**ABSTRACT**

Gene therapy involves the introduction of a normal, functional copy of a gene into a cell in which that gene is defective. This can be accomplished with a variety of viral vectors or nonviral administrations. While originally aimed at treating life-threatening diseases (inborn errors, cancers and hematological diseases like anaemias and thalassaemias), it is now considered for many conditions including dermatologic conditions (as epidermolysis bullosa, ichthyosis and xeroderma pigmentosa) and non dermatologic conditions (as acquired tissue damage, immunological disorders and systemic protein deficiency). It is also used in gene vaccination.

**KEY WORDS:** Gene therapy, Dermatology

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**INTRODUCTION**

The advances in researches in genetics have led to the increased interest in the field of gene therapy in both dermatologic and non-dermatologic conditions. Gene therapy was introduced to correct hereditary condition by replacing the absent or defective genes in these disorders. However, the use of gene therapy is not limited to the correction of genetic disorders, but also involves genetic vaccination, cancer treatment and immunomodulation.¹

**DEFINITION**

Gene therapy is broadly defined as using a vector to introduce a normal, functional copy of a gene into a cell in which that gene is defective, with the intention of altering gene expression to prevent, halt, or reverse a pathological process. Cells, tissue, or even whole individuals (when germ-line cell therapy becomes available) modified by gene therapy are considered to be transgenic or genetically modified.² Gene-based therapies depend on several critical elements. First, one must have a disease gene. Second, one must have a therapeutic gene. Third, gene therapy requires an efficient delivery system. This delivery system may be a virus or a formulated nucleic acid.³

**HISTORY**

It is important to remember that gene therapy is not a new idea. In 1963, Joshua Lederberg anticipated the interchange of chromosomes and segments. Less than 30 years later, the first clinical study using gene transfer was reported.¹ Rosenberg and his colleagues⁴ used a retroviral vector to transfer the neomycin resistance marker gene into tumor-infiltrating lymphocytes obtained from 5 patients with metastatic melanoma. These lymphocytes then were expanded in vitro and later re-infused into the respective patients. Showing that retroviral gene transfer was...
safe and practical, this study led to progressive studies. The first real case of gene therapy occurred in 1990, when a four-year-old patient with a severe immune system deficiency (adenosine deaminase enzyme [ADA] deficiency or bubble-boy disease) received an infusion of white blood cells that had been genetically modified to contain the gene that was absent in his genome.

TYPES OF GENE THERAPY
The two main types of gene therapy are reproductive or germ-line gene therapy and somatic cell gene therapy:

1. **Germ-line gene therapy**
   Germ-line gene therapy involves the introduction of corrective genes into reproductive cells (sperm and eggs) or zygotes, with the objective of creating a beneficial genetic change that is transmitted to the offspring. When genes are introduced in a reproductive cell, descendant cells can inherit the genes.

2. **Somatic cell gene therapy**
   Gene therapy of somatic cells, those not directly related to reproduction, results in changes that are not transmitted to offsprings. An example of gene therapy in somatic cells is the introduction of genes in an organ or tissue to induce the production of an enzyme. With somatic cell gene therapy, a disabled organ is better able to function normally. This technology has many applications to human health. One variant of somatic cell gene therapy is DNA vaccines, which allow cells of the immune system to fight certain diseases in a method similar to conventional vaccines.

Stem cell therapy involves the use of pluripotent cells, or cells that can differentiate into any other cell type. They are found in developing embryos and in some tissues of adult individuals. This therapy is similar to a conventional transplant, with the objective of regenerating or repairing a damaged organ or tissue. The procedure has a reduced probability of rejection because it uses the individual’s own cells. Stem cells differentiated into nerve cells could be used by patients suffering from paralysis, with the goal of helping them recovering movement. Similarly, muscle cells might be used to rejuvenate the cardiac muscles in cases of heart stroke.

TYPES OF VECTORS
Appropriate methods to deliver DNA used in gene therapy are vital. Gene therapy can be carried out using naked DNA delivered directly, however introducing isolated DNA molecules has a very low efficiency rate. To increase the efficiency of DNA uptake by the target cells, special molecules have been engineered. Molecules used to move recombinant DNA from one cell to another is called a vector. There are two general types of vector: viral and non-viral

1. **Viral vector**
   Viral vectors are the most effective vehicles of gene transfer because of their inherent ability to efficiently infect cells. The viruses possess a gene for production of the reverse transcriptase, an enzyme that transcribes RNA in DNA in the host cell.

Viral vectors have the advantage of achieving highly efficient gene transfer in vivo. Although replication deficient vectors are used, many concerns about safety of viral vectors are still present. Numerous viruses are under investigation for gene delivery, but the most commonly used viruses to target cutaneous tissue are retrovirus-
es, adenoviruses (AdV), adeno-associated viruses (AAV) and herpes simplex virus.

**Retrovirus**

Retroviruses have the longest history of use in gene therapy and are still the most frequently used therapy. A retrovirus is a special class of RNA viruses that can insert its nucleic acid into host cells. Retroviruses used in gene therapy are engineered so that any genes that are harmful to man are removed.\(^\text{10}\)

Retroviruses having RNA are converted by a ‘reverse transcriptase’ to DNA which attaches to host DNA by an ‘integrase’.\(^\text{11}\) They in turn break a growth regulator gene leading to uncontrolled growth. General design of retroviral vectors minimizes the potential to form a replication competent retrovirus (RCR). Vector construction should retain several elements that are important for the viral life cycle, such as RNA packaging signals and cis-acting viral sequences, such as 50- and 30- long terminal repeats (LTR).\(^\text{12}\) They include oncoretroviruses and lentiviruses.

The best example of an oncoretrovirus is the Moloney murine leukemia virus (MoMLV), whereas lentiviruses originate from human immunodeficiency virus (HIV).\(^\text{13}\) Both oncoretroviruses and lentiviruses delivered ex vivo provide the capacity for therapeutic gene expression in skin regenerated from transduced keratinocytes (KC) for several epidermal turnover cycles proving successful targeting of epidermal progenitor cells.\(^\text{14,15}\)

MoMLV vectors target dividing cells with a reasonably high degree of efficiency. They also lead to stable gene transfer because they integrate randomly into chromosomes of the target cell. A major disadvantage of MoMLV vectors is the risk of insertional mutagenesis caused by the integration of the retroviral genome into the host genome. Also, since retroviral vectors require dividing cells for successful transduction, they are not useful for targeting gene transfer to well-differentiated, quiescent cell types, such as in epithelial tissues.\(^\text{16}\)

HIV based viruses have a pronounced advantage over oncoretroviruses, namely the ability to infect nondividing cells owing to their ability to deliver the viral preintegration complex (PIC) across the nuclear membrane. Because epidermal stem cell populations have a low rate of mitotic activity, lentiviral vectors are more attractive for in vivo therapy used vectors for cutaneous gene transfer.\(^\text{17}\) Lentiviral vectors are more complex because of accessory proteins and sequences that allow nuclear import of viral PIC.\(^\text{18,19}\)

Additional genetic engineering of the targeting construct is directed to create self-inactivating (SIN) vectors including generation of a fusion 50 LTR promoter, to control therapeutic gene expression, and introduction of a deletion within the U3 region of the 30 LTR. This strategy is applicable for both lentiviral and MoMLV-derived vectors.\(^\text{8}\)

**Adenovirus (AdV)**

Adenoviruses can carry a larger DNA load than retroviruses, and are able to achieve high transduction efficiency in a variety of cell types including nondividing cells. These double-stranded DNA viruses can be rendered replication defective by substitution of the essential E1 gene without an apparent effect on viral growth. “Gutted” helper-dependent adenovirus is generated by stripping the majority of viral protein encoding genes leaving essentially inverted terminal repeats and the packaging sequence at the 50-end of the viral genome.\(^\text{20}\) Expression of AdV in the
skin is brief, lasting only about 2 weeks presumably attributable to lack of genomic integration and possibly delayed cytotoxic effects.\textsuperscript{21}

Vectors based on adenovirus type 5 (Ad5) are efficient at gene delivery to the skin, and despite their inflammatory and immunogenic properties, can lead to expression of therapeutic genes over a period of weeks in wounded epidermis.\textsuperscript{22,23}

Ad5 vectors are able to transduce both dividing and nondividing cells and facilitate highly efficient gene transfer. Importantly, Ad5 vectors only very rarely integrate into a chromosome, that is, they exist in a target cell nucleus in an epichromosomal location. Thus, if the target cell divides, only one daughter cell will receive the transferred gene, and with subsequent cell-division cycles, the gene will be dramatically diluted.\textsuperscript{23}

The main disadvantage of Ad5 vectors is that they induce a potent host-immune response. It is also important to recognize that different viral vectors will vary in their ability to transduce different cell types. Often, this reflects the presence or absence of cell membrane receptor proteins that mediate viral entry into the target cell.\textsuperscript{3}

\textbf{Adeno-Associated Virus (AAV)}

Adenoassociated viruses (AAV) are non-pathogenic parvoviruses having a single stranded DNA. They, like adenoviruses, can infect both dividing and quiescent cells like neuron, useful for treatment of brain, muscle and eye disease.\textsuperscript{9}

They can also infect KC, and wild-type AAV will replicate in a helper independent fashion in differentiating cells.\textsuperscript{24,25} But in the absence of the viral replication protein, AAV vectors do not usually integrate into chromosomal DNA and are diluted out of replicating cells in vivo. They may therefore be unsuitable for long-term transduction of the epidermis.\textsuperscript{26}

The nonpathogenic AAV-2 subtype of AAV, is a common gene therapy vector. It is characterized by stability of the viral capsid, low immunogenicity, the ability to transduce both dividing and nondividing cells, the potential to integrate site specifically and to achieve long term gene expression even in vivo. Proliferation depends on the presence of a helper virus such as AdV or herpes virus.\textsuperscript{25}

AAV-2 vectors transducer mainly ductal cells and require about 8-12 weeks to achieve maximal levels of transgene expression.\textsuperscript{27} AAV-2 vectors elicit only a modest immune response, and transgene expression in mice is quite stable.\textsuperscript{27,28}

Studies have shown that cutaneous transduction using AAV is possible both ex vivo and in vivo, although strong evidence for efficacy, duration, and vector integration is lacking.\textsuperscript{29,30}

Interestingly, packaging of an AAV vector with capsid serotype 6 increased KC transduction frequency 5 logs compared with the same vector packaged with capsid serotype 2. Therefore, recent improvements made in AAV vector design and production highlights the therapeutic potential of this vector in cutaneous gene therapy.\textsuperscript{31}

\textbf{Herpes Simplex virus}

Herpes simplex virus is a human neurotropic virus. Therefore, it is mostly required for gene transfer in the nervous system. Generally, the advantages of use of viral vectors include: transduction of neurons and glial cells, wide host range, large insert size up to 30kb, efficient infection and the newly engineered vector are avirulent in surrounding terminally differentiated cells. Its limitations are: short term expression, spreading of the infection to surrounding cell populations and its immunogenic nature.\textsuperscript{1}
Non-Viral vector

Targeting loss of function mutations is achieved by introducing a plasmid DNA (pDNA) or RNA encoding the gene of interest. Conversely, for gain-of-function mutations, therapies that reduce gene expression such as RNA interference and micro-RNA can be used. Moreover, recent developments in engineered nucleases to create breaks in the genome following repair based on homologous recombination using exogenous donor templates makes nonviral gene therapy vectors even more desirable to target monogenic diseases. These breaks can be generated by several methods: zinc finger nucleases, clustered regularly interspaced short palindromic repeats (CRISPR), or transcription activator-like effector nucleases (TALEN).

Nonviral gene transfer techniques possess several advantages including cost-effective production of large amounts of vector, low toxicity, low immunogenicity, and preferential safety compared with viral vectors, as there is no risk of RCR. Furthermore, nonviral gene transfer is usually characterized by transient gene expression and low transfection efficiency. Short-term gene expression may be desirable for wound healing or bone regeneration. Longterm gene expression can be achieved by selecting stable clones ex vivo. Nonviral gene transfer technologies display limitations in achieving efficient gene delivery and long-lasting gene expression.

The simplest and most straightforward gene delivery vehicle is pDNA. Plasmids are propagated in bacteria, therefore they contain a bacterial replication origin and a selection marker, a gene conferring antibiotic resistance. Tissue specific promoters, enhancers, splicing introns, and other regulatory elements of mammalian maintenance devices such as a locus control region, ensure that the therapeutic gene is adequately expressed in target human tissue. Inclusion of insulating elements on each side of the expression cassette ensure limited influence on other genes and flanking sequence with transposon elements that allow chromosomal integration of the entire transcription unit. To further improve the safety profile of pDNA, minicircle DNA lacking the bacterial backbone sequence, an antibiotic resistance gene, and an origin of replication were developed with greatly increased efficiency of transgene expression in vitro and in vivo.

The most desirable method of cutaneous DNA delivery is topical application, yet the stratum corneum (SC) prevents DNA transport across the phospholipid-rich layer. Topically applied naked pDNA in aqueous solution can reach the epidermis via hair follicles. The second method uses cationic lipids (so-called liposomes) to surround the plasmid DNA and is termed lipofection. Several methods were developed to cross the SC barrier, more invasive than topical application. Direct injection of interleukin-8 pDNA into porcine skin resulted in DNA uptake by KC and the appropriate biological response of neutrophil recruitment. Hypodermic needle use often causes pain and inflammation at the injection site; therefore, there is a need to develop needle free gene delivery strategies.

One of the methods that increases skin permeability is based on a ballistic pDNA projectile across the cutaneous barrier. The first account of successful needlefree pDNA delivery was reported in 1991 using a gene gun. pDNA covered gold particles 2-5 mm in diameter were shot into the skin driven by helium gas without evidence of skin injury and 10%-20% delivery efficiency.
Today other high-pressure flow methods are used, mainly for immunization purposes, such as liquid jet injection, which directs a pressurized liquid to make a pathway into the skin and epidermal powder immunization, which accelerates dried-powder vaccine particles into the skin at supersonic speed. The other methods of physical/mechanical gene delivery are sonoporation (ultrasound-mediated gene transfer), electroporation, and magneto-permeabilization. Sonoporation refers to transient porosities in the cell membranes induced by ultrasound (cyclic sound pressure with frequency range 20kHz) and uptake of DNA or drug microbubbles into the cells. Electroporation has been used for transdermal drug delivery by increasing skin permeability by applying an electric field, which surpasses the electrical capacity of the cell membrane. A combination of long, low voltage pulses is used for DNA transfer. The first successful in vivo pDNA electrotransfer was achieved in 1991 using newborn mice. To avoid unwanted electrode contact with the subject during electroporation, magnetic fields were generated. Magneto-permeabilization provides several advantages over electroporation: there is no need for invasive electrodes, it is more cost effective, and there is greater tissue penetration by the magnetic field. Microneedles (MN) have emerged as a potential new approach for minimally invasive delivery of epidermal gene transfer. The dimensions of MN are within the micron range and consequently their penetration, on topical application, is restricted to the most superficial layers of skin (i.e., the viable epidermis and papillary dermis). Such devices are widely used for vaccination and fall into at least four design categories: hollow, solid, coated, and dissolving. Coated and dissolving MNs incorporate drug within the body of the needle, providing simultaneous skin puncture and delivery.

To further enhance topical, dermal, or transdermal gene delivery efficacy, many cationic polymers have been studied both in vitro and in vivo. However, in recent years there has been a focus on nanoparticle (NP) biodegradable carrier systems. NPs vary in size from 1.5 to 1000 nm and are readily graftable with cationic polymers, nuclear localization signals, peptides, and polyethylene glycol to provide the ability to escape endosomes, navigate to the nucleus, target the site, and evade the immune system.

**TECHNIQUES OF GENE THERAPY**

**In vivo gene therapy**

The therapeutic gene delivered directly to the skin by microinjection, electroporation, gene gun and topical application. Disadvantages include: difficult targeting of host cell, introduction of therapeutic genes into a limited number of cells and transient expression of therapeutic gene.

- **a. Jet gun:**
  It is done through the introduction of a solution of DNA powered by gas such as CO2 into the host cells. It is good in local treatment of skin or breast cancer but may cause bleeding or bruising at the site of introduction.

- **b. Hydrodynamic gene transfer**
  It is pumping of a high pressure solution of DNA into veins or by using a catheter. It is a simple and safe method with efficient transfection (up to 40%), however, too large solution volumes are used for human.
c. Electroporation
It is electricity used to alter permeability of cell walls and cause gene transfer. Although it is safe and efficient method, yet it is rarely used because of difficulty of access with electrodes to treatment sites.55


d. Ultrasound
Herein, combined ultrasound energy and microbubbles are used to increase permeability of cell membrane to pDNA. It is safe and efficient with a good gene expression in vascular cells and muscles.56

e. Gene gun
It is a hand-held instrument that utilizes the ballistic particle-mediated delivery system to deliver genes into skin in vivo. It accelerates DNA-coated gold particles into target cells or tissues. Due to their small size, the gold particles can penetrate through cell membrane carrying the bound DNA into the cell. At this point, the DNA dissociates from the gold particles and can be expressed. Gene gun can successfully deliver genes into different mammalian cell types, however, it is physically limited by the degree of penetration into tissues.42

The advantages of gene gun over the other in vivo delivery system include: freedom from use of viruses and toxic chemicals, cell receptor-independent delivery, delivery of different sizes of DNA and the possibility of repetitive treatment.1

Ex vivo gene therapy
The therapeutic gene is transferred into the skin outside the body, precisely attacking the target cells (keratinocytes or fibroblasts). It carries many advantages over the in vivo technique such as precised introduction of the gene in correct type of target cells, less chance of immune reactions and the production of higher level of transduction.57

Gene therapy for skin diseases
Keratinocytes are the cells of choice for gene therapy in skin and systemic diseases because of their easy accessibility and rich vascularization.58 The genetically modified regions can be easily monitored and aberrant tissue can be surgically removed. Moreover, KC gene transfer has been considered as an alternative therapeutic approach for non dermatological conditions, and for systemic diseases. The chief cutaneous disorders where this technique is of the foremost importance are types of epidermolysis bullosa.59

Other areas of interest are xeroderma pigmentosum, X-linked lamellar ichthyosis,60 porphyrias, wounds and squamous cell carcinoma and melanoma.61

Epidermolysis Bullosa (EB)
EB is a family of inherited genetic blistering skin disorders associated with gene defects affecting gene expression of the basal epidermis. Fifteen genes and 13 proteins have been characterized and are responsible for the specific subtypes of this disease.62,63

There are three main types of EB; EB simplex (EBS), junctional EB (JEB), and dystrophic EB (DEB), each affects different levels of the epidermis. EBS is most often caused by dominant mutations in the genes encoding for keratin 5 or keratin 14, and is usually a milder phenotype than the other two forms of EB, with blisters mainly on areas of major trauma. JEB is caused by recessive mutations in the genes for collagen XVII, integrin a6b4, or laminin 332.8 Herlitz JEB is usually lethal within the first 2
years of life. Non-Herlitz JEB is characterized by chronic skin blistering, dental anomalies, and alopecia. DEB is attributable to mutations in the gene (COL7A1) encoding type VII collagen (C7), and can be recessive (RDEB) or dominant. The severe RDEB can result in chronic blistering and scarring, esophageal strictures, mitten deformity of the hands and feet, and early death from malnutrition, sepsis, or aggressive squamous cell carcinoma.

**Recessive Dystrophic Epidermolysis Bullosa (RDEB)**

Many different groups throughout the world have attempted to correct RDEB. One group has focused on exploring nonviral methods to target chromosomal integration of the transgene into KCs or fibroblasts. In 2010, using a retrovirus-based therapy, RDEB KCs were corrected with COL7A1 cDNA and longterm durable expression of C7 seen when grafting human skin onto an immunodeficient mouse model. A phase-1 clinical trial of ex vivo gene transfer in human subjects with RDEB using this retrovirus has been approved by the Food and Drug Administration (FDA).

Similarly, another group attempting ex vivo correction of DEB also created transplantable autologous skin equivalents using a retroviral vector grafted onto immunocompromised mice. Correction of spontaneous homozygous mutations was then shown in two canines with DEB. Woodley et al. injected a lentiviral vector expressing C7 into DEB skin grafted onto immunocompromised mice.

Several groups used antisense oligoribonucleotide (AON) therapy. Although it is thought that KCs are responsible for the majority of C7 production, there is debate as to whether KC or fibroblasts are the best target for DEB gene therapy. After transducing both RDEB KCs and fibroblasts, Woodley et al. showed that lentiviral-corrected RDEB fibroblasts could be used to create a skin equivalent to normal C7 expression.

**Junctional Epidermolysis Bullosa (JEB)**

In 2006, Mavilio et al. reported a successful ex vivo correction of LAMB3 gene using autologous skin grafts for a subject with nonlethal JEB using MLV retrovirus. No blisters, infection, immune response, or inflammation were observed. Using a model of lethal Herlitz JEB mice with homozygous LAMB3 mutations, Endo et al. attempted to perform in utero gene transfer. A lentiviral vector encoding for LAMB3 was injected into the amniotic space.

**Epidermolysis Bullosa Simplex (EBS)**

An AA V gene-targeting vector with promoter trap design targeting was used to correct the KRT14 gene in EBS KCs. Fully functional epidermis was seen for 20 wk post grafting onto mice. Short inhibitory RNAs (siRNAs) have been investigated as methods for disease correction for EBS.

**Pachyonychia congenita**

Pachyonychia congenita is a dominant negative disease stemming from a keratin mutation resulting in painful plantar keratoderma. In 2010, siRNA was used on one subject for 17wks. Regression of the callus and decreased tenderness were seen on the siRNA treated foot only.

**Ichthyosis**

**Lamellar ichthyosis**

Patients with lamellar ichthyosis (LI) have a defective barrier and abnormal differentiation of the epidermis because of a transglutaminase 1 deficiency. LI patient KC were transduced with a retroviral vector engineered to express trans-
glutaminase 1. Corrected KC were grafted on to immunodeficient mice, displaying normal phenotypes.\textsuperscript{74}

**Harlequin ichthyosis**
The gene ABCA12 is important for lipid secretion from lamellar granules; mutations in this gene result in harlequin ichthyosis (HI), which is often lethal. Corrective gene transfer was performed on KC from HI patients using a cytomegalovirus-based vector in vitro, which restored lamellar granule lipid secretion.\textsuperscript{75}

**Sjogren-Larsson Syndrome**
Sjogren-Larsson syndrome (SLS) is a disorder caused by a mutation in the gene ALDH3A2, which codes for fatty aldehyde dehydrogenase (FALDH) that catalyzes the oxidation of fatty alcohols into fatty acids. Using a recombinant AAV-2 vector, FALDH was transduced into SLS KC. Corrected KC appeared phenotypically normal with normal FALDH expression.\textsuperscript{76}

**Netherton syndrome**
Netherton syndrome is a genetic skin disorder in which mutations of the SPINK5 gene result in loss of a serine protease inhibitor LEKTI. Di et al. created a viral vector encoding for SPINK5. Transduced KC showed correction of LEKTI expression in vitro.\textsuperscript{77}

**Xeroderma pigmentosum**
Xeroderma pigmentosum (XP) results from a defective DNA repair mechanism involving nucleotide excision repair (NER). Cells without a functioning NER develop increased UV-induced damage, increasing mutagenesis and skin cancer development.\textsuperscript{78} Early attempts at gene therapy for XP were aimed at adenovirus-mediated fibroblast transduction. Later, researchers used a MLV-derived retrovirus to correct the NER mechanism in KC for one XP subtype. When exposed to UV irradiation, the corrected KC continued to correctly repair DNA with UV exposed cell survival comparable to wild-type KC.\textsuperscript{79}

**Wound healing**
The mechanism of impaired wound healing is often multifactorial including decreased levels of growth factors or growth factor receptors, defective function of dermal fibroblasts, or damaged nitric oxide synthetase.\textsuperscript{74} Diabetic mice who received an AAV expressing VEGF-A had increased VEGF-A expression and subsequently improved wound healing, compared with control.\textsuperscript{80} Using a nonviral method, VEGF was encoded in plasmid DNA in combination with a cationic dendrimer then injected subcutaneously into murine diabetic wounds, resulting in high levels of VEGF expression and complete wound healing within 6 days.\textsuperscript{81} A phase-1 clinical study of periwound injection of an adenovirus encoding PDGFB showed a decrease in the size of chronic venous leg ulcers within 1 month in 14/15 subjects.\textsuperscript{82} Keratinocytes treated with a plasmid encoding for EGF showed increased wound healing when compared with non-treated KC in a porcine model.\textsuperscript{83}

**Melanoma**
There are currently multiple clinical trials of gene therapy for melanoma. One study treated melanoma patients with autologous genetically modified lymphocytes expressing the cancer germ line gene MAGE-A3.\textsuperscript{84} A phase-I/-II study of an interleukin-2 (IL-2) intralesional injection mediated by adenovirus shows promise.\textsuperscript{85} There have also been several clinical trials using genetically engineered autologous T cells that express T-cell receptors against specific tumor antigens after retroviral transduction.\textsuperscript{86} Other metastatic melanoma trials used T-cell receptors.
to a different antigen, MART-1, and showed tumor regression.\textsuperscript{87}

**Gene therapy in non-dermatologic disorders**

**Prevention and repair of irradiation damage to salivary glands (SGs)**

Radiotherapy is used to treat the majority of head and neck cancers. 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. SGs are extremely sensitive to IR, and the mechanism of this damage is still not clear.\textsuperscript{88} Greenberger and Epperly\textsuperscript{89} have shown that administration of manganese superoxide dismutase-plasmid liposomes (MnSOD-PL) can provide mucosal IR protection in the lung, esophagus, oral cavity, urinary bladder, and intestine. Although the effects of MnSOD-PL on SG function have not been studied, this approach to prevent SG damage from IR appears promising.

**Infections of the Upper Gastrointestinal Tract**

HIV-1-infection can lead to significant morbidity.\textsuperscript{90} Oral candidiasis remains a common opportunistic infection observed among immunosuppressed patients. Histatin levels in saliva are reduced in HIV-1-infected patients. Transfer of the histatin-3 cDNA to SGs would result in an increased secretion of histatins in the oral cavity and might be useful in managing resistant candidal species.\textsuperscript{91}

**Autoimmune Disorders**

Sjogren’s syndrome (SS) is a common autoimmune disease that is characterized by the presence of a focal lymphoid cell infiltration in the salivary and lacrimal glands, although other organs may also be involved. The etiology of SS is unclear, and current treatment is only palliative.\textsuperscript{92} The transfer of genes encoding anti-inflammatory 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. Nonetheless, since we do not understand SS pathogenesis, this gene-transfer strategy is nonspecific and still requires considerable study.\textsuperscript{93}

**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. 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]). Many studies have involved transferring the cDNA for Epo using AAV-2 vectors encoding either human or rhesus Epo.\textsuperscript{94}

**Others**

The above mentioned uses are a few examples. Newer researches include malignancies like lung cancers, osteosarcoma and lymphomas, Alzheimer’s disease, sickle cell anaemia and thalassemia.\textsuperscript{74}

**Genetic vaccination**

Genetic application is an application of gene gun technology. Genetic vaccination is either pathogen vaccines or cancer vaccine. Immunization is achieved by introduction of DNA\textsuperscript{21} or mRNA\textsuperscript{95} into the skin leading to expression of the foreign antigen and subsequently the elicitation of an immune reaction. Possible advantage of genetic vaccine over live-attenuated, protein-purified and killed vaccine, include purity of antigen and higher effectiveness of immune elicitation. Human trials with DNA vaccinations against hep-
titis B, herpes simplex, HIV, influenza and malaria showed promising results.\textsuperscript{96}

The goal of cancer vaccination is to prime the host immune system against tumour cells. Many approaches had been done including introduction of foreign class I or class II MHC antigen genes to tumour cells, the use of vectors encoding tumour associated antigens as carcinoembryonic antigen,\textsuperscript{97} and the development of immunostimulatory techniques. Trials have been done in vaccines against prostatic cancer, breast cancers and several lymphomas.\textsuperscript{98}

**Limitations of gene therapy**

Since the first clinical gene-therapy trial was conducted, much attention and considerable promise has been given to this field. A major setback for the field occurred in September 1999, when a widely publicized death resulting from a gene-therapy trial was reported in an 18 years old boy who had a deficiency of ornithine transcarboxamylase, an important enzyme in the metabolism of ammonia. The gene therapy triggered a chain reaction in his immune system, resulting in hepatic and respiratory failure, and consequently, his death four days after being treated.\textsuperscript{99} Since then, all gene-therapy trials are now subjected to much tighter regulation by the National Institutes of Health (NIH) and FDA. Another challenge to gene therapy has been its ephemeral benefits to patients. Cure faded after a few months of therapy, and was followed by a return of the disease symptoms.\textsuperscript{13}

Gene therapy carries some disadvantages. The short lived nature of somatic gene therapy and the rapidly dividing nature of many cells prevent gene therapy from achieving long term benefits. Thus, patients will have to undergo multiple treatments. The introduced gene as well as viral vectors are protein structures and may be seen as foreign bodies by the immune system and an immune response would be started. In addition, genetic disorders caused by multiple genes will be difficult to treat.\textsuperscript{6}

**CONCLUSIONS**

Gene therapy is becoming a promising therapy for many dermatologic and non dermatologic disorders. This is done through the introduction of normal functioning gene to replace the pathologic one either by viral or non viral vectors. There are many ways to deliver the gene into the body including in vivo and ex vivo. While gene therapy is permissible for serious diseases of somatic origin, the prospects of using genetic interventions to improve the basic traits of humans is condemnable. Nevertheless, it has the potential to be the future of medicine and its possibilities must be explored.

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