Gene Therapy and its Application in Dermatology
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Abstract
Gene therapy is an experimental technique to treat genetic diseases. It is based on the introduction of nucleic acid with the help of a vector, into a diseased cell or tissue, to correct the gene expression and thus prevent, halt, or reverse a pathological process. It is a promising treatment approach for genetic diseases, inherited diseases, vaccination, cancer, immunomodulation, as well as healing of some refractory ulcers. Both viral and nonviral vectors can be used to deliver the correct gene. An ideal vector should have the ability for sustained gene expression, acceptable coding capacity, high transduction efficiency, and devoid of mutagenicity. There are different techniques of vector delivery, but these techniques are still under research for assessment of their safety and effectiveness. The major challenges of gene therapy are immunogenicity, mutagenicity, and lack of sustainable therapeutic benefit. Despite these constraints, therapeutic success was obtained in a few genetic and inherited skin diseases. Skin being the largest, superficial, easily accessible and assessable organ of the body, may be a promising target for gene therapy research in the recent future.

Key Words: Dermatology, gene therapy, genetic disease, vector

Introduction
Genetics plays an important role in several diseases. Some diseases have a definite genetic background whereas some others have genetic factors as an important etiological agent. In some of these diseases, the replacement of the defective gene by a corrected molecule forms a promising line of therapy. Nowadays, advancement in research in genetics has led to an increase in the interest in gene therapy. Gene therapy has been introduced to correct gene-related diseases by replacing the defective genes in these disorders. However, the use of gene therapy is not limited to the field of genodermatoses but also implicated in wound healing, genetic vaccination, cancer treatment, and immunomodulation.¹ This also helps in understanding the pathogenesis of a disease and its prevention.

Definition
Gene therapy is defined as the introduction of genetic material or nucleic acid into a cell to repair or compensate the activity of a mutated or abnormal gene or to synthesize beneficial proteins that help to prevent/halt, cure and/or reverse the genetic disease or restore the functional normal protein synthesis.²

History
Gene therapy was first conceptualized in 1972. The first successful gene transfer was shown on a trial in 1990 to treat adenosine deaminase deficiency-severe combined immunodeficiency (ADA-SCID).³ The first somatic gene therapy was documented for malignant brain tumors by using recombinant DNA technology which makes the tumor cells sensitive to drugs and causes the tumor cells to die.⁴

Types
Gene therapies are of two types, germ-line gene therapy (GGT) and somatic cell gene therapy (SCGT). In GGT, germ cells are modified by introduction of correct/functional genes into their genome. It is heritable and is passed onto the next generation. SCGT is the mainstream for basic research and is passed onto the next generation. Application of GGT in human being is still debatable due to insufficient knowledge about possible risks in future, ethical, and technical reasons.

Somatic cell gene therapy introduces a correct/functional gene in any cell other than the germ cell or undifferentiated stem cell. It is not heritable onto the next generation. SCGT is the mainstream for basic research and is common in clinical applications.

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research and application of gene therapy to treat gene-related diseases, cancer treatment, vaccination, and wound healing.

**Technique of gene therapy**

A cell, tissue, or even the whole individual (GGT) may be the target of gene therapy. The most common method of gene therapy is introduction of a correct gene into a nonspecific domain of the genome which replaces a nonfunctional gene. The transformed cell or corrected gene then starts to produce normal protein and restores the healthy phenotype on successive cell division. Gene therapies depend on a few critical elements. For gene therapy, there should be a diseased gene, a therapeutic gene, and an efficient delivery system or carrier called a vector. The vector may be a viral vector (genetically engineered virus) or nonviral vector (naked DNA or DNA complex). Viral vectors are modified in such a way that they can infect the cell to incorporate their gene to host cell chromosomes without creating a disease.

One of the novel approaches of gene therapy is to replace the defective gene through homologous recombination and/or selective reverse mutation to correct the defect and synthesis of normal mRNA. Different techniques of genetic correction include gene restoration, gene augmentation, and gene inhibition. Gene restoration is applied in the case of small point mutation by DNA/RNA chimeric molecule. Gene augmentation is the most common form of gene therapy. It is used to treat a condition where the functional gene is inhibited to produce a functional protein due to mutation. For example, erythropoietin–responsive anemia treated by gene augmentation. Gene inhibition is used to prevent overproduction of some proteins. There are two methods of gene inhibition, antisense nucleic acid (block translation by using antisense oligonucleotide) and ribozyme (down-regulate gene expression by inducing site-specific RNA cleavage). This specific ribozyme used in gene therapy is known as a hammerhead ribozyme and used in gene therapy. There are two types of approaches for delivery of the correct gene, in vivo and ex vivo techniques. In vivo technique directly delivers the correct gene into the target cell or tissue. The challenge in in vivo gene therapy is ensuring specificity and to reach the target cell pinpointly. Genetic material may be introduced directly by injection in a specific tissue, electroporation, gene gun, topical application to normal and wounded surfaces, and bioplastic insertion. In vivo gene delivery technique is used where isolation, in vitro culture of the cell and reinsertion of the genetically manipulated cell is not possible. In ex vivo technique defective cells are isolated from the patient, in vitro cell culture is performed and the cultured cells are infected by genetically modified vector for correct gene introduction. The genetically corrected cells are identified and are amplified in vitro; then they are inserted into the host. This technique introduces the correct gene in a correct target cell; as a result, there is less chance of an immune reaction, the higher level of transduction (introduction of foreign DNA in the eukaryotic cell by using a viral vector). But it is specific for those cells only that can be removed from the body.

**Gene editing**

Gene editing is a type of genetic engineering in which DNA is inserted, deleted, or modified in the genome of a living organism. In gene editing, a tailored nuclease induces a break in DNA double-strand at a targeted site followed by repair with nonhomologous end joining (NHEJ), causing imperfect repair or by homologous recombination (HR) resulting in perfect repair. Two decades ago meganucleases and zinc finger nuclease were used for breaking the DNA, but in recent days, transcription activator–like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeat/Cas9 (CRISPR/Cas9) are used for this purpose. This permits gene inactivation through NHEJ repair and correction of disease mutation by HR repair in various animal models much more easily. Significant achievements in gene editing for genetic skin disease have been obtained for dominant dystrophic, junctional, and recessive epidermolysis bullosa.

**Vector**

It is vital to select an appropriate method to deliver DNA. A special molecule has been engineered to increase the efficiency of DNA uptake by the target cells. The molecule used in gene therapy to move recombinant DNA from one cell to another is called a vector. Vector may be viral or nonviral and both of them can be used for in vivo and ex vivo techniques. Success of gene therapy depends on the proper selection of vector.

**Viral vector**

A number of viruses have been used in human gene therapy, including retrovirus, lentivirus, adenovirus, adeno-associated virus, herpes simplex virus, cytomegalovirus, and vaccinia virus. Among them, retrovirus is the most favorable one. Though the viral vectors have superior efficiency of gene transfer, the main drawback is virus–associated toxicity, complicated manufacturing process, and high cost [Table 1]. Genetic material for viral replication is replaced by the therapeutic gene. Virus carrying therapeutic genes infect the target cell, incorporate the correct gene into the target cell genome, which serves as a template for correct protein synthesis either temporarily or permanently.

**Retrovirus**

Till date retrovirus is the most commonly used vector for cutaneous gene transfer. It is of two types, i) oncoretrovirus, for example, Moloney murine leukemia virus (MMLV), ii) lentivirus originating from human
immunodeficiency virus (HIV). Lentivirus has the capability to infect nondividing cells by their ability to deliver pre-integration complex (PIC) across the basement membrane. Epidermal stem cells have low mitotic activity; hence it is more attractive for gene therapy related to dermatology. Oncoretroviruses are efficient for ex vivo gene delivery. It allows transduction of epidermal stem cells in reconstituted skin tissue, which is essential for long-term transgene (therapeutic gene that has been transferred to another genome) expression. During the process, insertional mutagenesis and silencing of the regulatory gene may lead to malignancy. To prevent this, few techniques are followed, like the incorporation of self-inactivating (SIN) vector which is constructed by introducing a fusion 5’ long terminal repeat (LTR) promoter, insertion of sequence like “beta-globin locus,” “control region,” or “Zinc finger nuclease” and introduction of a deletion within U3 region of 3’LTR.

**Adenovirus (ADV)**

It is a double-stranded DNA virus used as a vector in 23.3% cases worldwide. The main advantages of ADV are its DNA cannot be integrated to host DNA and floats freely in the nucleus, carry a large load of DNA than retrovirus, high transduction efficiency in all types of cells including quiescent cells. So, the viral genome including the therapeutic gene is not transmitted to descendants of target cell, making it unfit to treat a condition where gene therapy is needed for long duration or lifelong. Transgene expression of transduced keratinocytes persist for only 2 weeks, making it ideal for tissue repair and immunotherapy, with less chances of delayed cytotoxicity.

**Adeno-Associated virus (AAV)**

It is a nonpathogenic parvovirus with single-stranded DNA as genetic material. AAV-2 subtype of the virus has low immunogenicity, stable viral capsid, potential to integrate at specific site, ability to transduce both dividing and quiescent cells like neuron, muscle cell, and eye cells.

**Nonviral Vector**

Nonviral gene transfer possesses several advantages over viral gene transfer, such as cost-effectivity, low toxicity, low immunogenicity [Table 1]. The disadvantages of these vectors are transient gene expression and low transduction capacity (30%). To improve transduction capacity, gene gun or electroporation technique has developed to increase transduction rate to a higher value (56%). To improve transient gene expression, P-1 based artificial chromosome (PAC) construct has been developed and used to produce stable type VII collagen gene expression in dystrophic epidermolysis bullosa (DEB). It is useful for wound healing and bone regeneration. Uses of nonviral vectors are “loss-of-function” mutation by introducing a plasmid DNA (pDNA) or RNA; “gain-of-function” mutation by RNA interface and micro-RNA. Recent advancement in the field of nonviral gene therapy is to treat monogenic disease by homologous recombination after creating a break in the genome by engineered nuclease. These breaks can be generated by zinc finger nuclease, CRISPR and TALEN.

**Naked DNA**

Naked DNA refers to DNA without any associated histone protein, lipid, or any other molecule that protects it. Naked DNA plasmid and naked DNA-PCR products are also used in gene therapy. In plasmid DNA design, plasmids are propagated in bacterium; hence they have a bacterial replication origin, and a selection marker responsible for antibiotic resistance. A plasmid DNA has tissue-specific promoter, enhancer, splicing introns, locus control region that ensures that the therapeutic gene is adequately expressed in the human target tissue. Inclusion of insulating elements on each end of expression cassette ensures limited influence on other genes and flanking sequence with transposon element allows chromosomal integration of the entire transcription unit, making it safe and efficient. To increase the safety profile, minicircle DNA is developed, that lacks in bacterial backbone sequence, antibiotic resistance gene and this increases the efficiency of transgene expression in vivo and ex vivo.

Plasmid DNA can be delivered by different techniques like topical application, intradermal injection, gene gun (delivering gas, liquid or powder), sonoporation, electroporation, magneto-permeabilization, microneedling, and nano technology. In topical application, plasmid DNA is delivered by aqueous solution. DNA is transferred via hair follicle without affecting the phospholipid rich stratum corneum. Intradermal injection of pDNA incorporates gene mainly into keratinocyte and little into fibroblast and selectively expressed in upper and middle layers of epidermis. Gene gun is needle-free delivery of pDNA driven by helium gas into skin with 10%-20% delivery efficiency, without skin injury. High-pressure flow method, which directs pressurized liquid jet into the skin for immunization, dried-powder vaccine particle accelerates into the skin by epidermal powder immunization. Physical methods are sonoporation in which ultrasound-mediated transient porosities are created in the cell membrane to deliver gene and drug microbubble using sound frequency >20 kHz, electroporation in which long, low voltage pulse is used to surpass the electrical capacity of the cell membrane for transdermal delivery, magneto-permeabilization where the magnetic field is generated for delivery of protein encoded pDNA. Microneedling is minimally invasive epidermal gene transfer where efficacy is restricted up to the papillary dermis. It is widely used for vaccination.
Nanoparticle (NP) is a biodegradable carrier system of variable sizes ranging from 1.5-1000 nm used for gene silencing.\(^{40}\) Spherical nucleic acid and NP conjugate freely; the conjugate penetrates almost 100% keratinocytes \textit{in vitro} in mouse epidermis and within hours in the human epidermis after its application.\(^{41}\)

pDNA and minicircle DNA have low transfection (deliberate introduction of naked or purified nucleic acid into eukaryotic cell) efficiency \textit{in vivo} compared to viral vectors, limiting its effectiveness in gene therapy.

**Immune reaction**

Introduced gene and the viral vector both are foreign proteins for the recipient, hence able to induce an immune reaction. Initially CD4 and CD8 T-cells participate in immune reaction,\(^{42}\) on successive therapy, memory T-cell also takes part in it and makes the situation worse. Viral vectors stimulate dendritic cells (DC) through type 1 interferons and phosphatidylinositol 3-kinase pathway.\(^{43}\) Recent researches are focused to find out the way to prevent immune reaction. These are i) tolerogenic DC which are generated with the help of IL-10, NVP347 treatment and ligation of CD45RO/RB;\(^{44}\) ii) antibody formation against CD45RB\(^{45}\) and blockade of CD28 and CD40 pathway;\(^{46}\) iii) suppression of immune system by regulatory T-cell.\(^{47}\)

**Why skin is suitable for gene therapy?**

Being superficial, easily manipulatable, observable, and versatile organ, skin is suitable for gene therapy.\(^{48}\) Dermis is a highly vascular structure with an ability to synthesize and secret a lot of proteins in the circulation. If the skin is transduced with transgene of transferrin,\(^{49}\) erythropoietin, growth hormone,\(^{50}\) apolipoprotein \(E^{51}\) and factor IX,\(^{52}\) it secrets the gene product in circulation and its physiological effect can be enormous. Its ability to secret the gene product is further increased by modified progesterone receptor driven transcription.\(^{53}\) Keratinocyte is the target of choice for gene therapy. It is easily accessible for isolation, \textit{in vitro} tissue engineering\(^{54,55}\) and can be reconstituted into the subject, \textit{in vivo} experimental gene transfer\(^{56}\) and for monitoring of the modified genome. A study done by Katz \textit{et al.} reports that keratinocyte secretes 70 proteins into their surroundings.\(^{56}\) Transduced keratinocytes can synthesize cytokines, like IL-4, IL-6, IL-10, GM-CSF, IFN, monocyte chemotactic and activating factor and secret them into the circulation.\(^{57}\) Fibroblast, melanocyte, macrophage, endothelial cells are also used as a target of gene therapy.

**Gene therapy in Dermatological disorders**

Because of the comparative ease of gene transfer, the dermatological disorder is a favorite target of gene therapy. But, to date, the therapy is experimental and not yet in regular use. The autosomal recessive monogenetic disease is easier to treat than an autosomal dominant disease. Other than genetic diseases, skin cancer, chronic wound, and intractable inflammatory disease can also be treated by gene therapy.\(^{58}\) Candidate skin diseases that can be treated or can be conceptually managed by gene therapy are described below.

**Epidermolysis bullosa simplex (EBS)**

EBS is a genetically inherited blistering skin disease associated with defective gene expression of the basal keratinocytes. Fifteen genes and 13 proteins are responsible for the specific subtype of this disease.\(^{59}\) It is caused by a dominant mutation affecting \(KRT5\) and \(KRT14\) keratin genes. Oligonucleotide mediated gene correction technique is used to correct \(KRT\) 14 gene. AAV gene-targeting vector with promoter trap design technique\(^{60}\) was documented beneficial. Antisense technology and RNA interface has also been used. Treatment of EBS patients with heterozygous dominant-negative mutation by silencing\(^{61}\) or removing the defective allele with the help of short inhibitory RNA (siRNA) and spliceosome-mediated RNA trans-splicing (SMaRT) followed by correct gene insertion is promising but are still experimental.\(^{62,63}\)

**Junctional epidermolysis bullosa (JEB)**

JEB is of two types, Herlitz JEB (H-JEB) and non-Herlitz JEB (NH-JEB). \(LAMA3, LAMB3,\) and \(LAMC2\) genes mainly encode for three chains of laminine 5 \(\alpha\), \(\beta\) and \(\gamma\), respectively. \(LAMB3\) gene is mostly affected in H-JEB. MMLV retroviral vector was successfully used for \textit{ex vivo} correction of \(LAMB3\) gene by using autologous skin grafting in NH-JEB.\(^{64,65}\) Nonviral vector phiC31 integrase and transposon were used to correct JEB by cut and paste technique.\(^{66}\) Dillinger \textit{et al.} spliced out specific mutant exon to correct collagen 17 mutation of keratinocyte \textit{in vitro} by using SMaRT in NH-JEB.\(^{67}\) Laminine-5 and BP-180 deficient H-JEB had been corrected \textit{ex vivo} by using human skin graft/immune-deficient mouse xenograft model.\(^{68,69}\) Nearly 80% clinical correction was achieved with genetically modified skin graft in a patient of JEB.\(^{70}\)

**Dystrophic epidermolysis bullosa (DEB)**

DEB is due to mutation in the gene (\(COL7A1\)) encoding type VII collagen (\(C7\)) and may be of two types, dominant and recessive. Among these two, recessive DEB (RDEB) has dreadful complications, like scarring, esophageal stricture, mitten deformity of hands and feet, and early death due to sepsis or aggressive squamous cell carcinoma.\(^{71,72}\) Larger size of the defective gene is the major drawback of gene therapy in DEB. To produce durable, persisting type VII collagen, vector phiC31 bacteriophage integrase along with plasmid expressing \(COL7A1\), plectin cDNA transgene is used. Transgene integration is done into the chromosome of target keratinocytes\(^{73}\) and fibroblasts\(^{74}\) of RDEB. Previously
it was thought that keratinocyte is responsible for C7 at dermo-epidermal junction (DEJ). But the study done by Woodley et al. shows that it is possible to restore COL7A1 gene expression in RDEB skin by direct intradermal injection of either normal fibroblast or gene-corrected DEB fibroblast in vivo. A study done by Goto et al. showed retroviral gene transfer method for COL7A1 gene into DEB epidermal keratinocytes and dermal fibroblasts, which most efficiently expressed collagen VII at the DEJ. Fibroblast is a better target than keratinocyte, as it produces majority of type VII collagen. Ex vivo gene therapy for inherited skin disorders has been developed in several preclinical studies, with a focus on JEB and RDEB. For ex vivo correction of DEB, human keratinocyte transduced with retrovirus to create transplantable autologous skin on immunocompromised mice. Woodley et al. injected SIN lentiviral vector expressing human C7 in DEB skin grafted onto immunocompromised mice that reverses DEB phenotype for at least 3 months after single intradermal injection. Hence, efficient and long-term transfer of COL7A1 gene in vivo is possible by using an engineered lentiviral vector. Local injections of BM-MSCs (bone marrow-derived mesenchymal stromal cells) have shown transient restoration of type VII collagen at the dermal-epidermal junction. This corrected keratinocyte and reverse the DEB phenotype but AON degrade easily after injection into DEB keratinocyte. So, its therapeutic effect is short-lived. Murauer et al. used trans-splicing method by retroviral transduction of keratinocyte with 3′pre trans-splicing molecule to express C7 at the basement membrane. Recently Osborn et al. described gene-specific mutation in RDEB fibroblast by using TALEN novel genome-editing tool. This corrected fibroblast reprogrammed into inducible pleuripotent stem cell that had normal C7 expression. Three “off target” editing events were also documented in addition to “on target” correction of mutation, emphasizing the necessity of establishing the safety profile of such in situ approach. TALEN-mediated correction of a recurrent RDEB mutation was recently achieved by Chamarro et al.

### Netherton syndrome
It is a genetic disorder due to mutation of the SPINK5 gene responsible for the loss of serine protease inhibitor LEKTI. This causes premature degradation of cornodesmosome and defective keratinization. In vitro transduction of SPINK5 gene in human keratinocyte by using SIN lentiviral vector to correct expression of LEKTI in human skin graft/murine model was done by Di et al. Roedl et al. used AAV2 mediated viral vector to increase transduction of SPINK5 gene (75%) in wild type keratinocyte to restore LEKTI expression in nonscratch keratinocyte.

### Sjogren-Larsson syndrome (SLS)
It develops due to mutation of ALDH3A2 gene, which encodes the enzyme, fatty aldehyde dehydrogenase (FALDH). It is clinically characterized by ichthyosis, mental retardation, and spasticity. Recombinant AAV2 vector is used to transduce SLS keratinocyte, resulting in normal FALDH expression by phenotypically normal keratinocyte.

### Xeroderma pigmentosum
It is a genodermatosis due to defective nucleotide excision repair (NER) in DNA repair mechanism. Cell without functional NER are prone to UV-induced damage, mutation and development of skin cancer at a younger age (<30 years). MMLV derived retrovirus was used to correct the NER mechanism of keratinocyte DNA repair. On exposure to UV radiation, the corrected keratinocytes continue to repair DNA properly. Corrected keratinocyte holoclones also appear to convey long-term DNA repair while maintaining their DNA repair capabilities. Naldini et al. used a lentiviral vector for transduction into the postmeiotic cells due to its high transduction efficiency and stability. Adenovirus-mediated transduced fibroblast was also used to correct DNA repair XP gene therapy. Plasmid DNA vector should be used in the management of XP, to avoid the risk of insertional mutation.

### Ichthyosis
#### Lamellar ichthyosis (LI)
It is an autosomal recessive disorder of defective keratinization due to transglutaminase-1 (Tgase-1) deficiency. Keratinocytes of LI patient were transduced in vitro with an engineered retroviral vector to express correct Tgase-1, which restores defective cross linking of involucrin and filaggrin in immunodeficient mice. This process restores the epidermal barrier function of the immunodeficient mice.

#### X-linked ichthyosis
It develops due to steroid sulphatase (STS) deficiency, leading to accumulation of cholesterol sulfate known as “arylsulphatase C.” Gene encoding for STS is completely deleted in 90% of the cases and rest have partial
deletion. In vitro transfection of gene encoding STS causes partial correction of the phenotype with increased cell maturation. Retroviral vector-mediated transduction of STS gene to primary keratinocyte is associated with restoration of full-length STS protein expression and its enzymatic activity.[95] Early loss of transgene expression is more common with plasmid or nonviral naked DNA mediated transfection than retroviral vector-mediated transduction.

Herlequin ichthyosis (HI)
In HI, defect lies in ABCA 12 gene responsible for lipid secretion from lamellar granules of granular layer keratinocyte. Cytomegalovirus based in vitro gene transfer corrects the mutation and restores lipid secretion from lamellar granules.[96]

Bullous congenital ichthyosiform erythroderma (BCIE)/Epidermolytic hyperkeratosis (EH)
BCIE caused by mutation of keratin 1 (KRT1) and keratin 10 (KRT10) genes. It can be corrected by knocking out the mutated allele and expression of the dominant negative protein by using antisense probe. But the main drawback of gene therapy in BCIE is to find out a good antisense probe and varied location of the mutated gene.

Porphyria
It is a disorder of the hematopoietic system. Among various types, erythropoietic protoporphyria (EPP) is characterized by photosensitivity and protoporphyrin deposition in bone marrow, RBC, and other organs due to deficiency of ferrochelatase enzyme. Gene therapy has been tried with SIN lentiviral vector containing human ferrochelatase cDNA driven by human ankyrin-1/β-globin HS-40 chimeric erythroid promoter/enhancer in mice. Ferrochelatase gene was efficiently transferred to erythroid lineage of bone marrow and corrected all the clinical and biological alteration.

Malignancy
Melanoma
Recently different clinical trials were undertaken using T-lymphocyte to treat melanoma. Fontana et al. treated melanoma patients with genetically modified autologous lymphocytes expressing the cancer germ-line gene MAGE-A3. Increment in circulating anti-MAGE-A3 antibody was found in 3 out of 10 patients without any toxicity or adverse effect.[97] Dummer et al. gave adenovirus-mediated IL-2 intratranslecular injection for gene transfer, in patients of advanced solid tumor and metastatic melanoma. Interleukin-2 by stimulating T-cell proliferation induces complete/partial tumor regression.[98] Genetically engineered autologous T-cell was used to express T-cell receptor against NY-ESO-1 antigen to treat metastatic melanoma and metastatic synovial cell carcinoma. Clinical responses were seen in 5 out of 11 patients, with complete regression in 2 patients for at least 1 year.[99] Other clinical trials to treat metastatic melanoma used engineered T-cell receptor against different antigens, like MART-1[100] and gp100[101] with variable response.

Squamous cell carcinoma (SCC)
Replication-defective recombinant adenovirus-mediated transfer of the herpes simplex virus thymidine kinase gene (“suicide” gene) followed by administration of ganciclovir was used to treat human head and neck SCC successfully in nude mice model.[102]

Wound healing
Wound healing is a complex mechanism with a different sequence of inflammation and has no similarity with genodermatosis. Gene therapy can be applied to wound healing due to i) gene transfer is easier, as there is no epidermal barrier; ii) limited size of area of intervention; iii) therapy is required for a limited time (till healing). Indications of gene therapy are refractory burn wound, diabetic ulcer, vascular ulcer and decubitus ulcer, for rapid healing and to ensure tensile strength of newly formed skin and to avoid complications, like scarring, keloidal changes. Different techniques used for gene therapy are direct application of naked DNA, intraulcer injection, gene-gun delivery, electroporation, microvascular transfection and wound bed implantation. Liechty et al. used adenoviral vector encoding PDGF-β gene for treating ischemia impaired wound healing.[103] Collagen embedded PDGF-β DNA plasmid also promotes wound healing in rabbit.[104] Nonintegrative viral vector are ideal for gene therapy in wound. Diabetic mice who received AAV carrying VEGF-A gene, express increased VEGF-A and showed improved healing than who received AAV-LacZ control.[105] Intradermal injection of plasmid encoded TGF-β2 gene in diabetic mice, resulted in accelerated cell growth and wound healing.[106] Alogenic keratinocytes are treated ex vivo with plasmid encoded EGF gene, for overexpression of EGF to accelerate wound healing.[107] Nonviral gene transfer of TGF-1 increases granulation synthesis and keratin growth, promotes wound healing in diabetic ulcer of pig.[108] Sonoporation of minicircle VEGF 165 with 1 MHz sound pressure for wound healing in diabetic mice showed reasonable restoration of microarchitecture and wound closure.[109] Perilesional injection of adenovirus encoding PDGF-β in chronic venous leg ulcer showed reduction in size in 1 month.[110] Minicircle plasmid DNA encoding VEGF gene used in combination with cationic dendrimer injected subcutaneously into murine diabetic wound resulting in high level expression of VEGF and wound healing by 6 days.[111] Intradermal injection of sonic hedgehog gene by using biodegradable polyanoparticle in murine full-thickness wound promote expression of VEGF and chemokine stromal cell derived factor-1α significantly accelerate wound healing.[112] Different preclinical studies have been undertaken to assess the role of VEGF, iNOS and EGR-1 genes in vivo for re-epithelialization.

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Conclusion
To make gene therapy clinically available, future research work should focus on both vector design and delivery technique. The real challenges are to increase the effectiveness and durability of nonviral vectors and improve safety, avoid genomic integration and immune reaction associated with viral vectors. As adverse events can be easily monitored on skin it may represent a promising organ to explore these options. The procedure of initiating human gene therapy trials are extremely complex, permissions to be obtained from several authorities and bodies. The cost of any gene therapy trial is huge. In spite of these constrains, new gene therapy is persistently being developed and recently one gene therapy drug is approved in Europe for the treatment of lipoprotein lipase deficiency. We wish to see more gene therapy trials in dermatology and to obtain novel treatment options in different chronic, persistent and genetic dermatological disorders.

Abbreviations other than those described in the text
PCR: Polymerase chain reaction, NVP: N-Vinayl Pyrrolidone, IL: Interleukin, CD: Cluster differentiation, COL: Collagen, KTR: Keratin, SPINK: Serine peptidase inhibitor kazal, LEKTI: Lympho-epithelial kazal type related inhibitor, MAGE: Melanoma associated antigen encoding, PGDF: Platelet-derived growth factor, VEGF: Vascular endothelial growth factor, TGF: Transforming growth factor, EGF: Endothelial growth factor, IGF: Insulin like growth factor, iNOS: Inducible nitric oxide synthase; EGR: Early growth response.

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Conflicts of interest
There are no conflicts of interest.

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| Vector            | Advantage                                      | Disadvantage                                      | Use                           |
|-------------------|------------------------------------------------|---------------------------------------------------|-------------------------------|
| Lentivirus        | Sustain gene expression                        | Insertional mutagenesis                           | Replacement gene therapy      |
|                   | Acceptable coding capacity                      | Prone to recombination                            | Inherited genetic disorder    |
|                   | No mutagenicity                                 | Potential for silencing                           |                               |
|                   | High transduction efficiency in vitro and       | Expensive                                         |                               |
|                   | acceptable transduction efficiency in vivo      |                                                   |                               |
| Oncoretrovirus    | Sustain gene expression                        | Insertional mutagenesis                           | Replacement gene therapy      |
|                   | Acceptable coding capacity                      | Prone to recombination                            | Inherited genetic disorder    |
|                   | No mutagenicity                                 | Potential for silencing                           | Trans-splicing RNA repair     |
|                   | High transduction efficiency in vitro           | Cannot transduce non-dividing cells               |                               |
| Adenovirus        | Does not integrate into host genome             | Transient gene expression                         | Antitumor therapy             |
|                   | High transduction efficiency in vitro           | Immunogenic                                       | Chronic non-healing ulcer     |
|                   | High coding capacity                            | Expensive                                         |                               |
| Adeno-associated  | Sustain gene expression                        | Insertional mutagenesis                           | Homology directed mutation    |
| virus             | High transduction efficiency in vivo and ex vivo| Low coding capacity                               | repair                        |
|                   | Low immunogenicity                              | Immunogenic after repeated administration         | Inherited genetic disorder    |
|                   | Stable                                          | Expensive                                         | Systemic disease              |
| Nonviral (plasmid | Does not integrate into host genome             | Transient gene expression                         | Antitumor therapy             |
| DNA)              | High coding capacity                            | Risk of “Off target” genome editing               | Chronic non-healing ulcer     |
|                   | In vivo administration                          | Nonselective                                      | Vaccination                   |
|                   | No immunogenicity                               | Low efficiency                                    | Inherited genetic disorder    |
|                   | Stable                                          |                                                    | Homology directed mutation    |
|                   |                                                 |                                                   | repair and genome editing     |
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