulted in a significant increase in CF intestinal organoid swelling (a specific indicator of CFTR channel activity) compared with nontransduced controls, suggesting partial restoration of channel activity (70). Together, these preclinical results support the progression of the vector into a first-in-human clinical trial.

Introduction to the First-in-Human Trial of BI 3720931 (Lenticlair™ 1)

rSIV.F/HN-hCEF-soCFTR2 is now being developed as BI 3720931 in a first-in-human phase I/II trial (81). This trial will investigate safety, tolerability, and efficacy and is expected to begin in 2024. Adult males and females of nonchildbearing potential with a clinical diagnosis of CF who are genetically ineligible for CFTR modulator therapy will be recruited into the trial.

Phase I is an open-label dose-escalation trial (minimum of $n \geqslant 9$). Participants will receive a single low, medium, or high dose of orally inhaled, nebulized BI 3720931 (1:1:1) plus standard of care, with 24 weeks of follow-up. The primary endpoint is the occurrence of any drug-related treatment-emergent adverse event (AE) within 24 weeks. Secondary endpoints are occurrence of a treatment response, defined as a change from baseline ⩾5% $ppFEV_1$ within 8 weeks after dosing, absolute change from baseline in $ppFEV_1$ at Week 24 after dosing, and occurrence of dose-limiting toxicity up to Week 24 after dosing. Interim safety and efficacy results from a minimum of 8 weeks after dosing will inform dose selection for phase II.

Phase II ($n = 27$) is a randomized, double-blind, placebo-controlled, dose-expansion trial with a 4-week screening period, followed by randomization to a single dose of nebulized BI 3720931 (one of two dose strengths) or placebo plus standard of care (1:1:1) with 24 weeks of follow-up. The primary endpoint is absolute change from baseline in $ppFEV_1$ at Week 8 after dosing. Secondary endpoints include absolute change from baseline in $ppFEV_1$ and occurrence of serious AEs and drug-related treatment-emergent AEs up to Week 24 after dosing.

After trial participation, all participants will be asked to enter a separate extension trial for 15 years of follow-up (in accordance with regulatory guidance from the U.S. Food and Drug Administration) to investigate any potential delayed AEs (Lenticlair™-ON) (82). Participants may enter other investigational trials during this time pending eligibility criteria of these other trials.

Conclusions

Despite the unprecedented improvements in CF clinical care due to the introduction of CFTR modulator therapies, a need for disease-modifying treatments still exists for people with CF who are ineligible for, or intolerant of, these therapies. Various genetic approaches, including gene addition therapy, provide opportunities to address this unmet need. Lentiviral vectors have been successful for several indications and hold promise for CF gene therapy because of their long duration of gene expression, high packaging capacity, noninflammatory nature, and ability to transduce both dividing and nondividing cells. The rSIV.F/HN lentiviral vector has shown favorable preclinical safety and efficacy results, with a first-in-human trial expected to start in 2024. This approach has the potential to address the unmet need

in CF clinical care and help to close the widening health outcome gap between modulator-treated and non–modulator-treated people with CF. ■

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