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REVIEW

Antisense molecules: A promising new therapy for atopic dermatitis



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Abstract Atopic dermatitis (AD) is a common chronic inflammatory skin disorder affecting all age groups, especially children, with a prevalence of up to 20% globally. AD remains burdensome and incurable with current therapeutic strategies—ranging from trigger avoidance and skincare to medication—primarily address symptoms rather than disease modification, underscoring the imperative for innovative therapeutic paradigms. RNA-targeted therapies, particularly antisense molecules, have emerged as a transformative approach in precision medicine, with proven clinical success in diseases such as spinal muscular atrophy and familial chylomicronemia syndrome. These therapeutics achieve post-transcriptional regulation unattainable by conventional therapies, enabling direct targeting of messenger RNA (mRNA) and regulatory non-coding RNAs (ncRNAs) implicated in disease pathogenesis. Furthermore, skin is better suited to the antisense modulation due to the relatively easy access to target cells. Numerous studies have explored antisense-based targeting of key drivers in AD progression, yielding promising proof-of-concept results and prompting several early-stage clinical trials. This modality represents a paradigm shift in AD management—one that aligns with the broader revolution in RNA therapeutics reshaping modern medicine. This review critically examines the evolving role of antisense technology in AD, addressing both its mechanistic rationale and the translational challenges that must be overcome to realize its full clinical potential.

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1. Introduction

Atopic dermatitis (AD), a chronic heterogeneous inflammatory skin disease, can affect up to 20% of children and 10% of adults worldwide, with over 200 million people impacted. Its prevalence has increased in both developed and developing nations in recent decades¹. AD presents with a range of symptoms, from acute eczema-like eruptions such as erythema, papules, erosion, vesicles, and exudative lesions, to long-term skin thickening and lichenification². The most disruptive symptom is intense, persistent itching, which not only interferes with daily life but also causes sleep disturbances^{3,4}. Beyond skin issues, AD has multisystemic features, such as metabolic disease, including diabetes, cardiovascular disease, etc., as well as infections, malignancy, autoimmune diseases, and mental disorders⁵⁻⁷. Moreover, several studies indicate a positive correlation between the severity of AD and the incidence of these comorbidities^{5,6}. AD is highly heritable, and its onset often triggers other allergic conditions like asthma, allergic rhinitis, and eosinophilic esophagitis, with the risk increasing with AD severity⁸⁻¹⁰. About a third of AD patients may eventually develop asthma, and another third will have allergic rhinitis¹¹. As a lifelong condition, AD significantly impacts both patients and their families, reducing quality of life and placing a financial burden on society through rising healthcare costs and lost productivity¹².

The pathogenesis of AD involves complex interactions between genetic factors, epidermal barrier dysfunction, immune system abnormalities, epigenetic changes, and so on¹³. Although treatment strategies for AD have evolved, challenges remain in addressing clinical needs. Traditional treatment regimens, primarily based on topical corticosteroids (TCS), topical calcineurin inhibitors, phosphodiesterase-4 inhibitors, phototherapy, and broad-spectrum immunosuppressive agents, have been complemented in recent years by the advent of biologics and small-molecule drugs¹⁴. Notably, dupilumab, targeting the interleukin (IL)-4/IL-13 receptor, nemolizumab, an IL-31 receptor inhibitor, and Janus kinase (JAK) inhibitors have improved outcomes for moderate-to-severe AD¹⁵⁻¹⁷. However, a substantial proportion of patients still experience treatment resistance or partial responses, and novel targeted therapies may be associated with adverse effects such as blepharitis, head and neck dermatitis, or exacerbation of acne¹⁸. While current therapies can achieve short-term symptom control, long-term management of AD faces dual challenges: first, traditional immunosuppressants may increase the risk of long-term complications such as leukopenia, hepatotoxicity, and nephrotoxicity due to systemic exposure¹⁹; second, existing targeted therapies fail to fully block the multi-pathway pathogenesis of AD, resulting in persistently high recurrence rates, which underscores the necessity of developing long-term therapies targeting the root causes of AD.

Antisense molecule technology, as a focal point in contemporary biopharmaceutical research, offers a promising new direction²⁰. They are a class of therapeutic oligonucleotides designed to target disease-associated messenger RNA (mRNA), or regulatory non-coding RNAs (ncRNAs). And after base complementary pairing, they modulate the gene expression in various ways²¹ (Fig. 1). Based on the broad-spectrum action characteristics of ncRNAs in gene regulation, antisense molecules can systematically target gene expression networks related to AD, achieving coordinated intervention of multi-pathway nodes^{22,23}. This approach not only achieves long-term remission but also fundamentally alters the AD's course through epigenetic reprogramming²⁴.

More and more antisense drugs have gained approval for various conditions, such as fomivirsen²⁵, mipomersen²⁶, nusinersen²⁷, eteplirsen²⁸, inotersen²⁹, golodirsen³⁰, volanesoren³¹, viltolersen³², casimersen³³, tofersen³⁴, eplontersen³⁵ (Table 1²⁵⁻⁴¹). Several clinical trials are underway. CDR132L, a first-in-class microRNA (miRNA)-132 inhibitor, was safe and well-tolerated in a Phase Ib trial for chronic ischemic heart failure⁴². A Phase IIa clinical trial of BP1001, an antisense DNA (ODN) targeting growth factor receptor-bound protein 2, in combination with venetoclax and decitabine in patients with acute myeloid leukemia who are not candidates for intensive induction therapy is ongoing, with interim data showing encouraging efficacy and no drug-related toxicity (NCT02781883)⁴³. Bepirovirsen targeting all hepatitis B virus (HBV) mRNA was shown to continuously reduce hepatitis B surface antigen and HBV DNA in 9 to 10% of patients with chronic HBV infection in a phase 2b trial⁴⁴ (Table 2⁴²⁻⁴⁴). These examples highlight the potential of antisense therapies (Table 3^{21-24,45-52} provides a brief overview of different kinds of antisense molecules) as a novel and feasible therapeutic strategy for diseases like AD. Additionally, since the skin is accessible, antisense drugs can act directly on the lesion without the need for liver metabolism, which has a unique advantage for treating skin diseases^{53,54}. To become a reality for treating AD, however, therapeutic antisense strategy in AD is still in its infancy and must first clear some significant obstacles. This review focuses on the latest advances and ongoing efforts to bring antisense therapeutics to AD.

2. Causes and molecular pathogenesis of AD

2.1. Genetic defects

Typically, AD patients have a family history of atopic diseases. To date, a variety of AD susceptibility genes have been identified; the most important ones are those encoding structural and functional proteins of the epidermis and proteins that modulate the innate and adaptive immune responses⁵⁵. One key risk factor for AD is epithelial barrier dysfunction caused by mutations in the filaggrin (*FLG*) gene⁵⁶. *FLG*, a major protein in the stratum corneum (SC), aggregates keratin fibers to form a barrier that prevents moisture loss and protects against allergens⁵⁷. Deamination of the *FLG* produces a natural moisturizing factor, which can retain an adequate amount of moisture in the skin, maintain the skin pH value at a proper level, and then inhibit the expansion of *Staphylococcus aureus*⁵⁸. The *FLG* loss-of-function mutation will impair the skin barrier, leading to increased water loss, elevated skin pH, and greater permeability to allergens, which heighten the risk of AD^{59,60}. Additionally, roughly half of patients with moderate to severe AD had *FLG* with null mutations⁶¹. Genetic barrier defects also reduce the expression of other key structural proteins, such as keratin, loricrin, involucrin, and cladin-1, claudin-23, compromising the mechanical barrier function of the skin⁶². In addition, a European and multi-ancestry genome-wide association meta-analysis of AD reported two other genes significantly associated with AD. They were *S100A9* (OR = 1.36, *P* = 5.5E-212) and *RORC* (OR = 1.24, *P* = 3.1E-134), whereas the association of *FLG* with AD had an OR = 1.41, *P* = 1.4E-228⁶³. *RORC*, encoding the Th17-specific transcription factor retinoid-related orphan nuclear receptor gamma t, drives Th17 differentiation and synergizes with other factors to activate Th17-associated genes (e.g., *IL17A*, *IL17F*, and *IL23R*)⁶⁴. Th17 cells, which are closely associated with AD's pathogenesis, produce cytokines like IL-17A, IL-17F, and IL-23. These cytokines are elevated in

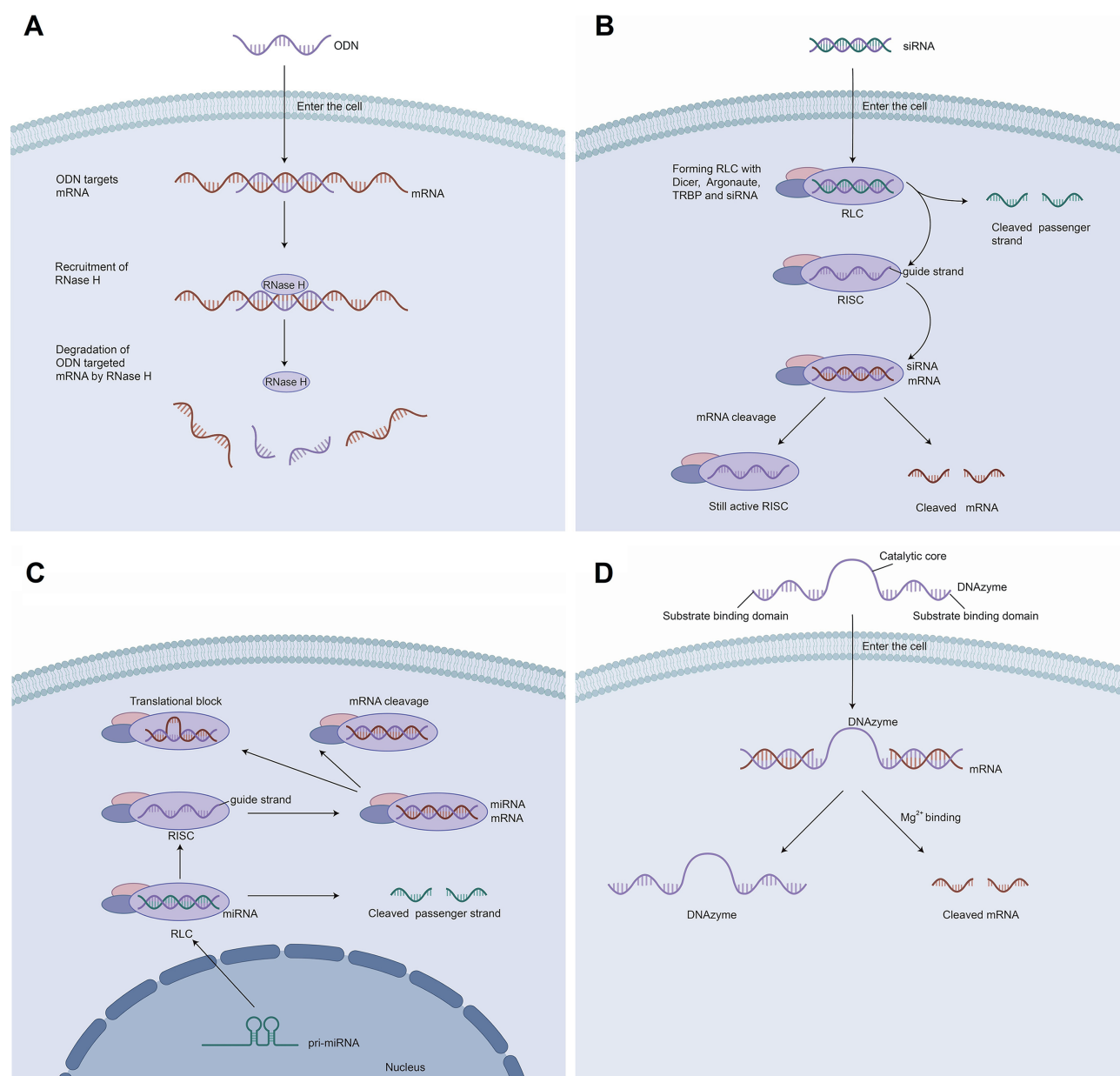


Figure 1 Mechanisms of oligonucleotide-induced regulation of gene expression. These four methods involve binding complementary oligonucleotides to the target RNA through base pairing, and thus all four methods essentially operate through an antisense mechanism. However, their downstream mechanisms differ significantly. (A) Antisense DNA (ODN) enter cells, bind to their target mRNA, and recruit ribonuclease H (RNase H), an enzyme that causes RNA cleavage. (B) *Via* endocytosis, siRNA enters the cytoplasm, where it combines with Dicer (RNase III endonuclease), Argonaute (RNase), and transactivation response element RNA-binding protein (TRBP) to form the RNA-induced silencing complex loading complex (RLC). To create a mature RNA-induced silencing complex (RISC), the RLC keeps the guide strand of siRNA and eliminates the passenger strand. Once the target mRNAs have sequences complementary to the siRNA guide strand, RISC may bind to them. Argonaute then induces cleavage of the mRNA and subsequent degradation by exonuclease. The RISC is a multiple-turnover enzyme, and thus the still-active RISC continues to bind to other target mRNA molecules. (C) In the nucleus, RNA polymerase II produce hairpin-containing pri mRNA, which is then processed by Drosha and di George critical region 8 proteins to form pre mRNA. Next, pre mRNA is exported to the cytoplasm, processed by Dicer into duplex, and loaded into the RLC for action. The mechanism of action is similar to that of siRNA. With the difference that the mature miRNAs are often not fully complementary to their targets and result in translational repression upon binding to the target mRNA. When a perfect match is made with the target, the miRNA exerts a cleavage effect. (D) The target RNA binds to the substrate binding domains of DNAzyme. After the recruitment of RNase H, the DNAzyme cleaves the target RNA in the presence of Mg^{2+} .

pediatric AD patients and positively correlate with disease severity⁶⁵. In murine models of AD, IL-17E upregulation stimulates the production of endothelin-1, a key pruritogenic mediator⁶⁶. Studies have demonstrated that IL-17 can induce the

expression of antimicrobial peptides (*e.g.*, IL-36 γ , S100A7, and human beta-defensin 2), chemokines including C-X-C motif chemokine ligand 8 and C-C motif ligand 5 (CCL5), as well as other cytokines like vascular endothelial growth factor, IL-8, and

Table 1 Antisense therapeutics approved by the FDA and EMA.

Drugs name	Target	Indication	Administration	Approval year	Ref.
ODN					
Fomivirsen	<i>CMV IE2</i>	Cytomegalovirus retinitis	Intravitreal	1998 ^{a2002}	25
Mipomersen	Apolipoprotein B-100	Hypercholesterolemia	Subcutaneous	2013 ^{a2018}	26
Nusinersen	<i>SMN2</i>	Spinal muscular atrophy	Intrathecal	2016	27
Eteplirsen	Dystrophin gene exon 51	Duchenne muscular dystrophy	Intravenous	2016	28
Inotersen	<i>TTR</i>	Hereditary transthyroxine amyloidosis	Subcutaneous	2018	29
Golodirsen	Dystrophin gene exon 53	Duchenne muscular dystrophy	Intravenous	2019	30
Volanesorsen	Apolipoprotein CIII	Familial chylomicronemia syndrome	Subcutaneous	2019	31
Viltolarsen	Dystrophin gene exon 53	Duchenne muscular dystrophy	Intravenous	2020	32
Casimersen	Dystrophin gene exon 45	Duchenne muscular dystrophy	Intravenous	2021	33
Tofersen	Superoxide dismutase 1	Amyotrophic lateral sclerosis	Intrathecal	2023	34
Eplontersen	<i>TTR</i>	TTR-mediated amyloidosis	Subcutaneous	2023	35
siRNA					
Givosiran	Delta-ALA synthase 1	Acute hepatic porphyria	Subcutaneous	2019	36
Lumasiran	<i>HAOI</i>	Primary hyperoxaluria type 1	Subcutaneous	2020	37
Inclisiran	<i>PCSK9</i>	Primary hypercholesterolemia/mixed dyslipidemia in adults	Subcutaneous	2020	38
Vutrisiran	<i>TTR</i>	hATTR-PN	Subcutaneous	2022	39
Nedosiran	<i>LDHA</i>	Primary hyperoxaluria type 1	Subcutaneous	2023	40
Patisiran	<i>TTR</i>	TTR-mediated amyloidosis	Intravenous	2018	41

hATTR-PN, hereditary transthyretin amyloid polyneuropathy; LDHA, lactate dehydrogenase A in hepatocytes, ALA, aminolevulinic acid.

^aWithdrawal of approval.

Table 2 The current preclinical antisense drugs (mentioned in the text).

Drugs name	Target	Indication	Administration	Phase	Ref.
miRNA antagonist					
CDR132L	miR-132	Chronic ischemic heart failure	Intravenous	Phase 1b	42
ODN					
BP1001	<i>Grb2</i>	Acute myeloid leukemia	Intravenous	Phase 2	43
Bepirovirsen	HBV mRNA	Chronic HBV infection	Subcutaneous	Phase 2b	44

HBV, hepatitis B virus.

Table 3 Characteristics of major antisense molecule families^{21-24,39-46}.

Antisense molecule	Antisense DNA (ODN)	siRNA	miRNA	DNAzyme
Structure	Linear ssDNA or dsDNA	Linear dsRNA	Linear ssRNA	ssDNA with two binding domains encircling a central catalytic domain
Length	15–20 nucleotides	21–25 nucleotides	19–25 nucleotides	30–35 nucleotides
Enzymes needed	Ribonucleases H1	RNA-induced silencing complex	No	No
Modifications	<i>e.g.</i> , 2'-O-Me, 2'-MOE and locked nucleic acids	<i>e.g.</i> , 3'-inverted thymidine	<i>e.g.</i> , 2'-F, 2'-O-Me and 2'-MOE	<i>e.g.</i> , 3'-inverted thymidine and PS
Advantages	High target affinity Extensive modification capacity → improved nuclease resistance and pharmacokinetics	Endogenously processed by Dicer Complete mRNA degradation	Multi-gene regulation Extensive modification capacity → enhanced drug properties	Low immunogenicity Enhanced cell penetration Ease of synthesis Minimal side effects
Disadvantages	Off-target effects Chemistry-dependent toxicity	Poor <i>in vivo</i> stability Inadequate cell uptake	Low specificity Unforeseen side effects	Mg ²⁺ -dependent

ss, single-stranded; ds, double-stranded; 2'-O-Me, 2'-O-methyl; 2'-MOE, 2'-O-methoxymethyl; 2'-F, 2'-fluoro; PS, phosphorothioate.

granulocyte-macrophage colony-stimulating factor, while also promoting Th2 immune responses^{67,68}. The Th17/Th22 pathway can induce the expression of S100A9, which is highly elevated in AD patients' serum and skin tissues, correlating strongly with the disease severity⁶⁹. Extracellular S100A9 acts as a pro-inflammatory "sirens", promoting neutrophil, monocyte, and lymphocyte chemotaxis *via* Toll-like receptor 4 (TLR4) and enhancing Th2 responses by inducing keratinocyte-derived IL-33 through the receptor of advanced glycation endproducts^{70,71}. However, no study has yet revealed a direct link between mutations in either of these genes and the development of AD.

2.2. Immune dysregulation

Immunologic factors are also important in the development of AD (Fig. 2). One theory suggests that impaired skin barriers lead to immune dysregulation, while another posits that immune abnormalities make the skin more vulnerable to environmental stimuli⁷². Anyway, targeting the immune system is crucial in the treatment of AD. The main immunological features of AD are Th2 cell activation and related cytokine expression (*e.g.*, IL-4, IL-5, IL-13, and IL-31), further promoting B cell proliferation, IgE synthesis, and eosinophilic activation¹³. The activation of T cells is regulated by multi-layered signaling mechanisms. Following antigen recognition by the T cell receptor, which generates the first signal, the downstream signal molecule, such as IL-2-

inducible tyrosine kinase, amplifies the signaling cascade within T cells^{73,74}. The second signal for T cell activation is provided by the interaction between CD86 on antigen-presenting cells and CD28 on T cells^{75,76}. IL-4 and IL-13 work by binding to IL-4 receptor type II, triggering the phosphorylation of JAK1 and tyrosine kinase 2 (TYK2), leading to the activation of signal transducer and activator of transcription 3 (STAT3) and STAT6, which enter the nucleus to initiate AD cytokine-related gene expression⁷⁷. Additionally, aberrantly expressed IL-10 in AD lesions activates the STAT1/STAT3 signaling axis *via* JAK1/TYK2, playing a pivotal role in regulating Th17/Th22 immune responses, which are more prominent in chronic AD. And these two signaling pathways can be inhibited by cytokine signaling transmission 1 (SOCS1) inhibitors⁷⁸.

Beyond the adaptive immune system, the innate immune system, serving as the skin's first line of defense, also plays a significant role in the development of AD. Keratinocytes, the primary cellular component of the epidermis, are key players in the skin's innate immunity⁷⁹. Upon exposure to external environmental stimuli, the epidermis is damaged, and keratinocytes secrete pro-inflammatory cytokines such as thymic stromal lymphopoietin (TSLP), IL-33, IL-25, and intercellular adhesion molecule-1 (ICAM-1), which promote the maturation and migration of eosinophils, Th2 cells, and other immune cells^{80,81}. Furthermore, macrophage-derived chemokine CCL22 is significantly elevated in the serum of AD patients and correlates with disease severity,

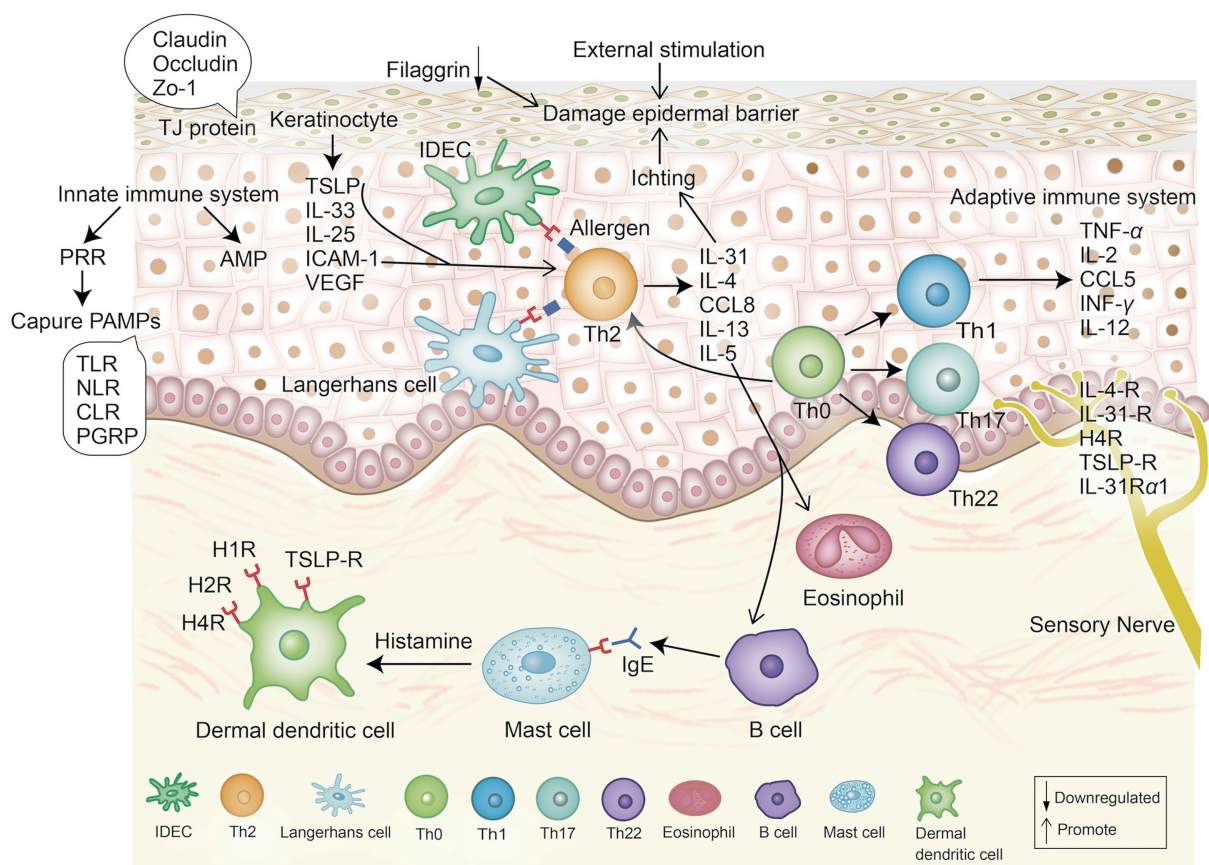


Figure 2 Immunological dysregulation of AD. Zo-1, zonula occludens protein-1; TJ, protein tight junction protein; PRR, pattern recognition receptors; AMP, antimicrobial peptides; PAMPs, pathogen-associated molecular patterns; TLR, Toll-like receptors; NLR, NOD-like receptors; PGRP, peptidoglycan recognition protein; TSLP-R, thymic stromal lymphopoietin receptor; H1R, histamine H1 receptor; VEGF, vascular endothelial growth factor; IDEC, inflammatory dermal dendritic cell; INF- γ , interferon gamma; TNF- α , tumor necrosis factor alpha.

helping recruit Th2 lymphocytes⁸²⁻⁸⁴. Pattern recognition receptors, which are mainly expressed on the surface of innate immune cells, identify pathogen-associated molecular patterns and help to trigger innate immunity^{85,86}. Pattern recognition receptors related to AD mainly include TLRs, NOD-like receptors (NLRs), C-type lectin receptors, etc⁸⁷. TLRs and NLRs are the main receptors against *S. aureus*, and damage to these receptors increases *S. aureus* colonization, which is common in AD⁸⁸. The adaptive immune response also regulates the innate immune system in AD, for instance, under the action of Th2 cytokines, antimicrobial peptides are suppressed, and dendritic cells also lose their ability to release anti-inflammatory IL-10 against bacteria⁸⁹⁻⁹¹. Therefore, therapies targeting the Th2 bias in AD may help restore balance to both the adaptive and innate immune responses.

2.3. Damaged epidermal barrier

AD often originates from skin barrier dysfunction, which is driven by the synergistic interaction between genetic defects (as previously mentioned), environmental factors, and inflammation, creating a vicious cycle of "barrier damage-environmental stimulus-immune activation-barrier deterioration"^{92,93}. In AD patients, ceramide levels are significantly reduced, correlating positively with disease severity⁹⁴. The activities of proteases secreted by *S. aureus* and house dust mites are markedly elevated in AD lesions, while the activity of endogenous protease inhibitors is insufficient, resulting in increased barrier permeability and amplified inflammation. In addition, the use of soap or detergent increases skin pH, together with the activation of serine protease, which accelerates the degradation of ceramide synthetase, leading to disorganized lipid layer structures and further compromising the hydrophobic barrier function⁹⁵. *In vitro* studies show that elevated Th2 cytokines can induce barrier defects even without genetic mutations by downregulating key epidermal proteins like FLG, loricrin, and involucrin, thus impairing barrier function⁹⁶. Notably, pruritus-related inflammatory cytokines such as IL-31 induce scratching behavior, further exacerbating physical barrier damage⁹⁷.

2.4. Epigenetic changes

The rapid rise in the prevalence of AD in recent years cannot be fully explained by the low probability of genetic mutations alone. Instead, environmental factors, such as exposure to house dust mite allergens, have emerged as significant contributors to AD development⁹⁸. Epigenetic mechanisms, including histone modifications, DNA methylation, and miRNA dysregulation, have been identified as key mediators of environmental influences on gene expression. Histone modifications such as acetylation induce tighter chromatin packing, thereby suppressing transcription, while deacetylation promotes chromatin relaxation and transcriptional activation. DNA methylation targets cytosine-phosphate-guanosine (CpG)-rich promoter sequences and inhibits gene expression by blocking transcription factor binding^{55,99}.

The interaction between miRNA therapy and important targets in the pathogenesis of AD has been extensively studied (Fig. 3). Sonkoly et al.¹⁰⁰ and Yao et al.¹⁰¹ identified 44 differentially expressed miRNAs (34 downregulated and 10 upregulated) in AD lesional skin compared to healthy controls. Notably, miRNA-155, one of the most upregulated miRNAs in AD, positively correlates with disease severity. miR-155 can activate the JAK/STAT pathway by targeting SOCS1 and exacerbate T cell activation by inhibiting cytotoxic T lymphocyte-associated antigen-4

expression^{100,101}. One study confirmed that miR-155-5p destroyed tight junction (TJ) protein by targeting protein kinase inhibitor¹⁰². Conversely, miR-143 is downregulated in AD lesions and directly targets *IL13RA1* mRNA, regulating the impact of IL-13 on epidermal barrier function^{100,103}. Another miRNA, miR-100-5p, inhibits the NLRP3 (NLR family, pyrin domain containing 3) signaling pathway by targeting forkhead box protein 3 (a transcription factor of the O subclass of the forkhead family), thereby reducing inflammation and pyroptosis¹⁰⁴. Additionally, miR-146a, known for its role in innate immune responses, suppresses the expression of immune-related factors in keratinocytes, such as IL-1 receptor-associated kinase 1 and tumor necrosis factor (TNF) receptor-associated factor 6, both of which are critical molecules in TLR signaling¹⁰⁵. Rebane et al.¹⁰⁶ further demonstrated elevated miR-146a levels in chronic AD lesions and identified novel targets, including CCL5 and caspase recruitment domain-containing protein 10, which mediate the degradation of nuclear factor kappa-B (NF- κ B) inhibitors. Yang et al.¹⁰⁷ reported reduced miR-124 expression in AD lesions, inversely correlated with elevated levels of *RELA*, *IL8*, *CCL5*, and *CCL8* mRNA. Recently, a study found that miR-147a is significantly downregulated in the serum and skin lesions of AD mice¹⁰⁸.

Furthermore, prenatal and postnatal environmental exposures can permanently alter the epigenome of offspring^{109,110}. For example, maternal tobacco exposure during pregnancy increases miR-223 expression in umbilical cord blood, reduces methylation levels at the TSLP 5'/CpG island, and alters DNA methylation patterns at the forkhead box protein 3 locus, leading to reduced Treg cell counts and heightened susceptibility to AD within the first three years of life^{99,111,112}.

2.5. Other aspects

Pruritus, one of the hallmark symptoms of AD, arises from a highly organized interplay among keratinocytes, the immune system, and the nervous system. Chronic itch in AD is primarily driven by non-histaminergic pathways¹¹³. TSLP directly activates sensory neurons expressing transient receptor potential ankyrin 1, thereby triggering itch signaling¹¹⁴. Additionally, Th2 cytokines such as IL-4 directly stimulate afferent neurons via the IL-4 receptor α and JAK1¹¹⁵. TSLP and substance P amplify this process by activating the production of Th2 cytokines, creating a feed-forward loop in which Th2 cytokines induce additional TSLP expression, perpetuating the itch¹¹⁶. Beyond peripheral sensitization mechanisms, dysregulation of central neural circuits also contributes to AD-associated pruritus. Peptidergic C fibers release neuropeptides such as substance P, exacerbating itch and neuroinflammation¹¹⁷. Moreover, non-peptidergic sensory neurons stimulated by IL-31 release brain natriuretic peptide, which transmits IL-31-mediated itch signals to the spinal cord, linking peripheral inflammation to central pruriceptive processing^{118,119}.

Dysbiosis of the skin microbiome, particularly the abnormal colonization of *S. aureus*, also plays a significant role in AD pathogenesis^{120,121}. Overgrowth of *S. aureus* exacerbates skin barrier dysfunction and inflammation through multiple mechanisms, such as the release of δ -toxin, which stimulates mast cell degranulation, and superantigens like enterotoxins, which activate T cells^{122,123}. Recent studies have emphasized the role of vascular endothelial growth factor in AD by promoting angiogenesis¹²⁴.

Specific transcription factors are integral to AD pathogenesis. Notably, NF- κ B not only enhances the transcription of Th2

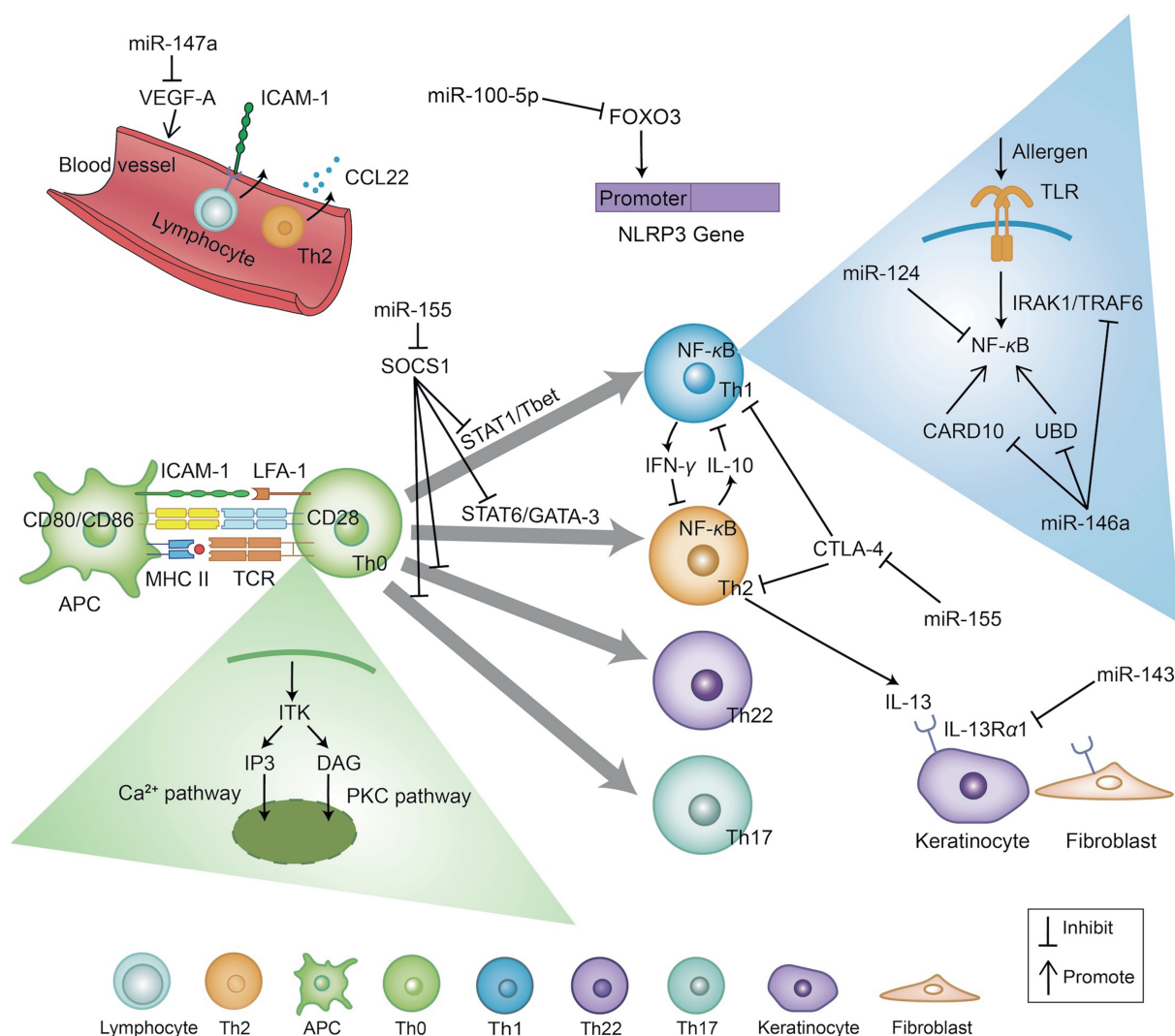


Figure 3 Antisense therapies based on the pathogenesis of AD. APC, antigen-presenting cells; ICAM-1, intercellular adhesion molecule-1; LFA-1, leukocyte function-associated antigen-1; MHC II, major histocompatibility complex II; TCR, T cell receptor; ITK, IL-2-inducible tyrosine kinase; IP3, inositol triphosphate; DAG, diacylglycerol; PKC, protein kinase C; SOCS1, suppressors of cytokine signaling 1; STAT1, signal transducers and activators of transcription 1; Tbet, T-box expressed in T cells; GATA-3, guanine adenine thymine adenine sequence-binding protein 3; NF- κ B, nuclear factor kappa-B; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; CARD10, caspase recruitment domain-containing protein 10; IRAK1, IL-1 receptor-associated kinase 1; UBD, ubiquitin D; TRAF6, tumor necrosis factor receptor-associated factor 6; CCL22, chemokine ligand 22; VEGF-A, vascular endothelial growth factor-A.

cytokines but also induces cytokines like interferon- γ (IFN- γ), modulating the delayed Th1-dominant allergic response in AD¹²⁵⁻¹²⁷. IFN- γ can upregulate cytokines such as CCL5, which recruits eosinophils and macrophages, and CCL8, which drives the recruitment of Th2 cells¹²⁸⁻¹³¹. Knockdown of *RELA* significantly reduces the levels of CCL5 and CCL8¹⁰⁷. Hwang et al.¹³² demonstrated that upregulated IL-31 levels in AD mouse skin lesions are directly associated with increased infiltration of nuclear factor of activated T-cells 1 (NFAT1)⁺CD4⁺ T cells, with NFAT1 enhancing IL-31 expression. Additionally, transmembrane protein 232, significantly elevated in both MC903-induced AD mouse models and human AD lesions, exacerbates inflammation by activating NF- κ B and STAT3 pathways¹³³. Guanine adenine thymine adenine sequence-binding protein 3 (GATA-3) and T-box expressed in T cells (Tbet) are master transcription factors for Th2 and Th1 cell differentiation and activation, respectively¹³⁴.

GATA-3 drives Th2 cells to produce IL-4, IL-5, and IL-13, while Tbet is essential for Th1 cells to produce IFN- γ ^{18,135,136}.

3. AD: Current therapeutic landscape

Topical anti-inflammatory treatments remain the cornerstone of AD symptomatic control. TCS, with its multiple mechanisms—anti-inflammatory, immunosuppressive, vasoconstriction promotion, and so on, has long been the first-line treatment for AD¹³⁷. As an alternative to TCS, topical calcineurin inhibitors (e.g., tacrolimus and pimecrolimus) exert their immunosuppressive effect by inhibiting the activation of T lymphocytes¹³⁸. Phosphodiesterase-4 inhibitors have demonstrated clinical efficacy in AD patients by enhancing anti-inflammatory cytokine production (e.g., IL-10) while concurrently suppressing pro-inflammatory mediators (IL-4, IL-13, IL-22) and chemokine synthesis^{139,140}.

For mild-to-moderate AD, these therapies provide transient relief, but disease recurrence often necessitates prolonged use, leading to dose-dependent adverse effects such as skin atrophy, increased susceptibility to infections, metabolic disturbances, and malignancies^{141,142}. In moderate-to-severe AD, topical agents exhibit minimal efficacy, necessitating escalation to systemic immunosuppressants like cyclosporine, a calcineurin inhibitor used systemically to treat AD¹⁴³, methotrexate, or azathioprine, which use their cytotoxicity to inhibit cells, particularly T lymphocytes, and exert anti-inflammatory effects by inhibiting activation and adhesion molecules^{144,145}. However, these approaches are plagued by non-specific immunosuppression, resulting in substantial off-target risks, such as renal toxicity, hepatosis, and leukopenia¹⁹. In recent years, the introduction of new targeted biologics and small-molecule drugs has brought new hope to AD patients. Dupilumab, a fully humanized monoclonal antibody that blocks the α -chain of IL-4 and IL-13 receptors, has a good curative effect in adults and children aged 6 years or older with moderate-to-severe AD and has been listed in many countries¹⁵. Nevertheless, in addition to being costly, it may suffer from conjunctivitis, herpes zoster, acne, or folliculitis¹⁴⁶. Nemolizumab, which targets IL-31 receptor α , has demonstrated promising efficacy in alleviating itching and improving symptoms in AD patients aged 12 years and older¹⁶, but the efficacy of this biologic was only temporary, suggesting that IL-31 has no upstream role in the pathogenesis of AD¹⁴⁷. JAK inhibitors such as abrocitinib, upadacitinib, and baricitinib have been approved for the systemic treatment of moderate-to-severe AD patients and have shown good therapeutic effects. However, this class of drug has been associated with hepatotoxicity, nephrotoxicity, and blood toxicity (including thrombocytopenia and lymphocytopenia), with black box warnings of increased mortality, malignancy, and major cardiovascular events¹⁴⁸. Despite the availability of these new treatments, a substantial proportion of patients further face treatment resistance or partial responses¹⁸. Allergen immunotherapy (AIT), also known as desensitization therapy, refers to the gradual increase of patient exposure to specific allergen doses, enabling the immune system to progressively adapt and develop tolerance, ultimately achieving the aim of desensitization¹⁴⁹. Several large randomized clinical trials¹⁵⁰⁻¹⁵² have reported that this strategy can importantly improve AD severity and the quality of life of AD patients.

However, AIT's adverse events increased, and no study addressed its potential long-term immunomodulatory effects. A critical limitation of current therapies is their inability to fully address the multi-pathogenic nature of AD, which encompasses dysregulated epidermal barrier function, Th2/Th1-driven inflammation, genetic and epigenetic changes, neuroimmune interactions, and other contributing factors¹³. This incomplete blockade of disease pathways perpetuates a "relapse upon discontinuation" cycle and further complicates long-term management. As a result, the development of safe and effective treatments that synergistically target upstream core disease drivers in AD remains a pressing medical priority, motivating researchers to investigate treatments such as oligonucleotide-based therapies (Fig. 4).

4. Antisense DNA and siRNA as therapeutic strategies for AD

ODNs and small interfering RNA (siRNA) have emerged as potent tools for the specific modulation of gene expression, gaining increasing acceptance for therapeutic applications (Table 1). While siRNAs demonstrate robust mRNA degradation capabilities in cellular cultures, their cell penetration lags behind ODNs and requires the use of cell-penetrating proteins^{21,153,154}. The *in vivo* administration of siRNAs often suffers from poor specificity^{155,156} and immunogenicity, which can lead to severe toxicity¹⁵⁷. To solve those problems, several chemical modification techniques for siRNAs, such as 2'-O-benzyl have been used¹⁵⁸. ODNs are generally preferred due to their superior stability in both cellular and cell-free environments, as well as their simpler synthesis^{159,160}. Nevertheless, the potential for ODNs to exhibit protein-binding activity must be carefully considered in their application¹⁶¹. Given their promise for AD therapy, substantial efforts have been directed toward developing these strategies. This section highlights key examples (Table 4^{103,105-108,132,133,162-183}).

Hertl et al.¹⁸⁴ demonstrated the efficacy of a phosphorothioate oligonucleotide (S-ODN) targeting the 3' untranslated region of *ICAM1* mRNA. At concentrations ranging from 0.2 to 1.0 $\mu\text{mol/L}$, S-ODN abolished ICAM-1 expression in up to 75% of keratinocytes. Their work emphasized the importance of lipid-based delivery systems and ODN backbone structure in optimizing

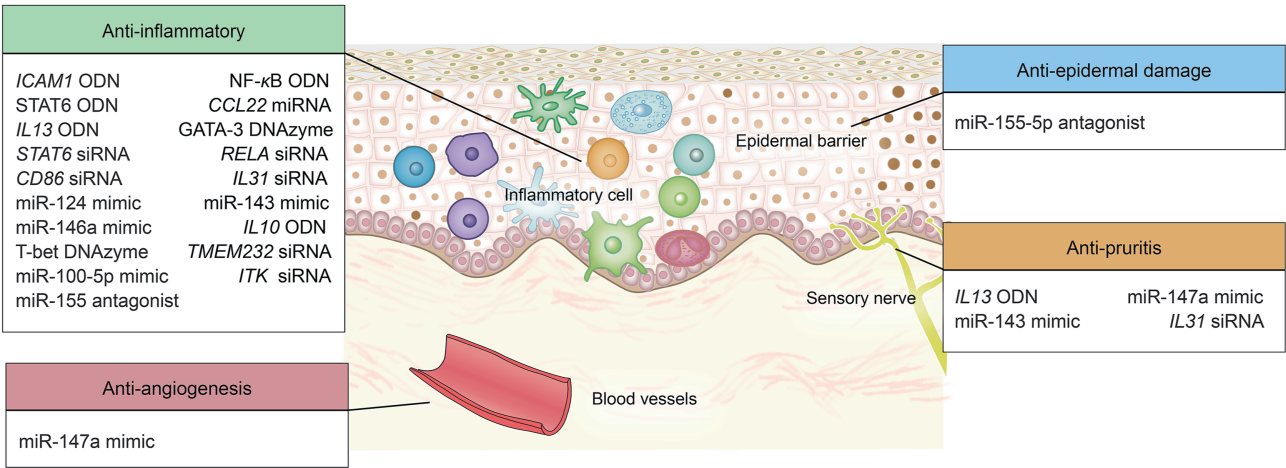


Figure 4 Pharmacological actions of antisense molecules as potential therapeutics for AD. ODN, antisense DNA; NF-κB, nuclear factor kappa-B; STAT6, signal transducers and activators of transcription 6; T-bet, T-box expressed in T cells; GATA-3, guanine adenine thymine adenine sequence-binding protein 3.

Table 4 Studies describing antisense molecules administered in AD therapy.

Antisense molecule	Target	Delivery vector	Administration	Model	Action	Adverse reaction	Ref.
ODN	<i>Icam1</i> ^b	Liposome Atelocollagen	Intravenous	Mice	Inhibit lymphocyte trafficking and activation	Unreported Immunostimulation/ Histological changes of liver and kidney	162
							163
	<i>Il10</i> ^b	/	Iontophoresis	Mice	Inhibit IL-10	Unreported	173
	NF- κ B ^b	/	Topical application	Mice	Regulate Th1 and Th2 immune responses	Local immunosuppression causes infection and cancer	164
	STAT6 ^b	HVJ—liposome	Subcutaneous/ Intramuscular Topical application	Patient	Regulate Th2 immune responses and IgE production	Unreported	165
				Mice		Unreported	170
				Patient			171
<i>Il13</i> ^b	Cationic liposome	Topical application	Mice	Inhibit IL-13	No obvious side effects	174	
siRNA	<i>Cd86</i>	Cream	Topical application	Mice	Inhibit T cell activation	Unreported	175
	<i>Stat6</i>	Cationic liposome	Subcutaneous	Mice	Regulate Th2 immune responses and IgE production	Th2/Th1 imbalances lead to increased risks of Th1-mediated disease progression	172
	<i>ITK</i>	/	Transfection	Cell	Inhibit T cell development, activation and function	Unreported	176
	<i>Rela</i>	Cell-penetrating peptide	Transfection/Topical application	Cell/mice	Regulate Th1 and Th2 immune responses	Unreported	166-169
	<i>Il31</i>	/	Transfection	Cell	Inhibit IL-31	Unreported	132
	<i>Tmem232</i>	/	Topical application	Mice	Activate the NF- κ B and STAT3 pathways	Unreported	133
	miRNA	<i>Ccl22</i>	Recombinant <i>Salmonella typhimurium</i>	Transfection/Or-al	Cell/mice	Inhibit Th2 cell recruitment	No obvious side effects
miR-146a		/	Transfection/Topical application	Cell/mice	Inhibit IRAK1, TRAF6,CCL5,CCL8, CARD10,UBD	Unreported	105,106
miR-155		/	Transfection	Cell	Inhibit SOCS1	Unreported	179
miR-143 ^a		/	Transfection	Cell	Inhibit IL-13R α 1	Unreported	103
miR-124 ^a		/	Transfection	Cell	Inhibit RelA	Unreported	107
miR-155-5p		/	Transfection/Su- bcutaneous	Cell/mice	Inhibit PKI	Unreported	180
miR-147a ^a		Exosomes	Transfection	Cell	Inhibit VEGFA and MEF2A-TSLP axis	Unreported	108
miR-100-5p ^a		OSA-CMCS hydrogel	Topical application	Mice	Reduce pyroptosis	Unreported	178
DNAzyme	<i>GATA3</i>	/	Transfection	Cell	Inhibit Th2 cell differentiation and activation	No off-targeted effects	181
			Topical application	Mice		Unreported	182
	<i>TBX21</i>	/	Transfection/Topical application	Patient Cell/mice	Inhibit Th1 cell differentiation and activation	Unreported	183 182

NF- κ B, nuclear factor kappa-B; HVJ, hemagglutinating virus of JAPAN; CARD10, caspase recruitment domain-containing protein 10; IRAK1, IL-1 receptor-associated kinase 1; UBD, ubiquitin D; SOCS1, suppressor of cytokine signaling-1; PKI, protein kinase A inhibitor; MEF2A, myocyte enhancer factor 2A; TRAF6, tumor necrosis factor receptor-associated factor 6; TLR2, Toll-like receptor 4.

^aExpression in AD patients is downregulated.

^bThe chemical modification of antisense molecule is phosphorothioate.

antisense efficacy and represented an early successful attempt to combine antisense ODN with lipid delivery systems for gene modulation. Further, atelocollagen-mediated delivery of S-ODN in inflamed mouse models showed over 50-fold greater efficacy compared to a single S-ODN infusion, suggesting enhanced systemic delivery and antisense efficacy¹⁶². Comparable results were observed in a murine AD model using S-ODN encapsulated with egg phosphatidylcholine and cholesterol¹⁶³. However, the absence of pharmacokinetic data—such as half-life ($t_{1/2}$), maximum blood concentration, and area under the curve—limits the ability to guide clinical dosing regimens, such as administration frequency and dose.

Nakamura et al.¹⁶⁴ investigated the efficacy of an NF- κ B decoy ODN ointment in NC/Nga mice. The results demonstrated significant improvements in clinical skin scores and histology, with reduced mast cell migration and increased apoptotic cells. This ointment exhibited fewer prominent local or adverse effects compared to TCS. In a clinical trial with 10 severe adult AD patients, 6 out of 7 patients with facial inflammation responded favorably to the 2% NF- κ B decoy ointment. Although the trunk lesions showed no improvement, suggesting a possible site-dependent effect, the mechanism of which needs to be further explored (e.g., skin thickness). Further phase II trial revealed optimal efficacy at the medium dosage¹⁶⁵. The NF- κ B target has also been validated in the siRNA strategy. Uchida et al. demonstrated effective *Rela* mRNA silencing in mouse dendritic cells using anti-*Rela* siRNA (siRela) complexed with the cell-penetrating peptide Tat, which improved nuclease resistance and transdermal delivery significantly¹⁶⁶. Further optimization with Tat and AT1002 significantly improved ear thickness, skin severity, cytokine levels, and serum IgE in AD mice, enhancing siRNA stability and delivery efficiency greatly¹⁶⁷. Another study employed siRela with a stearyl-oligopeptide OK-102, demonstrating effective *Rela* knockdown without immunotoxicity, highlighting its clinical potential¹⁶⁸. Ibaraki et al.¹⁶⁹ used siRela complexed with a peptide STR-CH2R4H2C and AT1002, showing increased cellular uptake, reduced TNF- α and IL-6 expression, and significant symptom relief in AD mice. Despite the availability of various cell-penetrating proteins, systematic comparisons of their stability, delivery efficiency, and toxicity are lacking, making it difficult to determine the optimal strategy and hindering the timely progress of clinical trials for AD.

In targeting STAT6, siRNA and ODN approaches exhibit distinct developmental trajectories. In the ODN paradigm, Yokozeki et al.¹⁷⁰ reported that subcutaneous STAT6 decoy ODN administration attenuated late-phase inflammatory responses in murine models through suppression of eosinophil/neutrophil infiltration and Th2 cell recruitment. This strategy demonstrated translational potential in a 7-patient clinical trial¹⁷¹ showing reduced refractory facial erythema and pruritus, though its broader applicability remains constrained by limited sample size and unverified long-term safety profiles. Hosoya's team¹⁷² screened three *Stat6* siRNA variants, identifying siRNA 3 as the optimal candidate through its superior suppression of STAT6 protein overexpression in dermal fibroblasts. Subcutaneous injection of siRNA 3 into the auricle of mice resulted in a marked reduction in contact hypersensitivity to trinitrochlorobenzene, dinitrofluorobenzene, and oxazolone, with histopathologic analysis confirming reduced dermal cell infiltration and edema. In summary, despite promising therapeutic prospects demonstrated by animal experiments, safety evaluations, and quantitative data on

$t_{1/2}$ and delivery efficiency of *Stat6* siRNA 3 remain incomplete. As for clinical studies, *Stat6* siRNA has yet to commence, and decoy ODN is still confined to the phase of localized application.

ODN therapy has also been studied for other AD-related targets. Arima et al.¹⁸⁵ used an ODN for mouse *Il10* mRNA (AS6), which significantly decreased the production of IL-10 in RAW264.7, a murine macrophage-like cell line. Sakamoto et al.¹⁷³ demonstrated that topical AS6 application in NC/Nga mice decreased IL-10 and its mRNA levels, leading to lesion resolution. The aforementioned study utilized AS6 derived from murine sources, and its safety and efficacy necessitate further validation through human cellular or clinical trials. Additionally, there was research using the *Il13* ODN/cationic elastic liposome combination, and the IL-13 secretion from Th2 cells was greatly inhibited. Treating the ovalbumin-sensitized murine models of AD with this complex significantly reduced the IL-13 production (by as much as 70% of the control) in the suffering skin regions as well as the IL-4 and IL-5 levels, alongside decreased inflammatory cell infiltration and skin thickness¹⁷⁴.

There have been significant efforts made to develop siRNA therapeutics for AD. Hwang et al.¹³² transfected NFAT1 siRNA into activated CD4⁺ T cells, and IL-31 expression levels were considerably decreased, suggesting the potential of NFAT1 siRNA to treat IL-31-mediated AD symptoms. A study has shown the therapeutic efficacy of cream-emulsified *Cd86* siRNA in NC/Nga mice, resulting in significant improvement of AD clinical signs, along with decreased serum levels of IL-4, IgE, and IgG¹⁷⁵. von Bonin et al.¹⁷⁶ have proved that siRNA-mediated inhibition of IL-2-inducible tyrosine kinase in primary human T cells effectively suppressed T cell receptor-induced lymphokine production. Topical application of transmembrane protein 232 siRNA significantly ameliorated MC903-induced AD-like lesions in mice, reducing AD severity scores, ear thickness, and inflammatory cell infiltration. Concurrently, the phosphorylation levels of RelA and STAT3 were significantly suppressed, and mRNA levels of AD-related cytokines (e.g., IL-1 β , IL-4, IL-5, IL-6, IL-13, IL-31, and IL-33) and chemokines (e.g., CCL17, CCL22) were notably downregulated¹³³.

5. Targeting key miRNAs in the pathogenesis of AD

Since miRNAs are very stable in tissues and biofluids^{186,187}, they can be identified with great specificity and sensitivity using high-throughput sequencing technology. Despite the fact that several miRNAs have been associated with AD, only a small number of them have been functionally validated in AD models to confirm their potential as therapeutic targets. Next, we review several key miRNA studies that highlight potential therapeutic benefits for AD (Table 4).

5.1. miRNA mimic

Zeng et al.¹⁰³ showed that forced expression of miR-143 mimic in normal human epidermal keratinocytes could reverse the IL-13-induced downregulation of FLG, loricrin, and involucrin. Yoon et al.¹⁷⁷ designed a recombinant strain of *Salmonella typhimurium* that expressed CCL22 miRNA, which downregulated CCL22 expression in activated lymphocytes. When mice with AD-like lesions were treated with *Ccl22* miRNA, CCL22 and IL-4 levels were suppressed, and IFN- γ levels were induced. Yang et al.¹⁰⁷ identified that miR-124 directly suppressed the expression of

RelA, suggesting its potential to regulate IFN- γ -induced cytokine production. And transfection of miR-124 mimics into IFN- γ -induced human keratinocytes significantly downregulated the levels of IL-8, CCL5, and CCL8.

In a research¹⁰⁵ using miR-146a mimics in TLR2-induced human keratinocytes, the expression of IL-8, CCL20, and TNF- α was considerably inhibited, further repressing the recruitment of neutrophils. Furthermore, even under homeostatic conditions, miR-146a still suppressed the expression of inflammatory mediators and weakened the TLR2 stimulation in nonstimulated keratinocytes. In MC903-induced AD mouse models, the expression of miR-146a was enhanced in wild-type mice, while miR-146a-deficient mice showed more obvious inflammation, characterized by increased dermal infiltrating cells and increased production of IFN- γ , CCL5, and CCL8¹⁰⁶. This suggests the potential of miR-146a to alleviate chronic skin inflammation in AD.

One study employed miR-147a-overexpressing exosomes in human epidermal keratinocyte (HaCaT) cells and human umbilical vein endothelial cells, which notably weakened inflammatory responses and cell apoptosis in HaCaT cells, and inhibited angiogenesis in human umbilical vein endothelial cells. In the mouse models of AD, miR-147a successfully inhibits pathological angiogenesis and inflammatory damage¹⁰⁸. A study¹⁷⁸ has evaluated the efficacy of miR-100-5p, which is highly expressed in extracellular vesicles (EVs) extracted from the mucus of *Pinctada fucata martensii*, for the treatment of AD. The results demonstrated that it had significant anti-inflammatory effects on HaCaT cells. Furthermore, *in vivo* experiments confirmed its ability to suppress inflammation and pyroptosis. Future research should focus on assessing the immunogenicity and other potential safety risks of these EVs as foreign substances in humans.

5.2. miRNA antagonist

In one study, transfection of miR-155 antagonists into cultured CD4⁺ T cells significantly reduced miR-155, Th17 cell differentiation, and IL17 expression, while markedly increasing SOCS1 expression¹⁷⁹. This highlights the potential of miR-155 antagonists to modulate Th17-mediated immune response in AD. Wang et al.¹⁸⁰ transfected miR-155-5p antagonists into HaCaT cells, protein kinase inhibitor, and TJ proteins (occludin, Zo-1, claudin) expression rose, whereas TSLP and IL-33 levels showed a marked decline. In the AD mouse models administered with miR-155-5p antagonists, molecular changes similar to the cell experiments were seen. Decreased Th2 cytokines release, inflammatory cell infiltration, and epidermal thickening were observed.

6. DNazymes: Targeting transcription factors in AD

Since their first demonstration of *in vivo* activity in 1999¹⁸⁸, 10–23 Dzs have shown significant therapeutic potential in pre-clinical studies across various animal models, positioning them as promising candidates for clinical applications^{181,189,190}. Their catalytic activity, which is independent of intracellular enzymes, allows for flexible design and broad applicability¹⁹¹. Up to now, Dzs against chronic inflammatory illnesses are under clinical investigation, with two Phase II trials being successfully conducted (NCT01743768 and NCT02129439). Dz hgd40, in particular, has emerged as a strong candidate for Phase III trials¹⁹². As more clinical data become available, Dzs may prove to be a safe and effective treatment. This section highlights the

progress of Dzs in AD therapy, focusing on their ability to target key transcription factors (Table 4).

The GATA-3-specific Dz hgd40, with 100% sequence identity between mice and humans. *In vitro*, hgd40 reduced GATA-3 protein and IL-13 production in human Th2-polarized CD4⁺ T cells compared to control Dzs¹⁸¹. Similarly, the human T-bet-specific Dz htd70 decreased T-bet and IFN- γ levels in human Th1-polarized CD4⁺ T cells¹⁸². *In vivo*, hgd40 mitigated oxazolone-induced skin swelling in AD mouse models, consistent with its efficacy in allergic asthma models, underscoring its broad therapeutic potential across target organs^{182,193}. To enhance delivery, Schmidts et al.^{194,195} formulated hgd40 in a water-in-oil-in-water emulsion, improving penetration and protecting Dzs. A phase IIa clinical trial evaluated this formulation in 25 patients with AD (NCT02079688), though results remain unpublished¹⁸³. For T-bet, the mouse-specific Dz mtd32 reduced skin swelling in both oxazolone-induced (mixed Th1/Th2 inflammation) and ovalbumin-induced (Th1-dominant inflammation) models¹⁸².

7. Challenges of using antisense therapeutic agents to treat AD

The above studies have shown that antisense molecules hold potential for treating AD; however, before putting them into clinical practice, several critical challenges require resolution, particularly in ensuring effective and safe delivery, prolonged target-specific action, and favorable pharmacokinetics *in vivo*. These issues are outlined below.

7.1. Inefficient delivery—instability, nucleases, and permeation

Although good effects of antisense molecules on gene regulation have been demonstrated, several major concerns remain—the instability, susceptibility to nuclease degradation, and low permeation efficiency. Recent efforts to optimize their efficiency have focused on chemical modifications and advanced delivery vectors (Table 4).

Chemical Modifications. To enhance stability and resistance to nuclease degradation, the backbone of antisense molecules has been modified with various chemical groups, such as phosphorothioate (PS), 2'-O-methyl, 2'-O-methoxymethyl (2'-MOE), 2'-fluoro, or phosphorodiamidate morphino (PMO)¹⁹⁶. And currently approved antisense drugs are dominated by 2'-MOE (*e.g.*, mipomersen²⁶, nusinersen²⁷, inotersen²⁹, volanesoren³¹ and tofersen³⁴) and PMO (*e.g.*, eteplirsen²⁸, golodirsen³⁰, viltolarsen³² and casimersen³³) modifications. In antisense therapeutics for AD, the most widely studied backbone variant is the PS. It not only protects the antisense molecules from degradation but also increases their hydrophobicity through sulfur atoms and further enhances their interaction with plasma proteins, making them have good pharmacokinetic properties¹⁹⁷. Nevertheless, this modification is also linked to a number of unfavorable side effects¹⁹⁸. In addition, further studies are needed to explore the role of other oligonucleotide modifications in the treatment of AD, such as second-generation chemical modifications, by modifying a ribose sugar based on the PS backbone, which have enhanced nuclease resistance and binding affinity, reduced toxicity, and immunogenicity. Third-generation chemical modifications that enhance target binding, such as nucleobase modifications (methylation on 5' cytosines), 2'-MOE, and constrained ethyl groups¹⁹⁹. Moreover, there has been a development of nucleic acids referred to as bicyclic and locked nucleic acids, which

exhibit remarkable stability in the biological fluids and much-enhanced hybridization capabilities to targets⁴⁵. However, the majority of modifications may also lead to changed toxicological profiles, necessitating a thorough examination of the adverse effects, such as the use of pharmacologically exaggerated doses to assess in rats²⁰⁰.

Delivery Vectors. Through chemical modifications, the stability of antisense molecules was greatly improved, but the permeation efficiency from the skin to the target cells was still unsatisfactory. As large, hydrophilic molecules, antisense molecules struggle to penetrate the skin barriers, including the SC and TJs^{201–204}. Increasing dosages is not a viable solution due to the risk of off-target effects. Physical enhancement techniques like electroporation, sonophoresis, and iontophoresis can promote transdermal delivery; nonetheless, less invasive and simpler topical administration strategies are needed since these procedures are intrusive and subject the skin to injury¹⁶⁶. Nanodelivery systems, which include lipids, polymers, peptides, nucleotides, and inorganics, are popular for treating AD with antisense molecules^{197,205}. The cationic elastic liposome has been shown to improve delivery as it is transformable enough to cross the epidermal barrier²⁰². When applied topically with ODNs, its positive charge helps reduce systemic toxicity by restricting the complex to the afflicted region and facilitates easier cell membrane penetration compared to neutral or negatively charged particles^{206,207}. It has already been reported that the atelocollagen/siRNA combination is very stable and effective in cell transduction²⁰⁸. Ritprajak et al.¹⁷⁵ designed an anti-stimulatory cream-emulsified siRNA for infants, and it turned out to be more effective than the atelocollagen strategy. Lee et al.²⁰⁹ created a poly lactic-co-glycolic acid nanoparticle-based siRNA delivery approach with little cytotoxicity and efficient cellular uptake. Furthermore, they combined the nanoformulation with an ablative laser and showed a significant improvement in epidermis delivery. Hamasaki et al.²¹⁰ identified new RNA interference (RNAi) drugs known as nkRNA1 and PnkRNA1, which have no immunogenicity and are more stable against nucleases than classical siRNA. In combination with stearyl-oligopeptide OK-102, these new RNAi drugs have shown improved cellular uptake and endosomal escape in AD mouse models. Another study utilized oxidized sodium alginate-carboxymethyl chitosan hydrogel as a delivery vector to administer EVs in AD mice. This hydrogel exhibited several desirable properties, including stability, self-healing capability, excellent biocompatibility, and sustained release of EVs at the wound site. Animal experimental results further indicated that the hydrogel-EVs combination significantly alleviated inflammation in mice¹⁷⁸. All in all, for AD's local antisense administration, the skin barrier penetration is largely enhanced with nanodelivery systems, but the target binding affinity still needs to be further improved.

7.2. Unsafe delivery—toxicity, target engagement, and immunogenicity

Toxicity. The administration methods of antisense drugs can significantly affect distribution and toxicity, which remains a key issue in their clinical applications. Common routes of administration include subcutaneous, intraperitoneal, intravenous, direct-to-site administration, etc²¹¹. When administered intravenously, systemic toxicity may occur. For instance, S-ODNs can stimulate

the immune system and cause histological alterations in the kidney and liver^{212–214}, while lipoplexes can cause the generation of pro-inflammatory cytokines^{215,216}. Other systemic symptoms include flu-like symptoms, thrombocytopenia, morphologic changes (e.g., columnar, shrubby, and sessile microglia), and so on^{217,218}. But unlike lipid-based delivery methods, polymer-based delivery systems, such as the ones employed with cyclodextrin polymer-based nanoparticle formulation containing siRNA and atelocollagen, do not seem to have the same immunostimulatory effects^{219,220}. Owing to skin accessibility, the local application can effectively treat skin conditions without the liver's first-pass metabolism. While it minimizes systemic side effects, it may still cause infection and cancer due to local immunosuppression, and local injection site reactions (e.g., itching)^{164,221}. Last but not least, it may be necessary to consider certain clinical side effects associated with the function of regulated genes. For example, a disruption in Th1/Th2 imbalance from STAT6 inhibition may predispose patients to Th1-mediated diseases¹⁷². Therefore, it is important to conduct a comprehensive assessment of the dysregulated network based on the individual condition of patients, rather than focusing on just one pathway. Overall, although two completed clinical trials reported no safety concerns^{165,171}, the safety profile of other antisense strategies in AD is mostly unclear, with one Phase IIa trial (NCT02079688) yet not been reported (Table 5).

Target engagement. Off-target effects of antisense drugs include three main aspects: non-target organ accumulation and unintended RNA or protein binding^{222,223}. One example is the finding that small hairpin RNAs produced by U6 RNA polymerase III may overwhelm the endogenous miRNA pathway and result in significant toxicity *in vivo*²²⁴. Enhancing target engagement at therapeutic doses is crucial for improving the efficacy and safety of antisense therapy. One efficient strategy is to conjugate antisense drugs with molecules (e.g., carbohydrates, antibodies, or aptamers) that bind specific cell membrane proteins²²⁵. For example, *N*-acetylgalactosamine coupled modification, which has high affinity with asialal glycoprotein receptor on hepatocytes, is one of the most widely used antisense drug delivery systems today, as seen in recently marketed drugs like givosiran³⁶, lumasiran³⁷, inclisiran³⁸, vutrisiran³⁹, and nedosiran⁴⁰ (Table 1). Using the inherent specificity of antibodies to cell surface receptors, Sela's team conjugates ODN targeting the microtubule-associated protein tau with blood–brain barrier penetrating antibodies acting as a brainshuttle, showing good *in vitro* activity and *in vivo* pharmacokinetic behavior. It opens up new avenues for the treatment of tau-related neurodegenerative diseases such as Alzheimer's disease²²⁶. AD is often accompanied by increased antibody release, which makes it easy to employ antisense drugs that are conjugated with specific antibodies to enhance target binding. Selective organ-targeted lipid nanoparticles (LNPs), such as targeting the lung, spleen, and liver, have been successfully investigated²²⁷. Additionally, drugs conjugated with LNPs have been approved, like patisiran⁴¹ (Table 1). Although LNP delivery systems have been widely studied in preclinical studies of AD, their cellular targeting may need further investigation for treating AD. The latest study constructed an RNA 3D structure prediction model named RhoFold + combined with deep learning, which can accurately and efficiently predict the tertiary and secondary structure of RNA compared with other algorithms, and may help screen highly specific antisense sequences to avoid the risk of

Table 5 Current AD clinical trials using antisense molecules.

Target	Dose	Primary end-point	Status	Details	Duration of exposure	Efficacy	Adverse reaction
NF- κ B	Qd/bid	/	Phase I completed	t.a. single-dose efficacy study/10 patients	1 month	Effective in facial inflammation, no relief from skin lesions on the trunk	Unreported
	/	Skin symptom scores	Phase II completed	t.a. multiple-dose-escalation efficacy/safety study	/	Improved symptom scores were observed in the moderate-dose group	Undiscovered
STAT6	Bid	EASI	Phase I completed	t.a. investigator-initiated, open-label pilot study/7 patients	4 weeks	Skin lesions and pruritus were clearly improved	Unreported
<i>GATA3</i>	10 mg bid	SCORAD	Phase IIa completed	t.a. single-center, randomized, vehicle-controlled, double-blinded efficacy/safety study/25 patients	15 days	Unreported	Unreported

NF- κ B, nuclear factor kappa-B; t.a., topical application; STAT6, signal transducers and activators of transcription 6; EASI, eczema area and severity index; SCORAD, scoring atopic dermatitis.

competition with RNA-binding proteins (such as splicing factors) or non-targeted RNAs²²⁸.

Immunogenicity. Innate immune response activation has been a crucial issue for the safety of antisense therapeutics. Antisense molecules introduced into cells are perceived as undesirable gene activity, which in turn stimulates the TLRs (TLR3, TLR7, and TLR8, etc.), RNA-dependent protein kinase, and so on²²⁹⁻²³⁴, followed by the activation of inflammatory cytokines, the IFN- γ reactions, and the alternative pathway of the complement system. Symptoms from inflammatory cytokine release can be managed with supportive therapies or extended infusion periods²³⁵, while complement activation is transient and resolves within 24 h without significant adverse effects²¹⁹. The innate immune system's activation depends on the length and sequence of antisense molecules. For example, synthetic siRNAs exceeding 30 bases with 5' triphosphates demonstrate enhanced activation potentials²³⁶⁻²³⁸. Additionally, endosomal TLRs may be activated by "danger" sequences, such as GU-rich sequences, 5'GUCCUCAA3' and 5'UGUGU3'²³⁹. Since the expression cassette-derived RNAi effectors are transcribed in the nucleus, they will not cross the endosomal compartment to activate TLRs²⁴⁰, but may still stimulate immunity *via* unmethylated CpG islands within the expression cassettes²⁴¹. While immunogenicity was a significant issue with the first-generation siRNA drugs²³⁰, substantial 2'-O-methyl base alterations in subsequent RNAi triggers have greatly solved this problem²⁴². For ODNs, polyethylene glycol (PEG) modification of their vectors has reduced immunogenicity in some diseases^{211,243}. Although antisense drugs exhibit generally low and clinically manageable immunogenicity, comprehensive monitoring remains essential for this novel therapeutic class. Given the diverse administration routes of these drugs, special consideration should be given to detecting anti-drug antibody subtypes in different samples (such as serum, cerebrospinal fluid, and tissue fluid) and their dynamic changes. To enable more holistic monitoring, samples should be collected and stored in the early stages of use, and immunogenicity should be investigated based on emerging clinical presentation and exposure data.

7.3. Pharmacokinetics

The pharmacokinetics of systemically administered oligonucleotides have been extensively studied, revealing key insights into their distribution, metabolism, and excretion. Using stem-loop quantitative real-time polymerase chain reaction, it was found that siRNA was significantly distributed in 7 target organs at 0.5 and 2 h post-intravenous injection. The liver exhibited the highest siRNA accumulation, followed by the spleen and kidney, while significantly lower levels were detected in the lung and heart. Notably, the duodenum and brain showed barely detectable amounts²⁴⁴. This organ-specific distribution aligns with previous observations of preferential renal uptake of PS-modified oligonucleotides. It has been demonstrated that the kidney plays a major role in the excretion of oligonucleotides, with the liver being the primary site of degradation²⁴⁵⁻²⁴⁸. While these characteristics position the kidney as a potential therapeutic target for antisense therapies, they also raise concerns about nephrotoxicity risk, which calls for periodic monitoring²⁴⁹. Due to their relatively small size and negative charge, antisense drugs weakly bind to plasma proteins and are rapidly excreted by the kidneys, which results in poor pharmacokinetics and raises questions about their future use in AD¹⁹⁷. Various carriers and modifications have been developed to enhance the size of antisense drugs, thus preventing glomerular filtration, improving their $t_{1/2}$, and exhibiting good gene regulation at therapeutic doses. ALN-VSP employs LNPs to encapsulate siRNA, achieving the circulatory integrity for most siRNA and reaching the plateau level in 6–7 h²⁵⁰. Classical PEG modification prolongs the $t_{1/2}$ by forming a peripheral hydration layer that prevents the clearance of the mononuclear macrophage system. The pharmacokinetics of Willebrand factor-targeted oligonucleotides conjugated to 40 kDa PEG were tested in monkeys with a half-life of 67 h after intravenous administration and a subcutaneous bioavailability of 98%. In blood samples taken 300 h later, the complex still showed 90% activity to inhibit Willebrand factor²⁵¹. A novel approach utilizing the long cycle properties of human serum albumin, oligodeoxynucleotides

targeting factor IXa were conjugated with the cysteine site of albumin, and the constructs showed high stability in serum-containing buffers and completely blocked factor IXa for 2 h²⁵². At present, though many targets in AD have been validated in the preclinical trials, it is urgent to study the pharmacokinetics, safety profiles, and toxicology of antisense drugs in the treatment of AD, to enter clinical trials as soon as possible. Additionally, virtual clinical trial platforms could simulate the pharmacokinetics of various drug delivery regimens, speeding up the identification of optimal antisense strategies for treating AD²⁵³.

8. Current considerations and future perspectives

The current development of antisense therapies often focuses on isolated pathways; however, the complexity of AD necessitates a combinatorial targeting approach. Emerging technologies, such as integrating antisense molecules with CRISPR-based gene-modulating tools like CRISPR-Cas9, may offer a promising avenue to simultaneously regulate RNA expression and repair mutated genes, and mixed-mode strategies that combine ODN with siRNA may significantly broaden the therapeutic scopes of AD^{254,255}. Furthermore, combining antisense therapies with skin microbiome regulators may provide a dual-dimensional approach to prevent disease recurrence by simultaneously targeting immune and microecological pathways. Except for miRNA, circular RNAs in ncRNAs exhibit functionality as miRNA sponges, translation templates, and transcriptional regulation, positioning them as high-value therapeutic targets and tools²⁵⁶. On the one hand, the ncRNA–miRNA–mRNA competing endogenous RNA network can be further improved to help identify hub nodes in AD-specific regulatory circuits for targeted antisense interventions, thereby reducing the risk associated with pan-targeted therapies²⁵⁷. On the other hand, engineered circular RNAs as miRNA sponges capable of sequestering multiple miRNAs have attenuated heart hypertrophy and preserved heart function in a mouse model of transverse aortic coarctation of heart disease, demonstrating their potential as a novel therapeutic tool that may be equally applicable in AD²⁵⁸.

Delivery Vectors is still being improved and optimized. For instance, the combination of Tat and AT1002 peptides with siRNA has shown promising efficacy in treating AD skin lacking SC^{166,167}. To penetrate the SC efficiently, Kawai et al.²⁵⁹ developed a lyotropic liquid crystalline system encompassing Tat and AT1002, and this system considerably boosted intact skin permeability compared to other combinations. Realizing the full potential of antisense therapies requires advancements in endosomal escape technologies. One promising strategy involves conjuring cationic peptides with neutral PMOs, which in clinical trials of Duchenne muscular dystrophy was 18 times more active when given monthly than the FDA-approved non-cationic eteplirsen PMO given weekly²⁶⁰. But this strategy doesn't work with siRNA and ODN, due to their anionic phosphodiester and PS backbones, which will form ionic aggregates with cationic peptides. Another innovative strategy employs photosensitive nanocarriers that generate reactive oxygen species under near-infrared light irradiation, enabling precise spatiotemporal control of antisense molecule release²⁶¹. However, repeated administration of nanocarriers often triggers antibody production, leading to rapid immune clearance²³⁵. To address this limitation, researchers have engineered nanocarriers coated with natural red blood cell membranes. This biomimetic approach not only evades immune

detection but also enhances biocompatibility, reduces visceral organ accumulation, and minimizes toxic side effects²⁶².

The heterogeneity of AD subtypes necessitates real-time monitoring of disease activity to guide antisense molecule selection and dosing. Stimuli-responsive hydrogel-based dynamic drug delivery platforms can be engineered to release antisense oligonucleotides in response to AD-specific biomarkers^{263,264}, such as elevated IL-31 levels or pathological pH variations in lesional skin. Significant advancements have been made in leveraging microneedle delivery systems for AD therapy. Illustratively, researchers have successfully engineered novel nanoantibiotics by integrating EVs with hyaluronic acid-based microneedles, demonstrating efficacy in treating AD-associated bacterial infections²⁶⁵. Future research should prioritize the development of advanced platforms incorporating wearable microneedle sensors. These platforms can detect molecular levels indicative of disease activity in real time and transmit the signals into a closed-loop monitoring system, which immediately initiates microneedle-mediated precision drug delivery after decision analysis by artificial intelligence. These platforms have shown good applications in the management of chronic diseases such as diabetes^{266,267}. By detecting cytokine fluctuations or RNA signatures, we will further explore this technology in AD antisense drug therapy to realize precision therapy. Similarly, the platforms can realize intelligent drug administration by monitoring the dynamic changes of organ-specific toxicity biomarkers. While the long-term therapeutic effects of antisense strategies offer advantages in addressing adherence issues, they also pose potential risks, particularly in vulnerable populations such as pregnant women. To mitigate these risks, high-affinity oligonucleotides that complement the siRNA guide chain have been developed to rapidly and efficiently reverse RNAi activity *in vivo*²⁶⁸.

Current clinical trials on the use of antisense molecules for the treatment of AD have shown encouraging results, yet further research is needed to fully realize their therapeutic potential. Future clinical trials should encompass larger and more diverse patient populations to ensure statistical significance and generalizability. Given that AD is a chronic condition, extending the duration of trials (beyond one year) is crucial for evaluating the long-term safety and efficacy. Subgroup analysis of patients can be performed based on age, disease severity, and genetic background, in which liquid biopsy techniques detecting RNA have been proven to successfully detect different stages of cancer, it can also be further developed in the future to classify AD patients into molecular subgroups, such as “barrier dominant” and “immuno-dominant”^{269,270}. This stratification will help elucidate differential antisense treatment responses. Future trials should include active control groups, such as the current standard treatments, which will provide clearer insights into the relative efficacy and safety of antisense molecules.

The production of antisense therapies necessitates advanced synthesis technologies, exceptional precision, and stringent batch-to-batch consistency, all of which contribute to high manufacturing costs and play a critical role in the development and commercialization of antisense therapies. Consequently, a comprehensive evaluation of the economic feasibility of these therapies within patient populations is essential to ensure their widespread clinical adoption. Existing drugs have achieved significant results through the “pay for results” model. For instance, Harvard Pilgrim Healthcare's partnership with Amgen shows that tiered pricing based on clinical metrics like the six-dimensional evaluation system for rheumatoid arthritis patients can shift the

payment standard from “drug usage” to “treatment effectiveness achieved”, significantly reducing costs. This mechanism also provides a replicable path for cost control of antisense drugs²⁷¹. Microfluidic chip technology has shown breakthrough progress in the field of antisense drug production automation, and its core advantages are reflected in two aspects: First, its precision fluid control reduces synthesis cycles to 1/22 of conventional methods, enabling unprecedented production efficiency; Second, the platform facilitates on-demand drug development, as evidenced by its success in creating antisense candidates targeting SARS-CoV-2 genomic mRNA for COVID-19 treatment and exon 46 of *DMD* pre-mRNA for Duchenne muscular dystrophy. These advances suggest that the technique could also help produce and develop antisense candidates to treat AD²⁷².

The therapeutic efficacy of antisense drugs in AD is intrinsically linked to patient-specific variables encompassing genetic and epigenetic polymorphisms, immune dysregulation patterns, and demographic determinants. Thus, personalized medicine should be a key focus in the development of antisense therapies. A precision medicine paradigm integrating multi-omics stratification (such as transcriptomics, epigenomics, and proteomics) with advanced computational techniques, including artificial intelligence-driven image analysis and natural language processing, enabling the elucidation of complex molecular networks and offering individual therapeutic targets in AD for antisense intervention, thereby enhancing therapeutic efficacy and minimizing the risk of adverse effects²⁷³. In addition, the use of animal models in dermatological research is limited not only for ethical considerations but also because of their inherent physiological disparities with human skin architecture. In contrast, advanced 3D bioprinting technology can print a variety of skin cells (such as melanocytes, endothelial cells, pericellular cells, and adipose cells) into a specific layer of the engineered skin constructs, achieving unprecedented biomimicry of native tissue and enhancement of regenerative function. Beyond replicating cutaneous physiology, this platform can harness precision medicine principles through patient-specific modeling: tumor microenvironment reconstruction using autologous cancer cells exemplifies its capacity for personalized drug discovery in oncology²⁷⁴. Such physiologically relevant models merit additional exploration for preclinical evaluation of antisense therapy in AD, enabling simultaneous assessment of target engagement efficiency, dermal penetration kinetics, and immunotoxicity profiles while maintaining patient-derived genetic signatures.

9. Conclusions

Contemporary therapeutic paradigms for AD predominantly adhere to classical single-target interventions, which provide transient symptom alleviation but frequently induce pharmacological dependence and tolerance. Moreover, the heterogeneity among patients and the spatio-temporal evolution of individual conditions make it difficult to treat all patients with only a few universal protocols, which poses a significant challenge for the treatment of AD. However, antisense molecule-based precision targeting strategies represent a novel therapeutic dimension for AD, with their multiple molecular intervention and disease modification capabilities offering potential breakthroughs beyond current therapeutic limitations. Unlike the easy production of antisense molecules, despite undergoing a complex screening process, aptamers were found not to bind to RNA targets in

practical applications^{275,276}. CRISPR-based gene-modulating tools targeting RNA have been developed recently, but they face challenges in the nuclear delivery of large Cas–gRNA complexes and may be more immunogenic than antisense molecules^{277–279}. Furthermore, the prolonged therapeutic effects of antisense drugs align with AD’s chronic nature, reducing treatment frequency burdens compared to short-acting JAK inhibitors. However, antisense therapy for AD remains in nascent stages compared to applications in other diseases. To accelerate clinical translation, the field must prioritize several critical pathways. Leverage multi-omics technologies to decode AD pathogenesis and identify core upstream therapeutic targets for individuals. Using microfluidic chip technology, RNA structure prediction models, and 3D bioprinting technology to develop and produce antisense drugs that can treat AD in a precise manner. Developing adaptive delivery platforms for antisense drugs to enable more efficient delivery and more precise control to suit AD’s progression. Despite many preclinical studies showing the great potential of antisense drugs for AD, their clinical trials have stagnated in the era of the boom in small nucleic acid drugs. Global consortia should prioritize multicenter trials targeting NF- κ B, STAT6, and GATA-3, constructing AD-specific molecular subgroups through integrating multi-omics like transcriptomics and skin barrier lipidomics. By establishing AD-specific antisense molecule design guidelines, this approach may herald a new era of precision, durable AD therapeutics.

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Author contributions

Jiayi Xue: study concept and design, drafting of manuscript, visualization, and figure design. Zhirong Yao: study supervision, drafting of manuscript. All authors read and approved the final version to be published.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used DeepSeek-R1 in order to improve the readability and language of the article. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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