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Review Paper

Gene Therapy: Viral And Non-Viral Vectors and Applications

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ABSTRACT

This document discusses viral and non-viral vectors used in gene therapy, emphasizing its qualities, applications, and limitations. The three most common viral vectors are adeno-associated virus (AAV), adenovirus (Ad), and lentivirus (LV). AAV vectors are frequently utilised because of their low immunogenicity, non-pathogenicity, and capacity to deliver long-term gene expression, especially in non-dividing cells. They have been effective in treating genetic diseases and certain malignancies, but limited gene capacity and potential toxicity at high dosages remain concerns. Adenoviral vectors have high transduction efficiency and gene packaging capacity, making them beneficial in cancer treatment and vaccine production. However, they only produce transient gene expression and frequently trigger robust immunological reactions. Stable and long-term expression is made possible by lentiviral vectors' capacity to integrate into the host DNA. Although they are frequently employed in ex vivo treatments like CAR-T cell therapy, hazards such insertional mutagenesis need to be taken into consideration. Polymers, lipids, peptides, and inorganic materials are examples of non-viral vectors that offer safer substitutes with reduced toxicity and immunogenicity. However, they typically encounter difficulties like poor stability and reduced gene delivery effectiveness. In general, non-viral technologies are starting to show promise as alternatives to viral vectors, which currently dominate applications.

INTRODUCTION

Viral and non-viral vectors:

AAV Vectors

AAV-mediated gene transfer has significant promise as a therapeutic method. The majority of the already produced AAV vectors are orientated at monogenic disorders, which fall under the category of uncommon diseases [1]. The FDA authorised gene therapy products based on two viral vectors, both AAV vectors: LUXTURNA (Spark Therapeutics, Inc.) for the treatment of patients with confirmed biallelic RPE65 mutation associated retinal dystrophy, and ZOLGENSMA

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for the treatment of paediatric patients under two years of age with spinal muscular atrophy (SMA) with bi-allelic mutations in the survival motor neurone 1 (SMN1) gene. The use of recombinant AAV serotypes with specific tropisms to administer cytotoxic therapy could also be considered a local antitumor therapy strategy. EBV+ B-cells are more susceptible to rAAV6.2 infection. Therefore, the inclusion of a functional suicide gene into the rAAV6.2 genome may serve as a suitable vector for the development of rAAV-based oncolytic therapy targeting focal EBV-bearing B-lymphoproliferative diseases [2]. Intracranial interferon-beta (IFN-) gene therapy based on the local delivery of AAV vectors was found to be successful in treating non-invasive orthotopic glioblastoma models and was also effective against migratory tumours [3]. AAV vectors have several important qualities that could be used for gene delivery in cancer therapy [4]. AAV was first identified as a contaminant in adenovirus preparations [5]. The AAV genome is composed of single-stranded DNA that is roughly 4.8 kilobases (kb) long. In addition, the AAV contains a tiny (~25 nm) icosahedral capsid consisting of three types of structural proteins: VP1, VP2, and VP3 [6]. AAVs are replication-deficient parvoviruses that have historically required co-infection with a helper adenovirus or herpes virus to establish efficient infection [7]. Currently, the AAV-Helper-free method is primarily used in clinical research. On their own, AAVs are assumed to be non-pathogenic and have yet to be definitively related to any known human diseases. AAVs have at least 12 natural serotypes, each with unique tissue tropisms (Table 3). This is mostly due to the distinct affinities of these serotypes for a variety of primary cell surface glycoprotein receptors and secondary receptors or coreceptors. For example, heparin sulphate proteoglycan is expected to be the major AAV-2 receptor. AAV's identified co-receptors include alpha V beta5 integrin, fibroblast growth factor receptor 1 (FGFR-1), and hepatocyte growth factor receptor (c-Met). The attachment of AAV-3 strain H depends on heparin, heparan sulphate, and FGFR-1 [8]. AAV is a non-enveloped virus that can be modified to deliver DNA to specific cells. The virus genome does not integrate into the host cell and instead produces episomal concatemers in the nucleus. These head-to-tail circular concatemers remain intact in non-dividing cells, such as neurones and cardiomyocytes, and are thus capable of expressing transgenes for several months [9]. AAV vectors also enable rather steady expression in dividing cells. Integration events may occur more frequently if an exceptionally high multiplicity infection is utilised or if the cell is infected with an adenoviral replicase. Dhwanil A et al. recently reported that chromosomal integrations occurred at an unexpectedly high frequency of 1-3% both in vitro and in vivo [10]. Furthermore, according to recent studies, excessive copy numbers of the AAV9 vector caused severe toxicity in animal models [11]. AAV vectors are typically preferred for in vivo gene therapy due to several advantages, including the ability to transduce both dividing and quiescent cells, robust in vivo transduction efficiency, long-term transgene expression in quiescent cells, tropism for specific tissues and cell types, relatively low immunogenicity, non-pathogenicity, and a history of clinical safety (Figure 1)

Table no. 1. The tissue tropism of different AAV serotypes and the representative clinical trials.

AAV Serotype	Tissue-Specific Tropisms	Key Pipeline	Disease	The Delivered Gene	The Clinical Trial Stage
AAV1	Muscle, heart, sketala muscle (induding cardiac muscle), rer tisse	Glybera	Lipoprotein deficiency	Lipoprotein lipase	Approved *8
AAV2	Central nervous system, muscle, liver, brain tisse	BIIB111	Hereditary ophthalmopathy	Rab escort protein 1	Phase III completed, suspended
AAV3	Muscles, liver, lung, eye	N/A	N/A	N/A	N/A
AAV4	Central nervous system, mucs, eye	N/A	N/A	N/A	N/A
AAV5	Lung, eye, central nerve system, vomer, pancreas	N/A	N/A	Coagulation factor VIII	N/A
AAV6	Lung, heart	SB-525	Hemophilia type-A	Coagulation factor VIII	N/A
AAV7	Muscle, heart, liver, eye	BIIB112	X-linked retinoschisis	Pigmenotd factor VIII	Phase III, suspended N/A
AAV8	Heart, muscle, lung(alveolar), central nervous system	PF-06939926	Duchenne muscular dystrophy	Truncated dystrophin	Phase III,
AAV9	Lung, heart	N/A	N/A	N/A	N/A
AAV-DJ	Liver, retina, lung, kidney	N/A	N/A	N/A	N/A
AAV9	Liver, eye, central nervou system, muscle	PF-06939926	Truncated dystdophin	Phase III, lurin	N/A
AAV9	Liver, retina, lung, kidney	N/A	N/A	N/A	N/A
AAV-DJ/8	Liver, eye, central nervos system, muscle	LYS-GM101	GM1 gangliosidase	Phase II	N/A
AAV11	Lung, heart, muscle, central sysstem	N/A	N/A	N/A	N/A
AAV12	Unknown	N/A	N/A	N/A	N/A
AAV13	Unknown	N/A	N/A	N/A	N/A

N/A *Not available.*: Withdrawal of the marketing authorization.

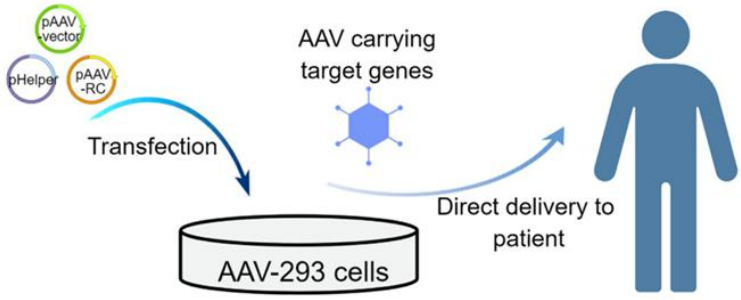

Figure 1. In vivo options for genetic disease treatment using AAV vectors are depicted schematically.

Ad Vectors

Ad is a huge, complex, non-enveloped, double-stranded DNA (dsDNA) icosahedral virus measuring 70 to 90 nm in size. Ad has an icosahedral protein capsid that holds a 26.45-kb linear, double-stranded DNA genome. There are around 100 serologically distinct varieties of adenoviruses, and 49 of them infect humans [12, 13]. Depending on their individual type, these viruses may bind to various cell surface proteins to promote their entry into the target cells [14]. Ads' great efficiency as gene therapy tools has resulted in the approval of over 450 protocols for clinical trials thus far [15]. Like AAV, Ad does not incorporate into the host DNA. Ad is the most effective gene delivery method for a variety of cell and tissue types. This is because most human cells have the primary adenovirus receptor and secondary integrin receptors, such as Coxsackie and Adenovirus Receptor (CAR), CD46, and desmoglein-2 (DSG-2), as well as the glycans GD1a and polysialic acid [16, 17]. Ad was the first DNA virus to undergo extensive therapeutic development, owing to its well-defined biology, genetic stability, enormous transgene capacity (up to 36 kb), and ease of large-scale production. In addition, Ad has side effects that are significantly milder than chemotherapy [18, 19]. Adenoviral vectors were first employed for brain cell transduction in the early 1990s [20]. Nonhuman canine adenovirus type 2 (CAV-2)-based vectors can deliver genes to neurones in the brain, spinal cord, and peripheral nervous system [21]. Adenovirus vectors can be classified into two types: replication-deficient viruses and replication-competent, oncolytic viruses (OVs) [18]. The most frequently employed adenoviral vector is the human Ad serotype 5, which is a common cold virus that circulates in humans with a seropositivity rate of 40-60% [22]. The deletion of the E1 and E3 genes caused this virus to be

replication-defective [23]. The other contemporaneous Ad vectors were developed from human adenovirus serotype 2 (HAd2) [24].So far, three generations of adenoviral vectors have been created. The first generation of Ad vectors was created by replacing the E1A/E1B region with transgenic cassettes of up to 4.5 kb in length. These Ad viral vectors could generate high-level innate inflammatory responses during the first 24 hours of transduction [25]. In the second generation of adenoviral vectors, the transgene capacity was further boosted by also removing the E2/E4 site. However, the overall production yield remained low due to the lower replication ability in producer cell lines [26]. The third-generation adenovirus vectors, also known as helper-dependent or gutless adenoviruses, have all viral sequences removed save for the ITRs and packing signal. These viral vectors have a significantly lower in vivo immune response than first- and second-generation adenovirus vectors, but maintaining good transduction efficiency and tropism [27]. Ad vectors are the most widely utilised vectors in cancer gene therapy. Ad vectors are also utilised in vaccinations to express foreign antigens [28]. Cancer remains the biggest cause of mortality globally, accounting for roughly 10 million deaths in 2020 [29]. The cumulative danger is linked with the tendency for cellular repair processes to become less effective as the individual grows older [30]. Cancer can be treated with surgery, radiation therapy, and/or systemic therapy (chemotherapy, hormone therapy, and targeted biological therapy). However, standard treatments, such as surgery, may cause side effects, including the inhibition of cellular immunity, reduction in the activity of natural killer cells, and reduction in the levels of anti-angiogenic substances [31-33]. Recently, viral vector gene therapy has attracted great attention as a unique therapeutic strategy for cancer because of the flexibility and effectiveness it provides [34, 35]. Most cancers, if detected at an advanced stage, cannot be treated using established therapy techniques. To increase tumour penetration and local amplification of the anticancer impact, oncolytic drugs were created, such as conditionally replicating adenoviruses (CRAds). Viral infection in tumour cells causes replication, oncolysis, and subsequent release of virus offspring. Importantly, this replication cycle enables significant local amplification of the input dose. In theory, CRAds will reproduce until all cancer cells are lysed [36]. On the other hand, oncolytic adenoviruses, like other kinds of oncolytic virotherapy, can elicit strong antiviral and anticancer immune responses in addition to tumour debulking. These viruses may convert a cool immunosuppressive tumour into one that is inflammatory [37, 38]. In other words, antitumor immunity is more significant than direct oncolysis because it allows the development of tumor-specific memory T cells [39]. In line with this, the 2018 Nobel Prize in Physiology or Medicine was awarded for the development of cancer therapy that inhibits negative immune regulation. Immune checkpoints (ICPs) play an important part in host defences aimed at eliminating harmful bacteria and microbial tactics, as well as managing the balance of tolerance, autoimmunity, infection, and immunopathology [40]. Antibodies targeting the T cell inhibitory checkpoint proteins, namely, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD1) protein, and the PD1 ligand (PDL1), have been approved for the treatment of a variety of cancers, including melanoma, non-small-cell lung cancer (NSCLC), head and neck cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma, and several types of tumours [41]. Adenoviral vectors may also be employed in therapeutic cancer vaccines. These vaccine adenoviral vectors can elicit both innate and adaptive immune responses in mammalian hosts

[42]. One example is ETBX-011, which was created to treat patients with malignancies that exhibit the carcinoembryonic antigen [43]. Another example is Ad-E6E7, which enhances the immune response against HPV-positive tumours [44]. In particular, significant progress has been made recently in using Ad-based vectors as a vaccine platform for HIV and cancer immunotherapy methods, as well as vaccination for other illnesses. The recent pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in the extraordinary creation of numerous vaccines. Among these vaccinations, Ad-vectored vaccines play a major part in global COVID-19 vaccine efforts. Examples include Ad26.COV2.S, ChAdOx1 nCov-19, Ad5 nCoV, and Gam-COVID-Vac vaccines, all of which have showed efficacy in guarding against symptomatic COVID-19 disease in humans [45]. Despite these accomplishments, the inherent and pre-existing immunity against Ad vectors remains a significant hurdle in the development and deployment of these vectors [46]. Furthermore, according to clinical records, the delivery of an adenovirus serotype 5 (Ad5) vector in a gene therapy trial caused deadly systemic inflammation in the victim [47]. One strategy to overcoming this barrier is to inject two or more antigenically different viruses in succession. This strategy would ensure that the specific immunity that emerges following the delivery of the first virus does not impair the therapeutic effects of the second virus [48]. Furthermore, many non-human advertising vectors have been proposed for development. Anurag Sharma et al. found that non-human adenovirus (Ad) vectors produced from bovine Ad serotype 3 (BAd3) or pig Ad serotype 3 (PAd3) may overcome pre-existing immunity against human Ad (HAd) [49]. Although adenoviruses are tissue-specific and flexible, an intravenous infusion of these viruses can cause severe liver injury, as has been shown in animal studies [50].In contrast to AAV, Ad has a short period of expression in vivo [51]. Ad vectors have been studied in rodents, primates, and humans, with varying results, highlighting the need for more detailed investigations into the natural history of Ad infection in humans, as well as questioning the value of animal models in determining the safety of viral vectors. However, establishing an ideal model that replicates human infection remains the primary goal of biomedical research.

LV Vectors

LVs belong to the orthoretroviridae subfamily of the genus retrovirus [52]. There are two major types of LVs: primary and non-primary [53]. The morphology and genomic organization of all LVs are identical in numerous ways: all LVs are pleomorphic spherical particles with sizes of roughly 100 nm [54]. A diploid genome consists of two single-stranded positive-sense RNA molecules. LV vectors typically contained the following required elements: 50 long terminal repeat (LTR) via the Y packaging signal, central polypurine tract/chain termination sequence (cPPT/CTS), Rev responsive element (RRE), and 30 LTR, containing the poly (A) signal [55]. A prior report went into detail into the classification and structures of various LVs.Currently, four generations of lentiviral vectors have been created. The first-generation lentiviral vectors included a considerable amount of the HIV genome and shown a high frequency of transfer of genetic material into the host cell [56]. The first-generation LV vectors featured the lentiviral accessory genes (vif, vpr, vpu, and nef), as well as the LV regulatory genes (tat and rev) [57]. In order to ensure greater safety, the second generation LV vectors were designed by deleting vif, vpr, vpu, and nef, which were present in the first generation

of LV vectors, as these are not necessary for the transfer of genetic material to the host cell [58]. Third-generation LV vectors are thought to be replication-inactive and self-inactivating vectors. This generation lacks the viral tat gene, which is required for wild-type human immunodeficiency virus type 1 (HIV-1) replication. Furthermore, the vector packing functionalities have been divided into three independent plasmids, rather than two, to lessen the danger of recombination during plasmid amplification and viral vector production. The vector is "self-inactivated" due to a changed 30 LTR, which inhibits the integrated genes from being repackaged. A heterologous coat protein [e.g., vesicular stomatitis virus G protein (VSV-G)] is utilised in place of the native HIV-1 envelope protein, and such vectors allow infection of a wide spectrum of host cell types. These factors make the third generation of LV vectors safer than the second generation LV vectors, allowing for the widespread use of the former [59]. The third-generation LV vectors make viral particles by combining four plasmids and a producer cell line. The purpose for incorporating four plasmids is to improve safety, because separating genetic components minimises the likelihood of recombination [60]. However, homologous recombination between the constructs is still conceivable because both transfer and structural packaging constructs contain the RRE region and a portion of the gag gene's packaging sequence. To address these problems, the RRE sequences were replaced with heterologous sequences that perform a similar function but do not require the REV protein. Codon optimisation is another way for fixing the aforementioned issue. These outlined answers resulted in the formation of the fourth generation of LV vectors. However, the titers had been compromised in the fourth-generation LV vectors, which has hampered their widespread adoption. [61]. LVs have several potential advantages over typical gene delivery techniques. Compared to adenoviral or adeno-associated vectors, neutralising antibodies are rarely developed against lentiviral vectors [62]. The most significant benefit of LV vectors is their ability to offer long-term and steady gene expression, which is critical for adolescents or paediatric patients; LV vectors are capable of infecting dividing/non-dividing cells, such as neurons [63] and osteocytes [64], and due to their relative low-immunogenic characteristics [65], LV vectors can accommodate constructions up to 9-10 kB in size. [66].LV vectors are primarily utilised in ex vivo gene therapies (Figure 3), such as those for B-cell acute lymphoblastic leukaemia (B-ALL) [67]. B-ALL is a clonal, malignant disease that starts in a single cell. B-ALL is characterised by the accumulation of blast cells that phenotypically resemble the normal stages of B-cell development [68]. B-ALL remains a prominent cause of non-traumatic death in children, and the majority of adults diagnosed with it die to the disease [69]. CAR-T therapy has produced outstanding clinical results in the treatment of B-ALL [70]. LVs are particularly interesting for developing CAR T-cells ex vivo because of their capacity to stably integrate relatively large DNA inserts [71]. Prior to transduction, CAR T-cell therapy isolates and activates the patient's T-cells, which might be CD4+ or CD8+. The CAR transgene is then delivered to activate T-cells using LV vectors and expanded. Finally, the generated CAR T-cells are formed in an adjusted buffer at a predetermined CD4+ ratio: CD8+ CAR T cells [72, 73].The current report does not provide a thorough description of modified CAR-T cells in cancer immunotherapy, but one can refer to other papers for that [74]. CAR-T cell therapy has opened up new possibilities for cancer treatment. In 2017, Novartis gained FDA approval for Kymriah (TM) (CTL019), a CAR-T cell treatment for children and young people with refractory or recurrent B-cell ALL. In 2019, the EMA approved Zynteglo, a

medicine used for the treatment of patients aged 12 years and older with transfusion-dependent _-thalassemia (TDT) who do not have a _0/_0 genotype and for whom haematopoietic stem cell (HSC) transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available. Zynteglo (betibeglogene autotemcel) is a genetically engineered autologous CD34+ cell-enriched population composed of HSCs transduced with the LV vector expressing the _A-T87Q-globin gene. LV vectors have also been established as effective gene transfer vehicles for human solid tumour cells, such as ovarian cancer cells, prostate cancer, and hepatocellular carcinoma [75].The major concerns associated with LV vector-based gene therapy include the possibility of generating replication-competent LVs during vector production, vector mobilisation by endogenous retroviruses in patient genomes, insertional mutagenesis that may lead to cancer, germline alteration resulting in transgenerational effects, and the spread of new viruses from gene therapy patients. LV vectors normally insert into host DNA as a single non-rearranged copy, and while these vectors are more stable and durable, the random insertion method still runs the danger of activating the malignant gene in the genome. Several Bluebird products based on lentivirus vectors have resulted in such situations in the clinic. For example, a patient was diagnosed with myelodysplastic syndrome [76]. Another patient developed acute myeloid leukaemia following treatment with LentiGlobin gene therapy [77]. Serious but tolerable side effects associated with CAR T-cell therapy for ALL patients include B-cell aplasia, tumour lysis syndrome, and cytokine release syndrome.

Essentially, the use of non-integrating LVs (NILVs) lowers insertional mutagenesis and the risk of malignant cell transformation caused by the integration of the lentiviral vectors [78]. As previously noted, the use of VSV-G modifies the inherent tropism of lentiviral vectors, allowing the infection of a broad range of host cell types [79], This means that targeting such viruses to certain cell types is problematic due to non-tissue-specificity [80,81]. However, the reported discrepancies could have been caused by differences in vector design, final formulation, immunomodulatory regimens (transient during vector administration), and surgical technique, among other things. With significant and detailed investigations on LV vectors in the last few years, this platform has been widely employed in both research and clinical trials. Although several issues remain to be resolved, safe and efficient LV vectors are seen as promising as a method for human gene therapy.

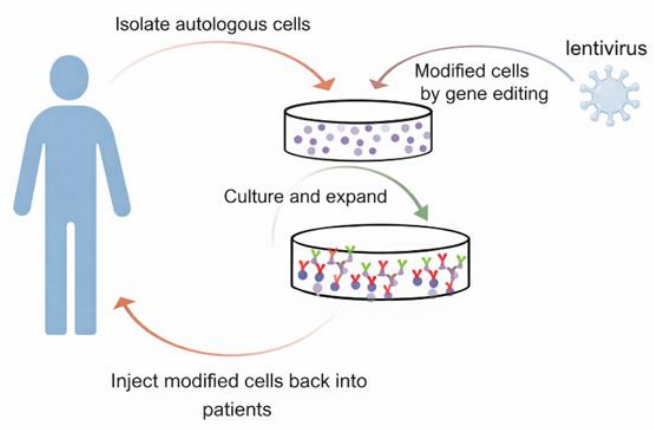

Figure 2. Schematic for the ex vivo strategies using LV vectors for treating genetic diseases. Cells are removed from the patient, genetically modified using LV vectors, and then returned to the patient

Other Viral Vectors

Other unidentified viral vectors include vaccinia, measles, herpes simplex, alphavirus, vesicular stomatitis, influenza, and baculoviruses. The vaccinia virus can preferentially replicate and propagate productively in tumour cells, resulting in oncolysis. Furthermore, its rapid viral particle production, broad host range, big genome size (about 200 kb), and safe handling make the vaccinia virus an ideal vector for gene therapy [82]. The measles virus is a non-integrating RNA virus with a lengthy track record of safety in humans when used as a vector for gene therapy. The measles virus provides a unique reprogramming platform for genomic modification-free iPSCs suitable for clinical translation [83]. The herpes simplex virus (HSV) has many benefits as a vector for delivering specific genes to the nervous system, including its vast size, wide host range, and ability to produce long-lived asymptomatic infections in neuronal cells [84]. Alpha-virus vectors are an appealing technique for gene therapy applications because they provide rapid and simple recombinant viral particle production and a wide spectrum of mammalian host cell transduction [85]. The vesicular stomatitis virus (VSV) is an appealing oncolytic virotherapy platform due to its powerful tumour cell-killing and immune-stimulating capabilities. However, this vector also poses several issues, such as poor systemic delivery, which may produce severe side effects, including neurotoxicity [86]. The influenza virus is a respiratory pathogen with a negative-sense, segmented RNA genome. The first reports of laboratory-created recombinant influenza viruses date back to the 1980s. Various gene alterations produce influenza viruses with reduced pathogenicity, increasing the safety profile of the influenza virus vector for application in cancer gene therapy [87]. Baculovirus has been routinely employed for several years to produce recombinant proteins in insect cells. It has also been evolved into safe and efficient vectors for gene transfer, providing advantages such as broad entry tropism and replication deficit in mammalian cells. [88]. Different viral vectors have various benefits. Because of their distinct properties, these viral vectors can be used in a variety of gene therapy applications. The currently approved viral vector-based gene therapy products are mentioned here. These viral vectors enhance the medications employed in illness therapies, making them more diverse and selective.

Non- Viral Vectors

Twenty-twenty marks another milestone in the realm of gene therapy. The FDA authorised Tecartus, the first cell-based gene therapy with a stage of enriching white blood cells, in July [89]. From the momentum of the first successful clinical trial of gene therapy in 1990 to the terrible death at the University of Pennsylvania in 1999, which halted work on the technique, gene therapy has come a long way to rise again in 2017, with three FDA approvals in a single year. Currently, there are ten gene therapy drugs on the market in the United States, with over 100 clinical trials enrolling patients on clinicaltrials.gov. The turbulence of gene therapy over the last 30 years has provided several learning opportunities and laid the groundwork for the newly rekindled optimism about the capacity of gene delivery systems to treat some of the most terrible diseases. While a few recent clinical trials have used non-viral vectors, most recent clinical studies use traditional viral vector systems, which are difficult to manufacture cost-effectively on a commercial scale [90]. As we all know, a vector is a carrier that transports genetic material to its intended

destination. It contributes significantly to the product's efficacy and safety. The use of a viral vector has always been fraught with controversy. Even though viral vectors are not known to cause any harm to patients, the tiny potential to trigger immunogenic responses and transgenic mis-insertion hazards, among other things, has led many in the field to continue seeking a non-viral delivery mechanism [91]. In recent years, polymers, lipids, inorganic particles, or mixtures of these have been extensively studied as non-viral vectors. Non-viral vectors have lower cytotoxicity, immunogenicity, and mutagenesis than viral vectors, enticing more researchers to investigate this promising delivery mechanism and advance the gene therapy field. However, non-viral vectors may not have optimal properties and have encountered significant problems, including gene transfer efficiency, specificity, gene expression length, and safety. Non-viral vectors have been a rapidly growing research issue in gene delivery.

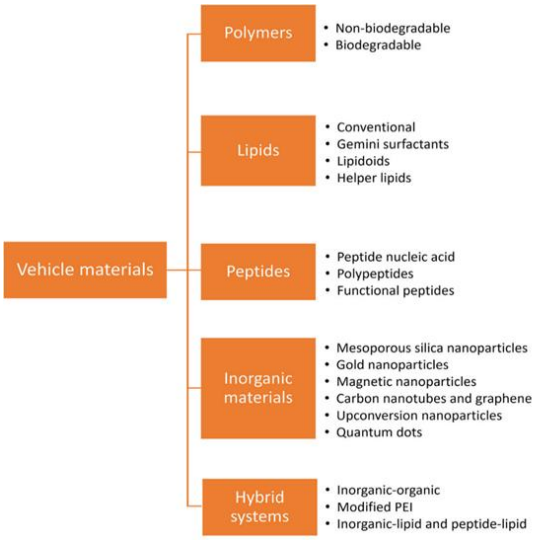

Figure 3. Vehicle materials

MATERIALS

Polymers

Gene therapy entails delivering genetic material into cells, transfecting it, and modulating gene expression. Cationic polymers have long been used as a non-viral gene therapy vector, and their diverse chemical structure and possible high loading capacity have piqued researchers' interest. They can neutralise the negatively charged genetic material, forming a complex (polyplex), and transporting the payload to the target cells.

Non-Biodegradable Polymers

Polyethylenimine (PEI) was the first polycationic polymer to be synthesised in both linear and branched forms for gene therapy in 1995. It has amine groups organised in a unique pattern on the polymer chain's backbone, allowing only partial protonation within the physiological pH range. However, in the endosome's more acidic compartment, more amine groups are protonated. The presence of charged PEI causes an osmotic effect ("proton sponge effect") that induces endosome burst, which is thought to improve transfection effectiveness. PEI's high buffer capacity is also useful for the endosomal escape of the gene payload [92]. Today, PEI is the gold standard for assessing the transfection effectiveness of non-viral vectors [93]. Despite being a high transfection non-viral vector, PEI may still have insufficient specificity and transfection. In addition, it is a non-biodegradable polymer that accumulates surrounding the cell and

causes cytotoxicity. Scientists have made great progress in understanding the mechanisms that cause these limits over the last few years. Clark et al. investigated the ability of PEI to escape late endosomal vesicles during intracellular distribution, as well as its interaction with endosomal lipids under osmotic stress, utilising model systems of monolayers and vesicles produced from a mixture of neutral and negative lipids. 1,2-dipalmitoylphosphatidylcholine (DPPC) and bis(monoacylglycerol)phosphate (BMP), respectively [94]. The findings supported PEI adsorption to DPPC/BMP membranes, which is a crucial factor in polyplex escape from endosomes. The model has provided a novel tool for investigating the impact of nonviral vectors on membrane stability and permeability. In addition to PEI, amine-terminated PAMAM is another cationic dendrimer. In fact, PAMAM is one of the most widely utilised dendritic carriers in biological applications, and it was the first to be used for gene delivery. One important downside of these typical dendrimers is their toxicity, which is mostly caused by the chemistry of the surface amine groups. Furthermore, polymethacrylates and polymethacrylamides are two significant types of important synthetic vinyl-based cationic polymers that can replicate the pH sensitivity, proton sponge theory, and buffering capabilities of PEI [95]. They have been continuously updated over the last 20 years in an attempt to improve gene delivery efficiency and reduce toxicity [93]. Although polymethacrylates are less cytotoxic than PEI, their application in gene therapy is currently limited due to their poor capacity to interact with membranes [96]. Poly (vinylimidazole) (PVI) is a water-soluble polymer derived from poly (1-vinylimidazole) and poly (4-vinylimidazole). At acidic pH levels, the imidazole group becomes protonated, altering the structure of PVI chains. PVI possesses extra biocompatibility, non-toxicity, and the ability to exit the endosome by activating the proton sponge mechanism, making it a developing non-viral vector [97]. Recently, alkylated poly (1-vinylimidazole) with varying chain lengths was studied for DNA complexation and transfection. Butylated PVI proved to be the most effective in HepG2 liver cancer cells. In addition, a folic acid-conjugated amine-containing poly (1-vinylimidazole) was found to successfully complex DNA and transfect cancer cells [98].

Biodegradable Polymers

Given that repeated administration is often necessary for gene therapy and less cytotoxicity is preferred, biodegradable polymeric vectors, either synthetic or natural, have a definitive advantage over non-biodegradable ones. The synthetic polymers have excellent chemical structure versatility and batch consistency, but may lack sufficient interaction with cells [99]. In contrast, the natural polymers have high biocompatibility but pose batch to batch variation problems due to the origin difference. Hence, to assure product quality, control strategies on the key attributes of natural polymers need to be put in place. The excipient companies sometimes use blending to meet the excipient specifications. In addition, appropriate tests and specifications are used to ensure consistent quality and reliable performance. For example, gel permeation/size exclusion chromatography (GPC/SEC) may be applied to measure the characteristics of the polymers. It will take a collaborative effort among pharma companies, excipient suppliers, the US Pharmacopeial Convention (USP), regulators, and the International Pharmaceutical Excipients Council (IPEC) to control, reduce, or minimize the possible negative impact of excipient variability on the natural excipients, including polymers [100]. Chitosan (CS) is a linear polysaccharide and one of the most abundant natural carbohydrate polymers. It is highly biodegradable,

biocompatible, and non-toxic. With an apparent pKa of 6.5, it is only soluble in acidic conditions where most of the amino groups are protonated to form a complex with the genetic material. It was found that chitosan with a high degree of polymerization (>50) could induce a significant opening of the tight junction between cells [101]. The surface of a chitosan carrier can also be modified or decorated with ligands to enhance cellular entry and specificity [102]. These properties have made chitosan an attractive non-viral vector for gene therapy. In recent years, chitosan-coated nanoparticles are actively studied as carriers for brain cancer gene therapy, where enhanced particle uptake was evidenced by human blood-brain barrier cerebral microvessel endothelial cells (hCMECs) via receptor-mediated endocytosis [103].

Poly (β-amino esters) (PBAEs) are a class of evolving non-viral vectors that have made significant advancements in the past 20 years. The class was first developed into linear PBAEs in 2000, but the development of this class transitioned to branched PBAEs in 2016 [104]. These amphiphilic polymers have shown robust transfection capabilities under challenging conditions as well as efficient endosomal escape properties. However, their application is limited due to forming self-assembled nucleic acid nanoparticles. Thus, they are insufficient to encapsulate proteins of various surface charges. In 2019, Green et al. synthesized a new hyperbranched PBAE containing both cationic and anionic charges. The structural change has offered the differentiation of polymer end-group hydrophobicity, affected protein complexation capabilities, as well as nanoparticle internationalization, and endosomal escape [105]. In the same year, Liu et al. synthesized highly branched PBAE containing biodegradable disulfide units in the HPAE backbone and guanidine moieties at the extremities. Those polymers delivered a minicircle DNA to multipotent adipose-derived stem cells and astrocytes, achieving high transfection efficiency [106]. Traditionally, polylactide (PLA) is a synthetic biodegradable polymer extensively applied to drug delivery. Its carboxylic acid hydrolyzes into lactic acid in vivo and rapidly converts to glucose eliminated from the body without adverse effects. In 2013, Jones et al. synthesized a cationic polylactide with tertiary amines to make it suitable for gene therapy [107]. Nowadays, PLA draws continued interest in targeted delivery through continual structure modifications. The full potential of polymer-based delivery systems has yet to be realized. In 2020, aminoglycosides, a class of naturally occurring and semi-synthetic antibiotics, have been investigated as new cationic polymeric vectors to facilitate the transfer of genes into cells [108].

Lipids

Lipids have been used to deliver genes for a long time. Most lipids consist of positively charged head groups which bind with the anionic phosphate groups of nucleic acids via electrostatic interactions to form lipoplexes. Due to the self-assembling lipid tail structures, lipoplexes are often present as liposomes, solid lipid nanoparticles, or lipid emulsions. Compared with other carrier materials, lipids are biodegradable, less toxic, and can incorporate hydrophilic and hydrophobic substances. The first FDA-approved small interfering ribonucleic acid (siRNA) treatment (Onpattro) utilized a lipid-based vector [109]. Another promising lipid-based siRNA therapy (inclisiran) for hyperlipidemia treatment was approved in the EU in December 2020 [110]. Phase 3 clinical trials have shown that inclisiran lowered the low density lipoprotein cholesterol

levels by 50% by subcutaneous administration every 6 months [111].

Conventional Lipids

Conventional lipids possess one head group on each molecule, which can be permanently or temporarily charged. The common head groups are ammonium, imidazolium, pyridinium, lysine, or arginine, etc. Meanwhile, the hydrophobic tails can be two saturated or unsaturated hydrocarbon chains or steroids [109]. The ability of hydrocarbon chain lipids to deliver nucleic acids has been widely explored, especially those with ammonium as head groups. Common examples include monovalent lipids such as N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium (DOTMA),

2,3-bis[[(Z)-octadec-9-enoyl]oxy]propyl-trimethylazanium (DOTAP), 2,3-di(tetradecoxy)propyl-(2-hydroxyethyl)-dimethylazanium (DMRIE), 1,2-dipalmitoyl-sn-glycero-3- phosphocholine (DPPC), and 1,2-dioleoyl-sn-glycero-3- phosphocholine (DOPC), or multivalent lipids such as 2,5- bis(3-aminopropylamino)-N-[2-[di(heptadecyl)amino]-2- oxoethyl]pentanamide (DOGS). While those lipids remain dominant as gene carriers due to their positive charges, they are relatively toxic and exhibit less than optimal in vivo behavior, e.g., short half-life. Therefore, the surface-modified ionizable lipids, such as 1,2-dioleoyloxy-3-(dimethylamino)propane (DODAP) or 1,2-dilinoleyloxy-3- dimethylaminopropane (DLin-DMA), were developed to overcome those shortcomings and achieve better efficacy. Those materials are neutral at physiological pH, allowing systemic delivery but can be positively charged to facilitate lipoplex formation with DNA and promote endosomal escape. It is worth noting that heptatriaconta-6, 9, 28, 31- tetraen-19-yl 4-(dimethylamino) butanoate (DLin-MC3- DMA) is the "gold standard" for siRNA delivery because of its superior gene silencing activity relative to its comparators [112] and was successfully applied in Onpattro, the first FDA-approved siRNA treatment. In addition to those hydrocarbon chain lipids, cholesterol and its derivatives are another category of lipids that can be used for gene delivery. One of the derivatives, DC-Chol, is now commercially available and has been utilized for cancer gene therapy in clinical trials [113].

Peptides

Peptides are short chains of 2–50 amino acid residues linked via peptide bonds. They are biocompatible and biodegradable and also can be rationally designed to serve as building blocks for self-assembling nanoscale structures [114]. The genetic material interacts with peptides either via conjugation or electrostatic forces to form peptiplexes which facilitates delivery. Peptide nucleic acid (PNA) conjugates are comprised of peptide moieties and nucleic acid moieties linked via covalent bonds. They are stable uncharged molecules, able to resist nuclease degradation and less labile to acidic and basic pH, as well as high temperature. Recently, Altrichter and Seitz prepared an antisense module based on peptide nucleic acids comprising a Smac mimetic compound (SMC) [115]. By incorporating cell-penetrating peptides, the SMC-PNA resulted in nearly complete downregulation of the cellular FLICE-like protein. Polypeptides can further be designed into dendrimers, which use amino acids as building blocks in the core, the branches, the dendrimer surface, or any combination of the three units. Peptide dendrimers may provide the necessary positively charged groups to complex with the genetic material, the likelihood to pass the cellular membrane, and the buffering capacity needed to escape endosomes. For example, a PLL dendrimer may utilize the flexible branched

dendrimer structure and the amino acid lysine in its core [116]. Recent studies have expanded the choice of amino acid from lysine to arginine or other substitutions, bringing additional benefits to the PLL dendrimer as a gene delivery vector. These efforts have changed the flexibility and charge distribution of the dendrimer, providing additional interactions with nucleic acids and increasing cellular uptake [117]. Dendrimer systems can also incorporate lipid or polymers to obtain greater efficacy. It has been demonstrated that transfection was enhanced by the addition of a polyol to the lipid/dendrimer hybrid or a polymer excipient, such as Polyvinylalcohol 18 (PVA 18) [118]. In addition to polypeptides, a number of functional peptides have also been developed. These peptides possess certain sequences in their structure, resulting in various benefits such as enhanced cell penetration or targeting. For instance, cell-penetrating peptides (CPP) are small peptides that can easily move across cell membranes and facilitate genetic material transport. As a commonly used CPP, Trans Activator of Transcription (TAT) protein was recently evaluated on solid tumors using the multicellular tumor spheroids as cell models. It showed that higher TAT concentrations significantly increased peptide uptake [119]. In addition to TAT, penetratin, GALA, transportan, and its derivatives such as PepFect and NickFect have also received attention for their cell penetration abilities [120]. Some other peptides can target specific cells by recognizing receptors at the cell surface, resulting in enhanced efficiency and reduced toxicity. Various targeting peptides have also been discovered or synthesized, such as the RGD peptide and transferrin (Tf). Additional Tf receptor–binding peptides are drawing interest to improve targeting capability, such as the T7 peptide. Gu et al. applied T7-modified polypeptide nanoparticles, CRDPEG-T7, to deliver the pDNA pPMEPA1 for bone metastatic prostate cancer treatment. They found the incorporation of T7 inhibited tumor growth and extended survival time of tumor-bearing mice [121].

Inorganic Materials

Inorganic materials are more stable than organic materials and have also been used as gene carriers. In fact, the first reported non-viral gene delivery was based on calcium phosphate, back in the 1960s [122]. Nowadays, the more commonly reported inorganic carriers include silica-based systems, such as mesoporous silica nanoparticles, gold nanoparticles, magnetic nanoparticles, carbon nanotubes, graphene, upconversion nanoparticles, and quantum dots [123]. Gold nanoparticles are relatively less toxic and can be prepared with polymeric and lipid carriers. Liu et al. constructed a gold nanoparticle composite for Parkinson's disease treatment, where pDNA was adsorbed onto the surface of positively charged gold nanoparticles and encapsulated into liposomes, followed by attaching a targeting NGF and DHA. It was noticed that this system exhibited significant neuroprotective effects for mice by improving both motor and non-motor dysfunction [124]. Carbon nanotubes are another attractive carrier, composed of single or multiple graphene sheets that can range in size from hundreds of nanometers to tens of microns. Carbon nanotubes facilitate gene penetration through the cell membrane independent of the endocytosis process of mammalian cells. With the help of molecular dynamics simulation, Liang et al. found that carbon nanotubes assisted nucleotide penetration through a lipid membrane by decreasing the free energy of this process [125]. In 2020, a single-walled carbon nanotube linked with siRNA from Caspase3 was synthesized to treat cardiovascular diseases. This gene carrier significantly improved transfection effi- ciency, resulting in greater Caspase3 gene silencing [126].

HYBRID VECTOR SYSTEMS FOR TRANSFECTION ENHANCEMENT AND CYTOTOXICITY REDUCTION

Non-viral vectors having an efficient gene transfection and low cytotoxicity have been a double-edged sword at the forefront of gene delivery. Some of these recent attempts to enhance transfection efficiency while lowering the cytotoxicity include inorganic-organic hybrid vectors, modified PEI vectors, inorganic-lipid vectors, and peptide-lipid vectors.

Inorganic-Organic Hybrid Vectors

Inorganic-organic hybrid vectors have been identified as an increasing trend in the new class of non-viral vectors, with either a platform or targeted gene deliveries, to various cell types. One attempt was to incorporate a mannitol-group into the vector. In 2020, Ma et al. were inspired by multi-hydroxyl compound mannitol being used as an osmolyte in the clinic [127]. They constructed biomimetic non-viral vectors with a controlled cellular uptake and consequent intracellular traf-ficking for the gene delivery and introduced for the first time mannitol-based calcium phosphate mannitol-alendronate (CaP-MA) organic-inorganic non-viral vectors. The new vectors with mannitol groups may simulate caveolaemediated cellular update and transfer the genetic payload in a non-destructive pathway and subsequently avoid gene degradation in the lysosome. As a result, these vectors are shown to be superior to the unmodified CaP nanoparticles in transfection, biocompatibility, and toxicity. Another attempt at cytotoxicity reduction was to use amine-free vectors to counter amine-associated toxicity [128]. Choi et al. have developed a non-conventional non-viral vector, using mesoporous silica nanoparticles (MSNs) as a biocompatible agent, to load siRNA in a sequential, cumulative, and directional way. It was established through calcium ion–mediated interconnection (calcium gluing) between phosphates of siRNA and the non-positively charged bare surface of MSN. The results suggested the potential of the nonpositively charged MSN-based calcium-gluing strategy as a general non-viral vector platform for RNAi delivery, which may open the door for additional investigation of its application in gene delivery.

Modified PEI Vectors

The most significant challenge for PEI vectors is cytotoxicity due to the presence of non-degradable amide bonds, causing accumulation and disruption of metabolic activity in normal cells. It is also known that the extent of cytotoxicity has a positive relationship with polymer molecular weight. Hence, a promising strategy to reduce toxicity lies in ligand-modified low molecular weight PEIs, such as those incorporating folic acid [129] or various functional peptides.Yu et al. have demonstrated that self-assembled nanoparticles (SNPs) prepared from cyclodextringrafted low molecular weight PEI (CD-PEI800) presented low cytotoxicity and a high transfection efficiency to Jurkat cells. They postulated that the cationic hydrogel generated from CD-PEI800 contributed to SNPs' enhanced gene encapsulation efficiency [130]. The first trisaminocyclopropenium-crosslinked linear PEI (PEI/TAC) nanoparticle vector was synthesized by Steinman et al. to lower the PEI toxicity in 2020 [131]. The vector particles with DNA were found to be smaller than those prepared with the unmodified PEI, which positively influenced cellular uptake. The cross-linking by TAC increased the cationic charge of the polymer, allowing binding to the genetic material and promoting endosomal escape, and resulting in enhanced overall transfection efficiency. Meanwhile, the decrease in the amount

of polymer introduced to the cell further reduced the toxicity effects.Another way to keep the PEI biocompatible and lower toxicity is through a combination of poly lactic-co-glycolic acid (PLGA) nanoparticles with arginine-modified PEI polymers (AnPn) [132]. The addition of AnPn significantly improved the nuclear localization of pDNA and successful gene expression in primary human astrocytes.

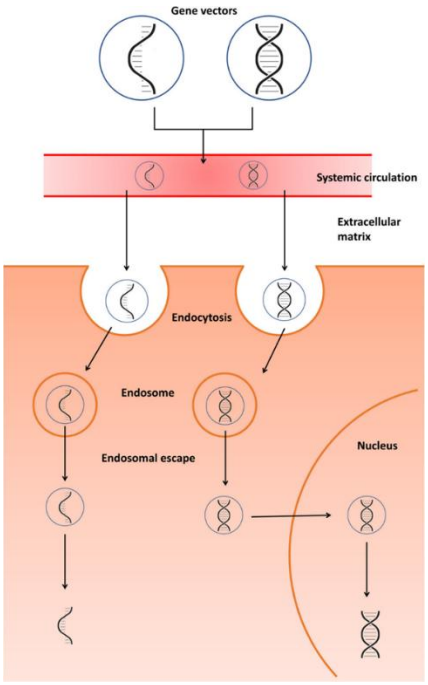

Figure 4. Delivery mechanism for non-viral gene vectors

Applications:

Acquired Tissue Damage

Prevention of Irradiation Damage to SGs

Radiotherapy is used to treat the majority of head and neck cancers. Most patients receive between 50 and 70 Gray (Gy) of irradiation (IR), which typically is divided into doses of 2 to 2.5 Gy/day, 5 days a week, for 5 to 7 weeks [133]. Unfortunately, normal SG tissue in the IR field is damaged, and patients suffer considerable morbidity from the IR induced salivary hypofunction. Therapeutic IR generates double-strand DNA breaks in target cells and also results in oxidative stress via the generation of potentially damaging free radicals. Cells that divide more rapidly (e.g., cancer cells) are usually considered more sensitive to IR. The relative radio sensitivity of a cell is cell-cycle dependent, with cells' being most radiosensitive in the G (2)-M phase. SGs are considered to be postmitotic, well-differentiated epithelial cells with a slow turnover rate. Therefore, it is expected that SGs would be relatively radio-resistant. However, SGs are extremely sensitive to IR, and the mechanism of this damage is still not clear [134].

Repair of SG Damage from IR

A major focus of our work has been to restore SG function in patients who have already received IR. For this goal, our strategy has used transfer of the aquaporin-1 (AQP1) complementary DNA (cDNA). AQP1 was the first water-channel protein discovered [135]. SGs present in the IR field show a dramatic loss of acinar cells; acinar cells are considered water-permeable secretory epithelia. Ductal cells typically survive the IR, but they are

considered to be relatively water-impermeable absorptive epithelia. We reasoned that duct cells in an IR-damaged SG would be capable of generating an osmotic gradient sufficient to allow the movement of water in a basal to apical direction, that is, into the lumen. We speculated that the gradient would be based on forming potassium bicarbonate in the lumen: potassium entering the lumen in exchange for a proton via a potassium-proton exchanger present in the apical membranes of duct cells. We further hypothesized that all that was lacking for the duct cells to secrete fluid in an IR-damaged gland was a facilitated water permeability pathway, a water channel protein. We constructed an Ad5 vector encoding human AQP1 (AdhAQP1) and showed that this vector leads to a dramatic increase in fluid secretion when administered 90 or 120 days after IR in rats or miniature to ~80% of control levels when measured 3 days after transduction[136]. A control Ad5 vector was without any significant effect on salivary flow. Additionally, after administration of AdhAQP1 to SGs, no significant toxicological effects were observed, that is, in multiple measured clinical chemistry and hematology values [137]. (Vector safety with some efficacy measures) clinical trial protocol to test AdhAQP1 in patients who received IR for head and neck cancer at least 5 years previously. Although Ad5 vectors only lead to transient gene expression, because of the generated immune response, we chose this type of vector because very little is known about human ductal cell physiology. We assume that human duct cells generally will be similar to those of rats and miniature pigs, that is, also able to generate an osmotic gradient and fluid flow as described above. However, we do not know that. In the event that human ductal cells are incapable of this response, the AdhAQP1 presence in the tissue will be relatively limited because of the immune response, which we consider an important safety consideration in the absence of any benefit. However, if irradiated human SGs are able to secrete fluid following AQP1 gene transfer, we have developed an AAV2 vector capable of mediating long-term AQP1 expression, and presumably, providing patients with the stable SG repair required. This vector includes the same promoter, AQP1 cDNA, and polyadenylation signal as AdhAQP1 [138].

Infections of the Upper GI Tract

Although rapid advances have been made in the detection, management, and biology of HIV-1, even today, oral candidiasis remains a common opportunistic infection observed among immunosuppressed patients. In HIV-1–infected patients, this can lead to significant morbidity [139]. Antifungal azole-type drugs are the principal management tool for such candidal infections. However, the occurrence of azole resistant *Candida* species necessitates the development of alternative treatment strategies. istatins are a family of histidine-rich, cationic peptides composed of up to 38 amino acids. They are secreted by the SGs of humans and some primates and are a major component of the innate host nonimmune defense system in the oral cavity against bacterial and fungal infections. The importance of histatins in azole-resistant candidiasis is twofold. Histatin levels in saliva are reduced in HIV-1–infected patients. Secondly, the mechanism of action of histatins in targeting candidal species is distinctly different from that of azole-type drugs. While azole drugs inhibit the synthesis of ergosterol, a major plasma-membrane sterol, histatins act by binding to the ergosterol present in the fungal membrane. We reasoned that transfer of the histatin-3 cDNA to SGs would result in an increased secretion of histatins in the oral cavity and be useful in managing azole resistant candidal species [140].In animal model studies, we successfully expressed histatin-3 in rat

SGs using an Ad5 vector (AdCMVH3). The concentration of histatin-3 in rat submandibular-gland saliva collected from treated rats 3 days after transduction with the AdCMVH3 was as high as 1 mg/ml, with a mean value of 302 g/ml. The fungicidal activity of the recombinant histatin-3 mediated by the AdCMVH3 vector was tested in vitro in a timed-kill assay. At a concentration of 100 g/ml, 90% of the azole-resistant *Candida* albicans were killed in 60 minutes [141].

Autoimmune Disorders

Sjogren's syndrome (SS) is the second most common autoimmune disease in the United States, affecting between 1 million and 4 million persons, primarily female (~90%). The etiology of SS is unclear, and current treatment is only palliative. SS is characterized by the presence of a focal lymphoid cell infiltration in the salivary and lacrimal glands, although other organs may also be involved. In the absence of any suitable conventional treatments, we have suggested that gene therapy may be beneficial for SS patients. We have hypothesized that transfer of immunomodulatory genes into SGs may reduce the autoimmune sialadenitis and lead to increased salivation as well as symptomatic relief. For example, the transfer of genes encoding antiinflammatory cytokines such as interleukin-10 (IL-10) or vasoactive intestinal peptide (VIP) could lead to a decrease in the expression of proinflammatory cytokines, and thus, protect SGs and preserve their secretory function [142].To test this hypothesis, we used a common animal model of SS, the female nonobese diabetic (NOD) mouse. We delivered the human (h) IL-10 and VIP cDNAs using AAV2 vectors because they provide stable transgene expression with little immune reactivity. Both AAVhIL-10 and AAVhVIP, as well as a control vector, AAVLacZ encoding galactosidase, were administered locally via retrograde cannulation of the submandibular glands. We compared salivary flow and sialadenitis ~8–12 weeks later. Administration of AAVhIL-10 led to preservation of salivary flow rates as well as a reduction of the focal autoimmune sialadenitis. Administration of AAVhVIP also resulted in a preservation of salivary flow; however, no reduction of the focal sialadenitis was observed with this transgene [143]. These initial studies show that immunomodulatory gene transfer may be useful in managing the autoimmune sialadenitis and resultant salivary hypofunction that occur in SS patients. Nonetheless, since we do not understand SS pathogenesis, this gene-transfer strategy is nonspecific and still requires considerable study.

Systemic Protein Deficiencies

As mentioned previously, SGs show several features that are common to many endocrine glands, particularly the ability to produce high levels of protein for export and the ability to secrete proteins into the bloodstream. We have suggested a therapeutic application to take advantage of these features: the treatment of systemic single-protein deficiency disorders, systemic single-protein deficiency disorders. Current treatment of these conditions involves the regular administration of a recombinant protein by bolus injection (e.g., insulin for diabetes mellitus and erythropoietin [Epo] for anemias related to chronic renal failure). For example, many of our studies have involved transferring the cDNA for Epo. Epo is produced in kidney epithelial cells and secreted by the constitutive secretory pathway into the bloodstream. In SGs, after gene transfer, much Epo is also secreted into the bloodstream [144].

CONCLUSION

The paper emphasizes how important it is to advance gene therapy using both viral and non-

viral vectors. Viral vectors including AAV, adenovirus, and lentivirus have great therapeutic promise and high gene delivery efficiency, particularly in the treatment of immunological illnesses, cancer, and genetic disorders. However, they have drawbacks like as toxicity, immunogenicity, and safety issues including insertional mutagenesis. Conversely, non-viral vectors, such as polymers, lipids, peptides, and inorganic materials, provide superior biocompatibility, reduced toxicity, and increased safety. Notwithstanding these benefits, they have drawbacks including reduced gene transfer efficiency and short gene expression lifetime. In general, the goal of ongoing research and the creation of hybrid systems is to maximise the advantages of both strategies while reducing their disadvantages. This development is anticipated to improve gene therapy's safety, effectiveness, and therapeutic applicability, making it a potential approach for the future treatment of a variety of illnesses.

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