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## RNA-based therapies for genodermatoses

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### **Abbreviations**

AON, antisense oligonucleotide; DEB, dystrophic epidermolysis bullosa; DMD, Duchenne muscular dystrophy; IVT mRNA, *in vitro* transcribed mRNA; PTC, premature termination codon; RDEB, recessive dystrophic epidermolysis bullosa; RTM, pre-mRNA *trans*-splicing molecule; siRNA, small interfering RNA.

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### **Abstract**

Genetic disorders affecting the skin, genodermatoses, constitute a large and heterogeneous group of diseases, for which treatment is generally limited to management of symptoms. RNA-based therapies are emerging as a powerful tool to treat genodermatoses. In this review, we discuss in detail RNA splicing-modulation by antisense oligonucleotides and RNA *trans*-splicing, transcript-replacement and genome editing by *in vitro* transcribed mRNAs, and gene knockdown by small interfering RNA and antisense oligonucleotides. We present the current state of these therapeutic approaches and critically discuss their opportunities, limitations and the challenges that remain to be solved. The aim of this review is to set the stage for the development of new and better therapies to improve the lives of patients and families affected by a genodermatosis.

### **Introduction**

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Genodermatoses – the inherited disorders of the skin – comprise a group of heterogeneous diseases. They display diverse clinical manifestations such as superficial epidermal and mucosal involvement, increased photosensitivity, inherited tumorigenesis and deep dermal trauma (1-3). Studies indicate that mutations in over 500 unique genes cause disorders with a distinct skin phenotype (4), which impedes the development of common therapeutic approaches. In comparison to other diseases the development of therapies for genodermatoses has its specific advantages and challenges. The direct accessibility of the skin, the ability to culture skin cells, and the possibility to reconstitute the organ *in vitro*, facilitate research. On the other hand, there are challenges such as multiple organ involvement, the large area that has to be treated, the avascular epidermis, and site-specific heterogeneity (5). Further, for some diseases, the *in vitro* organ reconstruction is too simplistic because it lacks immune cells and well-developed anchoring structures (6).

Therapies targeting the cause of genetic diseases can be divided into five groups: gene replacement, genome editing, protein replacement, cell-based and RNA-based therapies.

Classic viral vector-based gene replacement therapies aim to introduce correct cDNA copies of the defective gene into affected organs. To achieve this, these approaches utilize the specific tropisms viral vectors have for certain organs (7). The limited number of vectors efficiently targeting skin cells *in vivo*, poor vector transmission to the epidermis and the size of some transgenes (e.g. larger than the viral vector capacity) are limitations that impede development of gene therapy approaches for skin diseases (8, 9). At present, transplantation of gene-corrected skin grafts seems to be the most promising approach (10-14). In addition, genome editing is emerging as a powerful tool for gene and cell therapy (15). The technique has already been used to successfully correct skin cells *in vitro* (16, 17). However, the therapeutic potential of direct *in vivo* correction remains to be evaluated for genodermatoses and may appear to be challenging.

Systemic protein replacement therapy may be an alternative for a subset of genodermatoses and will likely be most effective for proteins naturally expressed in the dermal extracellular matrix or the dermal-epidermal junction (18). Epidermal delivery can be achieved by direct application onto wounds or by microneedle injections (19, 20).

Several genodermatoses caused by primary immunodeficiencies can be managed by HLA-matched bone marrow transplantations (21). It has been shown that bone marrow transplantation induced some transient symptomatic improvement also in connective tissue genodermatoses. Nevertheless, as the studies presented no clear evidence of wild-type protein synthesis, the exact underlying mechanism remains elusive (22, 23). The epidermis contains a pool of self-renewing stem cells sufficient to sustain organ homeostasis, which is evident from long-lasting mosaic patches that occur naturally in some genodermatoses (24, 25). However, bone marrow transplantation, presently performed without further cell and niche manipulation, does not seem to substantially increase the number of curative stem cells in the epidermis and its long-term potential is thus uncertain. The local application of corrected stem cells, on the other hand, might be a more efficacious approach (8, 26-28).

RNA-based therapies can overcome some of the difficulties that accompany these cell, gene and protein replacement therapies, as well as the unique challenges posed by treating skin. In the following sections we will introduce specific RNA-based therapies with their current and future applications for genodermatoses. Further, and importantly, we will critically discuss their advantages and limitations (summarized in Table 1). We will focus on approaches that directly utilize exogenously delivered RNA molecules. Post-transcriptional modification of RNA-transcripts by RNA-editing and modulation of translation of mutated mRNA transcripts by translational read-through are therapeutic options as well. However, for these approaches, the RNA is the therapeutic target rather than the therapeutic tool. To make a clear distinction between these two discrete

strategies, we have therefore chosen to place a review of the latter approaches in the Supplementary information (Supplementary Information).

### **Splice modulating therapies**

#### AON-mediated exon skipping

Antisense oligonucleotides (AON) are small pieces of modified DNA or RNA. They can be exploited to e.g. knock down gene expression or to manipulate splicing. For the latter, AONs hybridize to a mutated, in-frame exon during pre-mRNA splicing (Fig. 1 and Fig. S1). The targeted exon is no longer recognized by the splicing machinery and is skipped from the mature transcript. Subsequent translation of the generated transcript will result in an internally deleted protein.

AON-mediated splicing modulation is being pursued for dystrophic epidermolysis bullosa (DEB), which is caused by genetic deficiency of type VII collagen, encoded by *COL7A1* (29-31). In 2006, Goto *et al.*, (29) showed that *in vitro* transfection with a specific AON targeting the mutated exon 70 of *COL7A1* evoked *de novo* type VII collagen synthesis in patient keratinocytes. Subsequently, a single injection with this AON, directly into human skin-equivalents grafted onto the back of rats, partially restored type VII collagen synthesis. Although these first results were promising, the efficiency of type VII collagen restoration was low.

AON-mediated splicing modulation is currently in clinical trials for Duchenne muscular dystrophy and spinal muscular atrophy (32), and in preclinical stages for several other genetic conditions. The hope is that the experience gained from developing AON-based therapy for these diseases will help to significantly improve the exon skipping efficiency in *COL7A1* to reach therapeutically relevant levels

of type VII collagen synthesis (26, 31, 33, 34). Anticipating such advances in the development of an AON therapy for DEB, we envision that AONs will become attractive for other genodermatoses caused by mutant genes with a predominant in-frame exon organization. The greatest benefit from AON treatment is foreseen for patients with severe recessive diseases, for which even a low level of restored protein synthesis might yield significant phenotypic improvement (34-36).

Given that genodermatoses often affect multiple organs (4) a major advantage is that AONs can be administered systemically (37). Additional advantages are that AONs are easy to manufacture, have low toxicity and cause limited adverse events (38). Finally, AONs only restore protein synthesis in the cells that naturally express the protein, thereby avoiding concerns regarding organ and cell type specificity.

A limitation of AONs is that not all genes or mutations are suitable targets, as targeted exons need to be in-frame, and skipping should not remove amino acid sequences that are essential for protein function. Therefore, AON-based approaches need to be critically evaluated for each targeted gene, exon, and even mutation. This poses obvious challenges to drug development such as performing clinical trials and generating revenue from the product, when an AON can only be used for a few patients worldwide. Furthermore, due to turnover rate of AONs, transcripts and proteins, the effect of the therapy will be transient. Therefore, continuous treatment cycles will be needed, and it is not yet known whether life-long treatment with AONs would be tolerated and safe. Finally, the skipping efficiency, despite improvements in AON chemistry and formulation, may still remain too low for a therapeutic effect in some disorders.

#### RNA *trans*-splicing

In recent years, spliceosome-mediated RNA *trans*-splicing (SMaRT) has emerged as an attractive option for the repair of mutations on the mRNA level. SMaRT uses the cellular splicing machinery to

recombine an endogenous target pre-mRNA containing a mutation with an exogenously delivered pre-mRNA *trans*-splicing molecule (RTM) coding for part of the wild-type transcript (Fig.1 and Fig. S2). The RTM is composed of a binding domain, which confers target-specificity, and a 5', 3' or internal wild-type coding region to replace the part of the endogenous pre-mRNA containing the disease-causing mutation. After successful recombination, a hybrid full-length wild-type mRNA is generated, and wild-type protein synthesis restored. Proof-of-concept has been demonstrated in cell and animal models for a variety of human genetic diseases including Alzheimer's disease (*MAPT*) (39), muscular dystrophy (*DYSF* and *TTN*) (40, 41), hemophilia A (*FVIII*)(42), cystic fibrosis (*CFTR*)(43), hypertrophic cardiomyopathy (*MYBPC3*) (44), retinitis pigmentosa (*RHO*) (45), and epidermolysis bullosa (46-49).

The first preclinical proof-of-concept of the potential of *trans*-splicing came from studies in factor VIII hemophilia A-knockout mice (42). Low (< 5%) recombination frequency significantly hampered the therapeutic efficiency in the earlier studies. Since then, much effort has been put into optimization of the repair molecules. Recent studies have shown that the binding domain is the crucial factor in determination of the recombination frequency (50, 51). Further, a fluorescence-based RTM screening tool has been developed to optimize *trans*-splicing efficiency *in vitro* (50, 52, 53). Collectively, the studies have revealed that the most efficient binding domains hybridize to exon/intron junctions, thereby reducing *cis*-splicing due to the blockage of competitive splicing elements within the target region, and consequently enhancing *trans*-splicing (46, 48, 51).

Depending on the disorder, either *in situ* administration (54) or correction of epidermal stem cells and generation of skin grafts is conceivable. Most of the research on genodermatoses has so far focused on EB, for which RTMs have been successfully developed and optimized for preclinical studies on *PLEC*, *KRT14*, *COL17A1* and *COL7A1* (46-48, 50, 55).

The first correction of an EB-associated gene by RNA *trans*-splicing was achieved for the *PLEC* gene encoding the cytolinker protein plectin (49). Subsequently, proof-of-concept was obtained for repair of *COL7A1* (48). Replacement of a 3.3 kb 3' portion of the *COL7A1* mRNA harboring a pathogenic mutation led to restoration of full-length type VII collagen synthesis in cultured primary keratinocytes from a patient suffering from recessive DEB (RDEB). Further, when the corrected keratinocytes were used to generate skin equivalents that were subsequently transplanted onto mice, formation of type VII collagen-composed rudimentary anchoring fibrils was observed (48). Proof-of-concept has also been demonstrated for autosomal dominant disorders using SMaRT to exchange the first seven exons of the keratin 14 gene (*KRT14*), which resulted in a phenotypic reversion of patient cells *in vitro* (47).

A major advantage of *trans*-splicing is that it allows a reduction in size of the transgene to be delivered (i.e. only a portion of the cDNA is used instead of the full cDNA). This in turn allows a broader range of vectors to be used, which minimizes the risk of genetic rearrangements. An additional advantage of RNA *trans*-splicing is that the endogenous control of gene expression is maintained (56, 57). Still, however, there are challenges that have to be overcome before translation into the clinic can be realized. Concerns related to the safety of the vectors needed for RTM delivery or the RTMs themselves exhibiting unspecific *trans*-splicing have been raised and need to be carefully addressed. Therefore, future efforts should focus on investigating off-target events and further validating the safety of this approach. In the meantime, the most promising and safest strategy for a clinical application would be correction of stem cells followed by generation of skin grafts from single cell clones that have undergone careful safety profiling (10). Just as with AON-mediated splicing modulation, reaching clinically relevant *trans*-splicing efficiency may prove to be a hurdle.

### ***In vitro* transcribed mRNA-based therapy**

#### Transcript-replacement



Transcript-replacement therapy introduces mRNA transcripts into cells to drive synthesis of wild-type proteins (Fig.1 and Fig. S3) (58). *In situ* production of a protein in cells that naturally express this protein guarantees correct posttranslational modification, which helps overcome concerns about reduced functionality and immunogenicity of recombinant proteins (59). To create an active mRNA drug, a DNA template, based on wild-type or sequence-engineered cDNA (59, 60), has to be transcribed *in vitro* and a 5' cap and poly-A tail added (61) (Fig. S3). *In vitro* transcribed (IVT) mRNA can be further modified to enhance *in vivo* protein translation. Examples are modification of the cap and/or nucleosides to produce less immunogenic and more stable variants (61).

The potential of injecting pure mRNA to restore protein synthesis and revert disease phenotypes was recognized in the early 1990s (60, 62). However, due to inherent difficulties with the approach, such as rapid degradation of mRNA by RNases in the extracellular environment (63, 64), immunogenicity (59), and inefficient uptake of mRNA in non-immune cells (65) these studies did not lead to wider consideration of transcript-replacement as a therapeutic tool. Through increased knowledge of RNA and its recognition by the host, issues with degradation and immune activation have in part been overcome (59-61, 66). Nevertheless, low transfection efficiency remains a major hurdle (59) and improved transfection vehicles are needed. A few different delivery approaches are conceivable to target the skin. The dermis could be targeted by systemic administration because its deeper region is highly vascularized. Another option could be intradermal injections, since spontaneous calcium-dependent uptake of mRNA occurs in dermal cells (67). Because efficient systemic targeting of the avascular epidermis will be more difficult to solve, topical application of mRNA could be an alternative option (20). Achieving cell- and tissue-specific delivery is another challenge (68).

Transcript-replacement therapy is still in its infancy. We here illustrate its potential by a groundbreaking pre-clinical study on a monogenetic disorder affecting another squamous epithelium. Surfactant protein B (SFTPB) deficiency (OMIM 178640) is an autosomal recessive condition with

neonatal lethality due to respiratory failure (69). Direct administration of *Sftpb* mRNA to the lung under high pressure rescued conditional *Sftpb* knockout mice from lethality by synthesis of surfactant protein B (68). This success was achieved after optimizing both delivery of the mRNA to the lung and protein translation by mRNA modifications (68).

#### IVT mRNA-mediated genome editing

IVT mRNA can also be used for genome editing. Here, the IVT mRNAs encode site-specific nucleases, e.g. zinc fingers, transcription activator-like nucleases (TALEN) or CRISPR-Cas9 that can be designed to generate double-stranded DNA breaks at specific target sites, which are determined by the genomic sequence. To permanently correct a gene, an IVT mRNA encoding such site-specific nucleases can be provided to cells together with a DNA template. After double stranded breaks created by the nuclease, which is encoded by the IVT mRNA, the DNA template may be inserted between the breaks during repair by homologous recombination. The outcome of this approach is correction of the mutated gene, and restoration of wild-type protein synthesis (Fig.1 and Fig. S3) (59). Of note, when no correct DNA template is provided, the DNA repair system will repair the breaks through non-homologous end joining.

IVT mRNA-mediated genome editing has been used to correct *COL7A1* RDEB patient fibroblasts *in vitro* (70). Transfection with IVT mRNA encoding patient-specific TALEN promoted homology-directed repair, but with much lower efficiency than transfection with a plasmid encoding the TALEN. This indicates that mRNA-mediated gene editing is feasible in skin cells, but that further refinement is needed to make it an attractive alternative. *In vivo* proof-of-concept of the approach has been obtained from restoration of surfactant protein B synthesis in the above-mentioned conditional *Sftpb* knockout mouse (71).

A clear advantage of *in situ* genome editing is that the genome itself is corrected, which would theoretically require only a limited number of treatments to obtain long-standing effects. In addition, in contrast to direct IVT mRNA delivery, cell- and tissue-specific targeting is not a pre-requisite, because the natural regulation of gene expression is not changed. Furthermore, because the nuclease activity is limited in time due to the inherited instability of the transfected RNA, this is a safer option than vector-mediated transfer (59, 71). Still, safety issues from both potential off-target nuclease activity and viral vector-based DNA template delivery remain a concern. A yet unexplored challenge in a future clinical phase will again be the delivery efficiency of both IVT mRNAs and DNA templates to the target cells.

#### **Mutant gene/allele knockdown**

Small interfering RNAs (siRNAs) are double-stranded RNA molecules (frequently 20-25 base pairs long) that target mRNA for degradation utilizing the endogenous RNA induced silencing complex (RISC). They were first used in 1995 for silencing protein synthesis *in vivo* (72). Because siRNA may discriminate between two sequences differing by only one nucleotide, it constitutes a particularly interesting therapeutic option for dominantly inherited genodermatoses (Fig.1 and Fig. S4). Therefore, most work has been performed on the dominantly inherited diseases such as keratinopathies (reviewed in (73)) pachyonychia congenita (74) (*KRT6A*, *KRT6B*, *KRT16* or *KRT17*), epidermolytic palmoplantar keratoderma (75, 76), (*KRT9*), and on EB simplex (*KRT5*, *KRT14*) (77). A search on [www.clinicaltrials.gov](http://www.clinicaltrials.gov) for “siRNA” identified one genodermatosis study among 39 registered trials. This phase Ib trial for treatment of pachyonychia congenita was a single-patient, double-blinded, split-body, vehicle-controlled, dose-escalation trial (74). The trial evaluated the safety and efficacy of TD101, an siRNA specifically designed against the mRNA encoding the keratin 6a p.Asn171Lys mutant. Intradermal injections were performed in symmetric plantar calluses on opposite feet. Although the results were promising, with regression of the callus on the siRNA-treated side, the

intense pain experienced by the patient due to the injections was a significant concern. Thus, improved topical delivery methods will be necessary. siRNAs have also been pursued for dominant DEB (78, 79). The rationale there is to change the ratio between mutant and wild-type *COL7A1* transcripts by depleting the mutant version. This would result in increased synthesis of wild-type type VII collagen (80).

These siRNA strategies have the disadvantage of being mutation-specific. To overcome this, a mutation-independent strategy has been proposed (79). The strategy is based on administration of siRNAs to knockdown endogenous *COL7A1* mRNA in conjunction with a sequence-modified *COL7A1* cDNA that is not suppressed by the siRNA.

AONs can also be utilized to knockdown mutant mRNA transcripts. Although not yet described for genodermatoses, this approach is being evaluated for treatment of other genetic conditions like the neurodegenerative disorder Huntington's disease. In this disorder AONs are used to target single nucleotide polymorphisms that are in *cis* with the mutation to knockdown the mutant mRNA by activating RNase H-mediated degradation (81). The approach is applicable to an array of mutations and patients, and could thus also be relevant to several of the genes affected in dominant genodermatoses harboring single nucleotide polymorphisms with high minor allele frequencies. Alternatively, AONs could also be designed in a mutation-specific manner, but that comes with the same disadvantages as described in the section on exon skipping by AONs.

### **Future perspectives and concluding remarks**

It is clear that major advances are being made in the field of therapy development for genodermatoses. The surface area of the skin and the fact that many genodermatoses also manifest in other tissues complicate development of causal therapies. Here, the RNA-modulating therapies detailed in this review may have favorable properties because they generally use smaller tools than

the traditional gene and cell therapy approaches. These properties facilitate systemic delivery. For very small oligonucleotides topical delivery might be a possibility. Despite all the advancements made, there is still much room for improvement for RNA-based therapies. To facilitate this, good animal models are required. However, there are relatively few genodermatoses animal models that can be used to study RNA-modulating therapies (82), and many of the existing models are cDNA knock-ins, which cannot be used to optimize RNA modulating approaches (83). Thus, new genome DNA-based animal models are needed. Animal models might also shed light on treatment cycles. However, treatment regimens depend on many variables such as protein stability, the nature of the mutation, disease history and disease modifiers. Ultimately, treatment cycles may have to be determined for each disease, patient subset or even each individual patient independently.

For many disorders, the disease might evolve due to inflammation and organ damage, which activate secondary self-perpetuating mechanisms that are not a direct cause of the genetic defect (84). Consequently, it is foreseen that the potential benefits even of causal RNA-based therapies will largely depend on the timing of therapeutic interventions. In this regard, it is generally accepted that the therapeutic benefit is larger when patients are treated in the early stages of the disease (85). It may also be necessary to combine therapeutic approaches that restore protein function with, for instance, anti-inflammatory or anti-fibrotic drugs to maintain good quality of life.

Another challenge is that genodermatoses are rare diseases, and therefore, the number of patients in which therapeutic approaches can be tested is limited. This is even more complicated for the RNA-modulating therapies that are mutation-specific because they are only applicable to a subset of an already small group of patients. Each therapeutic compound is considered a separate medicinal product and must be evaluated separately. When patient numbers are very small, this poses obvious challenges. In this regard, a lot can be learned from the way the DMD community is trying to solve these issues in the development of the exon skipping approach. Here, several stakeholder meetings

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have been organized with academic researchers, regulators, patient representatives, and pharmaceutical companies to discuss the challenges and opportunities (86, 87). These discussions imply that it may be possible to have smaller trials for additional DMD exon skipping AONs once clinical benefit has been convincingly shown for multiple AONs. The discussions also made clear that it is critically important to be prepared for clinical trials. They further underscored the importance of clinical trial registries for rare diseases like those coordinated by TREAT-NMD (a clinical trial infrastructure network for neuromuscular disorders, <http://www.treat-nmd.eu/>) to facilitate selecting as many patients as possible to participate in clinical trials (88). Additionally, the insufficient understanding of the natural progression of many genodermatoses will likely impede translation of therapies into the clinic because it makes it challenging to define relevant trial endpoints. As more potential therapies are entering the clinical trial stage, there is an increasing demand for natural history studies for genodermatoses, which are now being planned and pursued (89).

The increased interest in rare diseases from newly formed disease-specific companies or large pharmaceutical companies is making the future brighter for affected individuals and their families. So where will RNA-based therapies for genodermatoses stand a decade from now? It is likely that by then a few approaches will be undergoing clinical trials. The top candidates for fast clinical implementation are therapies where studies in other diseases have paved the way, i.e. AONs and stop codon read-through therapies. Other approaches will likely take longer to develop, but they may on the other hand, yield greater clinical benefit. It is therefore imperative to continue pursuing research on all varieties of RNA-based therapies.

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### **Disclosures**

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### **Table and figure legends**

**Table 1.** Summary of the present status of RNA-based therapies for treatment of genodermatoses.

**Figure 1.** RNA-based therapy approaches for genodermatoses. Graphic summary of principles and major mechanisms of the RNA-based therapy strategies presented in this review. More detail on the specific approaches can be found in their specific sections in the text and in Figures S1-S5.

## Supplemental information

### Stop codon read-through

Mutations converting an amino acid into a premature termination codon (PTC) are found in one-third of all inherited genetic disorders (1). For genodermatoses, such as xeroderma pigmentosum and RDEB (2, 3), in which a relatively minor increase in protein synthesis significantly improves disease symptoms, chemically enhanced translational read-through of PTC is an attractive option. Small molecule drugs can create structural alterations in the ribosomal RNA that disrupt normal PTC recognition. As a result, the PTC is ignored, error-prone translation induced, i.e. translational PTC read-through occurs, and translation of mRNA continues beyond the PTC (Fig.1 and Fig. S5) (1). Clinical trials performed in other genetic diseases, e.g. cystic fibrosis and DMD, have led to cautious optimism (4-6). Aminoglycosides are the most prominent inducers of PTC read-through and most studies on read-through-based therapies have been performed with these compounds (1). Specifically for genodermatoses, aminoglycoside and non-aminoglycoside treatments have been shown to increase synthesis of xeroderma pigmentosum complementation group C protein in fibroblasts from patients with xeroderma pigmentosum - a genodermatosis accompanied by a 10,000-fold increased risk of skin cancer (7). Furthermore, for RDEB, aminoglycoside treatment led to *de novo* synthesis of type VII collagen and deposition of the protein at the dermal-epidermal junction zone in skin equivalents (8). Thus, the potential of read-through therapies for genodermatoses has already been recognized.

Aminoglycosides have known side-effects that involve renal and irreversible ototoxicity. Investigation of significantly less toxic non-aminoglycosides with stop codon read-through capacity is

being pursued (1, 9). One example is the compound PTC124 (Ataluren/Translarna) (10, 11) that has been tested in clinical trials for multiple diseases. Development is most advanced for Duchenne muscular dystrophy (DMD) (6, 12), for which it has received conditional marketing authorization from the European Medicine Agency for the use in ambulant DMD patients 5 years and older (13). In a recent phase III trial in DMD, the primary endpoint was not met, except in a prespecified subgroup (14) and further studies are needed to determine the usefulness of ataluren for genodermatoses. One study with this compound with several *COL7A1* and *KRT6A* stop codons could not confirm its read-through potential in a genodermatosis setting (15).

There are intrinsic factors related to the nature of the mutation and mutated proteins that may limit the use of read-through therapies. When nonsense-mediated mRNA is very efficient, there will be no or only a very limited number of target transcripts for the PTC inducing compounds. Furthermore, forced read-through of the mutated codon may evoke identity change of the original amino acid leading to either loss- or gain-of-function of the protein. Additionally, an increased error rate during translation (i.e. insertion of another amino acid than the wild-type one) may significantly impact the functionality of proteins, especially proteins with complex folding and organization. Furthermore, not only the nature of the stop-codon itself, but also the flanking sequence – especially the +1 position after the stop codon – is crucial in determining the read-through potential of stop codons and read-through compounds (16). Therefore, the feasibility and efficacy of induced-translational PTC read-through as therapy must be determined separately for each compound for each disease and each mutation.

### **RNA editing**

RNA editing is an approach that could have future therapeutic value. Therefore we will briefly introduce it, although it is currently in very early stages and not yet, to our knowledge, studied for genodermatoses. Therapeutic RNA editing does not employ RNA molecules to exert a therapeutic effect; it rather aims to correct mutant RNAs. RNA editing is a physiological post-transcriptional

process that increases transcript diversity by enzymatically modifying bases of RNA-molecules in thousands of genes (reviewed in (17)). The best-known example of endogenous RNA editing is A-to-I-editing, the deamination of adenosine (A)-bases into inosine (I)-bases, which is executed by a group of enzymes called the ADAR editases (adenosine deAminase acting on RNA). As the inosine is subsequently regarded as a guanosine (G), RNA editing has the potential to induce many A>G transitions and, consequently, to create different RNA versions of single RNA transcripts with different effects on the resulting protein. Successful attempts to direct the catalytic activity of the ADAR enzymes to specific adenosine bases have been carried out by conjugating the catalytic domain to guide RNAs, i.e. complementary RNA sequences to the target sequence (18). For instance, this approach has been performed *in vitro* to correct a stop codon in the *CFTR* gene (19). Many questions regarding the true clinical potential of RNA editing exist and the future studies will tell whether it could have therapeutic potential for genodermatoses.

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**Supplemental figure legends.**

**Figure S1. Principle of AON-based therapy.** (1) Small AONs (20-30 bp) are designed to target an in-frame exon that contains a mutation, in this case a pre-terminal stop codon (PTC). The AONs undergo *in silico* and *in vitro* selection to find one AON, or a combination of AONs, with the best performance. (2) After intracellular uptake *in vitro* or *in vivo* AONs bind to the pre-mRNA causing the interacting exon to be skipped during splicing. A mature mRNA lacking the targeted exon is

assembled, which allows translation of a shorter protein missing the amino acids encoded by the removed exon while retaining its functionality.

**Figure S2. Principle of *trans*-splicing-based therapy.** *Trans*-splicing is based on delivery of a pre-mRNA *trans*-splicing molecule (RTM) encoding parts of the transcript that need to be corrected. The RTM can contain the 5', 3' or an internal part of the cDNA. During assembly of pre-mRNA, a binding domain (BD) of the RTM binds to the target intron/exon region, inducing *trans*-splicing-mediated formation of a corrected hybrid transcript. The fusion transcript, comprising parts of the endogenous mRNA and the wild-type portion provided by the RTM, results in translation of wild-type protein.

**Figure S3. Principle of IVT mRNA-based therapy.** (1) A DNA template, a sequence-verified PCR product or a plasmid can be used for mRNA transcription. The template can either be a wild-type template or codon-optimized for improved translation. (2) RNA polymerases, usually SP6 or T7, are used for *in vitro* transcription. (3) After completion of transcription a 5' cap is added to the mRNA, the DNA template digested and the mRNA purified. (4) Two approaches of IVT mRNA-based therapy are relevant for genodermatoses. I: Transcript replacement, where the IVT mRNA is translated into wild-type protein after *in vivo* uptake into cells to transiently restore protein synthesis. II: Gene-editing using IVT-mRNA encoding tailored nucleases such as zinc finger nucleases, TALEN or CRISPR-Cas9, which after uptake in cells together with a DNA template (e.g. from viral vector or minicircle DNA) enables a sustained repair of the genomic DNA. .

**Figure S4. Principle of siRNA and AON-based mutant gene knockdown therapy.** A specific siRNA or AON is designed to target a dominant mutation or single nucleotide polymorphism *in cis* with the mutation. After cellular uptake the siRNA/AON specifically binds to the mutation-containing

mRNA. For siRNA, this binding induces the formation of a RNA-induced silencing complex (RISC) promoting cleavage of the siRNA-bound mRNA by RNases. For AONs, it activates RNase H cleavage of the targeted mRNA. As a consequence the wild-type mRNA transcript is enriched leading to relatively higher levels of wild-type protein being translated, thus diluting the dominant negative effect.

**Figure S5. Principle of stop codon readthrough.** The readthrough of a PTC can be enhanced by the treatment with small molecule drugs. The molecules bind to the ribosomal RNA and reduce specificity of the codon-anticodon pairing and thus drive error-prone translation. Translation of the wild-type transcript results in a full-length protein. Translation of the mutated transcript leads to premature stop of protein translation. After treatment with a small molecule, readthrough of the PTC is induced and translation of full-length proteins increased. The efficacy of the treatment depends on the mutation and sequence.

**Table 1**

| RNA-based therapy                                | Mechanism of action  | Delivery  | Advantages  | Limitations   | Disease  | Key references   |
|--|--|---|---|---|--|--|
| AON  | Induced skipping of mutated exon   | Systemic<br>Intradermal<br>Topical  | 1. Systemic administration / multiple routes of administration<br>2. Natural regulation of gene-expression kept<br>3. Advanced clinical development for other genetic diseases<br>4. Low toxicity and limited adverse events in clinical trials | 1. Not suitable for all exons and genes<br>2. Effect mutation dependent and difficult to predict  | EB   | Aartsma-Rus, 2010 (9)<br>Goto et al., 2006 (14)<br>Turczynski et al., 2012 (15)<br>van den Akker et al., 2009 (20)           |
| Trans-splicing                                   | Exchange of an mRNA part, carrying a mutation, by its wild-type copy using the endogenous splicing machinery   | <i>Ex vivo</i> (correction of skin cells and re-grafting)<br>intraepidermal / intradermal | 1. Delivered transgene is shorter than full-length transcript (facilitates cloning and delivery)<br>2. Natural regulation of transgene expression<br>3. Applicable for dominantly inherited disorders   | 1. Safety issues from viral vector (stable integration is needed to achieve high trans-splicing efficiency)<br>2. Safety issues from trans-splicing molecule (unspecific splice events) | EB   | Koller et al., 2014 (38)<br>Murauer et al., 2013 (34)  |
| IVT mRNA-mediated transcript-replacement therapy | Introduction of corrected mRNA transcripts to drive expression of wild-type proteins in vivo   | Systemic<br>Intradermal (Topical)   | 1. Correct posttranslational modification<br>2. Multiple routes of administration<br>3. Non-integrative   | 1. Multiple administration<br>2. Delivery to the skin<br>3. Inability to regulate tissue specific translation and protein expression levels   | -  | Kormann et al., 2011 (51)  |
| IVT mRNA-mediated gene editing                   | <i>In situ</i> gene repair with IVT mRNA coding for optimized zinc finger, transcription activator-like, or CRISPR-Cas9 nucleases together with a DNA template | <i>Ex vivo</i> (correction of skin cells and re-grafting)<br>Systemic<br>Intradermal      | 1. Natural regulation of gene-expression kept<br>2. Transient activity making it safer than vector-based approaches   | 1. Safety issues from nuclease activity and viral vector based DNA template delivery<br>2. Delivery to the skin   | EB   | Osborn et al., 2013 (54)<br>Mahiny et al., 2015 (55)   |
| Mutant gene/allele knockdown                     | Targeted-degradation of mutant transcripts   | Intradermal   | 1. Can be used for dominant disorders<br>2. Non-integrative<br>3. Natural regulation of gene-expression kept  | 1. Painful drug administration<br>2. Effect mutation dependent and difficult to predict   | Pachyonychia congenita, epidermolytic palmoplantar keratoderma, EB | Atkinson et al., 2011 (61)<br>Leachman et al., 2010 (58)<br>Leslie Pedrioli et al., 2012 (59)<br>Pendaries et al., 2012 (62) |
| Stop codon readthrough                           | Induced readthrough of PTCs  | Oral  | 1. Systemic administration<br>2. Natural regulation of gene-expression kept<br>3. Advanced clinical development for other genetic diseases  | 1. Toxicity<br>2. Only for PTCs<br>3. Increased translational inaccuracy  | Xeroderma pigmentosum, EB  | Cogan et al., 2014 (70)<br>Kuschal et al., 2013 (69)   |



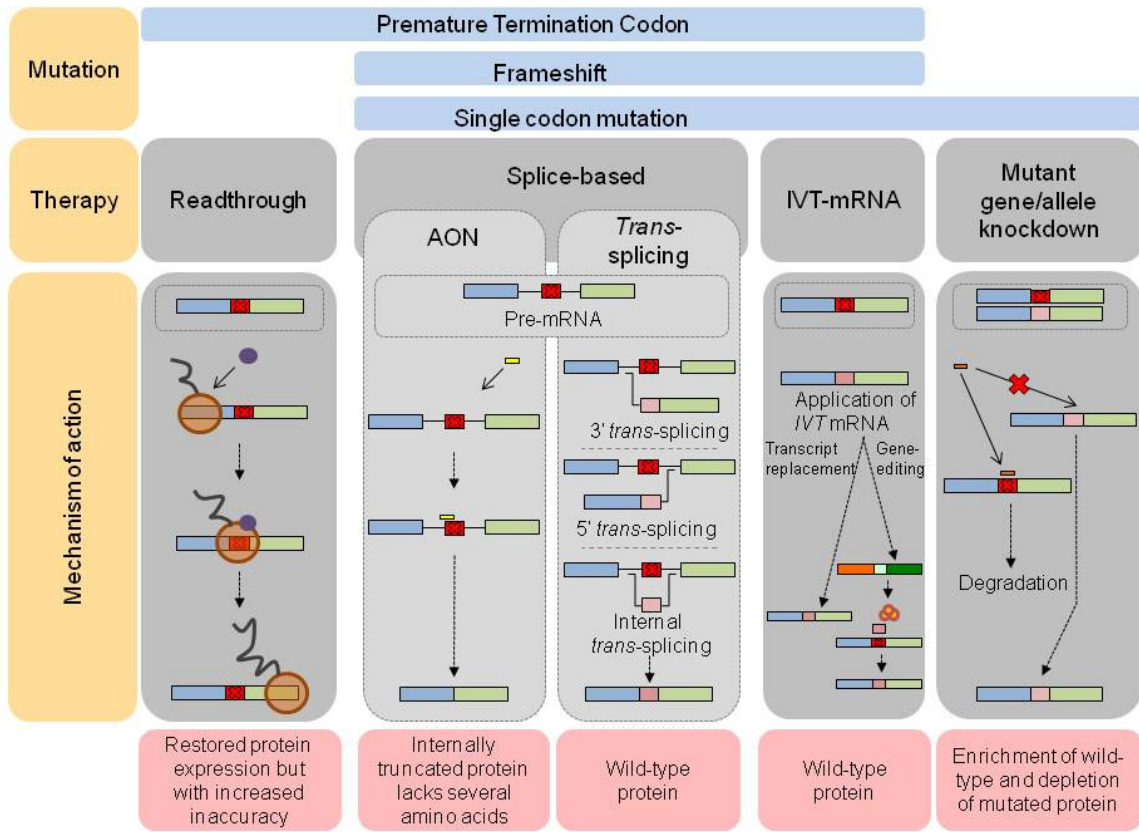


Figure 1