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Targeted immunological therapies in dermatology

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Targeted immunological therapies in dermatology

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Table of contents

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Editorial: Targeted immunological therapies in dermatology

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Editorial on the Research Topic
Targeted immunological therapies in dermatology

Cutaneous inflammation, particularly for chronic inflammatory diseases such as eczema and psoriasis are traditionally treated with corticosteroids to control ongoing inflammation. However, prolonged usage of steroids can lead to various side-effects such as thinning of skin, stretch marks or easy bruising. Prolonged suppression of the immune system may also result in secondary infections, driving more inflammation. Next-generation targeted immunological therapies can thus provide an alternative way for us to treat cutaneous inflammation in a more precise manner; these therapies are often wide-ranging in nature, encompassing biologics, small-molecule inhibitors, or RNA-based therapeutics.

Our Research Topic has two key focuses: firstly, we explore how the immune environment and response could contribute to disease pathogenesis; secondly, we dive into the different immunotherapies available for cutaneous diseases, and their associated efficacies, toxicities, and safety profiles.

In the first half of the topic, Chen et al. review how interplay between keratinocytes, the epithelial immune microenvironment and nerves may contribute to psoriasis pathogenesis. Separately, Yamamura et al. also undertake a comprehensive review of cytokines involved in pathogenesis of atopic dermatitis in both mice and humans, simultaneously taking into account publicly available negative clinical trial data to evaluate the importance of certain cytokines clinically.

Wang et al. further explore how actinic keratosis can be caused by factors such as chronic inflammation, oxidative stress, immunosuppression and human papillomavirus infection in addition to mutagenesis and prolonged ultraviolet radiation exposure. At the same time, Lim et al. use *in vivo* optical coherence tomography imaging of patients suffering from idiopathic itch to identify the cause as partial sweat duct obstruction, which resolves partially with retinoid treatment.

In the second half, the articles delve into the plethora of immunotherapies available for hair loss for alopecia areata and androgenetic alopecia; psoriasis and melanoma, discussing the associated efficacies, toxicities, and safety profiles. Hair loss is associated with collapse of immune privilege of the hair follicle, and Toh and Wang discuss how immunosuppression using Janus kinase (JAK) inhibitors, statins and a low dose of interleukin-2 to expand T regulatory populations can be used to restore immune privilege in the hair follicle.

Identifying latent infections such as tuberculosis, hepatitis or human immunodeficiency in patients suffering from alopecia areata is also crucial prior to initiating JAK inhibitor treatment, and Huang et al. conduct a retrospective screening study in Changsha, China to identify understand the frequency of such infections in the patient population.

To evaluate the efficacy of different immunotherapies for genital warts, condyloma acuminatum, which arises from human papillomavirus infection, Liu and Qi carry out a network meta-analysis comparing 8 different randomized controlled trials, subsequently concluding that treatment with the Bacillus Calmette-Guerin (BCG) vaccine is most efficacious, which could be due to the induction of the T helper 1 response to BCG which can aid in eliminating viral infection. Simultaneously, Ito et al. carry out a single-center retrospective study in Japan to understand how safe extended interval dosing of anti-programmed death-1 (PD-1) therapy is in Asian patients with melanoma by analyzing the immune-related adverse events. The long-term efficacy and safety of anti-interleukin-23 monoclonal antibody, guselkumab, for plaque psoriasis treatment was also assessed in a single-center retrospective trial in Chinese patients by Zheng et al.. Lastly, EGFRI are likely to induce skin irritation in the form of papulopustular rash through increased cytokine secretion to induce an inflammatory infiltrate. Hence, to obtain a better understanding of cutaneous toxicity associated with epidermal growth factor receptor inhibitors (EGFRI), Dan et al. carried out a disproportionality analysis on data from the FDA adverse event reporting system database (FAERS) for different types of EGFRIs and found that most adverse events occur within the first few days to 2 months.

This Research Topic highlights the transformative potential of targeted immunological therapies in treating cutaneous diseases. By delving into the mechanisms of disease pathogenesis and exploring innovative treatments like JAK inhibitors and monoclonal antibodies, the featured research underscores a shift toward more precise and effective interventions. These advancements promise not only reduced side effects but also enhanced efficacy in certain conditions, heralding a new era in dermatological care.

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YT: Writing—review & editing, Writing—original draft. NS: Writing—review & editing. HLT: Writing—review & editing, Supervision, Resources, Funding acquisition.

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Keratinoctye-neuro-immune-units (KNICUs): collaborative impact on the initiation and maintenance of psoriasis

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The skin is the outermost barrier that separates the human body from the external environment. In psoriasis, immune cells reside within or infiltrate the epidermis to form the epidermal (epithelial) immunological microenvironment (EIME) and engage in complex interactions with keratinocytes, nerves, and microbiota. The proposed hypothesis is that psoriasis is a chronic inflammatory disease mainly mediated by a specific inflammatory environment composed of keratinocyte–neuro–immune cell units (KNICUs). These KNICUs arise from the interaction between activated epidermal keratinocytes, nerves, immune cells, and the skin microbiota, forming a complex interaction framework. Multiple units gather to complete the circulatory and amplified loops, consequently serving as a group army to initiate and maintain psoriasis.

KEYWORDS

psoriasis, epidermal immunological microenvironment, keratinocyte, immunocyte, neuron

Highlights

- The immune and non-immune responses of the skin are composed largely of the EIME in the epidermis of psoriasis.
- Neuro-immune cell units (NICUs) have emerged as important structures in complicated processes such as inflammation in psoriasis.
- Keratinocytes are a trigger of inflammation in psoriasis.
- Psoriasis is mediated by a specific inflammatory environment composed of keratinocyte–neuro–immune cell units (KNICUs).

1. Introduction

The skin is the body's outermost barrier against environmental stressors, such as physical, chemical, and microbial agents. Within the skin, immune cells are present, encountering and responding to a myriad of inflammatory challenges (1). These cells are found in the epidermis, dermis, and subcutaneous fat. The epidermis comprises various cell types, such as keratinocytes, melanocytes, Langerhans cells (LCs), and other immune cells. Among those cells, LCs are considered the main skin-resident immune cells and antigen-presenting cells (APCs), located in the interfollicular epidermis and the epithelium of the hair follicles, while T cells, mainly $CD8^+$ T cells, dendritic cells (DCs), and macrophages,

are also involved in the network of APCs in recent research (2, 3). γδ T cells and $CD8^+$ resident memory T cells (T_{RMs}) are believed to be two specific T cells. $CD8^+$ T_{RM} cells comprise non-circulating cells and have been identified to be caused by skin inflammation, which can be found in the stratum basal and stratum spinosum; these cells have also been discovered in the non-lymphoid organs, including the skin, where they can persist for an extended period of time (1). Immune cell populations in the skin predominantly consist of DCs and macrophages. However, the specific subtypes of DCs and their precise roles in the development of diseases are not yet fully understood. Subset-defining markers could be achieved by advances in single-cell separation technology.

Another evolving theory is that the skin immune responses are composed largely of the EIME in the epidermis of psoriasis. Epidermal cells not only secrete their cellular contents but also detect foreign and danger signals and then produce specific immune responses (4). Subsequent DC responses are also significant for inflammation (5). In addition, the epidermis and other factors, especially the microbiota and the peripheral nerves, contribute to the components of EIME in chronic inflammatory diseases (6).

Psoriasis is a chronic immunogenetic skin disease that affects ∼1–3% of the population worldwide (7–9). However, the pathogenesis of psoriasis is still unknown. Historically, a prevailing theory indicates that excessive thickening of the epidermis in psoriasis is related to the abnormal proliferation of keratinocytes (10). Recently, neuroinflammation has been believed to be an important pathogenic element. Besides, the epidermis was considered the earliest stage of an inflammatory process (11), such as the pathogenic IL-17 loop. Keratinocytes are not only progenitors of the barrier but also participants in the innate immune system in the epidermis (1).

In contrast, the dermis is where immune cells and non-immune components interact (6, 12). However, recent research has identified the significance of keratinocytes as sensors of danger through the inflammasome systems in psoriasis (2). Furthermore, keratinocytes express several different pattern recognition receptors and produce a larger number of cytokines.

Neuro-immune cell units (NICUs) are anatomical units that exist at the interface of the immune and nervous systems, both in the states of health and when suffering from a disease (13). Clinical studies have identified that the peripheral nerves play a key role in the development of psoriatic lesions and that the injection of botulinum toxin type A is an effective treatment for psoriatic patients (14). In imiquimod-induced mice, skin inflammation was reduced by inhibiting the $TRPV1^+$ sensory nerves (15). Besides, silencing sensory neuron-expressed transient receptor potential channel 4 (TRPC4) can induce psoriatic itch in the same mouse model (16).

Therefore, it is necessary to consider that the inflammatory reactions in the epidermis could be the center of immune responses and, together with NICUs, can be taken as one complete cycle to initiate the pathogenesis of psoriasis. The interaction of the epithelial tissue, immune system, and nerve systems completes each inflammatory loop, forming the huge circulatory inflammatory cycles in psoriasis.

2. Subsections

2.1. Epidermal (epithelial) immunological microenvironment

The skin is composed of the epidermis, dermis, and subcutaneous fat. It is presumed that the immune responses of the skin are determined and tightly associated with EIME in the epidermis and papillary dermis. There are two functional phases of EIME in the epidermis. First, keratinocytes are the body's barrier and can recognize dangers and external agents. Second, keratinocytes can amplify the immune reactions through EIME and produce cytokines that stimulate subsequent inflammation (6). Similarly, cytokines secreted by the immune cells can also affect the activation of non-immune cells, which together form an inflammatory network cycle.

Recent studies have reported three types of APCs present in the epidermis: LCs, inflammatory dendritic epidermal cells (IDECs), and monocyte-derived LC-like cells (17). IDECs and LC-like cells have been shown to be present in both steady and inflammatory states, but they increase more under inflammatory conditions such as atopic dermatitis and psoriasis (17). However, some studies have illustrated that LC is one type of dendritic epidermal cell, which includes four types. Among these, aLC/migLC (active LCs/migratory LCs) from the psoriatic skin showed higher expression of inflammation-associated molecules (18). Epidermal DCs are $CCR2^+$ DCs, which are exclusively confined to psoriatic lesions and express different defining markers from dermal DCs (19). Other DCs found that $CD1c^+CD11b^+$ epidermal conventional type 2 DCs (cDC2s), $CD141^+$ cDC1s, and $BDCA2^+$ plasmacytoid DCs (pDCs) also played a significant role in psoriatic epidermis by using cyTOF (20). Thus, dendritic-like cells in psoriatic lesions are still inconsistent.

A review proposed that epithelial TRAF6, a type of ubiquitin E3 ligase, was associated with the immune responses and drove the inflammatory loops of IL-17 in the type 17 EIME in psoriasis, which also indicated the disordered homeostasis of epidermal NF-κB and MAPK signaling pathways. These were crucial for organizing the psoriatic EIME (4). Besides, neutrophil accumulation in the epidermis was the most important contributor to psoriatic EIME (6). The epidermal immune environment was more important and coincided with the inflammation occurring during psoriasis. EIME plays a dominant role in psoriasis, including psoriatic $CD1c^+CD11b^+$ epidermal cDC2s, $CD141^+$ cDC1s, and

Abbreviations: AMPs, antimicrobial peptides; APCs, antigen-presenting cells; cDCs, conventional DCs; CGRP, calcitonin gene-related peptide; DCs, dendritic cells; DETCs, dendritic epidermal T cells; eDCs, epidermal dendrite cells; EIME, epidermal (epithelial) immunological microenvironment; ELCs, epidermal lymphoid cells; IDECs, inflammatory dendritic epidermal cells; IL-17, interleukin-17; IARS, isoleucyl-tRNA synthetase; KNICUs, keratinocyte-neuro-immune cell units; LCs, Langerhans cells; NICUs, neuro-immune cell units; pDCs, plasmacytoid DCs; TLR, toll-like receptor; Tip-DCs, iNOS-producing DCs; TNF, tumor necrosis factor; T_{RMs}, tissue-resident memory T cells; TRPV1, transient receptor potential subfamily V member 1; TRPC4, transient receptor potential channel 4; TSLP, thymic stromal lymphopoietin.

BDCA2$^+$pDCs (20). However, the specific role of immune cells and keratinocytes in psoriatic EIME is still not fully understood.

2.2. Immune cells in psoriatic skin

2.2.1. DCs and epidermal DCs

A significant increase in DCs has been discovered both in the epidermis and the dermis in psoriatic lesions. DCs are typically classified into four groups: epidermal LCs, cDCs, plasmacytoid DCs (pDCs), and monocyte-derived DCs. Some types of DCs, such as TNF- and iNOS-producing DCs (Tip-DCs), slanDCs, and pDCs, are typically absent. However, in psoriatic skin, these DC types have been observed to primarily infiltrate the dermal component, but little is known about the specific classified types of DC in the epidermis of psoriasis (21). DCs have been believed to play a pivotal role in connecting innate immunity with adaptive immunity, which expresses TLRs and can sense danger signals from external agents. Besides, DCs are the source of IL-23 and TNF-a, which appear to have a central role in psoriasis pathogenesis (21). TLR stimulation can lead to the activation and maturation of DC, which are identified by the expression of costimulatory molecules and the secretion of cytokines (21).

Immune cell populations in the skin are dominated by DCs and macrophages (3). In an imiquimod-induced mouse model, in which MYD88 was only expressed by CD11c$^+$ immune cells, the mice exhibited distinct psoriatic phenotypes compared to wild-type mice. These findings suggest that CD11c$^+$ DCs are significant in psoriatic mice (22). Similarly, CD207$^-$ DCs could produce IL-23 to stimulate dermal T cells to secrete IL-17 and IL-22 (23).

eDCs are CCR2$^+$ DCs. They exceeded the number of LCs. The lesion would display an accumulation of neutrophils, the proliferation of keratinocytes, and the activation of T cells (19). These eDCs are capable of secreting IL-1β, IL-23, and TNF. Furthermore, research conducted by others has revealed the phenotypic and functional properties of eDCs and resident LCs in psoriasis. These cells play a role in amplifying the epidermal microenvironment through the secretion of IL-17 cytokines (24). eDCs have been observed to establish direct contact with hyperproliferative keratinocytes, a phenomenon that is strictly confined to active disease. Among the specific groups of eDCs involved, there may be cDC2s, CD141$^+$ cDC1s, and BDCA2$^+$pDCs (20). However, research on these eDC subsets in the context of psoriasis is still limited, emphasizing the need for further in-depth research in this area.

2.2.2. Langerhans cells

A growing body of evidence has brought the identity of LCs into question, suggesting that they may originate from macrophage subsets rather than DC lineages, as previously presumed (17). LCs are currently reported as a specialized lineage of tissue-resident macrophages (25). However, the specific role that LCs play in the pathogenesis of psoriasis remains controversial (21, 23, 26–29). One study illustrated that LCs have an anti-inflammatory role during the process of psoriatic disease (21).

Other studies have reported that the migration of LC in non-lesional psoriatic skin is dependent on the IL-17 stimulation of keratinocytes. The inhibition of IL-17 has been found to restore normal LC migration. Besides, LCs have been shown to produce IL-23 and are required for the development of IMQ-induced psoriasis-like lesions (27). These studies, along with others, indicate that LCs may be truly involved in the pathogenesis of psoriasis (30, 31).

2.2.3. Dendritic epidermal T cells

In the normal murine epidermis, the shape of several cell populations is included, including DETCs and epidermal lymphoid cells (ELCs) (32). DETCs are intraepithelial γδ T cells, which are identified by expressing Vγ5Vδ1 and have restrictive functions (33). The abilities of DETCs include inflammation modulation, cutaneous neoplasia protection, and skin wound healing (34, 35). The population of DETCs increases throughout adulthood until it reaches its peak, after which it remains stable (36). In the epidermis, DETCs could recognize a specific self-antigen that is restricted to damaged, stressed, or transformed keratinocytes (37). It is well-established that DETCs are potent IFN-γ-producing cells and that DETCs produce IL-17A upon TCR activation and in response to skin injury (38).

Human γδ T cells were only a small proportion of the total T cells in the dermis (2–9%) and the epidermis (1–10%), while in mice, DETCs account for more than 90% of epidermal T cells. DETCs are believed to negatively regulate αβ T cell-induced inflammation, thus contributing to local immune surveillance and immunoregulation, whereas αβ T cells promote skin tumor responses (39).

2.2.4. Tissue-resident memory T cells

T_{RMs} are a novel non-circulatory T-cell subset that provides protective memory responses, maintaining long-term residence in the barrier tissues. Cutaneous CD8$^+$ T_{RMs} reside in the epidermis and can last for approximately 1 year, whereas lung TRMs only last for 1 month. They are also considered to mediate autoimmune diseases such as vitiligo (40). The definition of T_{RMs} is the expression of CD69 in the absence of CD62L and the expression of T_{RM}-associated molecules such as CXCR6 and CD103 or the transcription factor Hobit (41). The characterized marker of T_{RMs} is CD44$^+$ CD62L$^-$ CD69$^+$. T_{RMs} can be distinguished from circulating lymphocytes by characteristically expressing CD69. Recent articles have reported that skin T_{RMs} in mice persist for more than 1 year. However, lung T_{RMs} last for only a few months (1). Although CD8$^+$ T_{RMs} have been well-defined, CD4$^+$ TRMs, especially skin-resident memory Th17 cells, are still being identified. T_{RMs} start a tissue-wide state of alert when recognizing their cognate antigen. They have the ability to recruit other immune cells to the site of infection (41).

In psoriasis, the skin affected by the condition displays an accumulation of IL-17-producing CD49a$^-$ T_{RMS} (42). T_{RMs} derived from the skin are enhanced in the circulation of patients with PsA compared to patients with psoriasis alone (43).

2.3. Keratinocytes in psoriasis

The previous pathogenesis of psoriasis was presumed to, in accordance with inflammatory exudates in the dermis, be mainly

composed of neutrophils, dendritic cells, T cells, and macrophages (44). The functions of the dermal immune cells have been widely explored, but the roles of epithelial cells have not been fully understood. Thus, we conclude that the two main roles of keratinocytes in psoriasis are as follows:

2.3.1. Keratinocyte responses in psoriasis

The epidermis has been reported to be involved in determining the type of immune responses (6). Within the epidermis, keratinocytes, which are components of the innate immune system, express specific receptors and secrete cytokines. These cytokines include IL-6, IL-10, IL-18, IL-33, other IL-1 family members, thymic stromal lymphopoietin (TSLP), and tumor necrosis factor (TNF). Keratinocytes release these cytokines when the skin is inflamed (45). In addition, keratinocytes produce innate inflammatory mediators such as antimicrobial peptides (AMPs). AMPs have been found to be present at high levels in psoriatic lesions, which is related to the lack of skin infection in these patients. Moreover, keratinocytes can produce the cathelicidin antimicrobial peptide LL37 and thus break the self-tolerance mechanisms in psoriasis patients (2).

2.3.2. Keratinocytes as a trigger of inflammation in psoriasis

Several studies have demonstrated that interlukin-17 (IL-17)-induced skin inflammation is targeted at keratinocytes (46). Th17 cytokines could activate keratinocytes in the inflammatory psoriasis loop and promote the release of more mediators such as CCL20. The production of neutrophil chemokines (CXCL1 and CXCL2) is one of the important roles of keratinocytes (47). In the pathogenesis of psoriasis, the DC responses might be stimulated by the abnormally dying keratinocytes in the stratum corneum. In addition, epidermal keratinocytes express several types of TLRs (48). TLR7 can be activated by stimulating TLR3 in keratinocytes, which is the most important signaling pathway in imiquimod-induced mice (49). Inhibiting the formation of isoleucyl-TRNA synthetase (IARS) in the epidermis may block the infiltration of immune cells in patients with psoriasis (50). A recent study by Moos et al. confirmed that the crosstalk between T cells, which produce IL-17, and keratinocytes facilitated the immune and non-immune responses that drove epidermal hyperplasia and cutaneous inflammation, which were critically dependent on keratinocytes. Since reduced inflammatory responses occurred only in mice with a specific deletion of IL-17RA in keratinocytes (10, 51), more and more evidence has shown that keratinocytes may trigger the progression of psoriasis (52). However, the specific role of other immune cells in psoriasis is still unknown.

2.4. Interactions between the nerve and immune system in the epidermis

Cutaneous innervation is critical in the peripheral nervous system (53). Epithelial neuro-immune cell units (NICUs) refer to specific anatomical locations. Neuronal and immune cells in NICUs can colocalize and functionally interact to regulate tissue pathology and physiology. NICUs have become an important structure for complicated processes such as inflammation, wound repair, and angiogenesis (13). In fact, in the epidermis, there is a colocalization of NICUs. Some studies have indicated that keratinocyte proliferation could result from cell–cell communication between keratinocytes and nerves by adding nerves to an HSE (54). Moreover, cutaneous nerve fibers interact with epidermal keratinocytes and immunocytes in the progression of psoriasis (55). Interactions between neurons and immune responses are mostly mediated by soluble factors such as neurotransmitters, neuropeptides, and cytokines due to keratinocytes having receptors such as neurotransmitters (e.g., acetylcholine, dopamine, adrenaline), neuropeptides, and neurotrophins, which are closely related to the psychoneuroimmunologic mechanisms (56).

2.5. Interactions between keratinocytes and nerve systemin in psoriasis

Cutaneous nerves consist of sympathetic and several sensory fibers, and the role of each fiber type remains unclear (15). The skin organ acts as both a sensory interaction place and a barrier. It is not only innervated by abundant sensory nerves but also by a smaller number of autonomic nerve fibers. Neurons form mesh-like bundles in the dermis, and there are abundant ends of nerves in the epidermis. A large fraction of dermal DCs' close contact with neurons in whole-mount immune staining indicate that neurons may regulate DC functions. To date, the functions of peripheral neurons in regulating immune responses in the skin have been unknown. However, through research involving the genetic and pharmacological ablation of TRPV1-expressing sensory neurons, it has become evident that neurons play a vital role in regulating cutaneous immune responses. This research has provided valuable insights into the regulatory role of neurons in the skin's immune system. For instance, after the pharmacological inhibition of $TRPV1^+$ sensory nerves, significant suppression of mouse skin inflammation induced by the TLR7 ligand by imiquimod occurs (15). Following nerve deletion, IL-23-producing DCs in the dermis are reduced in imiquimod-induced inflammatory skin, and thus, the IL-17-releasing γδ T cells are clearly decreased (57). $TRPV1^+$ sensory nociceptors directly recognize fungal agents in the *Candida albicans* infection model and then release calcitonin gene-related peptide (CGRP). Moreover, the production of IL-23 by $CD301b^+$ DCs could be upregulated by CGRP, which in turn drives dermal γδ T cells to produce IL-17A (1). These results illustrated the possible interaction between keratinocytes and neurosystemin in psoriasis patients.

3. Discussion

Based on the participation of EIME and NICUs in the pathogenesis of psoriasis, we proposed the concept of KNICUs in the epidermis, which could contribute to the establishment of an inflammatory environment where a large number of KNICUs interact with each other and subsequently perpetuate an inflammatory cycle in psoriasis. To substantiate this assumption,

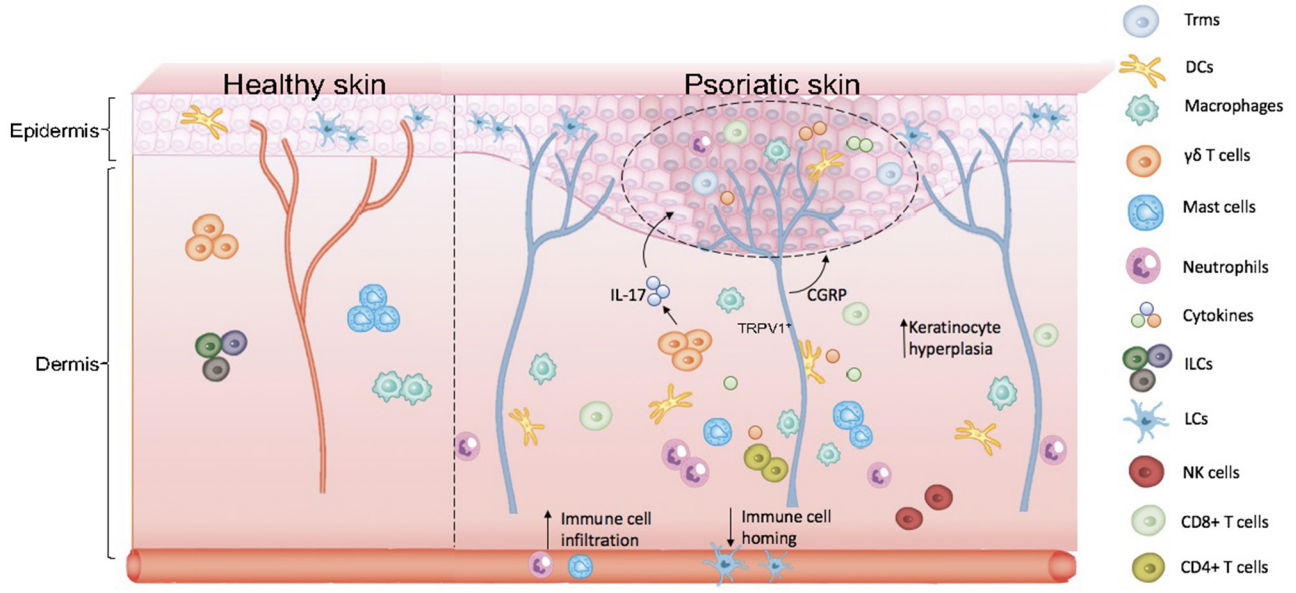

FIGURE 1
Skin anatomy and formation of keratinocyte–neuro–immune cell units, KNICUs. In psoriasis lesional skin loss, resident immune cells are largely activated. Dermal immune cells are immersed in the epidermis, with epidermal nerve fiber distribution increasing, which results in the abnormal proliferation of cells and the release of inflammatory factors. These cytokine factors, immune cells, and nerve fiber receptors—the three factors of the epidermis—form cell–neuro–immune cell units (keratinocyte–neuro–immune cell units, KNICUs). These constitute a complex feedback loop and cascade amplification effect, which plays a vital role in the development of psoriasis.

we need to fully understand the intrinsic mechanisms of KNICUs in all psoriasis types and phases, as shown in Figure 1.

In the past two decades, the pathogenesis of psoriasis has closely correlated with the functions of keratinocytes. Increasing evidence revealed that keratinocytes act as executors to actively participate in an immune-micro network interconnected by cytokines (56). The importance of keratinocytes in psoriasis leads to several new treatments and also raises new questions. For example, is it sufficient to target keratinocytes alone to induce psoriatic inflammation?

In the mouse model, TRPV1$^+$ neuron activation in the skin was enough to initiate local type 17 immunity that augmented local host defense in mice, and type 17 innate immune responses achieved regional anticipatory immunity through the nerve (58). In addition, TRPV1 knockout mice treated with IMQ suggest a significant reduction in skin inflammation and barrier defects (59). The evidence showed that the interaction between nerves and immune cells is tightly associated with imiquimod-induced mice. However, the role of type 17 cytokines in the positive feedback loop of sensory nerves in psoriasis is still not fully understood.

We may obtain a deeper understanding of the mechanisms of cutaneous immune responses and of the pathogenesis of psoriasis by evaluating these points. Immune cells could express receptors for molecules derived from neuronal cells to respond to neuronal signals. Moreover, reciprocally, receptors for immune-derived cytokines and neurotransmitters are expressed by neurons. Hence, immune cells and neurons may interact with keratinocytes in psoriasis. Cytokine factors, immune cells, and nerve fiber receptors, the three factors of the epidermis, may form cell-neuro-immune cell units (keratinocyte-neuro-immune cell units, KNICUs). These constitute a complex feedback loop and cascade amplification effect, which are important to the development of psoriasis.

Author contributions

X-YC and Z-YW: conceptualization and writing–original draft preparation. X-YM: funding acquisition. L-RY, YZ, and X-YM: writing–review and editing. All authors contributed to the article and approved the submitted version.

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References

1. Kabashima K, Honda T, Ginhoux F, Egawa G. The immunological anatomy of the skin. *Nat Rev Immunol.* (2018) 19:19–30. doi: 10.1038/s41577-018-0084-5

2. Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. *Nat Rev Immunol.* (2009) 9:679–91. doi: 10.1038/nri2622

3. Malissen B, Tamoutounour S, Henri S. The origins and functions of dendritic cells and macrophages in the skin. *Nat Rev Immunol.* (2014) 14:417–28. doi: 10.1038/nri3683

4. Dainichi T, Matsumoto R, Mostafa A, Kabashima K. Immune control by TRAF6-mediated pathways of epithelial cells in the eime (epithelial immune microenvironment). *Front Immunol.* (2019) 10:1107. doi: 10.3389/fimmu.2019.01107

5. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature.* (2007) 449:564–9. doi: 10.1038/nature06116

6. Dainichi T, Kitoh A, Otsuka A, Nakajima S, Nomura T, Kaplan DH, et al. The epithelial immune microenvironment (EIME) in atopic dermatitis and psoriasis. *Nat Immunol.* (2018) 19:1286–98. doi: 10.1038/s41590-018-0256-2

7. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol.* (2014) 32:227–55. doi: 10.1146/annurev-immunol-032713-120225

8. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med.* (2009) 361:496–509. doi: 10.1056/NEJMra0804595

9. Boehncke WH, Schon MP. Psoriasis. *Lancet.* (2015) 386:983–94. doi: 10.1016/S0140-6736(14)61909-7

10. Garzorz-Stark N, Eyerich K. Psoriasis pathogenesis: keratinocytes are back in the spotlight. *J Invest Dermatol.* (2019) 139:995–6. doi: 10.1016/j.jid.2019.01.026

11. Xiao T, Yan Z, Xiao S, Xia Y. Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. *Stem Cell Res Ther.* (2020) 11:232. doi: 10.1186/s13287-020-01755-y

12. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker J. Psoriasis. *Lancet.* (2021) 397:1301–15. doi: 10.1016/S0140-6736(20)32549-6

13. Godinho-Silva C, Cardoso F, Veiga-Fernandes H. Neuro-immune cell units: a new paradigm in physiology. *Annu Rev Immunol.* (2019) 37:19–46. doi: 10.1146/annurev-immunol-042718-041812

14. Nash MS. Known and plausible modulators of depressed immune functions following spinal cord injuries. *J Spinal Cord Med.* (2000) 23:111–20. doi: 10.1080/10790268.2000.11753518

15. Riol-Blanco L, Ordovas-Montanes J, Perro M, Naval E, Thiriot A, Alvarez D, et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature.* (2014) 510:157–61. doi: 10.1038/nature13199

16. Lee SH, Tonello R, Choi Y, Jung SJ, Berta T. Sensory neuron-expressed transient receptor potential channel 4 is a target for the relief of psoriasiform itch and skin inflammation in mice. *J Invest Dermatol.* (2020). doi: 10.1016/j.jid.2020.03.959

17. Otsuka M, Egawa G, Kabashima K. Uncovering the mysteries of langerhans cells, inflammatory dendritic epidermal cells, and monocyte-derived langerhans cell-like cells in the epidermis. *Front Immunol.* (2018) 9:1768. doi: 10.3389/fimmu.2018.01768

18. Liu X, Zhu R, Luo Y, Wang S, Zhao Y, Qiu Z, et al. Distinct human Langerhans cell subsets orchestrate reciprocal functions and require different developmental regulation. *Immunity*. (2021) 54:2305–20. doi: 10.1016/j.immuni.2021.08.012

19. Liu J, Zhang X, Cheng Y, Cao X. Dendritic cell migration in inflammation and immunity. *Cell Mol Immunol.* (2021) 18:2461–71. doi: 10.1038/s41423-021-00726-4

20. Zhou Y, Xu F, Chen XY, Yan BX, Wang ZY, Chen SQ, et al. The epidermal immune microenvironment plays a dominant role in psoriasis development, as revealed by mass cytometry. *Cell Mol Immunol.* (2022) 19:1400–13. doi: 10.1038/s41423-022-00940-8

21. Glitzner E, Korosec A, Brunner PM, Drobits B, Amberg N, Schonthaler HB, et al. Specific roles for dendritic cell subsets during initiation and progression of psoriasis. *EMBO Mol Med.* (2014) 6:1312–27. doi: 10.15252/emmm.201404114

22. Costa S, Marini O, Bevilacqua D, DeFranco AL, Hou B, Lonardi S, et al. Role of MyD88 signaling in the imiquimod-induced mouse model of psoriasis: focus on innate myeloid cells. *J Leukoc Biol.* (2017) 102:791–803. doi: 10.1189/jlb.3MA0217-054RR

23. Wohn C, Ober-Blobaum JL, Haak S, Pantelyushin S, Cheong C, Zahner SP, et al. Langerin(neg) conventional dendritic cells produce IL-23 to drive psoriatic plaque formation in mice. *Proc Natl Acad Sci USA.* (2013) 110:10723–8. doi: 10.1073/pnas.1307569110

24. Martini E, Wiken M, Cheuk S, Gallais Serezal I, Baharom F, Stahle M, et al. Dynamic changes in resident and infiltrating epidermal dendritic cells in active and resolved psoriasis. *J Invest Dermatol.* (2017) 137:865–73. doi: 10.1016/j.jid.2016.11.033

25. Doebel T, Voisin B, Nagao K. Langerhans cells - the macrophage in dendritic cell clothing. *Trends Immunol.* (2017) 38:817–28. doi: 10.1016/j.it.2017.06.008

26. Terhorst D, Chelbi R, Wohn C, Malosse C, Tamoutounour S, Jorquera A, et al. Dynamics and transcriptomics of skin dendritic cells and macrophages in an imiquimod-induced, biphasic mouse model of psoriasis. *J Immunol.* (2015) 195:4953–61. doi: 10.4049/jimmunol.1500551

27. Yoshiki R, Kabashima K, Honda T, Nakamizo S, Sawada Y, Sugita K, et al. IL-23 from Langerhans cells is required for the development of imiquimod-induced psoriasis-like dermatitis by induction of IL-17A-producing gammadelta T cells. *J Invest Dermatol.* (2014) 134:1912–21. doi: 10.1038/jid.2014.98

28. Sweeney CM, Russell SE, Malara A, Kelly G, Hughes R, Tobin AM, et al. Human ss-defensin 3 and its mouse ortholog murine ss-defensin 14 activate langerhans cells and exacerbate psoriasis-like skin inflammation in mice. *J Invest Dermatol.* (2016) 136:723–7. doi: 10.1016/j.jid.2015.12.011

29. Lee M, Kim SH, Kim TG, Park J, Lee JW, Lee MG. Resident and monocyte-derived Langerhans cells are required for imiquimod-induced psoriasis-like dermatitis model. *J Dermatol Sci.* (2018) 91:52–9. doi: 10.1016/j.jdermsci.2018.04.003

30. Kirby B. Langerhans cells in psoriasis: getting to the core of the disease. *Br J Dermatol.* (2018) 178:1240. doi: 10.1111/bjd.16596

31. Eaton LH, Mellody KT, Pilkington SM, Dearman RJ, Kimber I, Griffiths CEM. Impaired Langerhans cell migration in psoriasis is due to an altered keratinocyte phenotype induced by interleukin-17. *Br J Dermatol.* (2018) 178:1364–72. doi: 10.1111/bjd.16172

32. Almeida FF, Tenno M, Brzostek J, Li JL, Allies G, Hoeffel G, et al. Identification of a novel lymphoid population in the murine epidermis. *Sci Rep.* (2015) 5:12554. doi: 10.1038/srep12554

33. Heilig JS, Tonegawa S. Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature.* (1986) 322:836–40. doi: 10.1038/322836a0

34. Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, et al. A role for skin gammadelta T cells in wound repair. *Science.* (2002) 296:747–9. doi: 10.1126/science.1069639

35. Sharp LL, Jameson JM, Cauvi G, Havran WL. Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1. *Nat Immunol.* (2005) 6:73–9. doi: 10.1038/ni1152

36. Gentek R, Ghigo C, Hoeffel G, Jorquera A, Msallam R, Wienert S, et al. Epidermal gammadelta T cells originate from yolk sac hematopoiesis and clonally self-renew in the adult. *J Exp Med.* (2018) 215:2994–3005. doi: 10.1084/jem.20181206

37. Jameson JM, Cauvi G, Sharp LL, Witherden DA, Havran WL. Gammadelta T cell-induced hyaluronan production by epithelial cells regulates inflammation. *J Exp Med.* (2005) 201:1269–79. doi: 10.1084/jem.20042057

38. MacLeod AS, Hemmers S, Garijo O, Chabod M, Mowen K, Witherden DA, et al. Dendritic epidermal T cells regulate skin antimicrobial barrier function. *J Clin Invest.* (2013) 123:4364–74. doi: 10.1172/JCI70064

39. Silva-Santos B, Serre K, Norell H. γδ T cells in cancer. *Nat Rev Immunol.* (2015) 15:683–91. doi: 10.1038/nri3904

40. Mohammed J, Beura LK, Bobr A, Astry B, Chicoine B, Kashem SW, et al. Stromal cells control the epithelial residence of DCs and memory T cells by regulated activation of TGF-beta. *Nat Immunol.* (2016) 17:414–21. doi: 10.1038/ni.3396

41. Stark R, Wesselink TH, Behr FM, Kragten NAM, Arens R, Koch-Nolte F, et al. T RM maintenance is regulated by tissue damage via P2RX7. *Sci Immunol.* (2018) 3:e11. doi: 10.1126/sciimmunol.aau1022

42. Cheuk S, Schlums H, Gallais Serezal I, Martini E, Chiang SC, Marquardt N, et al. CD49a expression defines tissue-resident CD8(+) T cells poised for cytotoxic function in human skin. *Immunity.* (2017) 46:287–300. doi: 10.1016/j.immuni.2017.01.009

43. Leijten EF, van Kempen TS, Olde Nordkamp MA, Pouw JN, Kleinrensink NJ, Vincken NL, et al. Tissue-resident memory CD8+ T cells from skin differentiate psoriatic arthritis from psoriasis. *Arthritis Rheumatol.* (2021) 73:1220–32. doi: 10.1002/art.41652

44. Ogawa E, Sato Y, Minagawa A, Okuyama R. Pathogenesis of psoriasis and development of treatment. *J Dermatol.* (2018) 45:264–72. doi: 10.1111/1346-8138.14139

45. Zhou X, Chen Y, Cui L, Shi Y, Guo C. Advances in the pathogenesis of psoriasis: from keratinocyte perspective. *Cell Death Dis.* (2022) 13:81. doi: 10.1038/s41419-022-04523-3

46. Ha HL, Wang H, Pisitkun P, Kim JC, Tassi I, Tang W, et al. IL-17 drives psoriatic inflammation via distinct, target cell-specific mechanisms. *Proc Natl Acad Sci USA.* (2014) 111:E3422–31. doi: 10.1073/pnas.1400513111

47. Sawant KV, Sepuru KM, Lowry E, Penaranda B, Frevert CW, Garofalo RP, et al. Neutrophil recruitment by chemokines Cxcl1/KC and Cxcl2/MIP2: Role of Cxcr2 activation and glycosaminoglycan interactions. *J Leukoc Biol.* (2021) 109:777–91. doi: 10.1002/JLB.3A0820-207R

48. Jiang Y, Tsoi LC, Billi AC, Ward NL, Harms PW, Zeng C, et al. Cytokinocytes: the diverse contribution of keratinocytes to immune responses in skin. *JCI Insight.* (2020) 5:e142067. doi: 10.1172/jci.insight.142067

49. Lee HJ, Kang SJ, Woo Y, Hahn TW, Ko HJ, Jung YJ. TLR7 stimulation with imiquimod induces selective autophagy and controls Mycobacterium tuberculosis growth in mouse macrophages. *Front Microbiol.* (2020) 11:1684. doi: 10.3389/fmicb.2020.01684

50. Yan BX, Chen XY, Wang ZY, Cui YZ, Landeck L, Fu NC, et al. Mupirocin blocks imiquimod-induced psoriasis-like skin lesion by inhibiting epidermal isoleucyl-tRNA synthetase. *Cell Commun Signal.* (2022) 20:185. doi: 10.1186/s12964-022-00995-0

51. Moos S, Mohebiany AN, Waisman A, Kurschus FC. Imiquimod-induced psoriasis in mice depends on the IL-17 signaling of keratinocytes. *J Invest Dermatol.* (2019) 139:1110–7. doi: 10.1016/j.jid.2019.01.006

52. Ni X, Lai Y. Keratinocyte: a trigger or an executor of psoriasis? *J Leukoc Biol.* (2020) 108:485–91. doi: 10.1002/JLB.5MR0120-439R

53. Vidal Yucha SE, Tamamoto KA, Kaplan DL. The importance of the neuro-immuno-cutaneous system on human skin equivalent design. *Cell Prolif.* (2019) 52:e12677. doi: 10.1111/cpr.12677

54. Zbytek B, Mysliwski A, Slominski A, Wortsman J, Wei ET, Mysliwska J. Corticotropin-releasing hormone affects cytokine production in human HaCaT keratinocytes. *Life Sci.* (2002) 70:1013–21. doi: 10.1016/S0024-3205(01)01476-X

55. Chen SQ, Chen XY, Cui YZ, Yan BX, Zhou Y, Wang ZY, et al. Cutaneous nerve fibers participate in the progression of psoriasis by linking epidermal keratinocytes and immunocytes. *Cell Mol Life Sci.* (2022) 79:267. doi: 10.1007/s00018-022-04299-x

56. Pondeljak N, Lugović-Mihić L. Stress-induced interaction of skin immune cells, hormones, and neurotransmitters. *Clin Ther.* (2020). doi: 10.1016/j.clinthera.2020.03.008

57. van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol.* (2009) 182:5836–45. doi: 10.4049/jimmunol.0802999

58. Cohen JA, Edwards TN, Liu AW, Hirai T, Jones MR, Wu J, et al. Cutaneous TRPV1(+) neurons trigger protective innate type 17 anticipatory immunity. *Cell.* (2019) 178:919–32. doi: 10.1016/j.cell.2019.06.022

59. Zhou Y, Follansbee T, Wu X, Han D, Yu S, Domocos DT, et al. TRPV1 mediates inflammation and hyperplasia in imiquimod (IMQ)-induced psoriasiform dermatitis (PsD) in mice. *J Dermatol Sci.* (2018) 92:264–71. doi: 10.1016/j.jdermsci.2018.11.009

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In vivo imaging of patients with chronic pruritus of unknown origin reveals partial sweat duct obstruction with partial itch resolution upon retinoid treatment

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Background: Chronic pruritus of unknown origin (CPUO) is poorly understood and lacks effective treatment options.

Objectives: We aimed to elucidate abnormalities in the sweat apparatus of patients with CPUO, and to assess efficacy and safety of treatment with systemic retinoids.

Methods: An initial case–control study included 20 affected patients and five healthy controls, for whom heat and sweating were induced, either through a standardized exercise protocol or ingestion of hot water. *In vivo* high-definition optical coherence tomography, whole-body starch-iodine testing, and skin biopsy for immunofluorescence staining were done to evaluate for sweat duct obstruction. A subsequent retrospective cohort analysis included 56 patients with CPUO, seen at an Itch subspecialty clinic of a single tertiary referral centre, who failed conventional treatments and were treated with isotretinoin and/or acitretin from May 2014 to November 2020. Treatment response to retinoids was defined as a sustained reduction in itch score of ≥2/10. Safety was assessed by proportion stopping treatment due to side effects.

Results: *In vivo* imaging in 19 (95%) patients revealed features of partial keratinaceous sweat duct obstruction with statistically significant luminal dilatation compared to controls. Immunofluorescence studies of three patients' paired lesional/non-lesional biopsies revealed dermcidin accumulation within sweat glands coupled with dermcidin leakage in itchy skin. Fifty-six patients (mean [SD] age 55.2 [17.5] years, 69.6% male) were treated with systemic retinoids. Mean (SD) duration of itch was 116.3 (140.4) months and mean (SD) itch score was 8.2 (1.8). Forty-one (73.2%) initially received isotretinoin, and 15 (26.8%) acitretin. At three months, mean itch score reduced by 2.38 (95% CI -3.2 to –1.6, $p<0.0001$). Thirty-eight (67.9%) had a sustained response. Eight (14.81%) achieved an itch score of 0 or 1, with four stopping treatment for a mean (SD) of 318.5 (291.2) days without relapse. Eight (14.3%) stopped or switched retinoid due to adverse effects, with similar incidences between both retinoids, the commonest being dryness.

Conclusion: Based on novel findings from physiological imaging studies identifying partial keratinaceous sweat duct obstruction in CPUO, we instituted

systemic retinoid treatment to address the underlying pathology. In patients who failed conventional therapies, the treatment appears effective and safe.

KEYWORDS

acitretin, dermcidin, imaging, isotretinoin, pruritus, retinoids, sweat

Introduction

The point prevalence of chronic pruritus, defined as itch lasting for longer than six weeks, is estimated to be 13.5% in the general adult population, with the highest prevalence amongst the elderly (1). In 8–15% of affected patients, no causation or known associations are found, and these patients are diagnosed to have chronic pruritus of unknown origin (CPUO) (2). The pathogenesis of CPUO is poorly understood and there is a paucity of effective treatment options. A systematic review revealed an absence of evidence for efficacious interventions, such as topical emollients, cooling lotions, corticosteroids, antidepressants, antihistamines, anticonvulsants, and phototherapy (3). The medical and psychosocial morbidity of this disease cannot be over-emphasized, with 18.5% of patients reporting suicidal ideations due to pruritus in the course of their disease (2). Thus, it is imperative to identify the key pathogenic mechanisms and to uncover effective treatments for CPUO.

In our previous study on isolated hypohidrosis, where patients are unable to sweat on more than 40% of the body surface area, we found the presence of hypo-refractile material below the stratum corneum together with dilated sweat ducts that were not present in healthy individuals using high-definition optical coherence tomography (HD-OCT) (4). These findings suggested that the sweat ducts were obstructed with consequent pooling of sweat in the epidermis. Subsequent histological analysis of patients' skin biopsies revealed keratinaceous deposits in the superficial sweat ducts. More than 85% of these patients responded to treatment with systemic retinoids and remained well long term after stopping retinoid treatment, which was directed toward resolving the keratinaceous obstruction at the sweat orifices. Interestingly, more than 40% of these patients experienced itch, suggesting that itch could be associated with sweat orifical obstruction. Retinoids are known to reduce hyperkeratosis accounting for sweat duct obstruction, and have been demonstrated to reverse hypohidrosis and improve thermoregulation in congenital ichthyoses (5). This provides the scientific basis upon our investigation into the use of retinoids to resolve sweat duct blockage in itch.

Numerous previous studies have found the association between itch and sweat duct blockage. In 1947, Sulzberger published a finding of a blocked acrosyringium in a patient suffering from atopic dermatitis (AD), postulating that the blockage of the sweat duct is associated with the AD disease process (6). Similarly, parakeratotic plugging of the sweat duct was observed in lesional AD skin, with ductal distension following thermal initiation of sweating. More recently, Haque et al. and Allen et al. showed sweat duct blockage was caused by biofilm forming bacteria *Staphylococcus epidermidis* and is linked to miliaria, with 100% of 36 AD skin samples having blocked sweat ducts containing bacteria, together with associated inflammatory infiltrates (7, 8). Indeed, a commonly reported trigger of itch is the exposure to heat or initiation of sweating, during which there is stimulation of the sympathetic nervous system (9). Together, these studies suggest that obstruction of the acrosyringia can result in subclinical miliaria, inciting itching and scratching (8).

In our preliminary studies with non-invasive *in vivo* imaging using high-definition optical coherence tomography (HD-OCT) of individuals with CPUO, similar features of sweat duct obstruction were observed, akin to those observed in our patients suffering from hypohidrosis (4). We were therefore interested to study the relation between sweat duct abnormalities with reduction of sweat duct blockage using systemic retinoids. Hence, we aimed to elucidate abnormalities in the sweat apparatus of patients with CPUO using HD-OCT and immunofluorescence studies in biopsies from such patients and to investigate the efficacy and safety of treatment in CPUO patients using systemic retinoids to reduce sweat duct blockage in a retrospective cohort analysis. Supplementary Figure S1 depicts the respective studies performed on the subjects in our study.

Materials and methods

Clinical *in vivo* imaging

Patients with CPUO first underwent baseline noninvasive *in vivo* skin imaging using HD-OCT (Skintell®; Agfa, Belgium). Each image volume captures a 1.8 × 1.5 mm area of skin to a depth of 0.5 mm, in which slice and *en face* images can be simultaneously visualized, allowing for 3-dimensional assessment of the skin. Heat and sweat induction were subsequently performed by getting patients to exercise on a stationary bicycle for 20 min in a temperature- (30–33°C) and humidity-regulated (65–75%) room. For subjects who were unable to perform the exercise, heat stimulation was performed by asking them to drink hot water in a warm room (28–30°C), until they started sweating on any part of their body.

Subsequently, all the patients underwent repeat *in vivo* skin imaging using HD-OCT (Skintell®; Agfa, Belgium). These images were compared to HD-OCT skin images obtained from five healthy volunteers obtained at baseline and post-exercise. Four to six measurements of sweat duct lumen diameter and wall thickness were obtained for all participants, with the mean values for each subject recorded. One-way analysis of variance was used to assess for statistical differences in the means of the different patient groups.

Patients who could perform the cycling exercise also underwent a whole-body starch-iodine test, as described in our previous publication (10). This test is able to accurately identify if individuals are able to sweat on all skin areas. The presence of hypohidrosis is defined by the inability to sweat affecting >40% of body surface area, as previously described (11).

Three of the CPUO patients underwent two skin biopsies each, one obtained from an itchy region and another from an adjacent non-itchy region. Skin biopsies were cryosectioned and processed for indirect immunofluorescence staining with mouse monoclonal

antibody (G-81) labeling for dermcidin (Santa Cruz), an antimicrobial peptide produced in sweat glands and imaged using the EVOS slide scanner (Thermo Scientific) at 20x magnification.

Retrospective study on retinoid treatment

In relation to our preliminary findings, we had routinely instituted treatment of CPUO using systemic retinoids for patients who have failed conventional therapies, in the attempt to regulate abnormalities in keratinization at the skin surface (12, 13). We performed a retrospective cohort analysis of all patients with a diagnosis of CPUO, managed in our subspecialty Itch Clinic, treated with systemic retinoids over a 6.5-year period between 16 May 2014 and 6 November 2020. Twenty of these patients had undergone *in vivo* skin imaging, as described in the section above, based on their willingness to undergo additional investigations. We included only patients with at least 6 weeks of itch and no clinically evident primary dermatological abnormalities (except for scratch-induced skin lesions). All patients had failed treatment with topical emollients and steroids, and at least three types of antihistamines from different generations at three to four times the recommended daily dosage. The patients had been treated with isotretinoin and/or acitretin off-label for their itch. We excluded patients who were unable to provide a numerical itch score, those diagnosed with a primary dermatosis or systemic disease

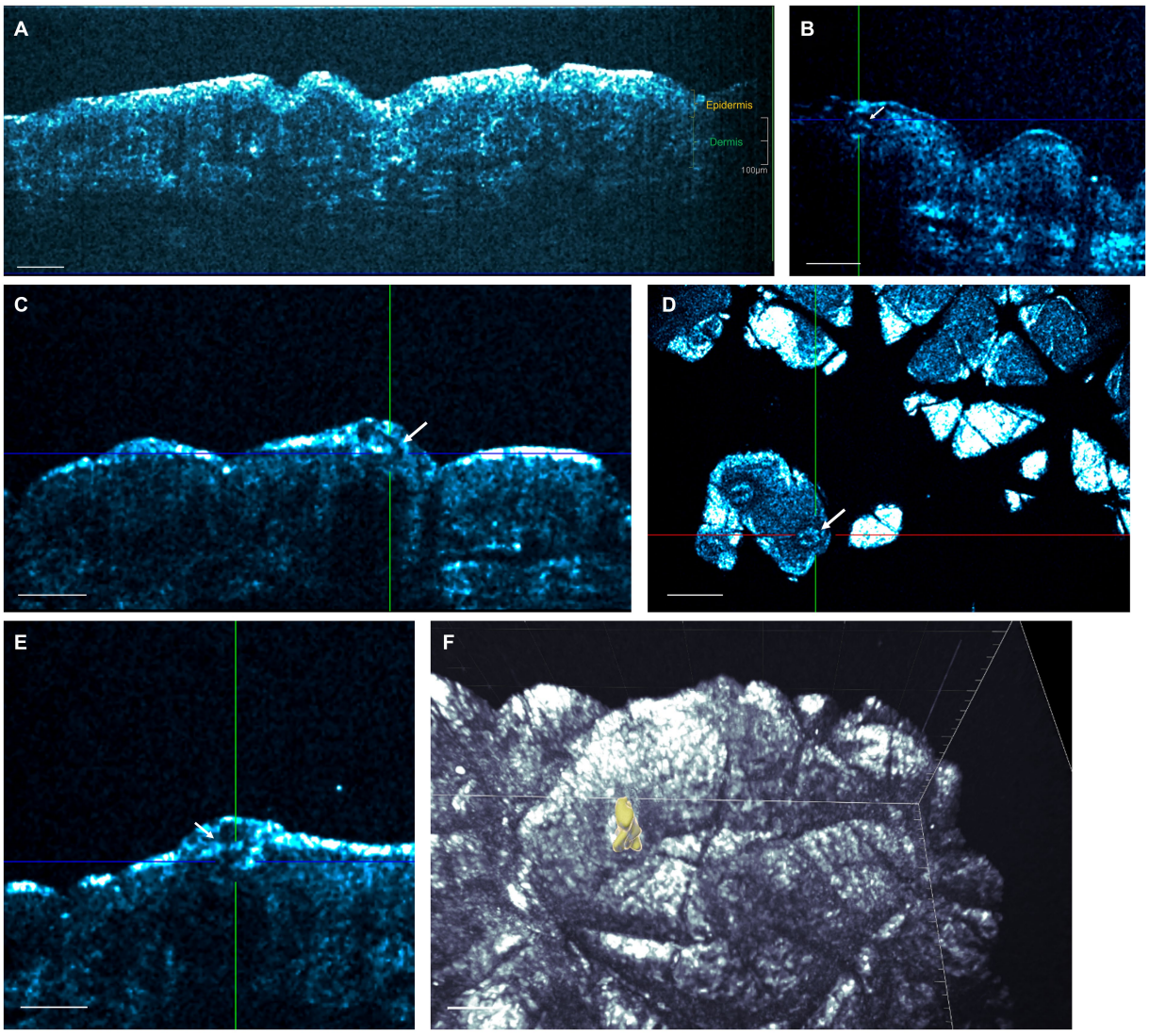

FIGURE 1
High-definition optical coherence tomography (HD-OCT) of sweat ducts. **(A)** *In vivo* HD-OCT slice view image of a healthy individual's abdominal skin. Sweat ducts are not normally visualized, except in images of the palms and soles. Scale bar; 200 µm. **(B)** A spiraling sweat duct within the epidermis (acrosyringium) in a patient with CPUO at rest. Scale bar; 100 µm. **(C)** Post-exercise slice image revealing a curved tubular structure in the epidermis representing a dilated acrosyringium. Scale bar; 100 µm. **(D)** In the corresponding *en face* image of **(C)**, tubular structures with thickened walls (one in crosshair), with a surrounding hypo-refractive bordering region indicative of fluid collection can be observed. Scale bar; 200 µm. **(E)** These dilated sweat ducts may present as subcorneal fluid collections, and the dilated sweat duct here is seen to extend into the dermis. Scale bar; 100 µm. **(F)** Three-dimensional rendering demonstrating a dilated and tortuous acrosyringium (yellow structure) in the epidermis. Scale bar; 30 µm. A more comprehensive view is available in Supplementary Video S1.

accounting for their pruritus, and those who did not return for at least one follow-up visit after starting systemic retinoids.

Patient demographics, disease characteristics, verbalization of suicidal ideation, retinoid treatment doses and duration, and adverse effects due to itch were collected. To assess the efficacy of retinoid treatment, we evaluated the itch score of each patient at each follow-up visit. The itch score reflects the patient's average intensity of itch over the prior 3 days on a 0–10 numerical rating scale. We defined treatment response as a reduction in itch score of two points or more, which is sustained during the treatment course. The itch score value of two was selected in accordance with the previously determined Minimal Clinically Important Difference in numerical rating of chronic itch (14). Safety was assessed by the number of patients who stopped either or both retinoids due to side effects.

All variables were summarized descriptively using counts and percentages for categorical variables and mean with standard deviation and median with range/interquartile range for continuous variables. Two sample t-test for continuous data and Fisher's exact test for categorical data were used to assess the difference. To evaluate how itch scores changed over time after retinoid treatment, a linear mixed-effects model was employed. The model for itch scores included time (t), number of retinoids used, baseline itch score, sex, race, and age when retinoid started as fixed effects, whereas a random intercept for subjects was included to account for within-subject correlations. A value of p less than 0.05 was considered statistically significant. Statistics were generated using R version 3.5.3. This study was approved for exemption under the application 2021/00785 from the National Healthcare Group Domain Specific Review Board.

Results

High-definition optical coherence tomography demonstrates sweat duct dilation and acrosyringial wall thickening after exposure to heat and sweating in subjects with CPUO

In order to visualize irregularities in the sweat duct apparatus, 20 subjects with CPUO underwent HD-OCT skin imaging and heat induction. Fifteen of these patients underwent heat induction by exercise and five, due to being elderly, underwent heat induction through ingestion of hot water in a warm room. HD-OCT identified features of sweat duct obstruction in 19 out of the 20 subjects (Figure 1; Supplementary Video S1), and these features were significantly more prominent post sweat-induction compared to baseline. These abnormalities were not found in healthy individuals. The most prominent features were dilation of the lumen and thickening of the wall of the acrosyringium, which is the spiraling intra-epidermal portion of the sweat duct. This was evidenced by larger sweat duct lumen diameters in affected patients after exercise and hot water stimulation, compared to healthy controls (respective means 37.2 µm and 37.3 µm vs. 22.3 µm, $p < 0.01$ and < 0.05 respectively) (Figure 2A). Concurrently, sweat duct wall thickness was increased in affected patients after exercise stimulation as compared to healthy controls but this did not reach statistical significance (means 17.5 µm vs. 14.7 µm, $p = 0.21$) due to high variability in sweat duct walls (Figure 2B).

The sweat duct dilation was found to form a collection of fluid under the stratum corneum. On the *en face* views, a surrounding hypo-refractive bordering region indicative of fluid accumulation could be observed. The dilation of the sweat ducts and exudation of fluid to the surroundings indicate presence of obstruction of the sweat orifice at the stratum corneum, which is comprised of keratin. We conducted the whole-body starch-iodine sweat testing for the 15 subjects who had exercised and found that they do not have generalized hypohidrosis. We thereby posit that partial, instead of complete, sweat duct obstruction occurs in patients with CPUO.

Immunostaining demonstrated sweat component accumulation within sweat glands with leakage into the skin

In order to further investigate associated pathology occurring in the sweat glands, we carried out immunostaining for dermcidin in skin biopsies of itchy versus non-itchy skin areas in three CPUO

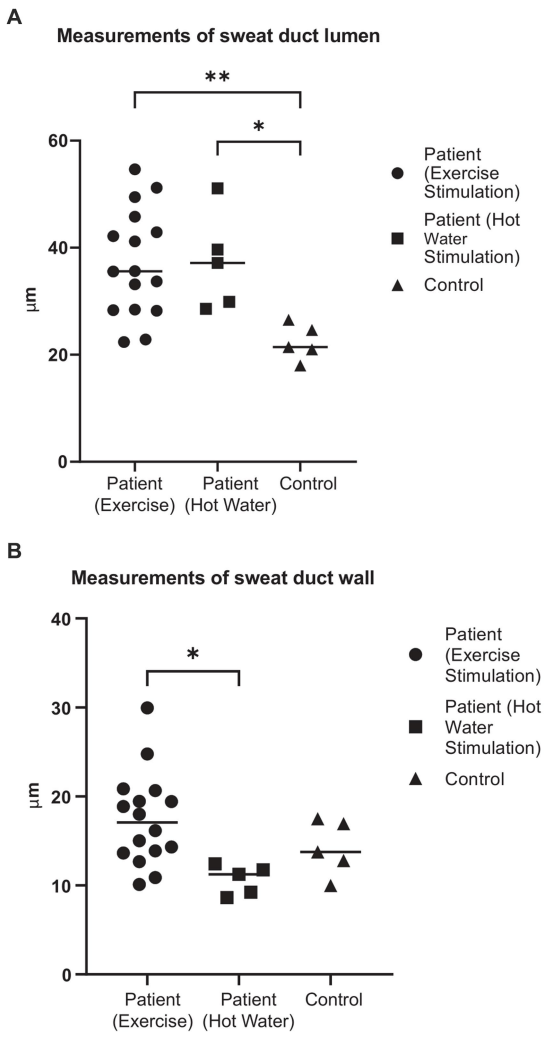

FIGURE 2
Measurements of sweat duct lumen and wall thickness from HD-OCT images. **(A)** Sweat duct lumen diameter (µm) after induction of heat and sweating in subjects with CPUO and healthy controls. *denotes $p < 0.05$, **denotes $p < 0.01$. **(B)** Sweat duct wall thickness (µm) after induction of heat and sweating in subjects with CPUO and healthy controls. *denotes $p < 0.05$.

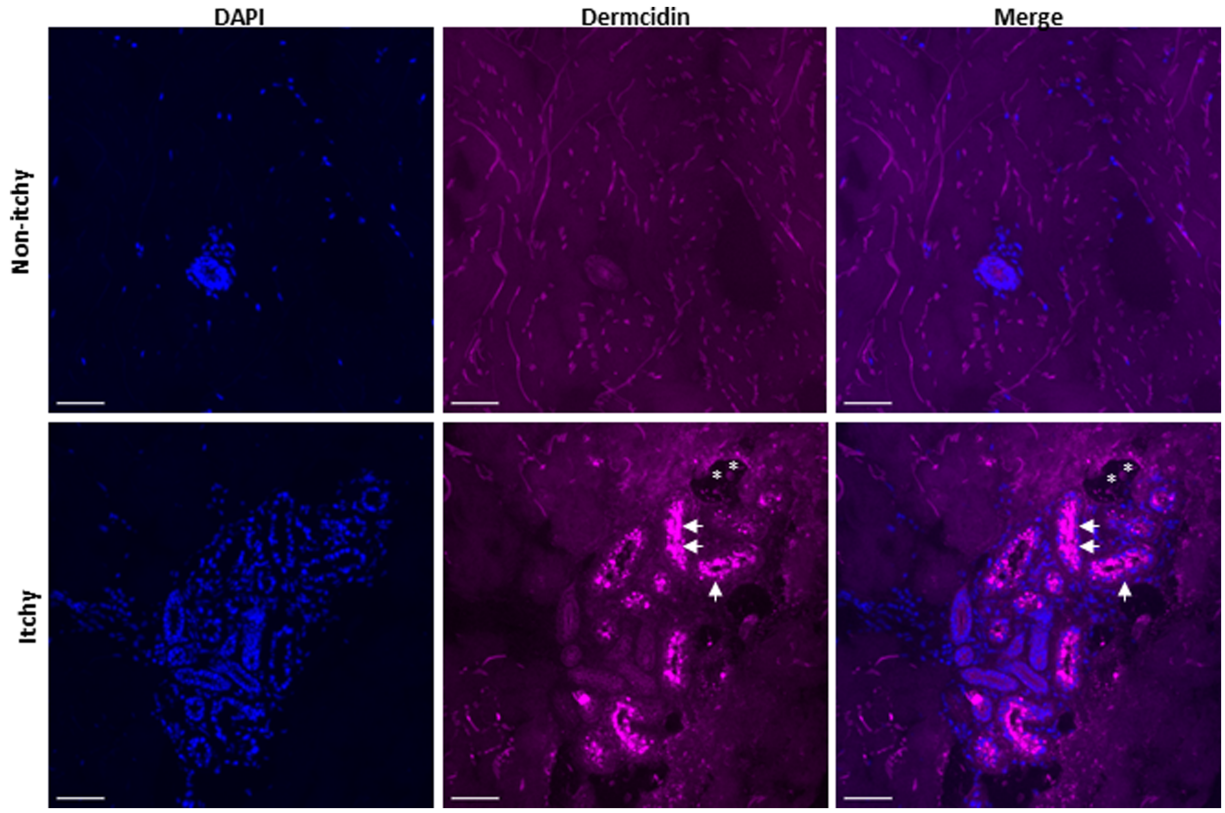

FIGURE 3
Immunofluorescence staining of sweat glands in a skin biopsy of non-itchy and itchy skin in a patient with CPUO. Scale bar; 50 μm. Images are representative of phenomena in three patients. The skin biopsies were cryosectioned to 10 μm thickness, fixed with paraformaldehyde and immunolabeled for nucleus (DAPI; blue), and dermcidin (magenta). Dermcidin, an antimicrobial peptide present in sweat, can be found accumulated within the secretory sweat glands (arrowheads) with some leakage (asterisk).

patients. Dermcidin is an antimicrobial peptide which is exclusively and constitutively expressed in eccrine sweat glands, and positive staining may be taken as a surrogate for the presence of sweat components (15). Immunostaining demonstrated accumulation of dermicidin in the sweat glands located within the dermis, together with leakage in some cases (Figure 3). Together with the HD-OCT findings above, we theorize that accumulated sweat leaks into the epidermis, irritating intra-epidermal nerve fibers, resulting in itch. Similarly, accumulated sweat which leaks into the dermis could potentially irritate nociceptive/pain nerve fibers, resulting in the prickly sensation that patients often concurrently experience.

Based on these findings indicating keratinaceous partial obstruction of sweat orifices, we treated patients with CPUO with oral retinoids, in the attempt to regulate abnormalities in skin surface keratinization (12, 13), followed by evaluating the change in itch severity experienced.

Analysis of retinoid efficacy and safety

A total of 56 patients were included in the final analysis, and the baseline characteristics of our study population are as outlined in Table 1. Most of our patients were older, male, and Chinese. The mean duration of itch before their first visit at the Itch Clinic was 116.3 months, and mean itch score at presentation was severe at 8.2. The lower limbs were the most common areas affected. To understand associated comorbidities, we also collected data on suicidal ideation and peripheral eosinophilia. Four (7.1%) patients voluntarily verbalized suicidal ideation due to itch. Thirteen (23.2%) patients had peripheral eosinophilia.

Nine patients were treated only with acitretin, 19 only with isotretinoin, and 28 were treated with both retinoids over the course of their follow-up (Table 2). The mean (*SD*) duration of retinoid treatment was 671.9 (397.6) days. Figure 4 and Supplementary Table S1 show the changes in itch scores after starting retinoids. There was a significant reduction in itch score over time, noticeable at 3 months of treatment, during which there was a mean reduction in itch score of 2.38 points ($p < 0.0001$), with the effect being sustained throughout treatment. Thirty-eight patients (67.9%) responded to treatment, defined as having an itch score two points or more lower than baseline, sustained during treatment. A total of eight patients (14.8%) achieved an itch score of 0 or 1 (Supplementary Table S2). Of these, four patients had stopped treatment for a mean of 318.5 days with sustained resolution of itch. Eight patients (14.3%), three on acitretin (8.11%) and five on isotretinoin (10.6%), stopped or changed retinoids due to adverse effects, the most common being dryness (Table 2).

Discussion/conclusion

Management of CPUO remains challenging, and at present, there exists no consistently effective treatment in the literature. Our results indicate that one cause of CPUO is partial sweat duct obstruction, likely

TABLE 1 Baseline characteristics.

Characteristics	Value
Age at first visit, years	
Mean (SD)	55.2 (17.5)
Minimum, maximum	16, 84
Age when systemic retinoid first started, years	
Mean (SD)	58.0 (18.2)
Minimum, maximum	19, 87
Sex	
Male, *n* (%)	39 (69.6)
Female, *n* (%)	17 (30.4)
Race	
Chinese, *n* (%)	48 (85.7)
Malay, *n* (%)	2 (3.6)
Indian, *n* (%)	6 (10.7)
Duration of itch before first visit, months	
Mean (SD)	116.3 (140.4)
Median (Q1, Q3)	48 (24, 180)
Minimum, maximum	1, 528
Baseline itch scores	
Mean (SD)	8.2 (1.8)
Minimum, maximum	3, 10
Areas affected, *n* (%)	
Face and scalp	32 (57.1)
Trunk	46 (82.1)
Upper limbs	49 (87.5)
Lower limbs	51 (91.1)
Verbalized suicidal ideation due to itch, *n* (%)	4 (7.1)
Eosinophilia, *n* (%)	13 (23.2)

SD, standard deviation.

TABLE 2 Treatment data.

Statistics	Value
Patients initially given acitretin, *n* (%)	15 (26.8)
Patients initially given isotretinoin, *n* (%)	41 (73.2)
Patients given acitretin during the course of treatment, *n* (%)	37 (66.1)
Patients given isotretinoin during the course of treatment, *n* (%)	47 (83.9)
Patients who responded to retinoids, *n* (%)	38 (67.9)
Patients who switched retinoids due to inefficacy, n (%)	**26 (48.2)**[†]
Eventually responded to retinoids, *n* (%)	13 (50.0)
Acitretin to isotretinoin, n (%)	**3 (5.36)**
Responded to isotretinoin, *n* (%)	1 (33.3)[‡]
Isotretinoin to acitretin, n (%)	**19 (33.9)**
Responded to acitretin, *n* (%)	10 (52.6)[§]
Acitretin to isotretinoin and back to acitretin, n (%)	**2 (3.57)**
Responded to third retinoid, *n* (%)	1 (50.0)
Isotretinoin to acitretin and back to isotretinoin, n (%)	**1 (1.79)**
Responded to third retinoid, *n* (%)	0 (0.00)
Isotretinoin to acitretin to isotretinoin to acitretin, n (%)	**1 (1.79)**
Responded to fourth retinoid, *n* (%)	1 (100)
Patients who stopped or changed retinoids due to side effects, n (%)	**8 (14.3)**
Acitretin, n (%)	**3 (8.11)**
Stopped retinoid therapy, *n* (%)	2 (66.7)
Changed to isotretinoin, *n* (%)	1 (33.3)
Side effects experienced	
Dryness, *n* (%)	2 (66.7)
Worsening of itch, *n* (%)	1 (33.3)
Isotretinoin, n (%)	**5 (10.6)**
Stopped retinoid therapy, *n* (%)	2 (40.0)
Changed to acitretin, *n* (%)	3 (60.0)
Side effects experienced	
Dryness, *n* (%)	3 (60.0)
Abdominal pain, *n* (%)	1 (20.0)
Mood swings, *n* (%)	1 (20.0)

[†]Includes four patients who also switched retinoids due to side effects.
[‡]Of the three patients who switched from acitretin to isotretinoin due to inefficacy, one defaulted follow-up and another did not respond.
[§]Of the 19 patients who switched from isotretinoin to acitretin due to inefficacy, one stopped treatment because of xerosis and eight have not returned for follow-up since.
The values on the indented lines following each bolded line are subsets of the bolded lines, and expressed as percentages of the bolded lines.

by keratinaceous deposits at the skin surface, with corresponding sweat accumulation and leakage into the dermis, and this is evident through the dilatation of the sweat duct upon heat induction observed through *in vivo* HD-OCT imaging, coupled with accumulation and leakage of sweat components into the dermis in immunostaining studies. Correspondingly, in our retrospective cohort, treatment of CPUO patients with retinoids to reduce hyperkeratinization, which could be linked to sweat duct obstruction led to a mean reduction in itch score of 2.38 points ($p < 0.0001$) at 3 months after starting treatment, with sustained effect throughout treatment. Thirty-eight patients (67.9%) responded to treatment, while eight patients (14.3%) achieved no or almost no itch. The results suggest that retinoids can be an effective therapeutic option for CPUO who have failed conventional treatment. By the end of the analysis period, four (7.1%) patients had exhibited a durable disease-free period after stopping treatment, suggesting that the systemic retinoids play a role in address the underlying pathology, rather than providing temporary control such as that conferred by immunosuppressant agents. Moving forward, we should assess for the presence of sweat duct pathology in patients with CPUO and consider the use of retinoids if detected. A low retinoid treatment dose, such as 10 mg daily, appears appropriate. Other clinical implications of the role of sweat gland dysfunction in CPUO would be the need for patient

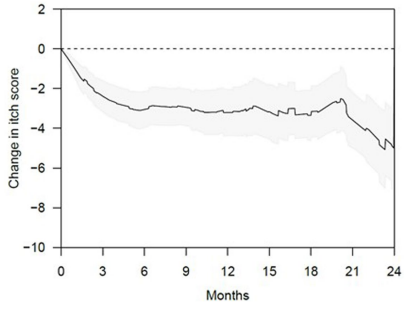

FIGURE 4
Change in itch scores after systemic retinoid therapy (graphical representation).

education to maintain a cool environment, wear loose fitting garments and avoid drugs known to induce hypohidrosis.

Isotretinoin and acitretin appear similar in terms of side effect profile. Of note, pregnancy is contraindicated for 3 years after taking acitretin, limiting its use in females of childbearing age, which made up a minority of the patients (8.93%) in our study population (16). The use of isotretinoin would be preferred in this subpopulation.

In our patient cohort with recalcitrant CPUO disease, 32.1% did not respond to either acitretin or isotretinoin. One reason may be the need to address existing secondary inflammation, in addition to the underlying cause. We postulate that the leakage of sweat into the skin, resulting from partial sweat duct obstruction, may trigger the innate immune cells, which include mast cells, basophils, and eosinophils. In our study, we found that 23.2% of our CPUO patients had blood eosinophilia. This is in keeping with a recent retrospective cross-sectional study of patients with chronic pruritus, in which eosinophilia was present in 17.9% of patients with normal-appearing skin and 39.7% of patients presenting with severe chronic secondary scratch lesions (17). Eosinophils are involved in host defense against exogenous antigens and pathogens, and their role in stimulating nerve cells and contributing to pruritus has been demonstrated in several studies (18). Subsequent to triggering of the innate immune system, activation of the adaptive immune system is expected to occur, with the primary involvement of the T-helper 2 pathway. Thus far, immuno-modulatory agents involving antagonism of the interleukins-4, −13 and −31 and the Janus kinase-signal transducer and activator of transcription pathway have been shown to be useful for controlling itch in various studies (19, 20). However, itch can recur after stopping such treatment because the underlying cause of itch was not addressed. Addition of such immune-modulatory agents to the treatment of underlying structural abnormalities in keratinization may help further optimize treatment outcomes.

CPUO is a disease with significant psychosocial burden. In our study population, 7.1% of patients voluntarily verbalized suicidal ideation due to itch. Expression of suicidal ideation has been associated with a four-fold increased risk for subsequent completed suicides, highlighting the importance of managing pruritus in this group of patients (21).

The limitations of our study are the lack of placebo control due to its retrospective nature, subjective reporting of itch score by patients and the high proportion of patients (60.7%) who remained on systemic retinoids at the conclusion of the study period. A prolonged period of follow-up, especially after cessation of retinoids, will be necessary to assess the proportion of patients with a durable disease-free period. We have only evaluated the use of acitretin and isotretinoin in this study, and other systemic retinoids have not been assessed.

In conclusion, based on the novel findings from physiological imaging studies identifying partial keratinaceous sweat duct obstruction in CPUO, we instituted systemic retinoid treatment to address the underlying pathology. In patients who failed conventional therapies, treatment with isotretinoin or acitretin appears to be an effective and safe therapeutic option.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The requirement of ethical approval was waived by the National Healthcare Group Domain Specific Review Board for the studies involving humans because the study posed no more than minimal risk to research subjects. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because none of the information collected affected the clinical decisions about the individual's care, and patients were not being deprived of clinical care to which they would normally be entitled.

Author contributions

SL: Data curation, Formal analysis, Writing – original draft. YT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. YZ: Data curation, Formal analysis, Writing – review & editing. XZ: Data curation, Formal analysis, Writing – review & editing. LN: Conceptualization, Project administration, Writing – review & editing. HT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1265148/full#supplementary-material

References

1. Weisshaar E, Dalgard F. Epidemiology of itch: adding to the burden of skin morbidity. *Acta Derm Venereol*. (2009) 89:339–50. doi: 10.2340/00015555-0662

2. Matterne U, Apfelbacher CJ, Loerbroks A, Schwarzer T, Buttner M, Ofenloch R, et al. Prevalence, correlates and characteristics of chronic pruritus: a population-based cross-sectional study. *Acta Derm Venereol*. (2011) 91:674–9. doi: 10.2340/00015555-1159

3. Andrade A, Kuah CY, Martin-Lopez JE, Chua S, Shpadaruk V, Sanclemente G, et al. Interventions for chronic pruritus of unknown origin. *Cochrane Database Syst Rev*. (2020) 1:CD013128. doi: 10.1002/14651858.CD013128.pub2

4. Chan MMH, Lim GH, Zhao X, Tey HL. Isolated hypohidrosis: pathogenesis and treatment. *Eur J Dermatol*. (2020) 30:680–7. doi: 10.1684/ejd.2020.3931

5. Haenssle HA, Finkenrath A, Hausser I, Oji V, Traupe H, Hennies HC, et al. Effective treatment of severe thermodysregulation by oral retinoids in a patient with recessive congenital lamellar ichthyosis. *Clin Exp Dermatol*. (2008) 33:578–81. doi: 10.1111/j.1365-2230.2008.02709.x

6. Sulzberger MB, Herrmann F, Zak FG. Studies of sweating; preliminary report with particular emphasis of a sweat retention syndrome. *J Invest Dermatol*. (1947) 9:221–42. doi: 10.1038/jid.1947.92

7. Allen HB, Vaze ND, Choi C, Hailu T, Tulbert BH, Cusack CA, et al. The presence and impact of biofilm-producing staphylococci in atopic dermatitis. *JAMA Dermatol*. (2014) 150:260–5. doi: 10.1001/jamadermatol.2013.8627

8. Haque MS, Hailu T, Pritchett E, Cusack CA, Allen HB. The oldest new finding in atopic dermatitis: subclinical miliaria as an origin. *JAMA Dermatol*. (2013) 149:436–8. doi: 10.1001/2013.jamadermatol.109

9. Silverberg JI, Lei D, Yousaf M, Janmohamed SR, Vakharia PP, Chopra R, et al. Association of itch triggers with atopic dermatitis severity and course in adults. *Ann Allergy Asthma Immunol*. (2020) 125:552–559.e2. doi: 10.1016/j.anai.2020.06.014

10. Lim JH, Choo W, Chang JH, Tey HL, Chong WS. Application of iodinated starch powder using an atomizer spray gun – a new and effective tool to evaluate hypohidrosis. *Skin Res Technol*. (2016) 22:370–4. doi: 10.1111/srt.12275

11. Lim JH, Kok WL, Bin Ali N, Chong WS, Tey HL. Hypohidrosis in individuals with exertional heat injury: a prospective open cohort study. *Dermatology*. (2016) 232:50–6. doi: 10.1159/000439108

12. Elias PM. Epidermal effects of retinoids: supramolecular observations and clinical implications. *J Am Acad Dermatol*. (1986) 15:797–809. doi: 10.1016/S0190-9622(86)70236-3

13. Torma H. Regulation of keratin expression by retinoids. *Dermatoendocrinol*. (2011) 3:136–40. doi: 10.4161/derm.15026

14. Reich A, Riepe C, Anastasiadou Z, Medrek K, Augustin M, Szepietowski JC, et al. Itch assessment with visual analogue scale and numerical rating scale: determination of minimal clinically important difference in chronic itch. *Acta Derm Venereol*. (2016) 96:978–80. doi: 10.2340/00015555-2433

15. Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, et al. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat Immunol*. (2001) 2:1133–7. doi: 10.1038/ni732

16. Ormerod AD, Campalani E, Goodfield MJ, Unit BADCS. British Association of Dermatologists guidelines on the efficacy and use of acitretin in dermatology. *Br J Dermatol*. (2010) 162:952–63. doi: 10.1111/j.1365-2133.2010.09755.x

17. Lehmann M, Cazzaniga S, Simon D, Perruchoud DL, Borradori L, Rammlmair A. Patterns among patients with chronic pruritus: a retrospective analysis of 170 patients. *Acta Derm Venereol*. (2020) 100:adv00068. doi: 10.2340/00015555-3405

18. Nakashima C, Ishida Y, Kitoh A, Otsuka A, Kabashima K. Interaction of peripheral nerves and mast cells, eosinophils, and basophils in the development of pruritus. *Exp Dermatol*. (2019) 28:1405–11. doi: 10.1111/exd.14014

19. Jeon J, Wang F, Badic A, Kim BS. Treatment of patients with chronic pruritus of unknown origin with dupilumab. *J Dermatolog Treat*. (2021) 33:1754–57. doi: 10.1080/09546634.2021.1880542

20. Misery L, Brenaut E, Pierre O, Le Garrec R, Gouin O, Lebonvallet N, et al. Chronic itch: emerging treatments following new research concepts. *Br J Pharmacol*. (2021) 178:4775–91. doi: 10.1111/bph.15672

21. Hubers AAM, Moaddine S, Peersmann SHM, Stijnen T, van Duijn E, van der Mast RC, et al. Suicidal ideation and subsequent completed suicide in both psychiatric and non-psychiatric populations: a meta-analysis. *Epidemiol Psychiatr Sci*. (2018) 27:186–98. doi: 10.1017/S2045796016001049

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Targeted immunotherapy for hair regrowth and regeneration

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KEYWORDS

targeted, immunotherapy, hair, alopecia, regrowth, regeneration

1. Introduction

Immunotherapy for skin conditions has a long and successful history. While the main strategy for treating inflammatory conditions is local or systemic immunosuppression, immunotherapy aims to stimulate parts or all of the immune system to bring about a therapeutic response. The immune system contains multiple, redundant avenues of checks and balances, and some immune cells have immunomodulatory or regulatory roles. These cells are targeted by immunotherapy in inflammatory skin conditions. Immunotherapy is effective in treating some hair loss disorders, supporting recent findings that the hair cycle is influenced by immune cells (1, 2).

The normal hair cycle consists of sequential growth (anagen), regression (catagen) and rest (telogen) phases. In humans, the anagen phase (9–10 years) reflects the growing hair shaft, while the end of telogen (2–3 months) is marked by hair shedding. When the hair cycle is disrupted, premature anagen-to-catagen transition results in majority of hair follicles (HFs) entering telogen and shedding concurrently, causing significant, noticeable hair loss.

The immune system is closely associated with the HF, with macrophages and mast cells described in the perifollicular dermis (3, 4). Skin-resident macrophages clear apoptotic cell fragments following catagen (5) and may also interact with hair follicle stem cells (HFSC) to maintain quiescence during telogen (1). The dynamic nature of the hair cycle results in a constant flux of antigens, both self and non-self, from the manufacture of the hair shaft and the infundibular connection to the outside world, respectively. To protect the hair bulb from inappropriate immune attack, the HF has evolved a status of relative "immune privilege" (IP). This was demonstrated elegantly by Billingham et al. decades ago showing that the presence of HFs in allografts was sufficient to prevent immune rejection of melanocytes in transplantation experiments on guinea pigs (6). Various mechanisms have since been proposed for the IP of the HF, with the most prominent one being a downregulation of MHC Class I expression (7). Hair loss disorders are associated with disruptions and changes in the immune milieu of the HF (8, 9), and conversely, immunotherapy has been utilized to promote HF regrowth and regeneration.

2. The immune system interaction with the hair follicle niche

With evidence of immune cells being associated with the HF, it is reasonable to hypothesize that changes in immune cell composition and activity around HFs can affect HFSC and the hair cycle. However, the direct influence of immune cells in promoting hair regrowth and regeneration in humans is still unknown. In mice, regulatory T-cells (T-regs) promote anagen re-entry via Notch signaling (10), and macrophages may release Wnt factors to stimulate anagen under certain conditions (11). Wound-induced HF neogenesis (WIHN)

is another phenomenon in rodents whereby brand-new HFs arise from scar tissue of a wound. Cotsarelis and Ito showed that relatively large wounds in the back skin of mice initially heal without HFs, but these scars are soon populated by *de novo* HFs via WIHN (12). Small cutaneous wounds in mice upregulate Wnt and Shh pathways (13), while larger wounds recruit dermal γδ-T-cells (14) and M2 macrophages (15) to promote WIHN. While the mouse's fur coat and human scalp hair differ in their stage of hair cycle, fundamental processes and pathways are likely conserved, and human HFs may respond in a similar manner to microtrauma.

In humans, crude immune stimulation has been shown to induce hair growth. Friction and irritation are known causes of hypertrichosis, and excessive hair growth occurs after limb fixation with plaster cast application (16, 17), and on burned skin borders (18). Allergic contact dermatitis to wig adhesives is reported to have a therapeutic effect in alopecia areata (AA) (19), forming the basis of topical immunotherapy for AA. Contact dermatitis introduces "antigenic competition" which recruits suppressor T-cells and macrophages, producing an immunomodulatory environment and dampening the autoimmune attack on HFs (20). These reports point to a role for microtrauma to elicit a permissive environment for hair regrowth and regeneration.

Newer treatment modalities for AGA harnessing the effects of microtrauma include platelet-rich plasma (PRP), microneedling and ablative and non-ablative lasers, which aim to reproduce the effects of wounding in areas of alopecia. In PRP, intra-dermal injection of activated platelets release growth factors near HFs (21), including transforming growth factor-β (TGF-β), epidermal growth factor, basic fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and insulin-like growth factor-1, which support the anagen phase of the hair cycle. The efficacy of PRP appears operator- and protocol-dependent, but has been shown in systematic reviews to promote hair regrowth modestly (22). In randomized, split-scalp studies of PRP vs. sham injections, hair density also increased among the controls, suggesting microtrauma alone may stimulate hair regrowth (23). Indeed, several groups have demonstrated that microneedling may be effective in treating AGA (24) and AA (25). Both ablative and non-ablative settings of Er:YAG lasers have been used in AGA and AA, with promising results (26–28).

The efficacy of these treatments supports the hypothesis that the microenvironment of HFSC can be manipulated to promote hair regrowth. Immune cells are likely involved in the process of microtrauma. The treatments above, however, are non-specific. They induce an irritant, allergic or wounding response that varies between patients, resulting in inconsistent effectiveness. We are still in the process of understanding this process, and fine tuning the procedures and patient selection to obtain the best possible clinical outcomes.

3. Targeted immunotherapy for hair regrowth

Targeted immunotherapy in promoting hair regeneration is expected to grow with understanding of the detailed control of the human hair cycle. The first mode of targeted immunotherapy has been applied for AA, as we know most about its immunopathology.

3.1. Immunosuppressive therapies with stimulatory action in other parts of the immune system or HFSC

3.1.1. JAK inhibitors

Janus kinase (JAK) inhibitors have revolutionized therapeutics in dermatology, successfully utilized in many inflammatory skin conditions including atopic dermatitis. The JAK-STAT pathway is an integral component of AA pathophysiology, downstream of interferon-gamma (IFN-γ) and interleukin (IL)-15 signaling.

Baricitinib is the first FDA-approved drug for AA in 2022. In two concurrent phase III randomized controlled trials (BRAVE-AA1 and BRAVE-AA2), baricitinib achieved SALT scores <20 at 52 weeks with a good safety profile (29). Baricitinib, which inhibits JAK1 and JAK2 signaling is effective in long-standing AA resistant to traditional therapies (30), besides being safe and effective in pediatric AA (31). Increasingly specific JAK inhibitors (JAK1 ivarmacitinib, JAK3 ritlecitinib, and JAK1/Tyk2 beprocitinib) (29, 32) are investigated for use in AA, making treatment more targeted.

Topical JAK inhibition not only reverses hair loss in AA mice, but also induces the telogen-to-anagen transition in disease-free C56Bl/6 mice (33). This suggests that the JAK-STAT pathway is also involved in normal hair cycles (34), leading to the discovery of a distinct subset of TREM2+ macrophages that maintain HFSC quiescence by secreting oncostatin M (1). Pharmacological, immunological and genetic inhibition of these macrophages sufficiently induced anagen in mice. Whether a similar mechanism is present in the human HF niche is unknown, but STAT3 is upregulated in AGA scalps (35).

The Wnt/β-catenin signaling pathway is the major pathway in activating DPCs, which are crucial in the hair bulb and bulge interaction for anagen initiation. Treatment of HFs with ruxolitinib, a JAK1/2 inhibitor, stimulates the expression of β-catenin mRNA, upregulating Wnt/β-catenin signaling. Further, proinflammatory cytokines of AA, namely IFN-γ-induced caspase-1, IL-1β, IL-15 and IL-18 are also suppressed by JAK1/2 inhibition (36). JAK inhibition in AA thus may have more than an immunosuppressive role, and may have immunotherapeutic roles in stimulation of DP and/or HFSC.

In IFN-treated dermal papilla (DP) culture, ruxolitinib was also shown to downregulate MHC class I expression, contributing to partial IP restoration. In addition, ruxolitinib stimulated several growth factors, including FGF7, that supported DP cell survival which translates to a hair growth-permissive microenviroment independent of its immunosuppressive properties (36).

The *in vivo* effects of JAK inhibition have yet to be evaluated, partly due to the challenges in analyzing and quantifying the hair cycle, as follicular units are asynchronous and hair cycle phases last months to years. Existing JAK inhibitors also penetrate skin insufficiently (37).

Other challenges to JAK inhibition include disease recurrence after discontinuation and balancing long-term usage against side

effects of infections, marrow suppression, transaminitis, and lipid abnormalities (38).

3.1.2. Statins

Statins have been proposed for treating various dermatologic conditions characterized by ingress of activated leukocytes into the skin, including AA (39). In a pilot study, a combination of simvastatin and ezetimibe reduced hair loss and resulted in stable remission in AA mice model, with an increase in FOXP3+ T-regs (40). Simvastatin may improve AA through multidirectional pro- and anti-inflammatory activities. These include increasing Th2 cytokine secretion, upregulating T-reg cells in mice (39), downregulating Th1 cytokine secretion via JAK-STAT pathway modulation (41), inhibiting leukocyte activation, adhesion and migration (42), activating Wnt/β-catenin signaling pathway (43), and downregulating reactive oxygen species production (44). However, larger placebo-controlled trials are still required, as these findings were not always reproducible by other authors (45).

3.2. Biologic therapies

Monoclonal antibody therapy is not as well established for AA as for dermatological conditions like psoriasis and eczema. Most TNF inhibitors are ineffective for AA, while dupilumab was found to be modestly effective in a Phase IIa trial (46). Although AA is associated with atopic dermatitis and psoriasis, treatment for the latter does not always improve AA (47), and may sometimes worsen hair loss (48).

The contrasting effects of biological therapies and JAK inhibitors in AA suggest that current targets of monoclonal antibodies (TNF-α, IL-17, IL-23 and IL-4 signaling) may support the HF immune privileged microenvironment, and an unbalanced blockade of these pathways leads to cytokine imbalance and AA (49). JAK-inhibition is more focused on immune mechanisms that share JAK-STAT pathways, which are more frequently associated with "active" T-cell directives on the HF.

Nivolumab is an anti-PD1 (programmed cell death-1) monoclonal antibody effective in many cancers, including melanoma. It releases the inhibition of autoreactive T-cells, allowing immune system clearance of tumor cells. This same mechanism has been reported to cause AA in these patients (50). The PD-1/PD-L1 pathway has also been implicated in T-cell exhaustion accompanying response to AA treatment with JAK-inhibitors (51). Exploring this pathway in AA may lead to new targets for biological therapy.

3.3. IL-2 complex treatment

A pilot study using low dose IL-2 to expand T-reg populations in severe AA showed initial promise (52). T-regs suppress autoreactive NKG2D+ T-cells that attack HFs and promote hair regrowth by inducing anagen. In mice models, intradermal injection of IL-2/anti-IL-2 antibody complex (IL-2c) efficiently stimulates T-reg proliferation by 8- to 10-fold in the skin. T-regs have also been shown to promote anagen via Jagged-1 expression (10).

A prospective randomized control study with low dose IL-2 was conducted which showed limited efficacy of this treatment (53). In murine models, while the fold ratio of CD8 T-cells over T-regs was also markedly reduced post-IL-2c treatment, CD8 T-cells remained around HFs, including NKG2D+ T-cells, in established AA mouse models (54). Despite no significant reduction in IL-10 or TGF-β secretion, the expanded T-regs were not sufficient to inhibit CD8 T-cell proliferation in established AA, resulting in neither anagen induction nor AA reversal. Further studies are warranted to investigate the role of IL-2/IL-2c in the treatment of AA. Early IL-2c therapy has been hypothesized to be effective in acute AA, which may slow disease progression but may require adjunctive treatments for more chronic, established cases.

3.4. Prevention or restoration of IP collapse

AA develops when the IP of the HF collapses, due to ectopic MHC class-1 expression induced by IFN-γ (55). *Ex vivo*-application of TGF-β1, α-MSH and the drug FK506 (Tacrolimus) have been found to suppress MHC Class I expression in cultured HF organ cultures, likely through suppressing mRNA transcription (56). α-MSH, which also has immunosuppressive properties, is also increased in AA lesional scalp post-UVA phototherapy (57). While systemic calcineurin inhibitors like ciclosporin have been effective in treating severe recalcitrant AA, topical tacrolimus/FK506 has proven to be less reliable (58), and exploring this method of restoring HF IP may further expand our immunotherapy repertoire.

3.5. Harnessing microtrauma

For AGA, there is currently a wide variety of modalities for inducing microtrauma to promote hair regrowth, including fractional lasers and microneedling. While these may be non-targeted, they enable more targeted drug delivery when combined with topical treatments like minoxidil or PRP. Release of growth factors with microtrauma [such as PDGF, VEGF, β-catenin, Wnt3a and Wnt10b (59)] has been postulated to lead to angiogenesis, dermal thickening, adipogenesis and HF stem cell activation to promote anagen.

Identifying the key factors and signaling pathways that promote microtrauma-induced hair regrowth will pave the way to the next phase of targeted immunotherapy to treat hair loss. Inclusion of these growth factors, or PRP, into customized microneedles is currently explored as a delivery method for treating AGA (60). These factors, as well as other signaling molecules like nucleic acids, membrane receptors or co-factors, can be packaged in exosomes to deliver a targeted signal of hair regeneration (61, 62).

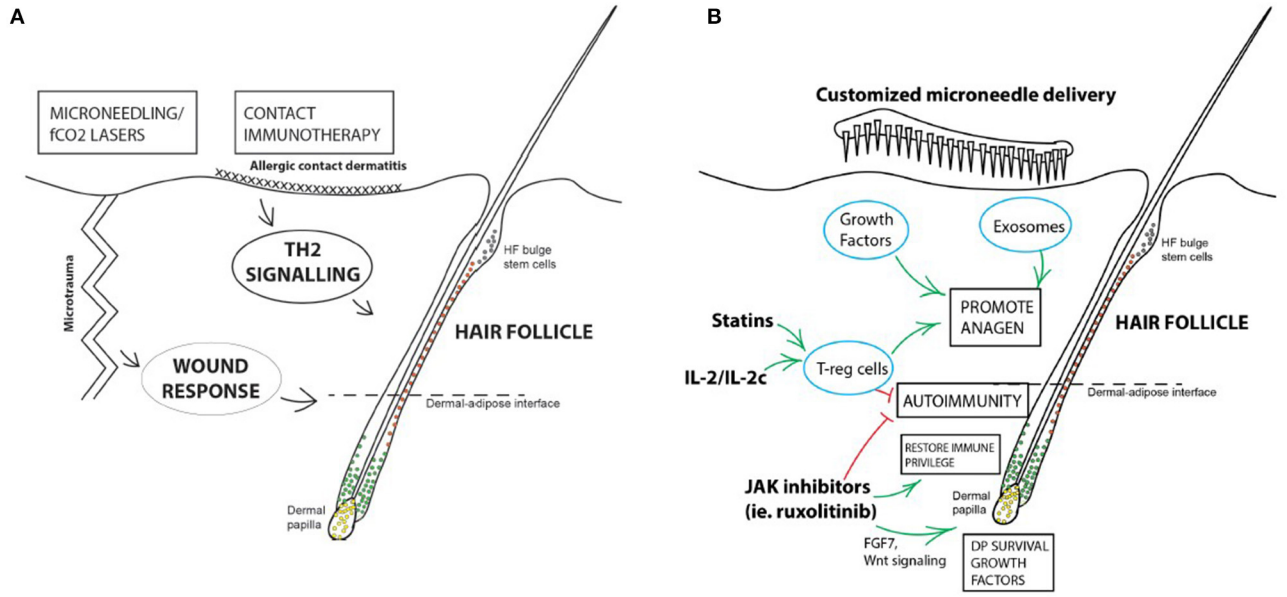

FIGURE 1
Summary of immunotherapy for hair loss disorders. **(A)** Current forms of immunotherapy are crude and non-targeted. Microtrauma produced by microneedling or PRP treatments are effective in promoting HF regeneration in AGA, and topical immunotherapy causing allergic contact dermatitis is an established treatment for AA. **(B)** Potential avenues for developing targeted immunotherapy for hair loss disorders. Some immunosuppressive treatments may have mechanisms of action independent of their suppressive effects, or direct stimulatory roles on the HF itself, like JAK inhibitors. JAK inhibition may have immunotherapeutic roles on the HF via upregulating the Wnt- β-catenin signaling pathway in DPCs, increasing FGF7 release which supports DP survival, and downregulation of MHC Class I expression in the HF, partially restoring IP. Stimulation of T-regs in the vicinity of the HF has both an immunomodulatory, as well as a direct pro-anagen effect on the HF. Targeted factors and signaling molecules can be packaged and delivered with bespoke microneedle patches or systems to mimic the effects of microtrauma to stimulate HF regrowth and regeneration.

4. Conclusion

Targeted immunotherapy (Figure 1) is a promising form of therapy for hair regrowth and regeneration, targeting immune cells that support and influence the hair cycle. Refining our current methods of immunotherapy will make these treatments more accessible to a wider population of patients suffering hair loss, with potentially fewer side effects. Further studies and controlled trials are required before they can be incorporated into clinical practice. If successful, targeted immunotherapy will provide hope for patients struggling with or have failed traditional treatments.

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References

1. Wang EC, Dai Z, Ferrante AW, Drake CG, Christiano AM. A subset of TREM2+ dermal macrophages secretes oncostatin M to maintain hair follicle stem cell quiescence and inhibit hair growth. *Cell Stem Cell.* (2019) 24:654–69. doi: 10.1016/j.stem.2019.01.011

2. Wang EC, Higgins CA. Immune cell regulation of the hair cycle. *Exp Dermatol.* (2020) 29:322–33. doi: 10.1111/exd.14070

3. Eichmüller S, Van Der Veen C, Moll I, Hermes B, Hofmann U, Müller-Röver S, et al. Clusters of perifollicular macrophages in normal murine skin: physiological degeneration of selected hair follicles by programmed organ deletion. *J Histochem Cytochem.* (1998) 46:361–70. doi: 10.1177/002215549804600310

4. Botchkarev VA, Paus R, Czarnetzki BM, Kupriyanov VS, Gordon DS, Johansson O. Hair cycle-dependent changes in mast cell histochemistry in murine skin. *Arch Dermatol Res.* (1995) 287:683–6. doi: 10.1007/BF00371743

5. Paus R, Van Der Veen C, Eichmüller S, Kopp T, Hagen E, Müller-Röver S, et al. Generation and cyclic remodeling of the hair follicle immune system in mice. *J Invest Dermatol.* (1998) 111:7–18. doi: 10.1046/j.1523-1747.1998.00243.x

6. Billingham RE. Transplantation immunity evoked by skin homografts and expressed in intact skin. *Adv Biol Skin.* (1971) 11:183–98.

7. Park JM, Jun MS, Kim JA, Mali NM, Hsi TC, Cho A, et al. Restoration of immune privilege in human dermal papillae controlling epithelial-mesenchymal interactions in hair formation. *Tissue Eng Reg Med.* (2022) 12:1–12. doi: 10.1007/s13770-021-00392-7

8. Sawaya ME, Bakshandeh H, Hordinsky MK, Penneys NS. CD44 expression in alopecia areata and androgenetic alopecia. *J Cutan Pathol.* (1994) 21:229–32. doi: 10.1111/j.1600-0560.1994.tb00265.x

9. Michel L, Reygagne P, Benech P, Jean-Louis F, Scalvino S, Ly Ka So S, et al. Study of gene expression alteration in male androgenetic alopecia: evidence of predominant molecular signalling pathways. *Br J Dermatol.* (2017) 177:1322–36. doi: 10.1111/bjd.15577

10. Ali N, Zirak B, Rodriguez RS, Pauli ML, Truong HA, Lai K, et al. Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell.* (2017) 169:1119–29. doi: 10.1016/j.cell.2017.05.002

11. Hardman JA, Muneeb F, Pople J, Bhogal R, Shahmalak A, Paus R. Human perifollicular macrophages undergo apoptosis, express Wnt ligands, and switch their polarization during catagen. *J Invest Dermatol.* (2019) 139:2543–6. doi: 10.1016/j.jid.2019.04.026

12. Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, et al. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. *Nature.* (2007) 447:316–20. doi: 10.1038/nature05766

13. Jiang S, Zhao L, Teklemariam T, Hantash BM. Small cutaneous wounds induce telogen to anagen transition of murine hair follicle stem cells. *J Dermatol Sci.* (2010) 60:143–50. doi: 10.1016/j.jdermsci.2010.10.008

14. Gay D, Kwon O, Zhang Z, Spata M, Plikus MV, Holler PD, et al. Fgf9 from dermal γδ T cells induces hair follicle neogenesis after wounding. *Nat Med.* (2013) 19:916–23. doi: 10.1038/nm.3181

15. Kasuya A, Ito T, Tokura Y. M2 macrophages promote wound-induced hair neogenesis. *J Dermatol Sci.* (2018) 91:250–5. doi: 10.1016/j.jdermsci.2018.05.004

16. Ma HJ, Yang Y, Ma HY, Jia CY, Li TH. Acquired localized hypertrichosis induced by internal fixation and plaster cast application. *Ann Dermatol.* (2013) 25:365–7. doi: 10.5021/ad.2013.25.3.365

17. Leung AK, Kiefer GN. Localized acquired hypertrichosis associated with fracture and cast application. *J Natl Med Assoc.* (1989) 81:65.

18. Shafir R, Tsur H. Local hirsutism at the periphery of burned skin. *Br J Plast Surg.* (1979) 32:93. doi: 10.1016/0007-1226(79)90004-3

19. Torchia D, Giorgini S, Gola M, Francalanci S. Allergic contact dermatitis from 2-ethylhexyl acrylate contained in a wig-fixing adhesive tape and its' incidental'therapeutic effect on alopecia areata. *Contact Dermatitis.* (2008) 58:170–1. doi: 10.1111/j.1600-0536.2007.01212.x

20. Happle R. Antigenic competition as a therapeutic concept for alopecia areata. *Arch Dermatol Res.* (1980) 267:109–14. doi: 10.1007/BF00416931

21. Gupta A, Bamimore M. Platelet-rich plasma monotherapies for androgenetic alopecia: a network meta-analysis and meta-regression study. *J Drug Dermatol.* (2022) 21:943–52. doi: 10.36849/JDD.6948

22. Zhang X, Ji Y, Zhou M, Zhou X, Xie Y, Zeng X, et al. Platelet-rich plasma for androgenetic alopecia: a systematic review and meta-analysis of randomized controlled trials. *J Cutan Med Surg.* (2023) 2023:12034754231191461. doi: 10.1177/12034754231191461

23. Chuah SY, Tan CH, Wang ECE, Tan KT, Chan RKW, Zhao X, et al. Efficacy of platelet-rich plasma in Asians with androgenetic alopecia: a randomized controlled trial. *Indian J Dermatol Venereol Leprol.* (2023) 89:135–8. doi: 10.25259/IJDVL_512_2021

24. English RS, Ruiz S, Do Amaral P. Microneedling and its use in hair loss disorders: a systematic review. *Dermatol Ther.* (2022) 12:1–20. doi: 10.1007/s13555-021-00653-2

25. Strazzulla LC, Avila L, Sicco KL, Shapiro J. An overview of the biology of platelet-rich plasma and microneedling as potential treatments for alopecia areata. *J Inves Dermatol.* (2017) 19:S21–4. doi: 10.1016/j.jisp.2017.10.002

26. McCarthy M, Day D, Talaber I. Hair regrowth and maintenance in alopecia universalis patient treated with nonablative Er: YAG laser. *Clin Case Rep.* (2021) 9:e04948. doi: 10.1002/ccr3.4948

27. Su YP, Wu XJ. Ablative 2940 nm Er: YAG fractional laser for male androgenetic alopecia. *Dermatol Ther.* (2022) 35:e15801. doi: 10.1111/dth.15801

28. Shokeir HA, Yousry A, Ibrahim SMA. Comparative study between topical steroid alone versus combined fractional Erbium: YAG laser with topical steroid in treatment of alopecia areata. *Arch Dermatol Res.* (2023) 315:241–7. doi: 10.1007/s00403-022-02417-0

29. Kwon O, Senna MM, Sinclair R, Ito T, Dutronc Y, Lin CY, et al. Efficacy and safety of baricitinib in patients with severe alopecia areata over 52 weeks of continuous therapy in two phase III trials (BRAVE-AA1 and BRAVE-AA2). *Am J Clin Dermatol.* (2023) 24:443–51. doi: 10.1007/s40257-023-00764-w

30. Wang Y, Liu T, Li S, Tang S, Lin P, Ding Y, et al. Efficacy and safety of baricitinib in patients with refractory alopecia areata. *Dermatol Ther.* (2022) 35:e15845. doi: 10.1111/dth.15845

31. Asfour L, Bokhari L, Bhoyrul B, Eisman S, Moussa A, Rees H. Treatment of moderate-to-severe alopecia areata in pre-adolescent children with baricitinib. *Br J Dermatol.* (2023) 12:ljad118. doi: 10.1093/bjd/ljad118

32. Zhou C, Yang X, Yang B, Yan G, Dong X, Ding Y, et al. A randomized, double-blind, placebo-controlled phase II study to evaluate the efficacy and safety of ivarmacitinib (SHR0302) in adult patients with moderate to severe alopecia areata. *J Am Acad Dermatol.* (2023) 1. doi: 10.1016/j.jaad.2023.02.063

33. Harel S, Higgins CA, Cerise JE, Dai Z, Chen JC, Clynes R, et al. Pharmacologic inhibition of JAK-STAT signaling promotes hair growth. *Sci Adv.* (2015) 1:e1500973. doi: 10.1126/sciadv.1500973

34. Wang E, Harel S, Christiano AM. JAK-STAT signaling jump starts the hair cycle. *J Invest Dermatol.* (2016) 136:2131–2. doi: 10.1016/j.jid.2016.08.029

35. Hassan AS, Al-Dhoun MQ, Shaker OG, AlOrbani AM. Expression of signal transducer and activator of transcription-3 in androgenetic alopecia: a case-control study. *Skin Pharmacol Physiol.* (2022) 35:278–81. doi: 10.1159/000525532

36. Kim JE, Lee YJ, Park HR, Lee DG, Jeong KH, Kang H. The effect of JAK inhibitor on the survival, anagen re-entry, and hair follicle immune privilege restoration in human dermal papilla cells. *Int J Mol Sci.* (2020) 21:5137. doi: 10.3390/ijms21145137

37. Kashetsky N, Turchin I. Utilization of topical ruxolitinib in dermatology: a review. *Skin Therapy Lett.* (2023) 28:8–13.

38. Yan D, Fan H, Chen M, Xia L, Wang S, Dong W, et al. The efficacy and safety of JAK inhibitors for alopecia areata: a systematic review and meta-analysis of prospective studies. *Front Pharmacol.* (2022) 13:950450. doi: 10.3389/fphar.2022.950450

39. Cervantes J, Jimenez JJ, DelCanto GM, Tosti A. Treatment of alopecia areata with simvastatin/ezetimibe. *Jo Invest Dermatol Symp Proc.* (2018) 19:S25–31. doi: 10.1016/j.jisp.2017.10.013

40. Kim YC, Kim KK, Shevach EM. Simvastatin induces Foxp3+ T regulatory cells by modulation of transforming growth factor-β signal transduction. *Immunology.* (2010) 130:484–93. doi: 10.1111/j.1365-2567.2010.03269.x

41. Youssef S, Stüve O, Patarroyo JC, Ruiz PJ, Radosevich JL, Hur EM, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature.* (2002) 420:78–84. doi: 10.1038/nature01158

42. Pruefer D, Scalia R, Lefer AM. Simvastatin inhibits leukocyte–endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. *Arterioscler Thromb Vasc Biol.* (1999) 19:2894–900. doi: 10.1161/01.ATV.19.12.2894

43. Palmer MA, Blakeborough L, Harries M, Haslam IS. Cholesterol homeostasis: Links to hair follicle biology and hair disorders. *Exp Dermatol.* (2020) 29:299–311. doi: 10.1111/exd.13993

44. Guasti L, Marino F, Cosentino M, Maio RC, Rasini E, Ferrari M, et al. Prolonged statin-associated reduction in neutrophil reactive oxygen species and angiotensin II type 1 receptor expression: 1-year follow-up. *Eur Heart J.* (2008) 29:1118–26. doi: 10.1093/eurheartj/ehn138

45. Loi C, Starace M, Piraccini BM. Alopecia areata (AA) and treatment with simvastatin/ezetimibe: experience of 20 patients. *J Am Acad Dermatol.* (2016) 74:e99–100. doi: 10.1016/j.jaad.2015.09.071

46. Guttman-Yassky E, Renert-Yuval Y, Bares J, Chima M, Hawkes JE, Gilleaudeau P, et al. Phase 2a randomized clinical trial of dupilumab (anti-IL-4Rα) for alopecia areata patients. *Allergy.* (2022) 77:897–906. doi: 10.1111/all.15071

47. Pagnanelli G, Cavani A, Canzona F, Mazzanti C. Mild therapeutic response of alopecia areata during treatment of psoriasis with secukinumab. *Eur J Dermatol.* (2020) 30:602–3. doi: 10.1684/ejd.2020.3866

48. Gallo R, Trave I, Parodi A. Massive acute alopecia of the scalp in a patient treated with dupilumab. *Acta Derm Venereol.* (2020) 100:1–2. doi: 10.2340/00015555-3549

49. Toussirot É, Aubin F. Paradoxical reactions under TNF-α blocking agents and other biological agents given for chronic immune-mediated diseases: an analytical and comprehensive overview. *RMD open.* (2016) 2:e000239. doi: 10.1136/rmdopen-2015-000239

50. Kim KH, Sim WY, Lew BL. Nivolumab-induced alopecia areata: a case report and literature review. *Ann Dermatol.* (2021) 33:284. doi: 10.5021/ad.2021.33.3.284

51. Dai Z, Sezin T, Chang Y, Lee EY, Wang EHC, Christiano AM. Induction of T cell exhaustion by JAK1/3 inhibition in the treatment of alopecia areata. *Front Immunol.* (2022) 13:955038. doi: 10.3389/fimmu.2022.955038

52. Castela E, Le Duff F, Butori C, Ticchioni M, Hofman P, Bahadoran P, et al. Effects of low-dose recombinant interleukin 2 to promote T-regulatory cells in alopecia areata. *JAMA Dermatol.* (2014) 150:748–51. doi: 10.1001/jamadermatol.2014.504

53. Le Duff F, Bouaziz JD, Fontas E, Ticchioni M, Viguier M, Dereure O, et al. Low-dose IL-2 for treating moderate to severe alopecia areata: a 52-week multicenter prospective placebo-controlled study assessing its impact on T regulatory cell and NK cell populations. *J Invest Dermatol.* (2021) 141:933–6. doi: 10.1016/j.jid.2020.08.015

54. Lee E, Kim M, Lee YJ. Selective expansion of tregs using the IL-2 cytokine antibody complex does not reverse established alopecia areata in C3H/HeJ mice. *Front Immunol.* (2022) 13:874778. doi: 10.3389/fimmu.2022.874778

55. Rückert R, Hofmann U, van der Veen C, Paus R, Bulfone-Paus S. MHC class I expression in murine skin: developmentally controlled and strikingly restricted intraepithelial expression during hair follicle morphogenesis and cycling, and response to cytokine treatment in vivo. *J Invest Dermatol.* (1998) 111:25–30. doi: 10.1046/j.1523-1747.1998.00228.x

56. Ito T, Ito N, Bettermann A, Tokura Y, Takigawa M, Paus R. Collapse and restoration of MHC class-I-dependent immune privilege: exploiting the human hair follicle as a model. *Am J Pathol.* (2004) 164:623–34. doi: 10.1016/S0002-9440(10)63151-3

57. Cuellar-Barboza A, Cardenas-de la Garza JA, Cruz-Gómez LG, Barboza-Quintana O, Flores-Gutiérrez JP, Gómez-Flores M. Local secretion of stress hormones increases in alopecia areata lesions after treatment with UVA-1 phototherapy. *Exp Dermatol.* (2020) 29:259–64. doi: 10.1111/exd.14077

58. Rallis E, Korfitis C, Gregoriou S, Rigopoulos D. Assigning new roles to topical tacrolimus. *Expert Opin Investig Drugs.* (2007) 16:1267–76. doi: 10.1517/13543784.16.8.1267

59. Kim YS, Jeong KH, Kim JE, Woo YJ, Kim BJ, Kang H. Repeated microneedle stimulation induces enhanced hair growth in a murine model. *Ann Dermatol.* (2016) 28:586–92. doi: 10.5021/ad.2016.28.5.586

60. Sun Y, Yang L, Du L, Zhou Y, Xu K, Chen J, et al. Duo-role Platelet-rich Plasma: temperature-induced fibrin gel and growth factors' reservoir for microneedles to promote hair regrowth. *J Adv Res.* (2023). doi: 10.1016/j.jare.2023.02.014

61. Wu J, Yang Q, Wu S, Yuan R, Zhao X, Li Y, et al. Adipose-derived stem cell exosomes promoted hair regeneration. *Tissue Eng Regen Med.* (2021) 18:685–91. doi: 10.1007/s13770-021-00347-y

62. Gangadaran P, Rajendran RL, Kwack MH, Jeyaraman M, Hong CM, Sung YK, et al. Application of cell-derived extracellular vesicles and engineered nanovesicles for hair growth: From mechanisms to therapeutics. *Front Cell Dev Biol.* (2022) 10:963278. doi: 10.3389/fcell.2022.963278

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Screening for latent infectious disease in patients with alopecia areata before initiating JAK inhibitors therapy: a single-center real-world retrospective study

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Introduction: Although there is growing evidence supporting the effectiveness of Janus kinase (JAK) inhibitors in treating alopecia areata, the high rate of recurrence following drug discontinuation has led to prolonged treatment courses and raised concerns about long-term safety. In clinical practice, caution should be exercised while using JAK inhibitors for various indications, and a comprehensive pre-treatment screening.

Methods: This study presents an analysis of screening data collected from real-world settings before the initiation of Janus kinase inhibitors in patients with alopecia areata. Investigators collected retrospective medical data characterizing patients' screening data. Data on demographic and clinical data, including age, sex, disease duration, severity of alopecia tool scale, history of prior treatment, and treatment regimen were recorded.

Results: In this cohort ($N = 218$), JAK inhibitors were initiated for 163 of 218 (74.8%) alopecia areata patients. The numbers of patients positive for antinuclear antibodies, hepatitis B surface antigen, hepatitis C virus antibodies, human immunodeficiency virus antibody, treponema pallidum hemagglutination assay, and thyroid-stimulating hormone were 32 (32/176), 10(10/218), 0 (0/218), 0 (0/218), 3 (3/218) and 9 (9/176), respectively. The number of patients with T-cell spot positive or imaging of the chest indicating tuberculosis was 37 (37/218).

Disccusion: Our data provide additional information on the safety profile of JAK inhibitors in patients with alopecia areata. As such, it is necessary and crucial to screen for JAK inhibitors before it is used, particularly for individuals with a high risk of tuberculosis, hepatitis B, and other infections.

KEYWORDS

alopecia areata, Janus kinase inhibitors, screening, tuberculosis, hepatitis

Introduction

Alopecia areata (AA) is a common autoimmune dermatosis characterized by nonscarring hairless patches that can involve most commonly the scalp or any hair-bearing part of the body (1), affecting approximately 0.1 to 0.2% of the population (2). With a recurrent or persistent course, AA can cause a severely reduced quality of life (3).

Although multifactorial in etiology, the mechanisms leading to AA are not fully understood but likely involve a combination of genetic predisposition and environmental triggers, which

may disturb the immune balance of the follicle niche and destroy the immune privilege, eventually leading to the auto-immune attack of the follicle bulb cells mediated by T cells (4). Evidence from the studies on mouse models of AA has shown that the positive feedback loop of interferon (IFN)-gamma and interleukin (IL)-15 activate target immune cells and amplify the inflammatory response *via* the JAK–STAT signaling pathway (5).

The management of AA is notoriously challenging, with high rates of therapeutic failures or relapses. However, in the past decade, the JAK inhibitors have revolutionized the treatment of AA. Among them, baricitinib and ritlecitinib have been approved by the US FDA as standard therapies for treating severe alopecia areata cases (6–8). They have improved the overall therapeutic outcomes of AA treatment and allowed patients to pursue a hair-normal life, especially for patients who have previously failed other systemic treatments (9). Unfortunately, due to drug availability and other reasons, the choice of JAK inhibitors in China is quite less. Currently, the only approved treatment for AA in China is baricitinib. Moreover, aside from the impressive treatment effects, clinicians still expressed concerns about the potential activation of latent infectious issues resulting from JAK inhibitors use, including tuberculosis (TB), hepatitis B virus (HBV), human immunodeficiency virus (HIV), etc.

To standardize the clinical application of JAK inhibitors, a consensus statement, formulated by 29-person experts in multiple disciplines, was published in 2020 (10). It recommends pre-treatment screening including routine laboratory tests (full blood count and blood biochemistry), hepatitis virus testing for HBV and hepatitis C virus (HCV), HIV testing, tuberculosis infection testing before initiation of JAK inhibitors. However, no data on the screening under JAK inhibitors in real-world settings have been available to date, and the evidence is lacking on the necessity of screening in patients prior to administering this class of small molecule drugs. Here, we conducted a retrospective study to describe the screening data before the initiation of JAK inhibitors for AA and aim to provide supporting evidence for the standardization of the clinical use of JAK inhibitors.

Methods

We conducted a retrospective study of all patients with AA prepared to treat with JAK inhibitors at our institution between February 2021 and October 2022. The study was approved by the institutional research ethics boards of Xiangya Hospital, Central South University (Changsha, China); approval number: 202303043.

The diagnosis of AA was confirmed by at least two dermatologists based on typical skin manifestations and results of dermoscopy. Patients with atypical manifestations (such as diffuse alopecia areata) were included only if confirmed by histologic findings. Patients with the unavailability of complete data were excluded. Patients who were pregnant or breast feeding or attempting to become pregnant were also excluded. All patients underwent screening including complete blood count, serum biochemical parameter, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibodies, human immunodeficiency virus(HIV)antibody, treponema pallidum hemagglutination assay (TPHA), antinuclear antibodies (ANA), thyroid-stimulating hormone (TSH), T-cell spot (T-spot) test, and chest computed tomography (CT) or chest x-ray film at baseline. Treatment was initiated based on shared decision-making between the patient and the medical specialist and regular follow-up laboratory testing (including complete blood count, lipid profiles, and liver and renal functions) was conducted in patients who continued treatment.

For each patient, demographic and clinical data, including age, sex, disease duration, extra-scalp manifestation, severity of alopecia tool (SALT) scale (11), history of prior treatment, and treatment regimen were recorded.

Statistical analysis

Descriptive statistics were summarized as number, percentage, mean and standard deviation (mean ± SD), and median and range.

Results

Of 232 patients evaluated, 218 patients were included in the study. Their characteristics are shown in Table 1. The sex distribution of study patients was 81 men (37.2%) versus 137 women (62.8%), median age was 29.0 years (IQR 20.0–40.5). The disease duration ranged from 2 to 277 months, with 59 patients more than 60 months. Except for 2 patients with<25% hair loss, most patients met the diagnostic criteria of moderate to severe AA, among which 61 patients were dignosed as alopecia totalis or alopecia universalis. In all, patients with body hair loss and nail involvement represented 109(50.0%) and 29(13.3%), respectively. In addition, systemic steroids and

TABLE 1 Patient characteristics.

Variable	*n* = 218
Age, year, median (range)	29 (6, 66)
Sex, n (%)	
female	81 (37.2)
male	137 (62.8)
Duration of disease, y, median (range)	25.5 (2, 277)
Duration of disease, m, n (%)	
<12	69 (31.7)
12 ~ 60	90 (41.2)
>60	59 (27.1)
SALT subclass, n (%)	
S1 = <25% hail loss	2 (0.9)
S2 = 25–49% hail loss	45 (20.6)
S3 = 50–74% hail loss	85 (39.0)
S4 = 75–99% hail loss	25 (11.5)
S5 = 100% hail loss	61 (28.0)
Body hair loss, n (%)	109 (50.0)
Nail involvement, n (%)	29 (13.3)
Previous treatments, n (%)	
Systemic steroids	110 (50.5)
Immunosuppressive agent[a]	10 (4.6)

[a]Including cyclosporine and methotrexate.

immunosuppressive agents were formerly used by 110(50.5%) and 10(4.6%), respectively.

Abnormal screening results were shown in Table 2. All patients were screened for infection-related examinations. A total of 31 (14.2%) patients presented positive for T-spot, among which only 1 patient showed signs indicating tuberculosis on chest radiograph. Of those presented negative for T-spot ($n = 187$), 6 patients showed signs of tuberculosis infection on chest radiograph. However, no patients reported tuberculosis infection-related clinical manifestations. Figure 1 shows the age distribution of patients screened for tuberculosis infection. The infection rate was 0, 11 and 43% in the groups with <18, 18–40 and >40 age groups, respectively. Moreover, the number of patients positive for HBsAg, HCV, HIV, and TPHA were 10 (4.6%), zero (0%), zero (0%), and 3 (1.4%), respectively. Five of the ten patients who were HBsAg positive had HBV DNA quantification positive. With regard to the TPHA-positive patient, syphilis serum antibody titers were not detected.

A total of 176 patients were screened for immunity-related examinations. ANA was positive in 32 patients (18.2%), of which 26, 5 and 1 patients had antibody titers of 1:80, 1:160 and 1:320, respectively. Among the patients with antibody titers of 1:80, the numbers of speckled, nucleolar and speckled-nucleolar patterns were 20, 4 and 2, respectively. Anti-double-stranded DNA (ds-DNA) antibody positivity was detected only in one patient with an antinuclear antibody titer of 1:320, who was subsequently diagnosed with systemic lupus erythematosus. Thyroid-stimulating hormone (TSH) abnormalities were detected in 9 patients (5.1%), of which 8 patients had a significant increase in TSH.

Chest imageological examinations were screened in all patients. The number of positive results was 17 (7.8%). Of these, the results were pulmonary inflammation, tuberculosis infection, pulmonary 4A nodules, pulmonary 4B nodules and pulmonary hypertension in 5, 7, 1, 3 and 1 patient, respectively.

Treatment options were also shown in Table 2. JAK inhibitors, conservative management, oral corticosteroids, immunosuppressive agents and withdrawl were initiated for 163 (74.8%), 25 (11.5%), 4 (1.8%), 12 (5.5%) and 14 (6.4%), respectively. Among those received JAK inhibitor, the most commonly prescribed targeted therapies were Tofacitinib ($n = 148$), followed by Baricitinib ($n = 10$), Abrocitinib ($n = 3$), and Jaktinib ($n = 2$). Despite the rapid development of novel JAK inhibitors, tofacitinib remains an effective and the longest-used JAK inhibitor for AA. As such, we often tend to prescribe tofacitinib. The detailed information on the approval status of the above drugs is provided in Supplementary Table S1. What's more, 36 patients abandoned JAK inhibitor due to signs indicating tuberculosis. Another reason patients discontinued JAK inhibitors is long-term security considerations.

TABLE 2 Abnormal screening results and treatment options of 218 patients.

Variable	Number	*n* (%)
Infection-related blood tests		
T-spot test	218	31 (14.2)
HBsAg	218	10 (4.6)
HCV antibodies	218	0 (0)
HIV antibodies	218	0 (0)
TPHA	218	3 (1.4)
Immunity-related blood tests		
TSH	176	9 (5.1)
ANA	176	32 (18.2)
ds-DNA antibody	176	1 (0.6)
Imageological examination		
CT[a]	218	17 (7.8)
Treatment options		
Tofacitinib	218	148 (67.9)
Baricitinib	218	10 (4.6)
Abrocitinib	218	3 (1.4)
Jaktinib	218	2 (0.9)
conservative management[b]	218	25 (11.5)
Oral corticosteroids	218	4 (1.8)
Immunosuppressive agents	218	12 (5.5)
Withdrawl	218	14 (6.4)

[a]Only meaningful reported results that affected treatment decisions were included.
[b]Including topical corticosteroids, intralesional steroids, and topical minoxidil. HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; TPHA, treponema pallidum hemagglutination assay; ANA, antinuclear antibodies; TSH, thyroid-stimulating hormone; CT, chest computed tomography.

Discussion

Driven by the lack of study describing the screening data of JAK inhibitors, we evaluated the blood examination and chest imaging screening data before initiation of JAK inhibitors for AA, providing supporting evidence for standardizing the clinical application of JAK inhibitors in practice. Overall, JAK inhibitors were initiated for 163 of 218 (74.8%) AA patients. The most common reasons for patients failing screening were tuberculosis and hepatitis B. Notably, we followed a possible association between AA and lung cancer. The chest CT screened 4 patients with lung-RADS category 4 nodules, 1 of whom was ultimately diagnosed with lung adenocarcinoma.

Latent tuberculosis infection (LTBI) is a state in which the host presents with immunoreactivity to tuberculosis antigens but without clinical and radiologic evidence of tuberculosis disease (12). It is estimated that the lifetime risk of reactivation in people with LTBI is 5–10%, while some drugs can aggravate this process (13). Notably, JAK inhibitors may reactivate latent tuberculosis by inhibiting macrophage function and causing granuloma structure dissolution. Chen et al. investigated the rate of reactivation of tuberculosis development in rheumatoid arthritis patients undergoing oral tofacitinib therapy (14). Among 28,099 patients undergoing the therapy, 79 (0.28%) patients developed active tuberculosis. Fifty three of the 79 patients were T-spot negative before treatment, while 5 were T-spot positive and developed tuberculosis in the case of combined isoniazid treatment. The 2015 American guideline for the treatment of rheumatoid arthritis suggests that patients with T-spot positive can start immunosuppressants after completion of at least 1 month treatment for anti-tuberculosis (15). China ranks as having the third-highest tuberculosis burden of any country in the world which can reasonably explain our findings (16). In the present study, 13.76% (30/218) of patients with AA were detected as LTBI. This rate was

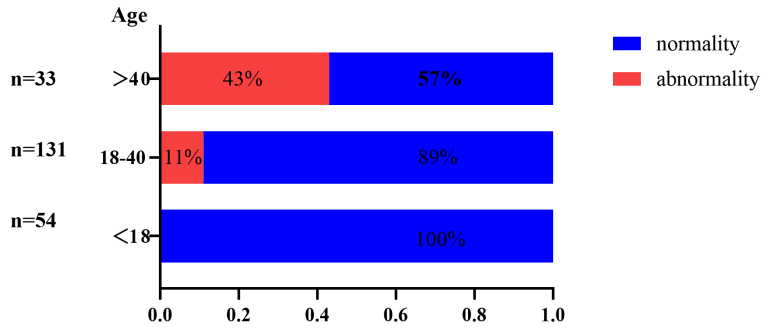

FIGURE 1
Screening abnormality rates for tuberculosis (%) in different age groups.

slightly lower than that in a previous LTBI report in the general population in the rural regions of China (LTBI rate is 18.8% (3,955/21022)) (17). However, we should emphasize that the 13.76% does not represent the prevalence of LTBI in AA becasue the majority of patients receiving other treatment were not screened for TB in real-life experience. Amony the 30 patients of LTBI, only 1 patient who refused LTBI prophylaxis treatment received tofacitinib treatment and reexamination of chest CT showed no obvious changes after 6 months. Despite the disease tends for AA to be prevalent in younger patients, the data that observed in larger, long-term studies of patients with rheumatoid arthritis strongly suggest a link between activation of tuberculosis and JAKi (18, 19). In additional, our data showed that the proportion of tuberculosis infections detected among patients over 40 years is 43%, which decreased to 11% in the 18–40 age group. As such, we recommend screening for TB infections on a routine basis before treatment with JAK inhibitors, especially for people over 40 years. These patients should also be closely monitored during the JAK inhibitors therapy to identify new tuberculosis infection or reactivation of latent tuberculosis (19, 20).

Hepatitis B virus (HBV) infection is a major global health issue, with 257 million chronically infected individuals and 887 000 HBV-related deaths in 2015 (21). In patients have previously had HBV infection, hepatitis can develop due to reactivation of the virus by the use of immunosuppressants (22). Thus, it is recommended that hepatitis B virus surface antigen (HBsAg) or hepatitis B virus core antibody (HBcAb) should be performed before treatment. Wang et al. had investigated the long-term outcome of reactivation of HBV (rHBV) development in rheumatoid arthritis patients undergoing tofacitinib therapy. Two of the 64 (0.03%) HBcAb positive patients developed rHBV, while 2 of the 6 (33%) HBsAg positive developed rHBV. It is worth noting that combined antiviral therapy could signifficantly reduce viral reactivation. In this study, only 1 patient with HBsAg positive/HBV DNA negative were treated with oral tofacitinib combined with entecavir and no HBV reactivation was observed during the follow-up.

Previous studies have shown a link between AA and autoimmune diseases, in particular thyroid diseases, suggesting the same genetic background and pathogenesis of these diseases (23). However, the latest meta-analyses agree that (24, 25) AA is not correlated with thyroid dysfunction and routine screening for thyroid diseases is not recommended. A retrospective study on children with AA screened for thyroid function also support this view (26). Among all included subjects, 9 patients (5.1%) presented an abnormal TSH serum level, of which 8 patients showed an elevated TSH concentration. This is consistent with a previous study by Thomas et al. (27). Antinuclear antibodies (ANA) are a group of autoantibodies targeting various antigen components within cells, which can be characterized in a variety of autoimmune diseases (28). However, there is no consensus on the relationship between ANA and AA. In this study, the number of ANA positive patients was 32, among whom only 1 patient was subsequently diagnosed as systemic lupus erythematosus. Considering the experience on clinical practice and our cohort data, it is reasonable to screen for thyroid function abnormalities and other autoimmune disease in patients with AA. What's more, it is not clear whether ANA or thyroid antibodies have an effect on the progresis of AA.

Currently, only a few studies have evaluated the association between AA and malignancy. A cohort study from Taiwan showed that the total cancer incidence of AA patients was slightly lower than that of the general population, especially in male patients (standardized incidence rate was 0.89). But the incidence of some types of malignancies, such as lymphoma (standardized incidence rate was 1.55), breast cancer (standardized incidence rate was 2.93), and urinary malignancies (standardized incidence rate was 2.95), was significantly higher, suggesting that there may be organ tendency between AA and cancer (29). However, a cohort study from Korea showed that the cancer incidence of AA patients is slightly higher than that of the general population (HR1.043; 95% CI1.022–1.065). After standardized age, sex and comorbidity, there are still significant differences in the risk of thyroid cancer, bladder cancer and prostate cancer (30). Further supporting evidence comes from another study showed the risk of cancer-related death of AA was higher than that of the general population. Especially, patients with alopecia universalis had a significantly higher risk of lung carcinoma-related death (HR2.16; 95% CI, 1.41–3.33) (31). In this study, a 32-year-old female with a lung-RADS category 4B nodule was diagnosed with lung adenocarcinoma after a biopsy. Our fingdings, along with previous research, suggest a possible association between AA and malignancy. Notably, JAK inhibitors may theoretically increase the risk of tumor occurrence or progression. However, there is currently no agreement on the cancer screening strategy before JAK inhibitors.

This study has some limitations, including a small sample size, a single-center study, and a single disease population. However, our study is the first to describe the screening data under JAK inhibitors, which has important implications for the awareness of the safety of

JAK inhibitors. Additionally, other laboratory tests such as complete blood count, routine urinalysis, fasting blood glucose and blood biochemistry were not described. Moreover, we did not collect comprehensive data about other risk factors associated with cardiovascular risk (e.g., obesity, current smoking history, and family history of coronary heart disease of premature onset) and family history of malignancy. However, the study population is largely composed of young or middle-aged individual who are less affected by biases related to comorbidities. Future larger studies are needed to evaluate the cost–benefit of such a screening approach.

Conclusion

In summary, we evaluated the screening data before initiation of JAK inhibitors for 218 AA patients in the real-world setting. The numbers of patients positive for ANA, HBsAg, HCV antibody, HIV antibody, TPPA and TSH were 32 (32/176), 10(10/218), 0 (0/218), 0 (0/218), 3 (3/218) and 9 (9/176), respectively. The number of patients with T-spot positive or pulmonary CT indicating tuberculosis was 37 (37/218). In developing countries, it is crucial to screen for JAK inhibitors before use, particularly for individuals with a high risk of tuberculosis, hepatitis B, and other infections.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the institutional research ethics boards of Xiangya Hospital, Central South University (Changsha, China). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

JH: Data curation, Writing – original draft. ZT: Data curation, Writing – original draft. YT: Data curation, Writing – original draft. WS: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1287139/full#supplementary-material

References

1. Gilhar A, Etzioni A, Paus R. Alopecia areata. *N Engl J Med*. (2012) 366:1515–25. doi: 10.1056/NEJMra1103442

2. Strazzulla LC, Wang EHC, Avila L, Lo Sicco K, Brinster N, Christiano AM, et al. Alopecia areata: disease characteristics, clinical evaluation, and new perspectives on pathogenesis. *J Am Acad Dermatol*. (2018) 78:1–12. doi: 10.1016/j.jaad.2017.04.1141

3. Liu LY, King BA, Craiglow BG. Health-related quality of life (HRQoL) among patients with alopecia areata (AA): a systematic review. *J Am Acad Dermatol*. (2016) 75:806–12. doi: 10.1016/j.jaad.2016.04.035

4. Bertolini M, McElwee K, Gilhar A, Bulfone-Paus S, Paus R. Hair follicle immune privilege and its collapse in alopecia areata. *Exp Dermatol*. (2020) 29:703–25. doi: 10.1111/exd.14155

5. Wang EHC, Khosravi-Maharlooei M, Jalili RB, Yu R, Ghahary A, Shapiro J, et al. Transfer of alopecia Areata to C3H/HeJ mice using cultured lymph node-derived cells. *J Invest Dermatol*. (2015) 135:2530–2. doi: 10.1038/jid.2015.176

6. FDA Approves First Systemic Treatment for Alopecia Areata, (2022). Available at: https://www.fda.gov/news-events/press-announcements/fda-approves-first-systemic-treatment-alopecia-areata

7. King B, Zhang X, Harcha WG, Szepietowski JC, Shapiro J, Lynde C, et al. Efficacy and safety of ritlecitinib in adults and adolescents with alopecia areata: a randomised, double-blind, multicentre, phase 2b-3 trial. *Lancet*. (2023) 401:1518–29. doi: 10.1016/S0140-6736(23)00222-2

8. King BA, Craiglow BG. Janus kinase inhibitors for alopecia areata. *J Am Acad Dermatol*. (2023) 89:S29–32. doi: 10.1016/j.jaad.2023.05.049

9. Liu LY, Craiglow BG, Dai F, King BA. Tofacitinib for the treatment of severe alopecia areata and variants: a study of 90 patients. *J Am Acad Dermatol*. (2017) 76:22–8. doi: 10.1016/j.jaad.2016.09.007

10. Nash P, Kerschbaumer A, Dörner T, Dougados M, Fleischmann RM, Geissler K, et al. Points to consider for the treatment of immune-mediated inflammatory diseases with Janus kinase inhibitors: a consensus statement. *Ann Rheum Dis*. (2021) 80:71–87. doi: 10.1136/annrheumdis-2020-218398

11. Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, et al. National Alopecia Areata Foundation. Alopecia areata investigational assessment guidelines--part II. National Alopecia Areata Foundation. *J Am Acad Dermatol*. (2004) 51:440–7. doi: 10.1016/j.jaad.2003.09.032

12. Zhou G, Luo Q, Luo S, Teng Z, Ji Z, Yang J, et al. Interferon-γ release assays or tuberculin skin test for detection and management of latent tuberculosis infection: a systematic review and meta-analysis. *Lancet Infect Dis*. (2020) 20:1457–69. doi: 10.1016/S1473-3099(20)30276-0

13. Cantini F, Nannini C, Niccoli L, Petrone L, Ippolito G, Goletti D. Risk of tuberculosis reactivation in patients with rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis receiving non-anti-TNF-targeted biologics. *Mediat Inflamm*. (2017) 2017:8909834. doi: 10.1155/2017/8909834

14. Cantini F, Blandizzi C, Niccoli L, Petrone L, Goletti D. Systematic review on tuberculosis risk in patients with rheumatoid arthritis receiving inhibitors of Janus kinases. *Expert Opin Drug Saf*. (2020) 19:861–72. doi: 10.1080/14740338.2020.1774550

15. Singh JA, Saag KG, Bridges SL Jr, Akl EA, Bannuru RR, Sullivan MC, et al. 2015 American College of Rheumatology Guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol*. (2016) 68:1–26. doi: 10.1002/acr.22783

16. Chakaya J, Petersen E, Nantanda R, Mungai BN, Migliori GB, Amanullah F, et al. The WHO global tuberculosis 2021 report - not so good news and turning the tide back to end TB. *Int J Infect Dis*. (2022) 124:S26–9. doi: 10.1016/j.ijid.2022.03.011

17. Gao L, Bai L, Liu J, Lu W, Wang X, Li X, et al. LATENTTB-NSTM study team. Annual risk of tuberculosis infection in rural China: a population-based prospective study. *Eur Respir J*. (2016) 48:168–78. doi: 10.1183/13993003.00235-2016

18. Zhang Z, Deng W, Wu Q, Sun L. Tuberculosis, hepatitis B and herpes zoster in tofacitinib-treated patients with rheumatoid arthritis. *Immunotherapy*. (2019) 11:321–33. doi: 10.2217/imt-2018-0113

19. Ji X, Hu L, Wang Y, Man S, Liu X, Song C, et al. Risk of tuberculosis in patients with rheumatoid arthritis treated with biological and targeted drugs: meta-analysis of randomized clinical trials. *Chin Med J*. (2022) 135:409–15. doi: 10.1097/CM9.0000000000001948

20. Samuel C, Cornman H, Kambala A, Kwatra SG. A review on the safety of using JAK inhibitors in dermatology: clinical and laboratory monitoring. *Dermatol Ther (Heidelb)*. (2023) 13:729–49. doi: 10.1007/s13555-023-00892-5

21. WHO Hepatitis B key facts. (2021).Available at: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

22. European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *J Hepatol*. (2017) 67:370–98. doi: 10.1016/j.jhep.2017.03.021

23. Chen CH, Wang KH, Lin HC, Chung SD. Follow-up study on the relationship between alopecia areata and risk of autoimmune diseases. *J Dermatol*. (2016) 43:228–9. doi: 10.1111/1346-8138.13165

24. Kinoshita-Ise M, Martinez-Cabriales SA, Alhusayen R. Chronological association between alopecia areata and autoimmune thyroid diseases: a systematic review and meta-analysis. *J Dermatol*. (2019) 46:702–9. doi: 10.1111/1346-8138.14940

25. Lee S, Lee H, Lee CH, Lee WS. Comorbidities in alopecia areata: a systematic review and meta-analysis. *J Am Acad Dermatol*. (2019) 80:466–77. doi: 10.1016/j.jaad.2018.07.013

26. Patel D, Li P, Bauer AJ, Castelo-Soccio L. Screening guidelines for thyroid function in children with alopecia Areata. *JAMA Dermatol*. (2017) 153:1307–10. doi: 10.1001/jamadermatol.2017.3694

27. Thomas EA, Kadyan RS. Alopecia areata and autoimmunity: a clinical study. *Indian J Dermatol*. (2008) 53:70–4. doi: 10.4103/0019-5154.41650

28. Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis*. (2014) 73:17–23. doi: 10.1136/annrheumdis-2013-203863

29. Chen CC, Chang YT, Liu HN, Chen YJ. Cancer risk in patients with alopecia areata: a nationwide population-based matched cohort study. *Cancer Med*. (2018) 7:2153–9. doi: 10.1002/cam4.1448

30. Lee JH, Song Y, Do Han K, Park YM, Lee JY, Park YG, et al. Cancer risk by the subtype of alopecia. *Sci Rep*. (2018) 8:9748. doi: 10.1038/s41598-018-28142-1

31. Lee S, Lee YB, Kim BJ, Bae S, Lee WS. All-cause and cause-specific mortality risks associated with alopecia Areata: a Korean Nationwide population-based study. *JAMA Dermatol*. (2019) 155:922–8. doi: 10.1001/jamadermatol.2019.0629

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The effectiveness of different immunotherapies in the treatment of condyloma acuminatum: a network meta-analysis of randomized clinical trials

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Background: The treatment of condyloma acuminatum (CA), especially the very persistent and recurrent CA, is currently the focus of our research. Immunotherapies have recently been shown to be well-tolerated and effective in treating warts, particularly refractory warts. However, there is still a lack of corresponding evidence-based medical evidence on the effectiveness of different immunotherapies in treating warts. The difference between network meta-analysis and meta-analysis is that network meta-analysis can be used to compare multiple treatments by combining direct and indirect evidence to assess the interrelationship between all treatments. We intend to compare the efficacy of different treatments for CA using a network meta-analysis.

Methods: PubMed, Cochrane Library and Embase from inception to June 1st, 2023 were searched using a computer. All articles on immunotherapies for CA were included. Stata MP17.0 software was used for data analyses.

Results: A total of 8 randomly-controlled trials involving 493 patients were included. Result showed that all treatment measures had a significant efficacy compared with the regular saline group (BCG (bacillus Calmette-Guérin vaccine) OR = 96.00, 95%CI: 10.35–890.58; MMR (measle, mumps and rubella vaccine) OR = 29.69, 95%CI: 7.47–118.04; Candida antigens OR = 27.34, 95%CI: 8.64–86.52; PPDs (purified protein derivatives) OR = 23.33, 95%CI: 6.75–80.60; VD3 OR = 21.36, 95%CI: 4.34–105.16 and purified protein derivatives (general) OR = 13.14, 95%CI: 3.38–51.12). The area under the curve (SUCRA) ranking results showed that the bacillus Calmette-Guérin vaccine had the highest total efficiency, which was 88.2%, with the rest in the order of measle, mumps and rubella vaccine, which was 68.9%, Candida antigens, which was 63.6%, purified protein derivatives, which was 52.9%, vitamin D3, which was 49.0%, purified protein derivatives (general), which was 27.4%, and saline, which was 0%.

Conclusion: In summary, we found that the bacillus Calmette-Guérin vaccine was superior to other treatments in terms of efficacy according to the SUCRA value.

KEYWORDS

acuminatum, HPV, immunotherapy, BCG, MRR, Candida antigen, PPD, vitamin D3

1 Introduction

Condyloma acuminatum (CA) is a cauliflower-shaped wart that appears on the genital area due to human papillomavirus (HPV) infection, which is most common among young people and is more common among women than men (1). Sexual transmission is the primary transmission mode of CA; the incubation period varies from 1 to 12 months, with an average of 2–3 months. There are also cases of auto-inoculation and vertical transmission. 90% of CA is caused by 2 low-risk subtypes, 6 and 11 (2), while some are also associated with other subtypes, such as 16, 18, 31, 33, and 35. Due to the moist environment of the genital area and excessive folds in the cavity, viruses have an easy chance to hide with a low spontaneous clearance by the body; warts are widely spread, which have a high recurrence rate. Some high-risk types, such as 16 and 18, may also lead to the development of cervical cancer, which imposes a specific psychological burden on the population. Currently, the treatment of CA, incredibly stubborn CA, is the focus of our research.

Traditional treatment modalities include chemical cautery, electrocautery, cryotherapy, surgical excision and laser removal, which can cause various adverse effects, such as pains, disfigurement and infections. In contrast, through immunotherapies, warts can be removed from the skin surface and the viruses can be eliminated by enhancing cell-mediated immunity. Recently, the intra-focal immunotherapy is a new treatment modality, such as measle, mumps and rubella (MMR) vaccines, purified protein derivatives (PPDs), the bacillus Calmette-Guerin (BCG) vaccines and the Candida antigens, which are well-tolerated and effective in the treatment of CA, particularly stubborn CA. MMR and PPDs have been reported to be most effective in achieving a complete clearance and long-term maintenance with reduced recurrence rates at the same site compared to other treatment modalities (3).

The exact mechanism of the intralesional immunotherapy has yet to be entirely understood, through which cell-mediated immunity to the HPV virus can be enhanced. The preliminary view is that the induction of a delayed hypersensitivity is the primary mechanism. Using immunotherapies, immediate cellular immunity can be increased by inducing a systemic T-cell-mediated response that increases the release of Th1 cytokines (IL-1 and IFN-γ) and the downregulation of Th2 cytokines (IL-10), leading to the clearance of warts (4). Hadeer et al. (5) found an elevated IL-18 level after PPD injection in warts, suggesting that this cytokine may play a role in PPD-induced wart clearance. Aktas (6) observed a complete remission among 70% of patients after 1 month of focal treatment with vitamin D; Shah (7) injected MMR vaccines locally into warts and found a complete remission among 72% of patients. Some researchers have found that immunotherapies have other advantages; for example, after a topical injection, warts further away from the injection site and those inside the mucosa that were not injected were found to shrink. They suggest that this may be due to a systemic immune response to local antigens, resulting in a widespread clearance of the HPV virus, which provides an excellent treatment idea for removing warts from problematic areas.

Although there is a wide range of immunotherapies available for treating CA, most are compared with saline placebo, and there is a lack of a direct comparison among the various immunotherapies. Many studies have demonstrated that network meta-analysis can analyze the data from randomized clinical trials to compare the effectiveness of multiple treatments simultaneously without disturbing each treatment. This study aimed to use a network meta-analysis to compare various immunotherapeutic treatments, so as to analyze their effectiveness in treating CA and provide more evidence for our future treatment.

2 Method

This study follows the extension statement on preferred reporting items for systematic reviews and meta-analyses (PRISMA) for reporting systematic reviews incorporating network meta-analyses of health care interventions.

2.1 Data sources and searches

PubMed, Cochrane Library and Embase were searched from databases from inception to June 1st, 2023 using a computer. All articles on immunotherapies for CA were included. A combination of free and subject words was used when searching the databases. Two researchers independently screened the literatures by title, abstract and full-text review, who discussed to resolve disputes they had encountered.

2.2 Selection criteria

The studies were screened for the following inclusion criteria: (1) Study type: randomly-controlled trials (RCTs); (2) Study population: patients diagnosed with CA; (3) Interventions: various immunotherapies, including MMR vaccines, PPD antigens, BCG vaccines, Candida antigens; (4) Outcome indicators: complete clearance rate of immunotherapies.

The studies were also screened for the following exclusion criteria: (1) Duplicate publications; (2) Literatures with only titles and abstracts; (3) Non-English literatures; (4) Academic conference literatures, secondary studies such as reviews or meta-analyses; (5) Literatures with unclear data or information descriptions.

2.3 Data extraction and quality assessment

We used standard data extraction forms to extract information, which included authors, year of publication, sample size, adequate sample size, interventions, follow-up time and outcome measures. We used standard criteria (Cochrane risk of bias tool) to assess the inherent risk of bias in trials. Two investigators independently undertook data extraction and quality assessment using a standardized approach. Any disagreement between the two investigators was resolved through consultations with each other.

2.4 Data analyses

First of all, we did pair-wise meta-analyses using StataMP 17. The odds ratio (OR) with a confidence interval (CI) of 95% was adopted as a representative measure of dichotomous outcomes. The statistical significance was set as $p < 0.05$. Secondly, a random-effects model network meta-analysis (NMA) was performed to calculate estimates

for the efficacy of different interventions against CA. For the outcome, we used a network graph to compare all treatments. Treatment strategies for each outcome were ranked according to the surface under the cumulative ranking curve (SUCRA) probabilities, SUCRA is a method for ranking the strengths and weaknesses of interventions, and a higher SUCRA probability of each simulation indicates a higher chance of being the best treatment regimen. We performed a cluster analysis to find the optimal intervention considering efficacy, and the interventions located in the upper left corner were superior to others.

3 Results

3.1 Retrieval results of literatures

According to the purpose of the study, 676 related articles were initially searched. After screening titles/abstracts and removing duplicate studies, the full text of 18 potentially-eligible studies was obtained. Ultimately, 8 randomized clinical trials were included in the quantitative data synthesis. Figure 1 illustrates the systematic literature search.

3.2 Basic characteristics of the included studies

A total of 493 patients were included in the 9 RCTs; the treatments involved were local injection of MMR vaccines, local injection of Candida antigens, local injection of normal saline, local injection of PPD vaccines, systemic injection of PPD vaccines, local injection of vitamin D3 and local injection of BCG vaccines (4, 8–15). The basic information of the literatures is shown in Table 1. Characteristics of included studies; MMR, measle, mumps and rubella vaccines. PPDs, purified protein derivatives. BCG, bacille Calmette-Guerin vaccines. VD3, vitamin D3.

3.3 Risk of Bias

Among the included articles, the random number table was used in 1 of them, random sampling was used in another, coin toss was used in another different from the above 2, and the remaining 5 studies were grouped according to the principle of randomization, but the specific random method was not described; 2 articles were single-blind, 1 was double-blind, and

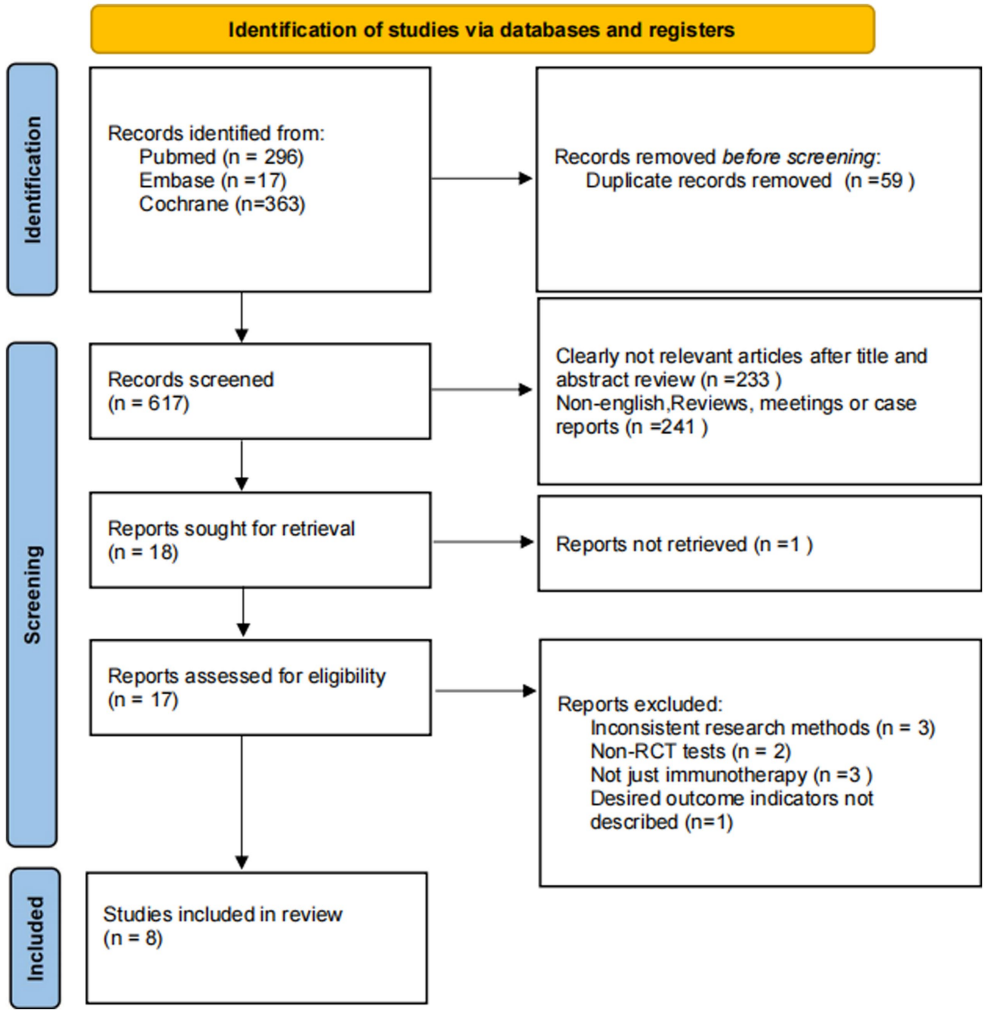

FIGURE 1
Search flow diagram.

TABLE 1 Characteristics of included studies.

Included studies	Age (years)	Treatment	Sample size	Effective size	Frequency	Treatment duration	Follow-up (months)	Outcome measures
Ahmad 2020 (7)	1–12	MMR	15	11	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
		Candida antigen	15	12	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
		intralesional saline	10	1	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
Noha Z 2022 (3)	21–63	PPD	40	26	Once every 2 weeks	Eliminate up to 4 times	6	complete clearance
		Candida antigen	40	25	Once every 2 weeks	Eliminate up to 4 times	6	complete clearance
Anant 2022 (8)	10–50	MMR	50	31	Once every 2 weeks	Eliminate or up to 3 times	6	complete clearance
		VD3	50	27	Once every 2 weeks	Eliminate or up to 3 times	6	complete clearance
Bayoumy 2011 (10)	20–35	PPD (general)	20	10	Once a weeks	Eliminate or up to 12 times	6	complete clearance
		intralesional saline	20	0	Once a weeks	Eliminate or up to 12 times	6	complete clearance
Nashwa 2023 (11)	18–60	MMR	23	18	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
		PPD	23	16	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
		PPD (general)	23	13	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
Ayman 2020 (12)	39–63	Candida antigen	30	24	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
		intralesional saline	20	3	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
Bahgat 2005 (13)	30–39	BCG	25	20	Once a week	Eliminate or up to 6 times	6	complete clearance
		intralesional saline	25	0	Once a week	Eliminate or up to 6 times	6	complete clearance
Nofal 2020 (14)	19–45	PPD	32	9	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance
		Candida antigen	32	12	Once every 2 weeks	Eliminate or up to 5 times	6	complete clearance

the blindness was not mentioned in the remaining 5 articles; the results of publication biases in studies contributing to outcomes are displayed in Figure 2. The risks of bias were generally low.

3.4 Network meta-analysis

3.4.1 Evidence network map

All the 8 included articles reported a complete clearance, involving 8 treatments, and their sample sizes and direct study evidences are shown in Figure 3. The node size indicates the number of patients randomized to a particular agent. The width of the line indicates the number of trials comparing two treatments. It was found that the samples of Candida antigens ranked the highest in this NMA.

3.4.2 Consistency test

Through an analysis of inconsistency tests on outcome results, it was found that the results of direct and indirect evidences for outcomes were consistent, which should be based on a consistency model ($p > 0.05$).

3.4.3 Results of the network meta-analysis

We used the frequentist method to analyze network meta, with OR as the effect size, and 95% CI inclusion 1 indicated that there

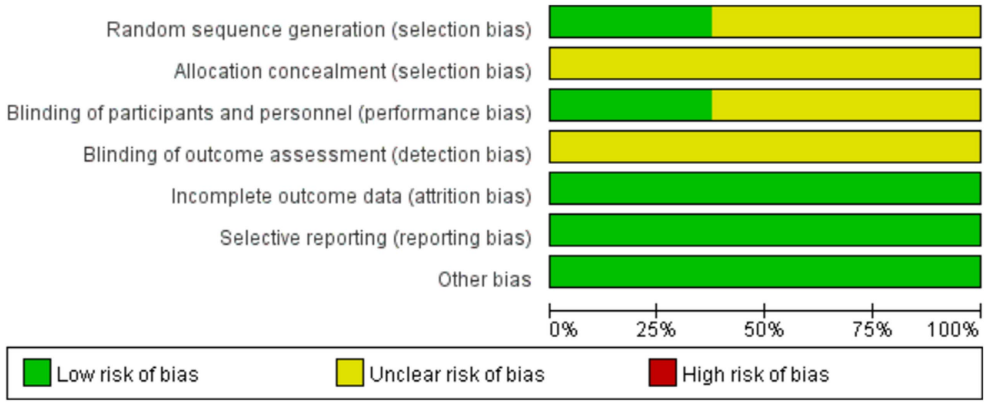

FIGURE 2
Risk of Bias Summary.

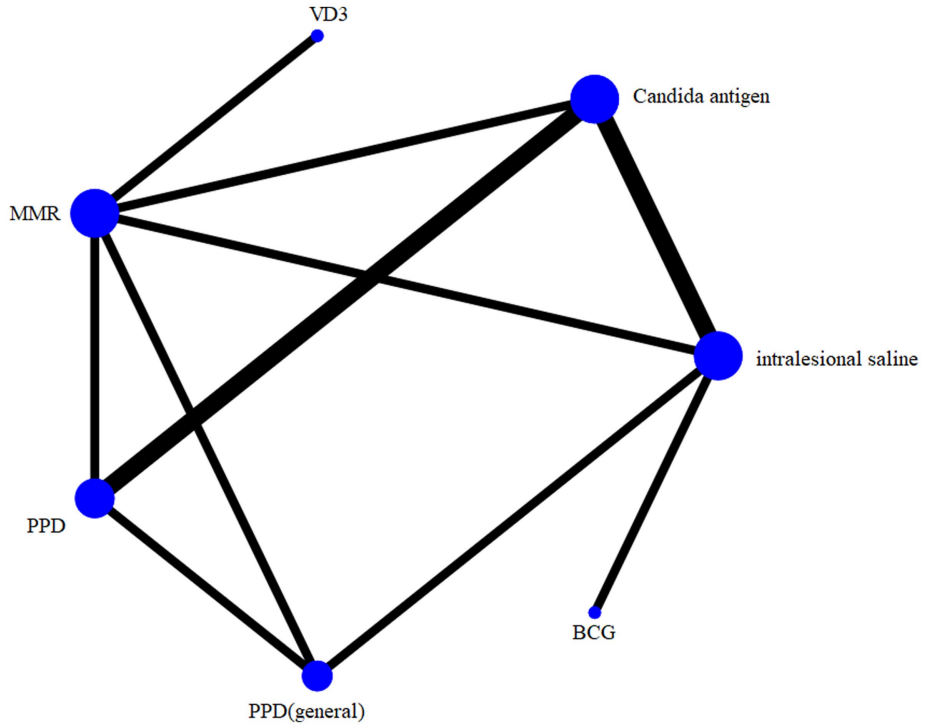

FIGURE 3
Network geometry.

was no statistic significance between the two groups. The frequentist method is based on a probabilistic interpretation of frequency, interpreting probability as a stable value of frequency after a large number of repeated trials. Using the frequency method can compare the advantages and disadvantages of treatments by means of a two-by-two comparison. Table 2 shows that all treatment measures have a significant efficacy compared with the standard saline group (BCG OR = 96.00, 95%CI: 10.35–890.58; MMR OR = 29.69, 95%CI: 7.47–118.04; Candida antigens OR = 27.34, 95%CI: 8.64–86.52; PPDs OR = 23.33, 95%CI: 6.75–80.60; VD3 OR = 21.36, 95%CI: 4.34–105.16 and PPDs (general) OR = 13.14, 95%CI: 3.38–51.12).

Table 2 the median of the posterior distribution based on 50,000 simulations was reported as odds ratio (OR), and the corresponding 95% credible intervals (CI) were obtained using the 2.5th and 97.5th percentiles of the posterior distribution, after adjusting for multiple arm trials. Treatments are reported in order of ranking of efficacy.

The frequentist method was used to rank for complete clearance probabilistically; efficacy rankings are based on cumulative SUCRA, with larger areas leading to better outcomes; results show that BCG vaccines 88.2% > MMR vaccines 68.9% > Candida antigens 63.6% > local injection of PPD vaccines 52.9% > vitamin D3 49.0% > systemic injection of PPD vaccines 27.4% > saline 0% in Figure 4.

TABLE 2 Results of network meta-analysis for primary outcomes.

BCG	0.31 (0.02, 4.25)	0.28 (0.02, 3.50)	0.24 (0.02, 3.11)	0.22 (0.01, 3.44)	0.14 (0.01, 1.86)	0.01 (0.00, 0.10)
3.23 (0.24, 44.43)	MMR	0.92 (0.31, 2.72)	0.79 (0.27, 2.26)	0.72 (0.32, 1.60)	0.44 (0.14, 1.40)	0.03 (0.01, 0.13)
3.51 (0.29, 43.11)	1.09 (0.37, 3.21)	Candida antigen	0.85 (0.45, 1.63)	0.78 (0.20, 3.00)	0.48 (0.15, 1.52)	0.04 (0.01, 0.12)
4.11 (0.32, 52.66)	1.27 (0.44, 3.67)	1.17 (0.61, 2.23)	PPD	0.92 (0.24, 3.44)	0.56 (0.19, 1.65)	0.04 (0.01, 0.15)
4.49 (0.29, 69.52)	1.39 (0.63, 3.08)	1.28 (0.33, 4.92)	1.09 (0.29, 4.11)	VD3	0.62 (0.15, 2.50)	0.05 (0.01, 0.23)
7.30 (0.54, 99.22)	2.26 (0.71, 7.15)	2.08 (0.66, 6.58)	1.77 (0.60, 5.21)	1.63 (0.40, 6.60)	PPD (general)	0.08 (0.02, 0.30)
96.00 (10.35, 890.58)	29.69 (7.47, 118.04)	27.34 (8.64, 86.52)	23.33 (6.75, 80.60)	21.36 (4.34, 105.16)	13.14 (3.38, 51.12)	intralesional saline

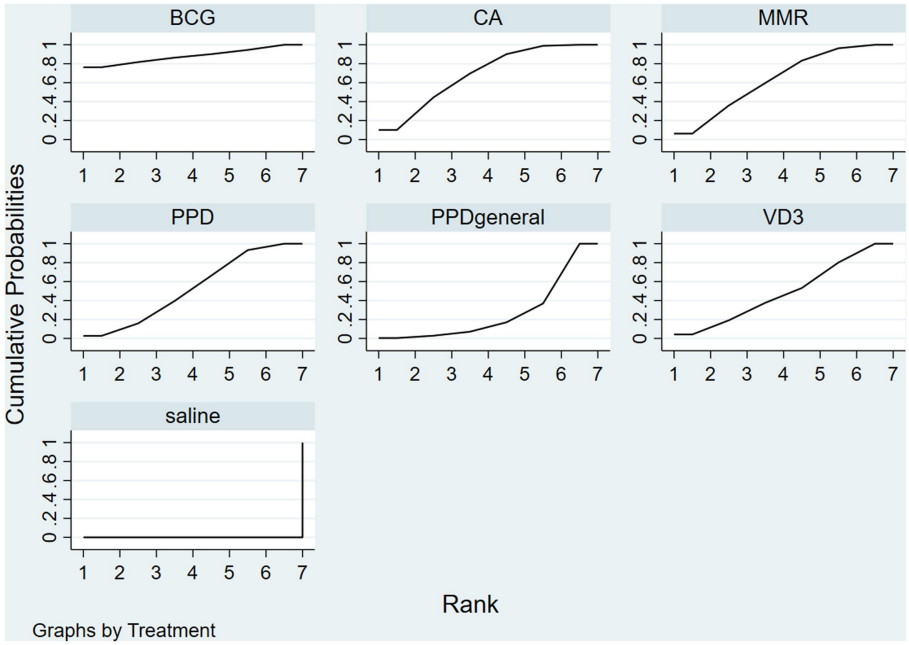

FIGURE 4
The frequentist method was used to rank for complete clearance probabilistically; efficacy rankings are based on cumulative SUCRA, with larger areas leading to better outcomes. Use this figure as an example, BCG has the largest area under the curve, indicating that it has the best treatment effect.

4 Discussion

This is the first NMA to compare the efficacy of all immunotherapies for CA, with 8 RCTs involving 493 patients included in the study. All treatment modalities showed a statistically-significant efficacy compared to placebo. According to the SUCRA results, the BCG vaccines are the most effective for CA among these immunotherapies.

Current medical treatments for CA are mostly topical with stimulant drugs such as imiquimod or cryotherapy to remove warts directly. Although the warts can be removed quickly, the recurrence rate is high, and the results are poor for specific areas such as the intravaginal, intra-urethral and intra-anal areas. The photodynamic therapy is often used to reduce the recurrence rate, but patients often have difficulty tolerating the pains associated with the treatment. Immunotherapies have recently been used to treat warts by injecting an immune extract directly into the warts to achieve their removal. In 1 study, the MMR vaccines were administered topically to 20 children with condyloma warts, 73% of whom showed a complete remission and no recurrence 6 months after the treatment (8). Mild erythema, edema and flu-like symptoms may occur during treatment, which rapidly subside after the administration of NSAIDs, which can provide more excellent relief to patients. Some reduction in warts without immunization has been found in some studies, which may be because local antigens stimulate the body to produce a systemic immune response. Intradermal forearm injections of PPD antigens were used in 1 study to treat 40 pregnant women with CA (11), 74% of whom had a complete wart clearance, reconfirming that antigenic treatment can act on distant warts with a good efficacy. Immunotherapies cannot be used to differentiate between HPV types and their high effectiveness against all types of warts. So topical immunotherapies can be an excellent treatment for areas where traditional lasers and cryotherapies are complicated options, such as the perineal area. Although local injections of immune extracts are commonly used to treat refractory warts, the choice of immunotherapy methods and the dose used are still challenging.

All immunotherapy treatments were more effective than saline, which was statistically significant. Immunotherapies have proven to be effective, which are a new option for our future treatment of refractory CA. In our results, BCG is more effective than immunotherapies. However, it is believed in other literatures that PPD and MMR vaccines may be considered first-line treatments for warts (16). Our analysis may be due to the fact that the literatures do not include randomly-controlled

trials of BCG for CA; our literatures show that MMR and PPD treatment is second only to BCG. To some extent, it also confirms the better efficacy of MMR and PPD treatment.

BCG is a live attenuated vaccine made from an artificially cultured suspension of non-toxic bovine *Mycobacterium tuberculosis*, which contains live, attenuated *mycobacterium bovis* and maintains a sufficiently high level of immunogenicity; the immune response it causes is a direct antigen–antibody reaction with a significant local response. However, PPDs are protein derivatives derived from *mycobacterium tuberculosis*, which causes cross-reactivity and a weak local response. This may also explain the superiority of BCG injections (77.8%) over PPD injections (23.8%).

The results show that local PPD injections (45.9%) are more effective than systemic PPD injections (23.8%); likely, PPDs are not immunogenic enough to elicit a strong systemic immune response to clear warts, but only cause a solid local immune response.

Different immunotherapies can be chosen based on patients' response to the antigens. A small quantity of antigens may be injected intradermally into the forearm of the test hosts for testing before immunotherapies, readings after 48–72 h and the sensitivity observed in the presence of erythema or hard nodules. If a patient shows little or no response to the antigens, the skin test is considered negative, indicating that the patient is likely to be unresponsive to the antigens and the treatment plan should be changed. However, as a novel therapy, immunotherapies have not yet undergone extensive clinical studies; more investigators and subjects need to be involved to further evaluate their efficacy and safety.

Laser and photodynamic therapies have gradually become main methods for reducing photodynamic recurrence. However, the guidelines state that the intralesional antigen immunotherapy has been used for the treatment of warts, and through conventional destructive therapies in combination with immunotherapies, the recurrence of genital warts can be significantly reduced (1). So for stubborn warts or areas where laser treatment is not convenient, we can take immunotherapies or combine them with lasers and drugs for a better treatment of warts.

There are some limitations in this study: (1) In addition, in some of the included trials, whether blinding was used was not mentioned or their randomization and allocation concealment processes were not adequately reported. The quality of the study is not described in great detail, especially in terms of allocation concealment, which is not clear from all the literatures. To reduce biases and make the results more reliable, researchers should follow the CONSORT reporting standards to improve the quality of reporting; (2) This study was focused on the analysis of the efficacy of immunotherapies on CA, which did not include comparative trials of other treatment methods, and immunotherapy approaches were relatively too new to be involved in a larger number of literatures compared to traditional treatments. (3) Due to the lack of data, we did not compare the ranking of the adverse effects or relapse rates among the various immunotherapies, which should also serve as an essential basis for the choice of treatment.

In summary, BCG has an excellent efficacy in the treatment of CA. Perhaps in the future, lasers or cryotherapy can be used to remove warts first, followed by local immunotherapies to achieve better results and reduce recurrence. Due to the quantitative and qualitative limitations of this study, future clinical studies with larger sample sizes, multiple centers and a higher quality will be needed to validate this conclusion.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sindhuja T, Bhari N, Gupta S. Asian guidelines for condyloma acuminatum. *J Infect Chemother*. (2022) 28:845–52. doi: 10.1016/j.jiac.2022.03.004

2. Zhan M, Tong Z, Chen S, Miao Y, Yang Y. Establishing a prediction model for the recurrence of condyloma acuminatum. *Eur J Med Res*. (2022) 27:183. doi: 10.1186/s40001-022-00816-7

3. Al-Qassabi A-M't, Al-Farsi F. Intralesional measles-mumps-rubella vaccine for genital warts: report of two cases with a review of literature. *Sultan Qaboos Univ Med J*. (2022) 22:413–6. doi: 10.18295/squmj.6.2021.093

4. Tawfik NZ, Eyada MMK, Abdel El Hamid RE, Halim HM. Intralesional injection of purified protein derivative versus Candida antigen in treatment of genital warts. *Dermatol Ther*. (2022) 35:e15762. doi: 10.1111/dth.15762

5. Korsa HHA, Nashaat H-A, Halim HM, Atwa MA, Mahmoud Marie R-S. Interleukin-18 serum level before and after intralesional immunotherapy with tuberculin purified protein derivative in patients with cutaneous and genital warts. *J Cosmet Dermatol*. (2022) 21:1–8. doi: 10.1111/jocd.15358

6. Aktas H, Ergin C, Demir B, Ekiz Ö. Intralesional vitamin D injection may be an effective treatment option for warts. *J Cutan Med Surg*. (2015) 20:118–22. doi: 10.1177/1203475415602841

7. Shah AN, Patel D, Ravishanker V. Measles, mumps and rubella vaccine as intralesional immunotherapy in the treatment of warts. *Int J Res Med Sci*. (2016) 4:472–6. doi: 10.18203/2320-6012.ijrms20160298

8. Nofal A, Alakad R. Intralesional immunotherapy for the treatment of anogenital warts in the pediatric population. *J Dermatol Treat*. (2022) 33:1042–6. doi: 10.1080/09546634.2020.1800573

9. Singh AK, Devi KN, Bist JS. A comparative evaluation of therapeutic response in warts to intralesional vitamin D3 versus intralesional measles, mumps, and rubella vaccine. *Dermatol Ther*. (2022) 35:e15813. doi: 10.1111/dth.15813

10. Elmaadawy EH, Shams SS, Hegab DS, Zaki RA. Pulsed-dye laser versus intralesional *Candida albicans* antigen injection in treatment of genital warts. *Acta Dermatovenerol Alp Pannonica Adriat*. (2019) 28:21–6. doi: 10.15570/actaapa.2019.5

11. Eassa BI, Abou-Bakr AA, El-Khalawany MA. Intradermal injection of PPD as a novel approach of immunotherapy in anogenital warts in pregnant women. *Dermatol Ther*. (2011) 24:137–43. doi: 10.1111/j.1529-8019.2010.01388.x

12. Othman NE, Safan FA, State AF, El-Desoky MM, El-Kamel MF. Immunotherapy for the treatment of female anogenital wart with purified protein derivative and measles mumps rubella vaccine: a comparative clinical and immunological study. *J Pharm Negat Results*. (2023) 14:640–9. doi: 10.47750/pnr.2023.14.02.80

13. Marei A, Alakad R, Wahid RM. Evaluation of intralesional Candida antigen in diabetic patients with multiple warts. *J Cosmet Dermatol*. (2021) 20:1248–53. doi: 10.1111/jocd.13718

14. Metawea B, El-Nashar AR, Kamel I, Kassem W, Shamloul R. Application of viable bacilli Calmette-Guérin topically as a potential therapeutic modality in condylomata acuminata: a placebo-controlled study. *Urology*. (2005) 65:247–50. doi: 10.1016/j.urology.2004.09.025

15. Nofal A, Soliman M, Hamdy F, Alakad R. Intralesional Candida antigen versus intralesional tuberculin in the treatment of recalcitrant genital warts: a comparative study. *J Am Acad Dermatol*. (2020) 82:1512–4. doi: 10.1016/j.jaad.2019.12.050

16. Salman S, Ahmed MS, Ibrahim AM, Mattar OM, El-Shirbiny H, Sarsik S. Intralesional immunotherapy for the treatment of warts: a network meta-analysis. *J Am Acad Dermatol*. (2019) 80:922–930.e4. doi: 10.1016/j.jaad.2018.07.003

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A real-world study on the safety profile of extended-interval dosing of immune checkpoint inhibitors for melanoma: a single-center analysis in Japan

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Background: Anti-programmed death-1 (PD-1) antibodies are the mainstay for the treatment of unresectable or high-risk melanoma. However, real-world data on the safety profile of their extended-interval doses (EDs) are limited, particularly in Asian patients with melanoma.

Materials and methods: In this single-center retrospective study, we analyzed the risks of immune-related adverse events (irAEs) among 71 Japanese patients (36 males; mean age, 65.0 years) who received anti-PD-1 monotherapy for melanoma at our institute. Patients who were administered ipilimumab prior to anti-PD-1 monotherapy were excluded. Patients were divided into three groups: canonical-interval dose (CD) group ($n = 50$, body weight-based dosing or 240 mg Q2W for nivolumab and body weight-based dosing or 200 mg Q3W for pembrolizumab), ED group ($n = 14$, 480 mg Q4W for nivolumab and 400 mg Q6W for pembrolizumab), and dose-switch (DS) group ($n = 7$, upfront CD followed by ED).

Results: The CD group received nivolumab more frequently in the metastatic setting. There were no significant differences in baseline characteristics among the three groups, including in sex, age, primary tumor site, tumor subtype, and follow-up period. irAEs occurred in 36.6% (26 patients) of all patients (32.0% of the CD group, 35.7% of the ED group, and 71.4% of the DS group), while severe (grade≥3) irAEs occurred in only two patients, both of whom were in the CD group. Most of the irAEs occurred during the first 6months of anti-PD-1 therapy and, interestingly, all of the irAEs in the DS group occurred before the switch (during the CD). There was no significant difference among the three groups in the probability of irAE estimated by the Kaplan–Meier method.

Conclusion: These findings may highlight the safety of ED of anti-PD-1 monotherapy in the treatment of Asian patients with melanoma.

KEYWORDS

acral melanoma, Asian population, nivolumab, PD-1, pembrolizumab

1 Introduction

Since the first introduction of immune checkpoint inhibitors (ICIs) for malignant melanoma, they have revolutionized the management of melanoma and led to dramatic improvements in patient survival (1–3). The application of ICIs has rapidly expanded to other cancers, hematologic malignancies, and sarcomas. Programmed death-1 (PD-1) is a key molecule of immune checkpoints and its inhibitors are now the mainstay of melanoma treatment in both metastatic and adjuvant settings (1–7). Clinical practice guidelines recommend anti-PD-1 therapy alone or with other drugs (e.g., ipilimumab) as first-line treatment for unresectable melanoma and high-risk advanced melanoma, particularly for BRAF wild-type melanoma (1–3). Melanoma subtypes differ between Caucasian and Asian populations, with Caucasians having more sunlight-related melanomas and Asians having more acral and nail melanomas (8–10). There are different genetic backgrounds for these subtypes, which can lead to differences in their biological behavior and response to antitumor therapy (11–15). There is evidence suggesting that acral melanoma is refractory to ICIs, and even non-acral cutaneous melanoma has a worse prognosis in Asians than in Caucasians under ICI therapy (16). These results indicate that response to ICIs varies depending on the tumor subtype and ethnicity.

Two anti-PD-1 inhibitors, nivolumab and pembrolizumab, have been approved for use in treating melanoma in Japan (2, 17). Nivolumab was initially used for every 2 weeks (Q2W) at a body weight-based dosing or a flat dosage of 240 mg, and later for its extended-interval dose (ED) of 480 mg every 4 weeks (Q4W). Pembrolizumab, on the other hand, was initially used for every 3 weeks (Q3W) at a body weight-based dosing or a flat dosage of 200 mg, and subsequently approved for its ED [400 mg every 6 weeks (Q6W)]. The approval of these drugs was based on pharmacokinetic data obtained from prior studies (18–21). While ED with anti-PD-1 antibodies would be convenient by reducing clinical visits, administering ED may be associated with increased risks for immune-related adverse events (irAEs). To date, only limited real-world evidence of the safety of ED has been obtained, particularly for Asian patients with melanoma (22–26). Is ED of anti-PD-1 monotherapy safe for Asians with melanoma? Is it necessary to initiate anti-PD-1 monotherapy with the canonical-interval dose (CD) and later switch to ED to reduce irAEs? This single-center retrospective study was conducted to answer these questions. Interestingly, no clear increase in irAEs or severe (grade ≥ 3) irAEs was observed in our cohort treated with ED compared with CD.

2 Methods

2.1 Ethics statement

We conducted this retrospective study in accordance with the concepts enshrined in the Declaration of Helsinki. This study was approved by Kyushu University Institutional Ethics Committee (30-363; 27 November, 2018). Written informed consent was received from the patients prior to their inclusion in the study.

2.2 Patients

The study included a total of 71 patients with malignant melanoma who received anti-PD-1 monotherapy (nivolumab and/or pembrolizumab) in a metastatic or adjuvant setting at the Department of Dermatology, Kyushu University (Fukuoka, Japan), between July 2014 and March 2023. Patients who received anti-CTLA4 therapy (monotherapy or in combination with anti-PD1 antibody) prior to anti-PD-1 monotherapy were excluded. Patients who received other anti-tumor treatments, including BRAF/MEK inhibitors, cytotoxic chemotherapy, and interferon β, prior to anti-PD-1 monotherapy were included. No patients underwent simultaneous anti-PD-1 plus any of these anti-tumor therapies including BRAF/MEK inhibitor therapy. At least three experienced dermatopathologists confirmed the diagnosis of all patients.

The following clinical and demographic data on all patients were retrieved from the patients' clinical records and analyzed: age at the initiation of anti-PD-1 monotherapy, sex, primary tumor site, tumor subtype, type of anti-PD-1 antibody, lines of treatment, types of irAEs and their grades (CTCAE v.5.0), and timing of irAEs. Two authors (T.I. and Y.K.-I.) independently reviewed the records of all patients included in this study and any discrepancy in the results that they recorded was resolved through discussion.

Patients were divided into three groups, namely, CD group, ED group, and dose-switch (DS) group. The CD group included patients who received the original doses of nivolumab (2 mg/kg Q3W, 3 mg/kg Q2W, and 240 mg Q2W) and pembrolizumab (2 mg/kg Q3W and 200 mg Q3W) throughout the course of anti-PD-1 monotherapy. The ED group included patients receiving ED (480 mg Q4W for nivolumab and 400 mg Q6W for pembrolizumab) from the beginning to the end of anti-PD-1 monotherapy. The DS group consisted of patients who started with CD and later switched to ED.

2.3 Statistical analysis

All statistical analyses were performed using GraphPad Prism version 8.3 (GraphPad Software, San Diego, CA, United States). To analyze the relationship among the three groups, chi-squared test and Kruskal–Wallis test were used for categorical and continuous variables, respectively. The Kaplan–Meier method and the log-rank test were used to estimate the probability of irAE. Patients who did not experience any irAE were censored at the last follow-up. A p-value of less than 0.05 was considered to indicate statistical significance.

3 Results

3.1 Patient clinicopathological data

Baseline characteristics of all 71 patients are shown in Table 1. All patients were Japanese (36 males and 35 females), with a mean age of 65.0 years (median, 69; range 30–86). Primary tumors were located on the skin of the extremities (43.7%), followed by non-skin lesions such as mucosa or viscera (25.4%), trunk skin (14.1%), head and neck skin (8.5%), and those of unknown origin (8.5%). Non-acral cutaneous melanoma was the predominant subtype (36.6%), followed by acral

TABLE 1 Baseline characteristics.

	All patients (*n* = 71)	Canonical-interval dose (*n* = 50)	Extended-interval dose (*n* = 14)	Dose switch[a] (*n* = 7)	*p* value
Sex, *n* (%)					0.369
Male	36 (50.7)	28 (56.0)	5 (35.7)	3 (42.9)	
Female	35 (49.3)	22 (44.0)	9 (64.3)	4 (57.1)	
Age, y					0.599
Mean (SD)	65.0 (14.0)	64.0 (15.0)	65.2 (12.5)	71.3 (5.8)	
Median (Min, Max)	69 (30, 86)	67 (30, 88)	67 (43, 83)	72 (63, 80)	
Primary site, *n* (%)					0.467
Head and neck	6 (8.5)	5 (10.0)	1 (7.1)	0 (0)	
Trunk	10 (14.1)	5 (10.0)	3 (21.4)	2 (28.6)	
Extremities	31 (43.7)	23 (46.0)	6 (42.9)	2 (28.6)	
Non-skin	18 (25.4)	14 (28.0)	3 (21.4)	1 (14.3)	
Unknown	6 (8.5)	3 (6.0)	1 (7.1)	2 (28.6)	
Tumor subtype, *n* (%)					0.844
Non-acral cutaneous	26 (36.6)	19 (38.0)	5 (35.7)	2 (28.6)	
Acral	20 (28.2)	14 (28.0)	4 (28.6)	2 (28.6)	
Mucosal	10 (14.1)	6 (12.0)	3 (21.4)	1 (14.3)	
Uveal	5 (7.0)	5 (10.0)	0 (0)	0 (0)	
Others/unknown	10 (14.1)	6 (12.0)	2 (14.3)	2 (28.6)	
Tumor stage, *n* (%)					<0.001
II	4 (5.6)	0 (0)	3 (21.4)	1 (14.3)	
III	15 (21.1)	6 (12.0)	6 (42.9)	3 (42.9)	
IV	52 (73.2)	44 (88.0)	5 (35.7)	3 (42.9)	
Treatment, *n* (%)					0.004[c]
Nivolumab	34 (47.9)	30 (60.0)	3 (21.4)	1 (14.3)	
Pembrolizumab	35 (49.3)	18 (36.0)	11 (78.6)	6 (85.7)	
Sequential[b]	2 (2.8)	2 (4.0)	0 (0)	0 (0)	
Line of treatment, *n* (%)					<0.001[d]
1st line[e]	36 (50.7)	32 (64.0)	2 (14.3)	2 (28.6)	
2nd line[e]	8 (11.3)	6 (12.0)	1 (7.1)	1 (14.3)	
3rd line or more[e]	2 (2.8)	2 (4.0)	0 (0)	0 (0)	
Adjuvant	25 (35.2)	10 (20.0)	11 (78.6)	4 (57.1)	
Treatment cycles					0.033
Mean (SD)	10.2 (10.0)	9.5 (8.2)	6.8 (4.7)	22.0 (19.2)	
Median (Min, Max)	8 (1, 62)	7 (1, 42)	7 (2, 16)	15 (3, 62)	
Follow-up period, w					0.224
Mean (SD)	91.8 (91.3)	91.5 (101.7)	73.4 (44.4)	131.0 (77.5)	
Median (Min, Max)	68 (4, 443)	52 (4, 443)	91 (6, 134)	130 (14, 259)	

[a]Switch from canonical interval dose to extended interval dose.
[b]Sequential use of nivolumab and pembrolizumab.
[c]Compared between nivolumab and pembrolizumab.
[d]Compared between metastatic and adjuvant setting.
[e]Metastatic setting.

melanoma (28.2%), mucosal melanoma (14.1%), and uveal melanoma (7.0%). Melanoma of unknown origin or unclassified type was found in 14.1%. Nivolumab monotherapy or pembrolizumab monotherapy was performed in 47.9 and 49.3% of the patients, respectively. Two patients received both nivolumab and pembrolizumab monotherapy in a sequential setting. Approximately 65% of patients received the therapy in a metastatic setting (49.3% as 1st line, 11.3% as 2nd line, and 2.8% as 3rd line or more) and 36.6% of patients in an adjuvant

setting. The mean follow-up periods after the initiation of anti-PD-1 therapy were 91.8 weeks (median, 68 weeks; range 4–443 weeks) for all patients, 91.5 weeks (median, 52 weeks; range 4–443 weeks) for the CD group, 73.4 weeks (median, 91 weeks; range 6–134 weeks) for the ED group, and 131.0 weeks (median, 130 weeks; range 14–259 weeks) for the DS group. There was no significant difference in the follow-up period among the three groups ($p = 0.224$).

Comparing the three groups (CD, ED, and DS), there were no significant differences in sex, age, primary tumor site, or tumor subtype (Table 1). Nivolumab was more frequently used in the CD group and pembrolizumab was more frequently used in the ED group and the DS group. In addition, a metastatic setting was more common in the CD group and an adjuvant setting was more common in the ED group and the DS group. There were significant differences in the AJCC tumor stage (8th edition) and treatment cycles among the three groups.

3.2 Adverse events

Comprehensive profiles of irAEs are summarized in Table 2. In total, 26 events of any grade occurred in the follow-up period, namely, 11 endocrinopathy-related events (thyroid dysfunction, adrenal dysfunction, and diabetes), along with 7 cutaneous, 2 pneumonitis, 2 fatigue, 1 hepatitis, 1 musculoskeletal, 1 ocular, and 1 gastrointestinal irAEs. Cutaneous irAE included 4 maculopapular rash, 1 psoriasiform dermatitis, 1 vitiligo, and 1 edema. Notably, only two severe (grade 3) irAEs (type 1 diabetes and hepatitis) occurred in all patients. Anti-PD-1 monotherapy was discontinued in two patients due to an irAE (grade 3 type 1 diabetes in one patient and grade 2 edema in the other), and the most common reason for terminating anti-PD-1 therapy was disease progression. The patient who experienced grade 3 hepatitis resumed the anti-PD-1 therapy after a temporary interruption. No patient switched back to CD.

In the CD group, a total of 16 irAEs including the two severe irAEs occurred, while no severe irAEs occurred in the ED group or the DS group. Interestingly, all five irAEs in the DS group occurred before the switch and no irAEs were identified during the subsequent ED period (escalation window).

3.3 Probability of irAE

Since most patients experienced only one irAE event or a second irAE at nearly the same timing as the first, we created Kaplan–Meier curves to compare the risk of irAEs among the groups (Figure 1). There was no significant difference in the probability of irAE (free of irAEs of any grade) among the three groups (Figure 1A). Considering the severe irAEs (grade ≥ 3), no significant difference was found among the three groups as well (Figure 1B). Most of the irAEs occurred during the first 6 months after the initiation of anti-PD-1 monotherapy (mean, 18.8 weeks; median, 12 weeks; range, 2–79 weeks).

4 Discussion

In this retrospective study, we found that ED had safety comparable to that of CD. Overall, 32.0% of patients treated with CD experienced an irAE of any grade, while the corresponding value was 35.7% for the ED group. Severe irAEs of grade 3 or more occurred exclusively in the CD group. Most of the irAEs occurred during the first 6 months after the initiation of anti-PD-1 therapy. All of the irAEs in the DS group occurred before the switch to ED.

The use of ICIs has significantly impacted the clinical practice of medical oncology. Despite their first introduction as traditional body weight-based dosing regimens, simulation pharmacokinetics studies showed that weight has only a minor effect on the distribution of ICIs; therefore, flat ICI doses became standard (27–29). Data from clinical trials also indicate that ICIs with ED (pembrolizumab 400 mg Q6W and nivolumab 480 mg Q4W) offer similar outcomes and safety as CD schedules (200 mg Q3W and 240 mg Q2W, respectively) (18, 20, 21).

TABLE 2 Adverse events.

	All patients ($n = 71$)		Canonical-interval dose ($n = 50$)		Extended-interval dose ($n = 14$)		Dose switch[a] ($n = 7$)			
	Total events		Total events		Total events		Total events		Escalation window[b]	
Adverse event	Any grade	Grade ≥ 3	Any grade	Grade ≥ 3	Any grade	Grade ≥ 3	Any grade	Grade ≥ 3	Any grade	Grade ≥ 3
All events, *n* (%)	26 (36.6)	2 (2.8)	16 (32.0)	2 (4.0)	5 (35.7)	0	5 (71.4)	0	0	0
Endocrinopathy	11	1[c]	5	1[c]	3		3			
Skin	7		5		1		1			
Pneumonitis	2		1				1			
Fatigue	2		2							
Hepatitis	1	1[d]	1	1[d]						
Musculoskeletal	1		1							
Ocular	1		1							
Gastrointestinal	1				1					

[a]Switch from canonical interval dose to extended interval dose.
[b]Period after switch from canonical interval dose to extended interval dose.
[c]Type 1 diabetes, Grade 3.
[d]Grade 3.

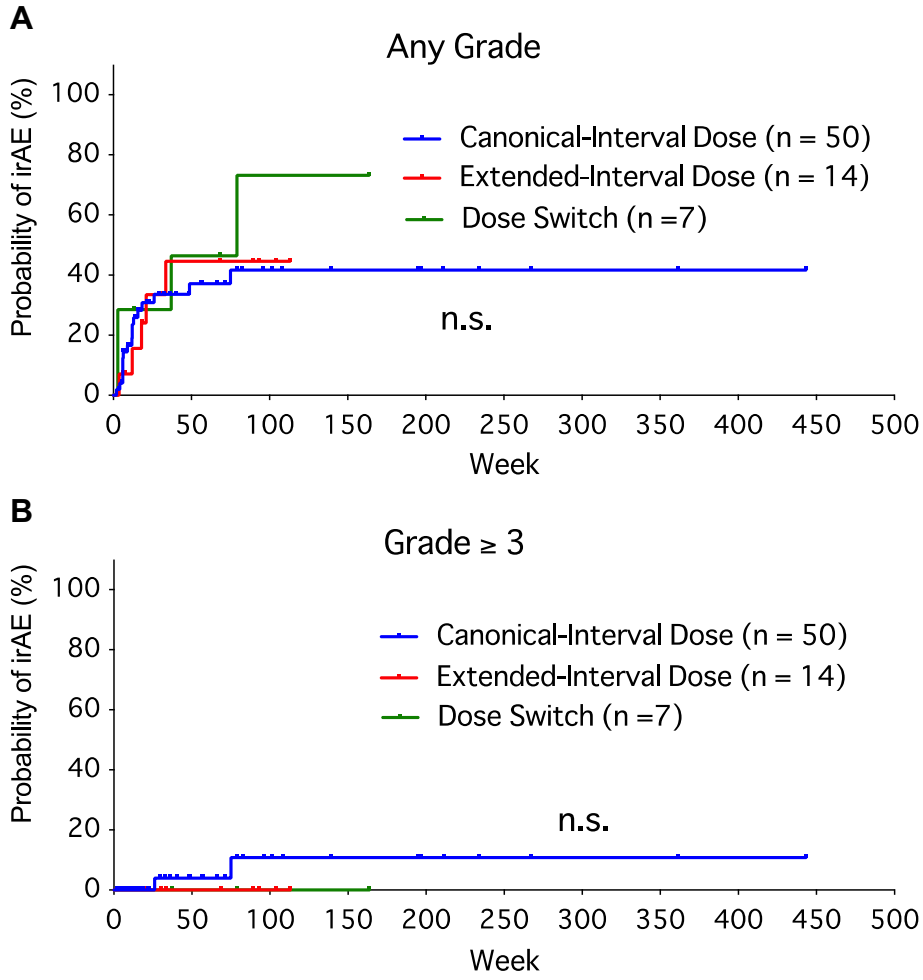

FIGURE 1
Kaplan–Meier curves of the probability of immune-related adverse events (irAEs) among canonical-interval dose group, extended-interval dose group, and dose-switch group. **(A)** Any grade of irAEs. **(B)** Severe (grade ≥ 3) irAEs.

However, there have been scarce real-world data on the safety profile of ED, particularly for Asian patients with melanoma (22–26). A retrospective study in Japan examined 45 patients with non-small cell lung cancer treated with pembrolizumab. All patients started at the CD and switched to ED after a median of six cycles of CD. New irAEs or the deterioration of existing ones occurred in 37.8% within three cycles of ED after switching, and the authors concluded that the ED may induce new irAEs (particularly pneumonitis) during the first few cycles after the switch, even in patients who had received stable treatment at CD (22). Another study from Japan retrospectively investigated the safety of ED of nivolumab and pembrolizumab across 69 patients with various solid cancers (including 21 melanomas) (23). Among 60 patients who switched to ED, 13 patients (21.7%) developed irAEs after the switch, seven of whom (53.8%) did so during the first ED cycle. These two studies may highlight the potential safety risk of ED.

In contrast, some recent reports have suggested that ED has a comparable safety profile to CD (24–26). A single-center analysis in the Netherlands compared the safety and efficacy between CD ($n = 88$) and ED cohorts ($n = 117$) with non-small cell lung cancer. Toxicity leading to dose reduction or discontinuation of treatment was not increased in the ED cohort (treatment was permanently discontinued due to irAEs in 4.3% of those on ICI treatment with ED) (24). Another study multicentrically recruited patients ($n = 91$) to analyze the safety of ED of ICIs for non-small cell lung cancer (25). After a median follow-up of 10.7 months on ICIs, only 4.3% of patients discontinued the treatment permanently, while 16% interrupted the treatment due to irAEs. More recently, a large cohort study on 812 patients with solid cancer (including 456 melanomas) was reported (26). Patients had received at least one cycle of monotherapy with ED after switching from CD or were treated upfront with ED. Out of 550 patients who started ICIs with CD and switched to ED, 225 (41%) developed irAEs of any grade and 17 (3%) those of grade 3 or more during CD, whereas irAEs of any grade and grade 3 or more were experienced by 155 (36%) and 20 (5%) patients after switching to ED, respectively. A lower probability of any grade irAEs was associated with switching to ED (adjusted odds ratio, 0.83; 95% confidence interval, 0.64–0.99; $p = 0.047$), whereas no significant difference was noted for ≥grade 3 events (adjusted odds ratio, 1.55; 95% confidence interval, 0.81 to 2.94; $p = 0.18$). The authors concluded that switching ICI treatment from CD and ED did not increase the incidence of irAEs. Our data, suggesting the unnecessity of dosing switch, align well with these

studies (22–26). However, the reason behind the conflicting results (22–26) regarding the safety of extended dosing is unclear. One potential explanation could be the different irAE profiles among the cancers, such as frequent pneumonitis in lung cancer and vitiligo in melanoma.

ED may have several potential disadvantages such as less monitoring for clinical progression, negative impact on detecting irAEs. Increased economic cost is another potential disadvantage because the treatment will be stopped upon disease progression regardless of when the last dose was received, potentially leading to drug waste in the bloodstream, more likely in the ED group than the CD group (30). Occasional case reports of severe irAEs after dose switch have been published (31). Careful monitoring can help overcome these potential disadvantages and highlight clear benefits of ED.

Besides the potential biases inherent in the retrospective nature of this study and its small sample size especially in the ED and DS groups, a limitation of this study was the inability to analyze the efficacy profile of ED due to the significant involvement of adjuvant therapy. In addition, caution should be taken when interpreting our results due to the frequent use of ED in the adjuvant setting.

In conclusion, we have provided further insights into the safety profile of ED in the treatment of melanoma. Based on our data, the risk of irAEs was not increased with ED compared with CD. Dose switch with upfront CD followed by ED may not be necessary to reduce irAEs. With careful monitoring, especially during the early phase of anti-PD-1 monotherapy, the use of ED should be a safe and convenient strategy for treating melanoma in both adjuvant and metastatic settings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Kyushu University Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

TI: Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Writing – original draft. YK-I: Data curation, Methodology, Writing – review & editing. FO: Methodology, Resources, Writing – review & editing. TN: Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. NCCN Guidelines Version 2.2023 Cutaneous Melanoma. (2023). Available at: https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf (Accessed August 19, 2023).

2. Nakamura Y, Asai J, Igaki H, Inozume T, Namikawa K, Hayashi A, et al. Japanese dermatological association guidelines: outlines of guidelines for cutaneous melanoma 2019. *J Dermatol.* (2020) 47:89–103. doi: 10.1111/1346-8138.15151

3. Garbe C, Amaral T, Peris K, Hauschild A, Arenberger P, Basset-Seguin N, et al. European dermatology forum (EDF), the European Association of Dermato-Oncology (EADO), and the European Organization for Research and Treatment of Cancer (EORTC). European consensus-based interdisciplinary guideline for melanoma. Part 2: treatment - update 2022. *Eur J Cancer.* (2022) 170:256–84. doi: 10.1016/j.ejca.2022.04.018

4. Wada-Ohno M, Ito T, Furue M. Adjuvant therapy for melanoma. *Curr Treat Options in Oncol.* (2019) 20:63. doi: 10.1007/s11864-019-0666-x

5. Muto Y, Kambayashi Y, Kato H, Fukushima S, Ito T, Maekawa T, et al. Adverse events associated with postoperative outcomes of adjuvant anti-PD-1 antibody therapy in both acral and non-acral cutaneous melanomas: a multicenter, observational, *post hoc* analysis study. *J Dermatol.* (2023). doi: 10.1111/1346-8138.16912

6. Muto Y, Kambayashi Y, Kato H, Fukushima S, Ito T, Maekawa T, et al. Postoperative adjuvant therapy for 120 melanoma patients, including acral and mucosal subtypes: a multicenter, observational study of two-year follow-up results. *Br J Dermatol.* (2023) 189:476–8. doi: 10.1093/bjd/ljad183

7. Muto Y, Kambayashi Y, Kato H, Fukushima S, Ito T, Maekawa T, et al. Adjuvant anti-PD-1 antibody therapy for advanced melanoma: a multicentre study of 78 Japanese cases. *Acta Derm Venereol.* (2022) 102:adv00756. doi: 10.2340/actadv.v102.678

8. Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, et al. Whole-genome landscapes of major melanoma subtypes. *Nature.* (2017) 545:175–80. doi: 10.1038/nature22071

9. Sakaizawa K, Ashida A, Uchiyama A, Ito T, Fujisawa Y, Ogata D, et al. Clinical characteristics associated with BRAF, NRAS and KIT mutations in Japanese melanoma patients. *J Dermatol Sci.* (2015) 80:33–7. doi: 10.1016/j.jdermsci.2015.07.012

10. Ito T, Hashimoto H, Kaku-Ito Y, Tanaka Y, Nakahara T. Nail apparatus melanoma: current management and future perspectives. *J Clin Med.* (2023) 12:2203. doi: 10.3390/jcm12062203

11. Namikawa K, Ito T, Yoshikawa S, Yoshino K, Kiniwa Y, Ohe S, et al. Systemic therapy for Asian patients with advanced BRAF V600-mutant melanoma in a real-world setting: a multi-center retrospective study in Japan (B-CHECK-RWD study). *Cancer Med*. (2023) 12:17967–80. doi: 10.1002/cam4.6438

12. Nakamura Y, Namikawa K, Yoshino K, Yoshikawa S, Uchi H, Goto K, et al. Anti-PD1 checkpoint inhibitor therapy in acral melanoma: a multicenter study of 193 Japanese patients. *Ann Oncol*. (2020) 31:1198–206. doi: 10.1016/j.annonc.2020.05.031

13. Fujisawa Y, Namikawa K, Yoshino K, Kiniwa Y, Ito T, Kato H, et al. Combined use of nivolumab and ipilimumab in Japanese patients with melanoma: a multicentre retrospective study of 111 cases. *Br J Dermatol*. (2023) 189:223–50. doi: 10.1093/bjd/ljad114

14. Inozume T, Namikawa K, Kato H, Yoshikawa S, Kiniwa Y, Yoshino K, et al. Analyzing the relationship between the efficacy of first-line immune checkpoint inhibitors and cumulative sun damage in Japanese patients with advanced BRAF wild-type nonacral cutaneous melanoma: a retrospective real-world, multicenter study. *J Dermatol Sci*. (2023) 110:19–26. doi: 10.1016/j.jdermsci.2023.03.008

15. Wada M, Ito T, Tsuji G, Nakahara T, Hagihara A, Furue M, et al. Acral lentiginous melanoma versus other melanoma: a single-center analysis in Japan. *J Dermatol*. (2017) 44:932–8. doi: 10.1111/1346-8138.13834

16. Bai X, Shoushtari AN, Betof Warner A, Si L, Tang B, Cui C, et al. Benefit and toxicity of programmed death-1 blockade vary by ethnicity in patients with advanced melanoma: an international multicentre observational study. *Br J Dermatol*. (2022) 187:401–10. doi: 10.1111/bjd.21241

17. Furue M, Ito T, Wada N, Wada M, Kadono T, Uchi H. Melanoma and immune checkpoint inhibitors. *Curr Oncol Rep*. (2018) 20:29. doi: 10.1007/s11912-018-0676-z

18. Long GV, Tykodi SS, Schneider JG, Garbe C, Gravis G, Rashford M, et al. Assessment of nivolumab exposure and clinical safety of 480 mg every 4 weeks flat-dosing schedule in patients with cancer. *Ann Oncol*. (2018) 29:2208–13. doi: 10.1093/annonc/mdy408

19. Bi Y, Liu J, Furmanski B, Zhao H, Yu J, Osgood C, et al. Model-informed drug development approach supporting approval of the 4-week (Q4W) dosing schedule for nivolumab (Opdivo) across multiple indications: a regulatory perspective. *Ann Oncol*. (2019) 30:644–51. doi: 10.1093/annonc/mdz037

20. Zhao X, Shen J, Ivaturi V, Gopalakrishnan M, Feng Y, Schmidt BJ, et al. Model-based evaluation of the efficacy and safety of nivolumab once every 4 weeks across multiple tumor types. *Ann Oncol*. (2020) 31:302–9. doi: 10.1016/j.annonc.2019.10.015

21. Lala M, Li TR, de Alwis DP, Sinha V, Mayawala K, Yamamoto N, et al. A six-weekly dosing schedule for pembrolizumab in patients with cancer based on evaluation using modelling and simulation. *Eur J Cancer*. (2020) 131:68–75. doi: 10.1016/j.ejca.2020.02.016

22. Higashiyama RI, Yoshida T, Yagishita S, Ohuchi M, Sakiyama N, Torasawa M, et al. Safety implications of switching pembrolizumab dosage from 200 mg every 3 weeks to 400 mg every 6 weeks in patients with advanced NSCLC. *J Thorac Oncol*. (2022) 17:1227–32. doi: 10.1016/j.jtho.2022.06.010

23. Morimoto K, Yamada T, Morimoto Y, Ishikawa T, Asai J, Fujihara A, et al. A real-world study on the safety of the extended dosing schedule for nivolumab and pembrolizumab in patients with solid tumors. *Int Immunopharmacol*. (2022) 108:108775. doi: 10.1016/j.intimp.2022.108775

24. Hijmering-Kappelle LBM, Hiltermann TJN, Bensch F. Safety and efficacy of extended interval dosing for immune checkpoint inhibitors in non-small cell lung cancer during the COVID-19 pandemic. *Clin Lung Cancer*. (2022) 23:143–50. doi: 10.1016/j.cllc.2021.12.005

25. Pierre C, Goter T, Lena H, Le Guen Y, Ricordel C. Safety of extended-interval dosing strategy of immune checkpoint inhibitors for advanced NSCLC. *J Thorac Oncol*. (2023) 18:e14–6. doi: 10.1016/j.jtho.2022.09.006

26. Cantini L, Paoloni F, Pecci F, Spagnolo F, Genova C, Tanda ET, et al. Safety of extended interval dosing immune checkpoint inhibitors: a multicenter cohort study. *J Natl Cancer Inst*. (2023) 115:796–804. doi: 10.1093/jnci/djad061

27. Sheng J, Srivastava S, Sanghavi K, Lu Z, Schmidt BJ, Bello A, et al. Clinical pharmacology considerations for the development of immune checkpoint inhibitors. *J Clin Pharmacol*. (2017) 57:S26–42. doi: 10.1002/jcph.990

28. Freshwater T, Kondic A, Ahamadi M, Li CH, de Greef R, de Alwis D, et al. Evaluation of dosing strategy for pembrolizumab for oncology indications. *J Immunother Cancer*. (2017) 5:43. doi: 10.1186/s40425-017-0242-5

29. Zhao X, Suryawanshi S, Hruska M, Feng Y, Wang X, Shen J, et al. Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3-mg/kg dosing regimen in patients with advanced tumors. *Ann Oncol*. (2017) 28:2002–8. doi: 10.1093/annonc/mdx235

30. Goldstein DA, Ginsberg GM, Hofnung-Gabbay D, De Abreu LR, Loong HH, Goh BC, et al. Multinational analysis of estimated health care costs related to extended-interval fixed dosing of checkpoint inhibitors. *JAMA Netw Open*. (2023) 6:e230490. doi: 10.1001/jamanetworkopen.2023.0490

31. Pous A, Izquierdo C, Cucurull M, Sánchez S, Lezcano C, Domenech M, et al. Immune-checkpoint inhibitors for lung cancer patients amid the COVID-19 pandemic: A case report of severe meningoencephalitis after switching to an extended-interval higher flat-dose nivolumab regimen. *Transl Lung Cancer Res*. (2021) 10:1917–23. doi: 10.21037/tlcr-20-1315

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Long-term efficacy and safety of guselkumab in Chinese patients with moderate-to-severe plaque psoriasis

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Background: Randomized controlled trials indicated guselkumab, the first anti-interleukin-23 monoclonal antibody, is efficacious in plaque psoriasis. However, guselkumab's performance in real life is scarcely examined, especially in China.

Objectives: This work aimed to assess the long-term effectiveness of guselkumab in actual clinical practice in China.

Methods: A retrospective study was performed for plaque psoriasis cases administered guselkumab in Shanghai Skin Disease Hospital between January 2020 and September 2022.

Results: A total of 37 patients were included (29 men, 78.4%), with a mean follow-up period of 72.3 ± 26.7 weeks (range of 12–108 weeks). At baseline, clinical examination revealed a mean PASI of 12.3 ± 7.1, a mean BSA of 17.1 ± 18.1, and a mean DLQI of 7.7 ± 4.3. Twenty-two (62.9%) and 17 (48.6%) cases achieved PASI 90 and PASI 100 responses at week 28. From weeks 60 to 92, >80% of cases achieved PASI 90 and PASI 100 responses. Regarding safety, no cases of serious AEs were recorded. A total of nine cases (24.3%) had different abnormal results in *HBV markers*, and two were T-SPOT positive. There was no hepatitis B virus or tuberculosis outbreak in these patients.

Conclusion: This real-life study confirmed the long-term efficacy and safety of guselkumab in daily clinical practice.

KEYWORDS

Chinese patients, efficacy, guselkumab, plaque psoriasis, real-life, safety

1 Introduction

Guselkumab represents an anti-interleukin (IL)-23 monoclonal antibody that reduces inflammation in psoriatic lesions by directly inhibiting IL-23/Th17 signaling (1). It is approved for the treatment of moderate-to-severe plaque psoriasis in cases eligible for systemic therapy or phototherapy (2). In a 48-week phase III, randomized, double-blind, placebo-controlled, active-comparator trial, the safety and efficacy of guselkumab combined with adalimumab or placebo were assessed in adults with moderate-to-severe plaque psoriasis. At week 16, 85.1% of cases administered guselkumab achieved an Investigator Global Assessment (IGA) of 0/1, and 65.9 and 6.9% in the adalimumab and placebo groups, respectively ($P < 0.001$). Psoriasis Activity Severity Index 90 (PASI 90) was achieved at week 16 in 73.3% of cases administered guselkumab, and 49.7 and 2.9% in the adalimumab and placebo groups, respectively ($P < 0.001$) (3). In real life, a retrospective study assessed

patients with moderate-to-severe psoriasis administered guselkumab in the Psoriasis Care Center of Dermatology at the University Federico II of Naples between June 2019 and December 2021 (4). A total of 44 patients were included (28 men, 63.6%; mean age of 59.0 ± 10.2 years). Significantly improved PASI and body surface area (BSA) were detected at each follow-up (PASI decreased from 13.9 ± 8.1 to 0.9 ± 0.7 at week 52, and BSA decreased from 24.3 ± 19.6 to 1.3 ± 1.4, both $p < 0.001$). Only three (6.8%) cases discontinued guselkumab treatment for secondary inefficacy. No cases of serious adverse events were recorded. Real-life studies seem to confirm guselkumab's efficacy and safety in a real-world setting, while cases in China have been rarely assessed (5).

Thus, we carried out a single-center, retrospective trial to assess the long-term efficacy and safety of guselkumab in Chinese patients, and some of whom were previously administered one or more biologic agents in a real-life setting.

2 Materials and methods

A retrospective study was carried out to assess patients with moderate-to-severe psoriasis administered guselkumab in the Psoriasis Care Center of Dermatology at the Shanghai Skin Diseases Hospital between January 2020 and July 2022. Inclusion criteria were (a) diagnosis of moderate-to-severe plaque psoriasis by a dermatologist for at least 6 months and (b) guselkumab administration for at least 12 weeks.

For each case, the following data were collected at baseline: age, sex, psoriasis duration, BSA, PASI, Dermatology Life Quality Index (DLQI), body mass index (BMI), comorbidities, family history of psoriasis, and past and current psoriasis therapies.

At each follow-up visit (weeks 4, 12, 28, 44, 60, 76, and 92) psoriasis severity indexes (PASI and BSA) were assessed as well as adverse events (AEs). At baseline and follow-up, routine blood tests [blood count with formula, transaminases, creatinine, azotemia, glycaemia, erythrocyte sedimentation rate, C reactive protein, total cholesterol and triglycerides levels, protein electrophoresis, Hepatitis B Virus (HBV) markers, anti-Hepatitis C Virus (HCV) markers, and mycobacterium tuberculosis T cell enzyme-linked immunospot tuberculous test (T-SPOT)] were collected. This study followed the Declaration of Helsinki, and all patients provided signed informed consent.

3 Statistical analysis

Statistical analysis was carried out to examine the statistical significance of clinical improvement. Clinicodemographic data were analyzed using descriptive statistics. Continuous data are mean ± standard deviation, and categorical variables are number and proportion. The Mann-Whitney *U*-test and the *t*-test were performed to compare values obtained at different times during treatment for categorical and continuous variables, respectively. $P < 0.05$ was considered statistically significant. GraphPad Prism 8.0 was used for data analysis.

4 Results

A total of 40 patients administered guselkumab in our department were enrolled. A final number of 37 (92.5%) cases (29 men, 78.4%) were included in the study (Table 1). Mean patient age was 44.1 ± 10.6 years (range of 24–65 years), and the mean disease duration was 16.8 ± 10.5 years. A family history of psoriasis was found in 8.1% of cases (3/37). A total two patients (5.4%) also had psoriatic arthritis. Regarding comorbidities, the most common were *abnormal HBV markers* (9, 24.3%), followed by obesity (7, 18.9%), T-SPOT positive (2, 5.4%), dyslipidemia (2, 5.4%), hypertension (1, 2.7%), diabetes (1, 2.7%), hyperuricemia (1, 2.7%), and abnormal kidney function (1, 2.7%) (Table 1). A total of 23 cases (62.2%) had previous treatment with at least one conventional systemic treatment, including methotrexate, cyclosporine, and narrow band (Nb)-ultraviolet B (UVB) phototherapy, while 12 (32.4%) had previous exposure to one or more biologic agents. A total of nine (24.3%) patients had failed anti-tumor necrosis factor-α (anti-TNFα), four (10.3%) had failed secukinumab, and two (5.4%) had failed ustekinumab (Table 1).

At baseline, clinical examination revealed a mean PASI of 12.3 ± 7.1, a mean BSA of 17.1 ± 18.1, and a mean DLQI of 7.7 ± 4.3. Regarding PASI, a statistically significant improvement in PASI value was detected at week 4 (7.0 ± 4.9, $p < 0.001$), with a PASI score improvement of 43.1%. At week 12, a PASI 75 response was achieved by 21 (56.8%) patients, with PASI < 3.0 in 20 (54.1%) subjects. From weeks 44 to 92, >90% of patients had achieved a PASI 75 response. Especially, at weeks 60 and 76, a PASI 75 response was found in 96.3 and 95.5% of cases, respectively. At week 28, a PASI < 3.0 response was achieved by 27 (77.1%) patients. From weeks 44 to 92, >85% of patients had achieved a PASI < 3.0 response. Similarly, at weeks 60 and 76, a PASI < 3.0 response was found in 92.6 and 90.9% of cases, respectively. A total of 22 (62.9%) and 17 (48.6%) patients reached PASI 90 and PASI 100 responses at week 28. PASI 90 and PASI 100 responses were found in >80% of cases between weeks 60 and 92. At week 76, a total of 20 (90.9%) patients had achieved PASI 90 and PASI 100 responses. A statistically significant improvement was also found for BSA at each follow-up (10.1 ± 11.0 at week 4, $p < 0.001$; 2.7 ± 5.6 at week 28, $p < 0.0001$; 1.5 ± 6.9 at week 60, $p < 0.0001$; up to 1.9 ± 6.4 at week 92, $p < 0.0001$) and DLQI at each follow-up (4.4 ± 3.3 at week 4, $p < 0.001$; 1.2 ± 2.6 at week 28, $p < 0.0001$; 0.3 ± 1.2 at week 60, $p < 0.0001$; 0.6 ± 1.7 at week 92, $p < 0.0001$). All psoriasis index results are detailed in Table 2 and Figure 1.

Till now, 11 patients (29.7%) have interrupted the treatment. One case was lost to follow-up at week 20, one needed chemotherapy and interrupted the treatment at week 36, two voluntarily withdrew from the treatment at week 44, one was operated on and had to end the treatment at week 68, and one left to study abroad and stopped the treatment at week 68. A PASI 100 response was found in 83.3% of cases between weeks 20 and 36. However, the patient who lost follow-up had achieved a PASI 90 response at week 12. Another five patients interrupted the treatment between weeks 28 and 52 due to inefficacy. One patient with previously failed treatment with TNF inhibitors and ixekizumab discontinued the treatment at week 28, and three cases previously administered TNF inhibitors discontinued the

10.3389/fmed.2023.1285972

TABLE 1 Baseline patient data.

	History of biological treatments		
	Total	Biological treatments	No biological treatments
Total number of patients (F/M)	37 (8/29)	12 (2/10)	25 (6/19)
Age (Mean SD, years)	44.1 ± 10.6	45.2 ± 8.5	43.6 ± 11.6
Disease course (Mean SD, years)	16.8 ± 10.5	18 ± 10.9	16.7 ± 10.6
Involvement of the scalp, *n* (%)	21 (56.8%)	8 (66.7%)	13 (52%)
Involvement of nails, *n* (%)	20 (54.1%)	8 (66.7%)	12 (48%)
History of PsA, *n* (%)	2 (5.4%)	1 (8.3%)	1 (4%)
Family history of psoriasis, *n* (%)	3 (8.1%)	2 (16.7%)	1 (4%)
PASI score (Mean SD)	12.3 ± 10.6	13.4 ± 8.2	11.8 ± 6.6
BSA score (Mean SD)	17.1 ± 18.1	21.1 ± 20.5	15.3 ± 16.9
DLQI score (Mean SD)	7.7 ± 7.3	8.3 ± 4.7	7.4 ± 4.2
BMI, *n* (%)			
Underweight	1 (2.7%)	1 (8.3%)	0 (0%)
Normal weight	20 (54.1%)	6 (50%)	14 (56%)
Overweight	14 (37.8%)	5 (41.7%)	9 (36%)
Obese	2 (5.4%)	0 (0%)	2 (8%)
Comorbidities, *n* (%)			
Positive HBV markers	9 (24.3%)	4 (33.3%)	5 (20%)
Positive T-SPOT	2 (5.4%)	1 (8.3%)	1 (4%)
Dyslipidemia	2 (5.4%)	2 (16.7%)	0 (0%)
Hypertension	1 (2.7%)	0 (0%)	1 (4%)
Diabetes	1 (2.7%)	0 (0%)	1 (4%)
Hyperuricemia	1 (2.7%)	1 (8.3%)	0 (0%)
Abnormal kidney function	1 (2.7%)	1 (8.3%)	0 (0%)
Previous treatments, *n* (%)			
Acitretin	11 (29.7%)	4 (33.3%)	7 (28%)
Methotrexate	10 (27%)	4 (33.3%)	6 (24%)
UB-UVB	11 (29.7%)	3 (25%)	8 (32%)
Etanercept	2 (5.4%)	2 (16.7%)	
Adalimumab	4 (10.8%)	4 (33.3%)	
Infliximab	6 (16.2%)	6 (50%)	
Secukinumab	4 (10.8%)	4 (33.3%)	
Ustekunumab	2 (5.4%)	2 (16.7%)	
Ixekizumab	1 (2.7%)	1 (8.3%)	

BMI, body mass index; PASI, Psoriasis Area and Severity Index; BSA, body surface area; DLQI, dermatology life quality index; HBV, hepatitis B virus; SD, standard deviation; PsA, psoriatic arthritis; UB-UVB, Narrow Band-UVB; T-SPOT, mycobacterium tuberculosis T cell enzyme-linked immunospot tuberculous test.

treatment between weeks 44 and 52 (Table 3). A PASI 75 response was achieved by two cases at week 20, indicating an ineffective rate of 13.5% in this study.

Moreover, bio-experienced cases (patients who have had any biologics input) (10 men, 83.3%; mean age of 45.2 ± 8.5 years, range of 35–65 years) showed a PASI superior to the bio-naïve cases (patients who have not had any biologics input) (19 men, 76%; mean age of 43.6 ±11.6 years, range of 24–65 years) at baseline (13.4 vs. 11.6, $p = 0.485$). From weeks 4 to 28, bio-experienced cases had less pronounced PASI score improvement than the bio-naïve group (35.1 vs. 46.6%, at week 4; 64.2 vs. 75%, at week 12; 71.6 vs. 90.5%, at week 28). However, no differences were detected between the two groups after week 28. Additionally, less bio-experienced patients achieved PASI 75 and PASI < 3.0 than the bio-naïve cases at weeks 12 and 28, and differences in PASI 90 and PASI 100 were found at weeks 12, 28, 44, and 60 (Figure 2). In this study, the ineffective rate was higher in the bio-experienced patients (33.3%) compared with bio-naïve patients (4%).

Furthermore, this study population had high body mass index values (overweight + obese $>$ 43.2%). Cases with BMI ≥ 25 group (15 men, 93.8%; mean age of 42.3 ± 10.4 years, range of 24–61 years) showed higher PASI scores than those with BMI < 25 group (14 men, 66.7%; mean age of 45.5 ± 10.8 years, range of 33–61 years) at baseline (14.3 vs. 10.8,

10.3389/fmed.2023.1285972

TABLE 2 Psoriasis assessment at baseline and weeks 4, 12, 28, 44, 60, 76, and 92.

Week	0	4	12	28	44	60	76	92
PASI	12.3 ± 7.1	7.0 ± 5.0	3.5 ± 3.8	2.0 ± 3.7	1.0 ± 2.4	0.8 ± 2.8	0.8 ± 3.0	1 ± 3.0
		$p < 0.05$	$p < 0.001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
BSA	17.1 ± 18.1	5.2 ± 7.2	5.2 ± 7.2	2.7 ± 5.7	1.4 ± 6.0	1.52 ± 7.2	1.4 ± 5.6	1.4 ± 5.6
		$p < 0.05$	$p < 0.05$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
DLQI	7.7 ± 7.3	1.7 ± 2.0	1.7 ± 2.0	1.2 ± 2.6	0.3 ± 1.2	0.3 ± 1.4	0.4 ± 1.4	0.4 ± 1.4
		$p < 0.001$	$p < 0.001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

PASI, Psoriasis Area and Severity Index; BSA, body surface area; DLQI, dermatology life quality index.

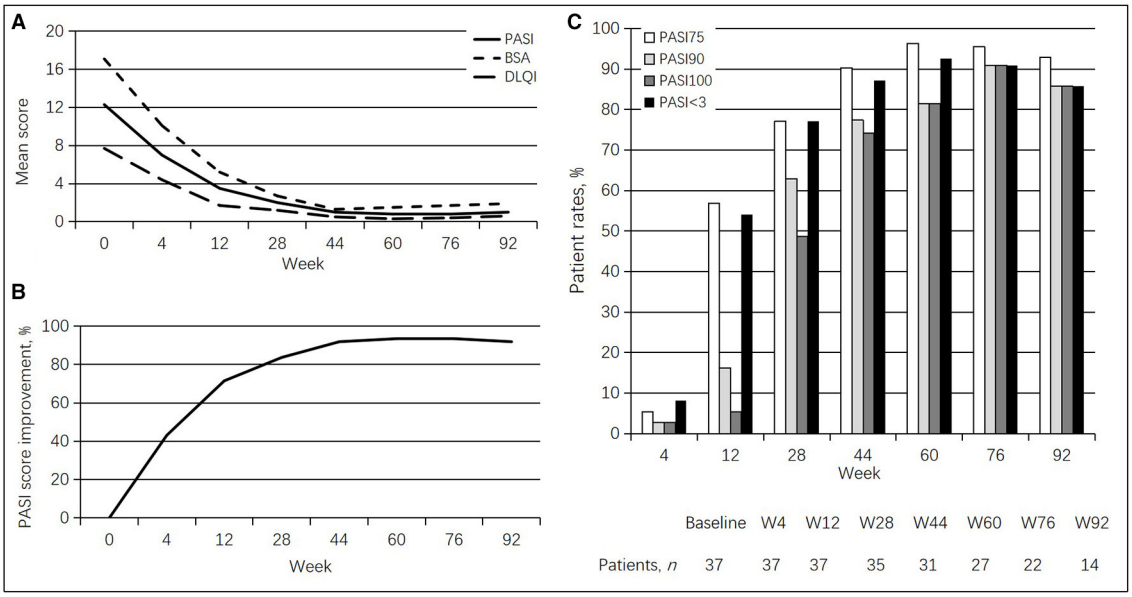

FIGURE 1
Guselkumab's efficacy in psoriasis patients. **(A)** Changes in mean PASI, BSA, and DLQI scores. **(B)** Percentages of mean PASI score improvement. **(C)** Rates of patients achieving PASI 75, PASI 90, and PASI 100 responses at different times.

$p = 0.167$). From weeks 12 to 92, cases with a BMI < 25 had more pronounced PASI score improvement compared with the BMI ≥ 25 group. The BMI < 25 group achieved PASI 75, PASI 90, PASI 100, and PASI < 3.0 responses to a greater extent than the BMI ≥ 25 group from weeks 28 to 92 (Figure 3).

Regarding safety, nine patients (24.3%) had different abnormal results in *HBV markers at baseline*. A total of five cases were anti-HBc positive; two were anti-HBe and anti-HBc positive; one was HBsAg and anti-HBc positive; and one was HBsAg, anti-HBe, and anti-HBc positive. HBV-DNA was normal at baseline. At present, except for one patient who discontinued the treatment for inefficacy, the remaining patients have completed the follow-up. A total of two cases were T-SPOT positive. However, they had no history of tuberculosis or abnormal manifestations of chest computed tomography. There was no hepatitis B or tuberculosis outbreak during follow-up. No cases of serious AEs, injection site reactions, candida infection, malignancy, or cardiovascular events were reported.

5 Discussion

Biologic application in psoriasis has improved beyond expectations in the therapeutic response in cases with moderate-to-severe psoriasis, with excellent safety features (6). PASI 90 and PASI 100 responses are considered the treatment goals in moderate-to-severe psoriasis, suggesting complete or nearly complete clearance, generally related to significant improvement in health-related quality of life. As a human monoclonal antibody targeting the p19 subunit of IL-23, guselkumab was the first antibody of its class to be approved for treating moderate-to-severe plaque psoriasis in adults (7–10). In this study, guselkumab was administered as a 100-mg subcutaneous injection at weeks 0 and 4, and then at 8-week intervals. The current patients had a mean PASI of 12.3 ± 7.1 and a mean disease duration of 16.8 ± 10.5 years at baseline, which corroborated the mean baseline PASI of 13.7 with an average evolution time for their psoriasis of 20 years reported by Rodriguez Fernandez-Freire L et al. in Spain (11). This study achieved a PASI 75 response below that reported

10.3389/fmed.2023.1285972

TABLE 3 Detailed information from some ineffective patients.

No.	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Sex	Male	Male	Female	Female	Male	Female	Male
Age (years)	48	40	45	36	35	40	35
Disease course (years)	22	29	27	26	21	14	12
Involvement of the scalp	Y	Y	N	N	N	Y	N
Involvement of nails	Y	Y	N	N	N	Y	N
Family history of psoriasis	N	N	Y	N	N	N	N
BMI	29.4	22.9	20.8	19.8	16.3	32.7	25.2
HBV markers	Anti-HBe(+) Anti-HBc(+)	N	N	N	N	N	N
Positive T-SPOT	N	N	N	N	N	N	N
Previous systemic	ACI/MTX/	ACI/MTX/	INX	N	INX	N	N
Treatments	ADA/INX	ADA/SEC/IXE					
BSA score (Week 0)	50%	14%	6%	7%	6%	70%	10%
DLQI score (Week 0)	14	5	10	6	6	16	7
PASI score Week 0	31.4	6.8	11	6.8	7.2	35	12
Week 12	18	2.4	10	3.4	3	16	7
Week 20	15	12	11	3.4	1.5	13	5
Week 28	16	UST	11	IXE	1.5	10.7	5
Week 44	Interrupt		SEC		5.5	12	3
Week 52					UST	13.6	3
Week 84						11	3

ACI, acitretin; ADA, adalimumab; BMI, body mass index; BSA, body surface area; DLQI, dermatology life quality index; HBV, hepatitis B virus; INX, infliximab; IXE, ixekizumab; MTX, methotrexate; PASI, Psoriasis Area and Severity Index; T-SPOT, mycobacterium tuberculosis T cell enzyme-linked immunospot tuberculous test; SEC, secukinumab; UST, ustekinumab.

in a previous study (12 weeks); differences were also detected in PASI 90 and PASI 100 responses at week 12 between the two populations. However, there were no differences in PASI 75, PASI 90, or PASI 100 responses following 36 weeks of treatment. Differences in guselkumab's efficacy at the early stage might depend on regions, but more data are required for confirmation. Moreover, a previous study population had a high BMI (overweight + obese > 89.1%) (11). In the present study, the ratio was 43.2%. However, no relationship between treatment effectiveness and weight was found. In this work, patients had a mean follow-up period of 72.3 ± 26.7 weeks (range of 12–108 weeks). The results suggested that guselkumab has excellent long-term effectiveness.

Furthermore, there were 12 cases with previously failed treatment with other biologic agents in this study; we found that less bio-experienced cases achieved a PASI 75 response than bio-naïve cases at 12 and 28 weeks. A total of five patients interrupted the treatment for inefficacy, including four who had previous treatment with other biologic agents. One case with previously failed treatment with both TNF inhibitors and ixekizumab discontinued the treatment at week 28; meanwhile, three cases previously administered TNF inhibitors discontinued the treatment at weeks 44–52. Similarly, Hung et al. (12) reported that previously administered anti-IL-17 cases had less substantial PASI improvement than biologic-naïve cases at the early stage, suggesting that biologic treatment may reduce guselkumab's effectiveness. However, Bonifati et al. (13) examined 9 patients who switched to guselkumab upon anti-IL-17 failure, demonstrating a significant PASI improvement after 3 months of treatment. In a short-term trial of 55 patients (11), 27 cases previously administered anti-IL-17 still had confirmed guselkumab's effectiveness. In addition, guselkumab discontinuation for inefficacy was found in only one patient. Finally, the effectiveness and safety of guselkumab were examined in 44 patients with previously failed anti-IL-17 treatment in a real-life setting up to 52 weeks (4). Significantly improved PASI [PASI decreased from 13.9 ± 8.1 to 0.9 ± 0.7 ($p < 0.001$) at week 52] and BSA [BSA from 24.3 ± 19.6 to 1.3 ± 1.4 ($p < 0.001$)] were detected, and only three cases (6.8%) discontinued the treatment for secondary inefficacy. These data indicated biologic exposure could not influence guselkumab's efficacy.

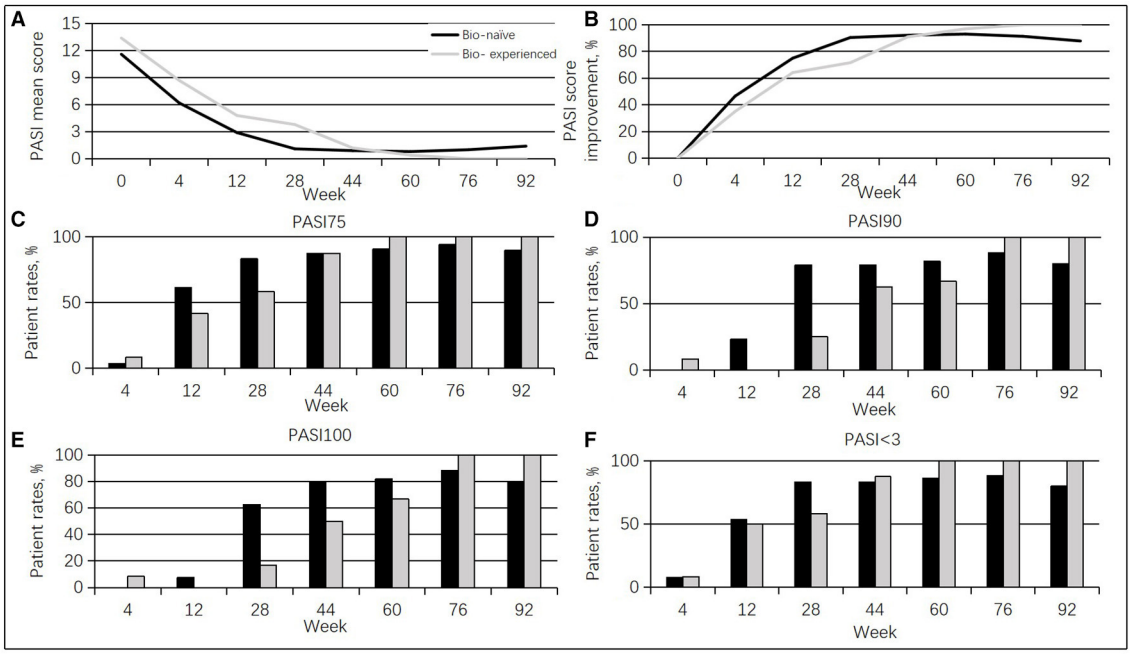

FIGURE 2
Guselkumab's efficacy between the bio-naïve and bio-experienced cases. **(A)** Changes in mean PASI scores. **(B)** Percentages of mean PASI score improvement. **(C)** Rates of cases achieving a PASI 75 response at different times. **(D)** Rates of cases achieving a PASI 90 response at diverse times. **(E)** Rates of cases achieving a PASI 100 response at diverse times. **(F)** Rates of cases achieving a PASI < 3.0 response at diverse times.

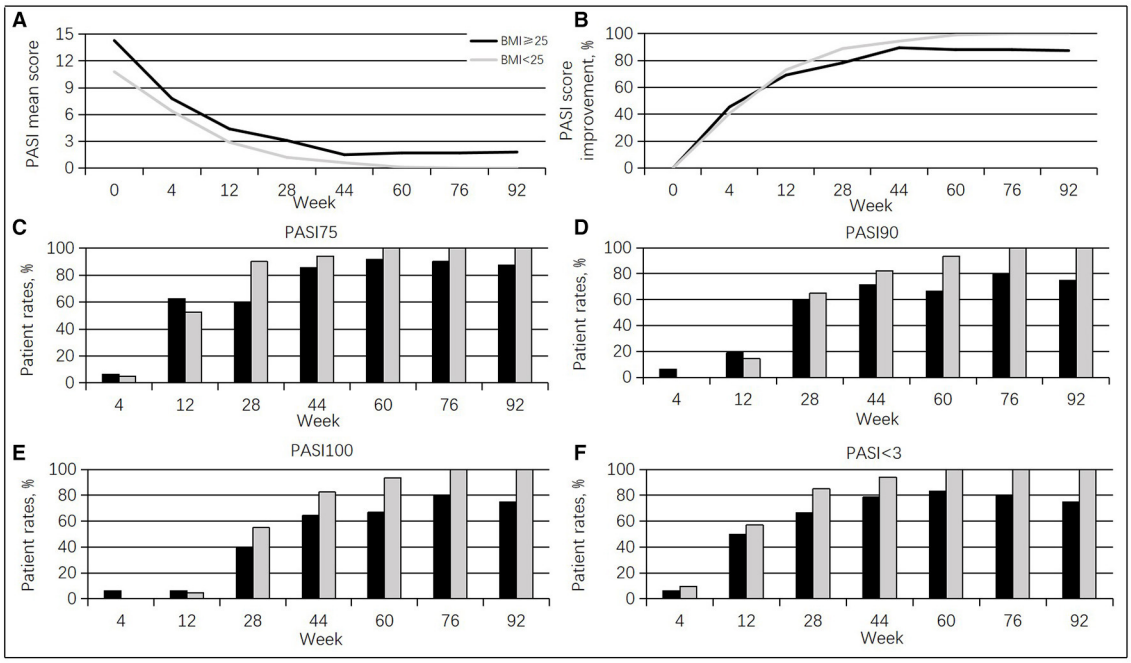

FIGURE 3
Guselkumab's efficacy between the BMI ≥ 25 group and the BMI < 25 groups. **(A)** Changes in mean PASI scores. **(B)** Percentages of mean PASI score improvement. **(C)** Rates of cases achieving a PASI 75 response at different times. **(D)** Rates of cases achieving a PASI 90 response at diverse times. **(E)** Rates of cases achieving a PASI 100 response at diverse times. **(F)** Rates of cases achieving a PASI < 3.0 response at diverse times.

The safety of guselkumab was confirmed since no cases of serious AEs were reported. In this study, nine cases had different abnormal results in the *HBV markers*, and two cases were T-SPOT positive. However, there was no hepatitis B or tuberculosis outbreak in the examined patients. In agreement, randomized controlled trials and real-life studies have reported

that guselkumab has an excellent safety profile (4, 14, 15). The most common adverse event was infection, mostly respiratory tract infections. No patients discontinued the treatment for AE in this study.

The specific pathogenesis of psoriasis remains undefined, although the IL-23/IL-17 axis is considered to play an important role (1). Particularly, IL-23 is a dimer comprising a specific subunit, p19, and a p40 subunit, which is also found in IL-12. These cytokines may activate two T-cell types, including helper T-cell types 1 and 17, which release psoriatic cytokines such as IL-17, interferon-γ, TNF-α, and IL-22 (16, 17). This may explain why some patients previously administered anti-IL-17 still showed an excellent response to guselkumab. Certainly, further investigation is required to help the clinician select the best treatment option based on the patient's particularities, ensuring the highest odds of achieving and maintaining expected clinical outcomes.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Institutional Review Board of Shanghai Skin Disease Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JZ: Conceptualization, Data curation, Formal analysis, Investigation, Writing—original draft, Writing—review & editing. WC: Data curation, Formal analysis, Writing—review & editing. XY: Resources, Writing—review & editing. NY: Resources, Writing—review & editing. YD: Funding acquisition, Resources, Writing—review & editing. YG: Project administration, Resources, Supervision, Validation, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, et al. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J Immunol.* (2002) 168:5699–708. doi: 10.4049/jimmunol.168.11.5699

2. Markham A. Guselkumab: first global approval. *Drugs.* (2017) 77:1487–92. doi: 10.1007/s40265-017-0800-7

3. Blauvelt A, Papp KA, Griffiths CE, Randazzo B, Wasfi Y, Shen YK, et al. Efficacy and safety of guselkumab, an anti-interleukin-23 monoclonal antibody, compared with adalimumab for the continuous treatment of patients with moderate to severe psoriasis: results from the phase III, double-blinded, placebo- and active comparator-controlled VOYAGE 1 trial. *J Am Acad Dermatol.* (2017) 76:405–17. doi: 10.1016/j.jaad.2016.11.041

4. Megna M, Potestio L, Ruggiero A, Camela E, Fabbrocini G. Guselkumab is efficacious and safe in psoriasis patients who failed anti-IL17: a 52-week real-life study. *J Dermatol Treat.* (2022) 33:2560–4. doi: 10.1080/09546634.2022.2036674

5. Yang J, Hu K, Li X, Hu J, Tan M, Zhang M, et al. Effectiveness and safety of guselkumab for the treatment of psoriasis in real-world settings at 52 weeks: a retrospective, observational, multicenter study from China. *Dermatol Ther.* (2023) 13:477–85. doi: 10.1007/s13555-022-00869-w

6. Megna M, Balato A, Napolitano M, Gallo L, Caso F, Costa L, et al. Psoriatic disease treatment nowadays: unmet needs among the "jungle of biologic drugs and small molecules". *Clin Rheumatol.* (2018) 37:1739–41. doi: 10.1007/s10067-018-4090-6

7. Mälkönen T, Nuutinen P, Hallinen T, Soini E, Nissinen R, Wennerström C, et al. Guselkumab treatment outcomes and persistence in a nationwide real-world cohort of patients with plaque psoriasis. *Acta Derm Venereol.* (2022) 102:910. doi: 10.2340/actadv.v101.910

8. Reich K, Gordon KB, Strober BE, Armstrong AW, Miller M, Shen YK, et al. Five-year maintenance of clinical response and health-related quality of life improvements in patients with moderate-to-severe psoriasis treated with guselkumab: results from VOYAGE 1 and VOYAGE 2. *Br J Dermatol.* (2021) 185:1146–59. doi: 10.1111/bjd.20568

9. Blauvelt A, Tsai TF, Langley RG, Miller M, Shen YK, You Y, et al. Consistent safety profile with up to 5 years of continuous treatment with guselkumab: pooled analyses from the phase 3 VOYAGE 1 and VOYAGE 2 trials of patients with moderate-to-severe psoriasis. *J Am Acad Dermatol.* (2022) 86:827–34. doi: 10.1016/j.jaad.2021.11.004

10. Reich K, Armstrong AW, Langley RG, Flavin S, Randazzo B, Li S, et al. Guselkumab versus secukinumab for the treatment of moderate-to-severe psoriasis (ECLIPSE): results from a phase 3, randomised controlled trial. *Lancet.* (2019) 394:831–9. doi: 10.1016/S0140-6736(19)31773-8

11. Fernandez-Freire LR, Galan-Gutierrez M, Armario-Hita JC, Perez-Gil A, Ruiz-Villaverde R. Guselkumab: short-term effectiveness and safety in real clinical practice. *Dermatol Ther.* (2020) 33:e13344. doi: 10.1111/dth.13344

12. Hung Y, Lin Y, Chiu H, Huang Y. Impact of previous biologic use and body weight on the effectiveness of guselkumab in moderate-to-severe plaque psoriasis: a real-world practice. *Ther Adv Chronic Dis.* (2021) 12:12. doi: 10.1177/20406223211046685

13. Bonifati C, Morrone A, Cristaudo A, Graceffa D. Effectiveness of anti-interleukin 23 biologic drugs in psoriasis patients who failed anti-interleukin 17 regimens. *A real-life experience Dermatol Ther.* (2021) 34:e14584. doi: 10.1111/dth.14584

14. Reich K, Papp KA, Armstrong AW, Wasfi Y, Li S, Shen YK, et al. Safety of guselkumab in patients with moderate-to-severe psoriasis treated through 100 weeks: a pooled analysis from the randomized VOYAGE 1 and VOYAGE 2 studies. *Br J Dermatol.* (2019) 180:1039–49. doi: 10.1111/bjd.17454

15. Reich K, Armstrong AW, Foley P, Song M, Wasfi Y, Randazzo B, et al. Efficacy and safety of guselkumab, an anti-interleukin-23 monoclonal antibody, compared with adalimumab for the treatment of patients with moderate to severe psoriasis with randomized withdrawal and retreatment: results from the phase III, double-blind, placebo- and active comparator-controlled VOYAGE 2 trial. *J Am Acad Dermatol.* (2017) 76:418–31. doi: 10.1016/j.jaad.2016.11.042

16. Kim J, Krueger JG. The immunopathogenesis of psoriasis. *Dermatol Clin.* (2015) 33:13–23. doi: 10.1016/j.det.2014.09.002

17. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med.* (2009) 361:496–509. doi: 10.1056/NEJMra0804595

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Dermatologic toxicities in epidermal growth factor receptor: a comprehensive pharmacovigilance study from 2013 to 2023

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Epidermal growth factor receptor inhibitors (EGFRIs) induced cutaneous toxicity is a common adverse event (AE), although it is not as severe as major cancers, we still need to pay enough attention to them. Therefore, it is necessary to evaluate the diversity of EGFRI class drugs. The objective of this study was to conduct a scientific and systematic investigation into the correlation between EGFRI and cutaneous toxicities. The data accessed from the FDA adverse event reporting system database (FAERS) encompass a time frame spanning from January 2013 to March 2023. By utilizing reporting odds ratios (RORs), information components (ICs), proportional reporting ratios (PRRs), and chi-squared (χ^2), the relationship between drugs and adverse reactions was evaluated through disproportionality analysis. Within the FAERS database, a total of 29,559 skin adverse events were recorded. A robust indication of the correlation between EGFRI and elderly patients (≥65 years) was identified. Among EGFRIs, erlotinib accounted for the largest proportion of skin adverse events (39.72%). Rash, dry skin, and pruritus ranked top among all preferred terms, and signals such as rash, skin lesions, and acneiform dermatitis were detected in every single drug. Clinicians should guide patients customize the treatment plan for each patient.

KEYWORDS

epidermal growth factor receptor inhibitors, cutaneous toxicity, pharmacovigilance, data mining, FAERS

1 Introduction

Epidermal growth factor receptor (EGFR) belongs to the receptor tyrosine kinase family (ErbB) that regulates tumor cell differentiation, survival, and proliferation. The EGFR family is also known as the EGFR tyrosine kinase family, which includes four receptor tyrosine kinases, such as EGFR/HER1, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4. Among them, EGFR is the first cell surface receptor found to be directly related to tumorigenesis. EGFR is also known as HER1, ErbB1, mutation, or overexpression generally cause tumors. Human epidermal growth factor receptor 2 (HER-2), a receptor that exists on the surface of breast cancer cells and is closely related to the occurrence and development of breast cancer (1–4). Epidermal growth factor receptor inhibitors (EGFRIs) are now well established as effective

agents for the treatment of various cancers (5), which include monoclonal antibodies(mAbs): cetuximab, necitumumab, and panitumumab; tyrosine kinase inhibitors (TKIs): afatinib, dacomitinib, erlotinib, gefitinib, and Osimertinib; and Tyrosine multikinase inhibitors: lapatinib and vandetanib (6–8).

EGFRI has shown significant curative effect, changing the prospects for non-small cell lung cancer (NSCLC) (9), metastatic colorectal cancer (10), breast cancer (11), thyroid cancer (12), and rectal cancer (rectal cancer) (13). In the IPASS study, first-line treatment with gefitinib significantly prolonged progression-free survival in patients with lung adenocarcinoma compared with paclitaxel plus carboplatin (14). The National Comprehensive Cancer Network (NCCN) has recommended mAbs for the first-line treatment of patients with wild-type RAS metastatic colorectal cancer (mCRC) (15). EGFRI can also be combined with other drugs to treat cancer, such as the combination of EGFRI and immune checkpoint inhibitors (ICI) in the treatment of non-small cell lung cancer (NSCLC) (16).

However, a first-line cohort study of icotinib in 152 patients with EGFR-mutated advanced NSCLC reported a major safety profile, with rash and paronychia occurring in 43.4 and 5.9% of patients (17), respectively. The indications of EGFRIs continue to expand to different cancers and different stages of the disease. Skin diseases can be divided into early and late stages, with the former being better known and usually easier to prevent and treat. Later in treatment, other skin toxicities such as xerosis and eczema, with accompanying pruritus, may occur (18). And due to its specific mechanism of action, cutaneous toxicity has become the most common adverse drug reaction (ADR) of EGFRIs, which usually leads to a decrease in the quality of life of patients. Gefitinib-induced cutaneous toxicity led to dose discontinuation in 6.9% of patients (19). In one survey, 10% of patients admitted to skipping doses due to side effects (20). More attention and awareness of adverse event-induced EGFR inhibitor-related cutaneous adverse events is needed for prevention and treatment.

FAERS is a free and public voluntary database for collecting post-marketing drug adverse event information and is often used to carry out signal mining research on drug adverse events. The FAERS database can receive more than one adverse event information report about drugs and medical equipment every year. The adverse drug event information is spontaneously reported by drug manufacturers, hospital medical staff, pharmacists, and other professionals, as well as patients. Therefore, the information in the FAERS database is from the real world. Real-world safety data on EGFRI-related skin AEs are currently lacking, and there are inherent differences in activity and dermal toxicity among EGFRIs. This study outlines the safety profile of EGFRI through pharmacovigilance analysis and provides guidance for clinicians and patients so as to be familiar with how to prevent or improve skin AEs.

2 Methods

2.1 Data extraction

This study retrospectively mined and analyzed the skin toxicity AEs of EGFRI in FAERS from 1 January 2013 to 31 March 2023 through data mining, and a total of 41 quarterly report documents were screened, although a large proportion of these data comes from the United States, Europe, and the Asia-Pacific region, which also account for a large proportion. The following seven types of files make up the FAERS database: patient demographic and administrative information (DEMO), drug/biological information (DRUG), adverse events (REAC), patient outcomes (OUTC), reporting sources (RPSR), reporting drug therapy start and end dates (THER), and indications (INDI). These tables can be considered distinct domains. For instance, the tables within DEMO belonging to different years and quarters can be categorized as a single domain. Once the structure is standardized, tables from the same domain can be merged together. By integrating the data of specific years and quarters in each domain to facilitate subsequent management and analysis. In addition, there is a special file category named DELETED, which include the information about the CASEID of expurgated reports. These files exist in several certain quarters. All types of documents can be accessed from the FDA website.[1]

According to the recommendation of FDA, we removed duplicate records by two steps: (1) selecting the greater PRIMARYID when the CASEID and EVENT_DT were the same and (2) selecting the latest EVENT_DT when the CASEID were the same. Then selected the drugs whose "ROLE_COD" fields are "PS" (Primary Suspect). Upon completing the screening process, we proceed with selecting six major file categories: DEMO, DRUG, REAC, OUTC, THER, and INDI. These categories serve as individual tables that can be linked through the common primary keys "PRIMARYID" and "CASEID" to consolidate patient and medication information. The DEMO, REAC, and OUTC tables are merged using the aforementioned primary keys. Additionally, the DRUG, INDI, and THER tables possess primary keys such as "DRUG_SEQ," "INDI_DRUG_SEQ," and "DSG_DRUG_SEQ," respectively. Although these primary keys serve the same purpose, they have different variable names across the tables. By unifying the primary keys in these three tables, they can be merged together so that, the two large tables are merged into a single consolidated table for comprehensive analysis. This merging process is based on the two primary keys, "primaryid" and "caseid." Finally, removed the wrongly uploaded report according to the CASEID in the DELETED folder (see Figure 1). SAS 9.4 (SAS Institute Inc., Cary, NC, United States) was used to integrate and process the raw data, and RStudio software was used to calculate the signal value for each group of clinical characteristics and visualize.

2.2 Target drugs and AEs

Our search in the FAERS database was conducted specifically for FDA-approved EGFRIs available on the market, encompassing mAbs (cetuximab, necitumumab, and panitumumab), TKIs (gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib), VEGF/VEGFR inhibitors (vandetanib), and EGFR inhibitors/HER2 inhibitors (lapatinib) (Supplementary Table S1) by using trade and generic names listed in the National Center for Biotechnology Information (NCBI).

Adverse events with EGFRI-induced cutaneous toxicity in the FAERS database were defined as cases where the treatment regimen

1 https://fis.fda.gov/extensions/FPD-QDE-FAERS/FPD-QDE-FAERS.html

included drugs in the EGFRIs class and a skin-related adverse reaction in the SOC classification occurred. The SOC consists of six High Level Group Terms (HLGT), which includes epidermal and dermal conditions (MedDRA code 10014982), pigmentation disorders (MedDRA code 10035023), skin and subcutaneous tissue disorders NEC (MedDRA code 10040790), skin and subcutaneous tissue infections and infections (MedDRA code 10040792), skin appendage conditions (MedDRA code 10014982), and skin neoplasms malignant and unspecified (MedDRA code 10040785). All PTs were selected under the six HLGTs. Furthermore, the FAERS database recorded a single adverse event report for EGFRI in relation to cutaneous toxicity as one instance of data, despite the possibility of multiple adverse event reports being filed for the same patient, owing to the database's structural and variable characteristics.

2.3 Time to onset

We analyzed the occurrence time of EGFRI-induced cutaneous toxicity. The occurrence time is the interval between START_DT (the time start therapy) and EVENT_DT (adverse event occurrence date). Incorrectly entered reports were excluded according to two exclusion criteria: (1) The value of START_DT or EVENT_DT is miss, (2) EVENT_DT is incorrect (START_DT later than EVENT_DT). The Kaplan–Meier curve can present the first quantile time, the median time, and the third quantile time and can be used to describe the changes in the incidence of AE. Discriminate the statistical difference in AE occurrence time between different EGFRI drug treatment regimens, mainly using the Kruskal–Wallis test and Wilcoxon rank-sum test to calculate.

2.4 Statistical analysis

2.4.1 Disproportionality analysis

Disproportionality analysis is one of the most basic data mining methods for adverse drug reaction monitoring, which can also be called case-non-case analysis. Disproportionality analysis compared selected ADR proportions for a single drug or drug class with the same ADR proportions reported for other drug groups (21). There are two primary types of proportional imbalance methods that exist nowadays. One method is based on frequency, called the frequency method, and the other is based on Bayes' theorem, also known as the Bayesian method. The former method, comprising the reporting odds ratio method and the proportional reporting ratio method, offers the advantages of low computational complexity, low time consumption, and independence from the need for *a priori* information in the model. Additionally, it exhibits resilience to non-selective underreporting of drugs or adverse drug reactions, thus not impacting the calculated ROR values in comparison with the patient population experiencing ADRs. However, it cannot be calculated when the denominator is zero, and it is easily affected by individual values. When the frequency is small, the statistics fluctuate greatly. The advantages and disadvantages of the PRR method are similar to those of the ROR method, but there is a fundamental difference. The ROR method calculates the statistic as

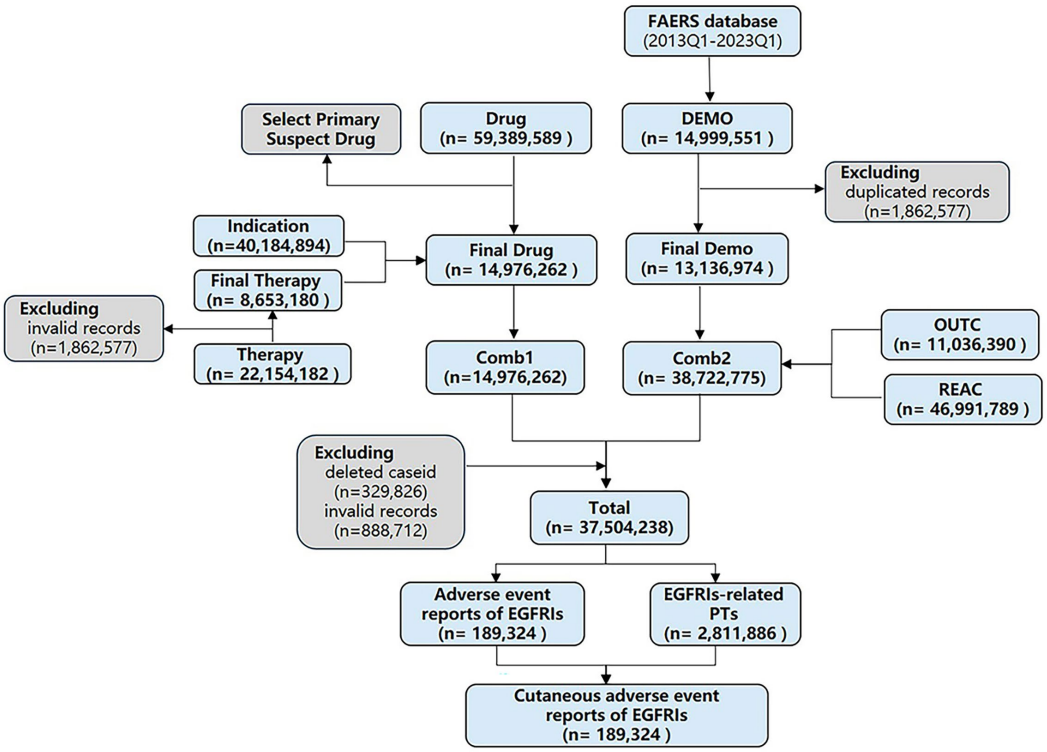

FIGURE 1
The flow diagram of selecting EGFRI-associated AEs from FAERS database.

the odds ratio (OR), while the PRR method calculates the relative hazard ratio (RR). The chi-squared test can be used in conjunction with the PRR method. The BCPNN method not only considers the information of probability asymmetry but also considers the information of the overall sample, which is more flexible and stable than the frequency method. In addition, since the model variable is not settled, the prior distribution corresponding to the variable will use a different distribution according to the change of the data, so there is no restriction on conditions for usage. Given the absence of a universally accepted "gold standard," it is important to recognize that each method possesses its own unique set of advantages and disadvantages concerning its applicability and feasibility in varying scenarios. Therefore, in practical applications, the four methods should be combined to comprehensively evaluate the results of pharmacovigilance signals.

The detailed information for calculating the AE reports of the target drug and other drugs can be found in 2×2 Matrix Table (Table 1). In order to investigate potential correlations between EGFRI and cutaneous toxicities, four data mining methods were employed: the reporting odds ratio (ROR) method (22), proportional reporting rate (PRR) method, chi-squared (χ^2) method (23), and the Bayesian confidence propagation neural network's (BCPNN) information component (IC) method (24).

2.4.1.1 Reporting odds ratio method

The ROR can be calculated using the following formula

$$ROR = \frac{a/c}{b/d} = \frac{ad}{bc}$$

Calculating the standard error of ln (ROR) and 95% confidence interval for the ROR involves the following steps

$$SE(lnROR) = \sqrt{\left(\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}\right)}$$

$$95\%CI = e^{\ln(ROR) \pm 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$$

2.4.1.2 Proportional reporting rate (PRR) method

The PRR measure can be expressed as

$$PRR = \frac{(a)/(a+b)}{(c)/(c+d)}$$

2.4.1.3 Chi-squared (χ^2) method

The χ^2 can be expressed as

$$\chi^2 = \Sigma(O-E)2/E$$

where O represent the observed count ($O = a$), and E represent the expected count [$E = (a+b)(a+c)/(a+b+c+d)$].

2.4.1.4 BCPNN method

The calculation of its variance can be derived from Bayes' theorem as

$$E(ICij) = \log_2 \frac{(c_{ij}+\gamma_{ij})(C+\alpha)(C+\beta)}{(C+\gamma)(c_i+\alpha_i)(C_j+\beta_j)}$$

$$V(ICij) = \frac{\frac{C-c_{ij}+\gamma-\gamma_{ij}}{(C_{ij}+\gamma_{ij})(1+C+\gamma)} + \frac{C-c_i+\alpha-\alpha_i}{(c_i+\alpha_i)(1+C+\alpha)} + \frac{C-c_j+\beta-\beta_i}{(c_i+\beta_j)(1+C+\beta)}}{(\log 2)^2}$$

where

$$\gamma = \gamma_{ij}\frac{(C+\alpha)}{(C_i+\alpha_i)}\cdot\frac{(C+\beta)}{(C_j+\beta_j)}$$

and $\gamma_{ij} = 1; \alpha_i = 1, \alpha = 2, \beta_j = 1, \beta = 2$, C is the total number of reports in the database, C_{ij} the number of combinations between an EGFRI drug (i) and the dermatologic toxicities drug reaction (j), C_i the total number of reports on EGFRI drugs (i) in the database and C_j the total number of reports on the dermatologic toxicities ADR (j) in the database.

The calculation of IC can be obtained as.

$$IC = \log_2\left[\frac{P(Drug|ADR)}{P(ADR)}\right] = \log_2\left[\frac{P(Drug,ADR)}{P(Drug)P(ADR).}\right]$$

There are diverse criteria for signal detection; ROR025 and IC025 represent the lower limit of the 95% confidence interval of ROR and IC. The signal was defined as positive if ROR025 was greater than 1 or IC025 was greater than 0. Besides, the signal was defined PRR is not less than 2 and chi-squared is not less than 4. Three or more cases should be met for all the criteria.

TABLE 1 2*2 matrix table for EGFRI-induced dermatologic toxicities.

	Cutaneous toxicity-related reports	Non-cutaneous toxicity-related reports
Target drug-related reports	a	b
Not target drug	c	d

TABLE 2 Demographic information of EGFRI-induced adverse events.

		Cutaneous toxicities AEs	Cutaneous toxicities AEs			
		With EGFRIs	With all other drug	IC025	ROR025	PRR (χ^2)
		(29559)	(2978563)			
Sex	Male	10,805(36.55%)	775,936(27.89%)	**0.35**	**1.45**	1.31(**776.80**)
	Female	17,047(57.67%)	1,780,249(63.98%)	−0.18	0.75	0.90(**180.45**)
	TS	0(0.00%)	13(0.00%)			
	Unisex	0(0.00%)	20(0.00%)			
	Unknown	5(0.02%)	752(0.03%)			
	Miss*	1,589(5.33%)	216,711(7.68%)			
Age	Elderly	9,954(33.67%)	494,970(18.63%)	**0.81**	**2.16**	1.81(**3480.90**)
	Adult	8,846(29.92%)	1,230,517(43.96%)	−0.58	0.53	0.68(**1302.52**)
	Adolescent	14(0.05%)	111,167(3.97%)			
	Child	17(0.06%)	49,713(1.78%)			
	Infant	2(0.06%)	3,779(0.14%)			
	Neonate	0(0.00%)	2004(0.07%)			
	Miss*	10,726(36.29%)	844,142(31.44%)			
Outcome	Other Serious	11,501(38.91%)	865,428(31.10%)	**0.33**	**1.44**	1.25(**565.19**)
	Hospitalization	4,216(14.27%)	322,919(11.61%)	**0.24**	**1.22**	1.23(**175.60**)
	Life-threatening	414(1.40%)	29,162(1.04%)	**0.30**	**1.24**	1.34(**34.18**)
	Disability	340(1.15%)	46,099(1.66%)	−0.63	0.71	0.69(**44.97**)
	Death	1,518(5.18%)	32,041(1.15%)	**2.09**	**4.63**	**4.48(3846.70)**
	RI	32(0.11%)	4,893(0.18%)	−1.21	0.43	0.62(**7.55**)
	Congenital anomaly	20(0.07%)	1992(0.06%)	−1.77	0.29	0.95(0.06)
Country	US	17,042(57.32%)	1,872,444(67.30%)	−0.27	0.61	0.85(**466.01**)
	JP	3,399(11.20%)	49,843(1.79%)	**2.53**	**6.79**	**6.34(14164.07)**
	CN	856(2.90%)	19,696(0.71%)	**1.86**	**3.86**	**4.04(1857.719)**
	FR	637(2.16%)	76,531(2.75%)	−0.48	0.71	0.77(**41.24**)
	DE	569(1.93%)	53,631(1.93%)	−0.14	0.91	0.99(0.10)
	BR	566(1.91%)	30,742(1.10%)	**0.64**	**1.59**	1.71(**162.85**)
	CO	510(1.72%)	11,095(0.40%)	**1.91**	**3.96**	**4.27(1210.078)**
	IT	462(1.56%)	33,427(1.24%)	**0.18**	**1.14**	1.25(**22.21**)

IC, information component; IC025, the lower end of the 95% confidence interval of IC; ROR, reporting odds ratio; ROR025, the lower end of the 95% confidence interval of ROR; PRR, proportional reporting ratio; χ^2, chi-squared. Bold values: index that meet the criteria for signal detection. *Unreported or lost reports.

3 Results

3.1 Descriptive analysis

After processing, the FAERS database recorded a total of 37,504,238 data between 2013 and 2023. Among these records, a total of 189,324 adverse events attributed to EGFRIs were reported, with 29,559 reports specifically associated with cutaneous toxicity. Demographic information about the patients is summarized in Table 2.

In all reported cases of cutaneous toxicity induced by EGFRI, females accounted for a higher percentage than males (57.67% versus 36.65%). But by further analysis, signal was only detected in males (ROR025 = 1.45, IC025 = 0.35, PRR = 1.31, χ^2 = 776.80). Significant differences were found among various subgroups in the study, indicating a greater proportion of elderly (> 65 years old, 33.67%) compared with non-elderly (29.92% for adults, 0.05% for adolescents, and 0.06% for children), and the difference is statistically significant (ROR025 = 2.16, IC025 = 0.81, PRR = 1.81, χ^2 = 3480.90), which probably be attributed to degenerative changes in the elderly. Other serious medical events, hospitalization, and life-threatening emerged as the most commonly reported outcome events. Other serious medical events (ROR025 = 1.44, IC025 = 0.33, PRR = 1.25, χ^2 = 569.19), hospitalization (ROR025 = 1.22, IC025 = 0.24, PRR = 1.23, χ^2 = 175.60), life-threatening (ROR025 = 1.24, IC025 = 0.30, PRR = 1.34, χ^2 = 34.18), and death

(ROR025 = 2.09, IC025 = 4.63, PRR = 4.48, χ^2 = 3846.70) indicate the life-threatening nature of potential EGFRI-related cutaneous toxicity. The United States (57.32%) represented the most frequently mentioned regions or countries, with Japan (11.20%), China (2.90%), France (2.16%), and Germany (1.93%) behind.

For all reports concerning EGFRI-related cutaneous toxicity, age and sex were analyzed separately for each class of EGFRI to investigate their relationship (Table 3). Signals were detected for all single agents in the elderly population, with a greater proportion of signals detected in males compared with females among all single drugs.

Across all EGFRI regimens, discernible differences were identified in specific adverse events associated with cutaneous toxicity. The top reported reports were rash (8,040 cases, 27.20%), dry skin (1934 cases, 6.54%), itching (1722 cases, 5.83%), acne like dermatitis (1,274 cases, 4.31%), acne (1,198 cases, 4.05%), alopecia (1,102, 3.73%), paronychia (1,004, 3.40%), erythema (809, 2.74%), skin lesions (651, 2.20%), skin exfoliation (611, 2.07%), and skin toxicity (591, 2.00%) (Table 4). Collectively, these comprised 64.07% of the total.

3.2 Cutaneous toxicity AE profile in treatment protocols

Overall, while not all EGFRIs exhibited associations with cutaneous AEs, signals were identified when analyzing each drug individually in relation to cutaneous toxicity AEs. Among the analysis of all EGFRIs and individual EGFRIs, erlotinib and panitumumab similarly demonstrated the most robust statistical association with EGFRI-related cutaneous AEs according to the value of IC025, ROR025, and PRR (χ^2). Panitumumab has higher IC025, ROR025, and PRR than erlotinib, but erlotinib has more counts and higher χ^2 than panitumumab (Table 5).

IC025, PRR, and ROR025 between PTs for drugs and adverse events are depicted in Figures 2–4, respectively. Erlotinib had the broadest range of cutaneous AEs, with 74 PTs monitored for signals ranging from blood blister (IC025 = 0.06, ROR025 = 1.26, PRR = 2.53) to dermatitis acneiform (IC025 = 5.16, ROR025 = 43.80, PRR =48.64). A total of 51 PTs were found to be significantly associated with afatinib treatment, ranging from eyelid disorder (IC025 = 0.04, ROR025 = 20.3, PRR = 5.44) to paronychia (IC025 = 6.83, ROR025 = 221.43, PRR = 171.51). A total of 48 PTs were found to be significantly associated with cetuximab, ranging from nail bed disorder (IC025 = 0.02, R0R025 = 4.50, PRR = 14.03) to radiation skin injury (IC025 = 6.09, R0R025 = 264.61, PRR = 260.27). A total of 70 PTs were monitored as signals for panitumumab ranging from skin hyperpigmentation (IC025 = 0.10, R0R025 = 1.31, PRR = 2.63) to dermatitis acneiform (IC025 = 6.74, R0R025 = 168.01, PRR = 182.12). Lapatinib had 32 PTs monitored as signals ranging from photosensitivity reaction (IC025 = 0.07, ROR025 = 1.24, PRR = 2.38) to onychalgia (IC025 = 3.23, ROR025 = 47.70, PRR = 67.91). Osimertinib had 25 PTs monitored as signals ranging from Stevens–Johnson syndrome (IC025 = 0.34, ROR025 = 1.36, PRR = 2.10) to paronychia (IC025 = 4.59, ROR025 = 36.50, PRR = 32.12). And PTs detected in gefitinib, vandetanib, dacomitinib, and necitumumab were 21, 16, 11, and 6, respectively. Signs of rashes, skin lesions, and acneiform dermatitis were detected in all drugs.

3.3 Time to onset

In total, 7,933 EGFRI-related cutaneous toxicity AEs were reported. Displayed in Figure 5 are the Kaplan–Meier curves, illustrating the onset time of adverse events (AEs) for different EGFRIs. The corresponding risk table, situated at the bottom of Figure 5, presents the number of individuals followed at each time point. Significantly different AE onset times among the various EGFRIs were observed following the Kruskal–Wallis test, with a value of p of less than 0.0001.

The median time to onset was 25 days, accompanied by an interquartile range (IQR) spanning from 7 to 108 days. Detailed information regarding the time to onset of cutaneous toxicity AEs for each specific EGFRI can be found in Table 6. Necitumumab exhibited the shortest median time to AE onset, recorded at 9 days with IQR of 5–16 days. Conversely, gefitinib demonstrated the longest median time to AE onset, reported as 57 days, with an IQR of 14–182 days.

Based on the Wilcoxon rank-sum test, pairwise comparisons were conducted to assess difference among different drugs. In terms of the time to onset of AEs, panitumumab demonstrated a significantly shorter duration than gefitinib, but not significantly shorter than vandetanib and erlotinib. Conversely, lapatinib was significantly shorter than osimertinib and cetuximab. As for dacomitinib, it did not show a significant difference compared with erlotinib (Figure 6).

4 Discussion

We analyzed EGFRI-related adverse events in the FAERS database by ROR, PRR, chi-squared, and BCPNN methods and identified associations and specificities between EGFRI and related skin toxicity AEs to delineate the safety profile. Launch dates for EGFRIs vary, with gefitinib first being on the market in 2003. However, based on clinical use, second- and third-generation tyrosine kinase inhibitors are currently the most used and widely available, which are listed after 2012. Redundant data in the analysis amplify the likelihood of probability errors in the results. Encompassing both currently utilized and previously marketed EGFRIs, we conducted pharmacovigilance studies utilized over 30 million records to investigate EGFRI-related skin toxicity within a specific time period.

Most clinical trials of EGFRIs assessed clinical outcomes, although some assessed AEs but not in sufficient detail, and only the grading degree and brief descriptions of these AEs have been provided. The published phase III studies did not include detailed information on the prevention of cutaneous toxicity. On the other side, the impact on pathology-related QoL is so far unclear. Prior investigations have presented evidence indicating that EGFRI escalates the risk of toxicity at the organ system level, encompassing pulmonary toxicity (25)and cardiotoxicity (26).

The analysis of AE time intervals reveals that for most EGFRI-related skin toxicity AEs, they occur within the first few days to 2 months after administration. Subsequently, based on the Kaplan–Meier curves, the incidence rate gradually descends. Adverse events persist in patients throughout the subsequent treatment, extending for months or even years (27). The median time from post-administration to AE occurrence, representing the point at which the AE incidence reaches 50%, was similar for cetuximab, lapatinib, and osimertinib, occurring on day 7 after administration. However, considering the

TABLE 3 The signals of cutaneous adverse events in EGFRI-related age and sex groups.

			Afatinib	Cetuximab	Dacomitinib	Erlotinib	Gefitinib	Lapatinib	Necitumumab	Osimertinib	Panitumumab	Vandetanib
Age group	Elderly	Count	1,501	1,388	71	3,548	475	773	47	935	1,590	120
		IC025	**1.26**	**0.81**	**0.12**	**0.58**	**1.30**	**0.15**	**0.69**	**1.11**	**0.90**	**0.20**
		ROR025	**3.69**	**2.19**	**1.48**	**1.71**	**4.08**	**1.21**	**2.37**	**3.09**	**2.41**	**1.28**
		PRR (χ^2)	**2.53(1388.05)**	1.86(**550.06**)	1.93(**31.67**)	1.54(**669.17**)	**2.74(525.18)**	1.20(**26.17**)	**2.36(36.79)**	**2.32(699.79)**	1.97(**759.30**)	1.41(**14.26**)
	Adults	Count	1,098	1,648	119	2,137	275	991	37	499	1776	266
		IC025	−0.44	−0.17	**0.54**	−1.40	−0.76	**0.03**	−0.89	−1.06	−0.17	**0.19**
		ROR025	0.63	0.85	**1.85**	0.26	0.47	**1.10**	0.46	0.35	0.85	**1.50**
		PRR (χ^2)	0.79(**58.91**)	0.95(**5.02**)	1.38(**12.76**)	0.40(**1951.25**)	0.68(**41.22**)	1.10(**10.23**)	0.80(1.92)	0.53(**207.52**)	0.94(**5.94**)	1.34(**22.94**)
Sex group	Male	Count	947	2,468	88	3,954	261	33	78	465	2,203	233
		IC025	**0.02**	**1.10**	**0.31**	**0.18**	−0.15	−4.56	**0.94**	−0.49	**0.83**	**0.70**
		ROR025	**1.05**	**4.05**	**1.60**	**1.21**	0.91	0.03	**4.73**	0.66	**2.64**	**2.47**
		PRR (χ^2)	1.08(**5.21**)	**2.22(1647.55)**	1.60(**19.78**)	1.16(**83.13**)	1.02(0.15)	0.06(**507.43**)	**2.62(78.25)**	0.78(**30.07**)	1.84(**862.90**)	1.92(**107.40**)
	Female	Count	2,106	1,260	105	7,788	625	1879	19	1,533	1,466	160
		IC025	−0.02	−1.11	−0.61	−0.05	−0.05	**0.44**	−2.53	**0.06**	−0.98	−1.07
		ROR025	1.05	0.24	0.48	0.94	1.06	**6.13**	0.07	**1.30**	0.27	0.26
		PRR (χ^2)	1.05(**4.98**)	0.50(**639.08**)	0.84(3.24)	1.00(0.01)	1.07(3.18)	1.45(**269.14**)	0.28(**35.02**)	1.13(**22.37**)	0.54(**578.82**)	0.56(**55.98**)

This table shows the signals of patients' sex and age with dermatologic toxicities using EGFRIs. Count, number of records. ROR, reporting odds ratio; IC, information component; IC025, the lower limit of the 95% confidence interval of IC; ROR025, the lower end of the 95% confidence interval of ROR; PRR, proportional reporting ratio; χ^2, chi-squared. Bold values: indexes that meet the criteria for signal detection.

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TABLE 4 Distribution of dermatologic toxicities related to EGFRIs drugs.

Dermatologic toxicities	*N*	Percentage
Rash	8,040	27.20
Dry skin	1934	6.54
Pruritus	1722	5.83
Dermatitis acneiform	1,274	4.31
Acne	1,198	4.09
Alopecia	1,102	3.73
Paronychia	1,004	3.40
Erythema	809	2.74
Skin disorder	651	2.22
Skin exfoliation	611	2.07
Skin toxicity	591	2.00
Others	10,390	35.48

TABLE 5 Signals for overall and each class of EGFRIs drugs with dermatologic toxicity AEs.

	(a)	(b)	(c)	(d)	ROR025	IC025	PRR(χ^2)
Total	29,559	159,765	2,782,327	34,858,945	**2.29**	**1.05**	**2.11 (17043.8)**
Afatinib	3,157	19,586	2,810,304	34,997,549	**1.93**	**0.85**	1.87**(1269.95)**
Cetuximab	3,974	21,361	2,807,912	34,997,359	**2.24**	**1.03**	**2.11 (2321.60)**
Dacomitinib	196	1,274	2,811,806	35,017,320	**1.65**	**0.62**	1.79**(68.85)**
Erlotinib	12,267	57,154	2,807,667	34,953,508	**2.62**	**1.22**	**2.38 (9720.43)**
Gefitinib	922	9,345	2,811,142	35,009,187	**1.15**	**0.17**	1.21**(33.05)**
Lapatinib	2,043	13,185	2,810,833	35,004,535	**1.85**	**0.78**	1.80**(732.53)**
Necitumumab	106	512	2,811,849	35,018,129	**2.09**	**0.88**	**2.31 (78.54)**
Osimertinib	2,147	29,111	2,809,648	34,989,690	1.04	−0.01	1.01(1.07)
Panitumumab	4,294	19,167	2,810,531	34,996,604	**2.70**	**1.25**	**2.46 (3720.20)**
Vandetanib	453	3,130	2,811,669	35,015,344	**1.63**	**0.62**	1.70**(130.82)**

(a) The number of skin AEs reported for EGFRIs. (b) The number of any other AEs reported for EGFRIs. (c) The number of any skin AEs for other drugs. (d) The number of other AEs reported for other drugs. ROR, reporting odds ratio; IC, information component; IC025, the lower limit of the 95% confidence interval of IC; ROR025, the lower end of the 95% confidence interval of ROR; PRR, proportional reporting ratio; χ^2, chi-squared. Bold values: indexes that meet the criteria for signal detection.

third quartile time, which indicates the time required for AE incidence to reach 75% after administration, cetuximab required 50 days, osimertinib took 64 days, and lapatinib necessitated the longest duration at 154 days. It signifies that the risk of skin-related AEs in long-term use of lapatinib is low, possibly owing to its metabolic mechanism. Lapatinib has a broad metabolic distribution, and although multiple metabolite forms are excreted, only one can inhibit EGFR (28). Among mAbs, panitumumab exhibited the longest duration, the median time to AE onset was 42 days for panitumumab and cetuximab had shorter median times of 9 and 19 days, respectively. This observation may be attributed to the fact that panitumumab belongs to the IgG2 isotype and primarily functions by blocking EGFR without participating in immune mediation. On the contrary, IgG type 1 antibodies like cetuximab mediate cytotoxicity mediated by cell that is antibody-dependent (29, 30). In addition, despite being the latest mAbs drug selected for this study, necitumumab exhibited the earliest onset time of AEs, with the first quartile time observed on day 5 after administration, and the third quartile time recorded on day 16. Remarkably, the elder had a significant signal associated with cutaneous toxicity AEs (IC 025 = 0.81, ROR 025 = 2.16, PRR = 1.81, χ^2 = 3480.90). The studies indicate an increased susceptibility among the elder to the development of skin AEs after treatment with EGFRI. According to previous investigations, more frequent and severe cutaneous toxicity was observed in elderly patients (31), which may be due to differences in pharmacogenomics or pharmacodynamics in the elderly. EGFRI drugs are mainly metabolized by CYP3A4, CYP3A5, and CYP1A1 in the cytochrome P450 enzyme family (32, 33), age-related changes may impact these metabolic pathways, leading to decreased activity observed in some elderly (34, 35). Patients taking EGFRI drugs, which are predominantly prescribed for NSCLC, may exhibit gender differences that are influenced by their distinct indications, EGFR has a greater mutation probability in female with NSCLC (36, 37).

In addition, rash and acneiform dermatitis had the strongest adverse reaction signal and the most widespread distribution. EGFRI-induced rashes vary in severity. The frequency and severity of rashes increased with antibodies compared with low-molecular-weight TKIs, which can be attributed to the process of antibody-mediated receptor

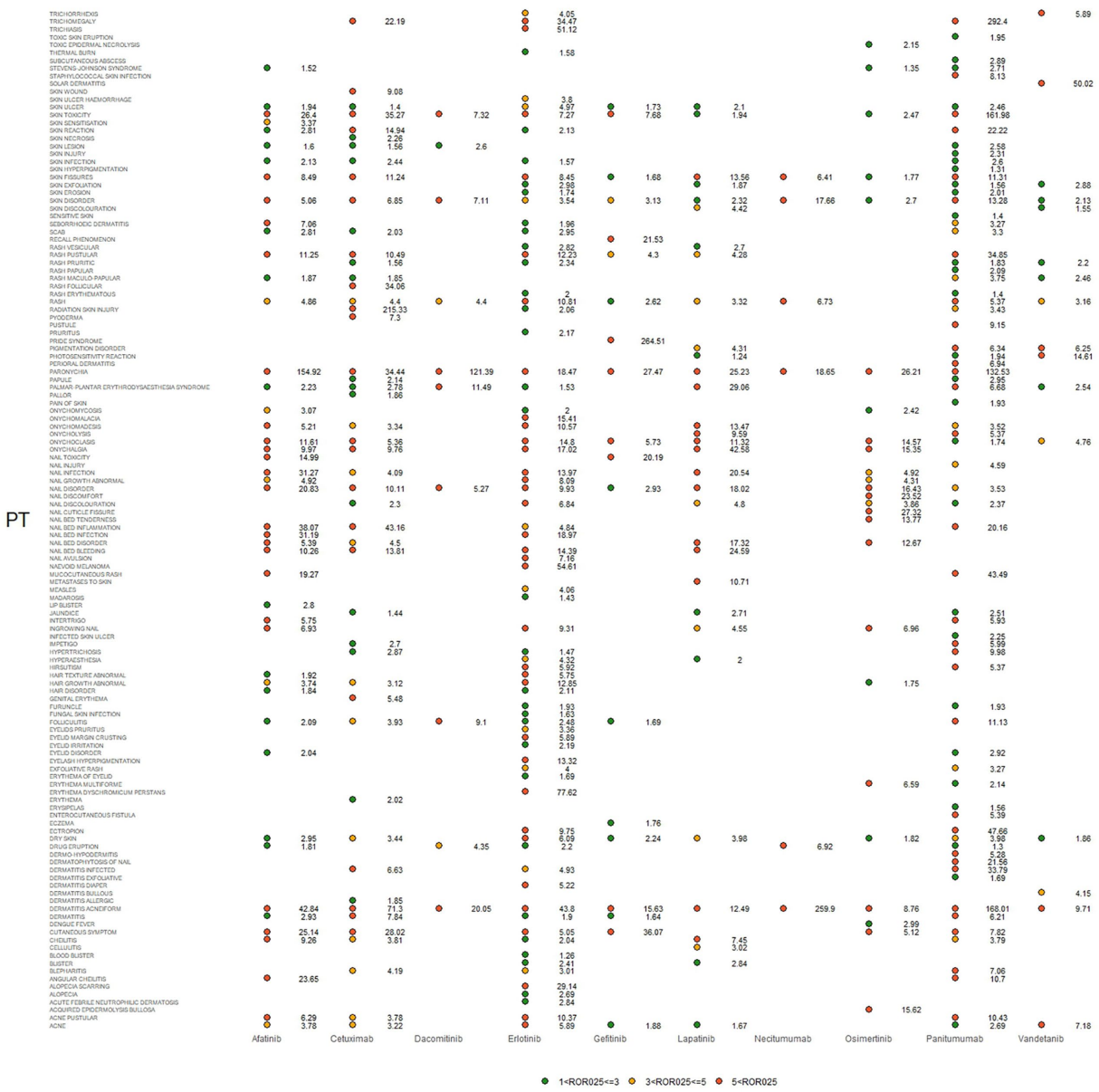

FIGURE 2
Adverse events (AEs) related to dermatologic toxicity associated with epidermal growth factor inhibitor (EGFRI) drugs exhibited signals indicated by reporting odds ratios (RORs). PT, preferred term. The lower end of the 95% confidence interval of ROR, denoted as ROR025, served as a threshold for identifying signals. ROR025 greater than 1 was deemed a signal.

internalization. This is a pivotal mechanism to abolish activation of receptor and may lead to enhanced inhibition of the EGFR signaling pathway (38). Dermatitis acneiform, characterized as a major cutaneous toxicity event, exhibits a signal across 10 drugs, with the most pronounced signal observed for panitumumab (IC025 = 6.75, ROR025 = 168.01, PRR = 182.12, χ^2 = 68808.86). Controversially, previous studies using several different EGFR drugs have demonstrated a link between rash and clinical efficacy. Skin rash appears to be a surrogate marker of clinical benefit (39). However, as described elsewhere, additional analyzes are required to describe the correlation between rash occurrence and overall treatment response (40). In addition to the commonly mentioned adverse reactions in the instructions of EGFRI drugs, some new signals have been identified. Afatinib and erlotinib have been associated with drug eruptions, while cetuximab, erlotinib, and panitumumab have been linked to skin radiation lesions. Furthermore, lip blistering has emerged as a new signal for afatinib and cheilitis for cetuximab. Gefitinib has been linked to PRIDE syndrome and recall phenomenon. Osimertinib has been associated with toxic epidermal necrolysis and epidermolysis bullosa acquired 3. Panitumumab has been associated with intestinal fistula and perioral dermatitis. However, the cases of these signals are rare and the influence of other drugs or alternative therapies should be considered.

The mechanism by which EGFRI induces skin irritation has been described differently. Low-molecular-weight TKIs block phosphorylation of the cytoplasmic domain, while mAbs

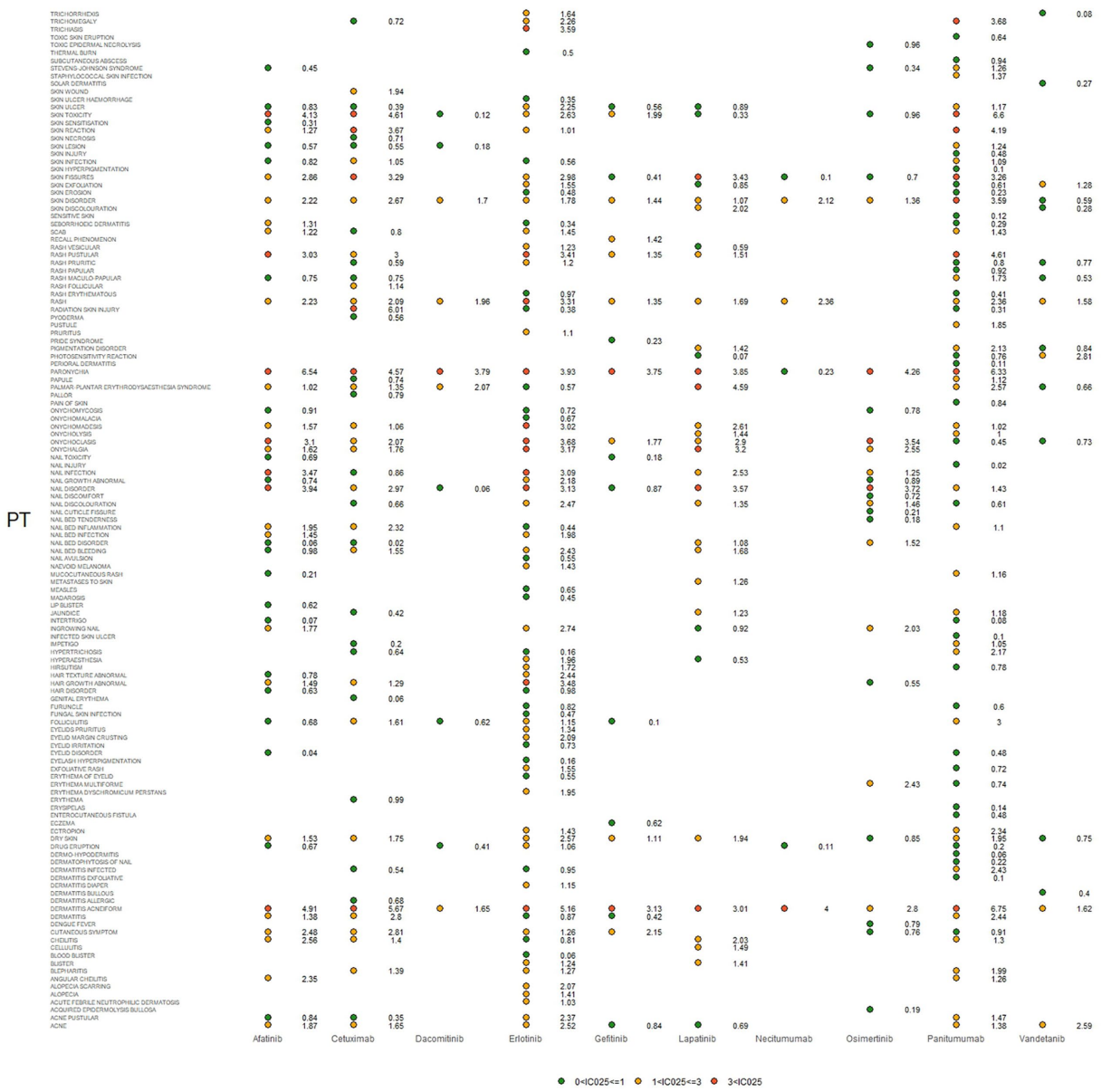

FIGURE 3
Signals were observed for epidermal growth factor inhibitor (EGFRI) drugs in relation to detailed adverse events (AEs) associated with dermatologic toxicity. PT, preferred term. The lower end of the 95% confidence interval of IC, denoted as IC025, served as the threshold for identifying signals. IC025 greater than 0 was deemed a signal.

competitively inhibit ligand binding to the extracellular domain, exerting their activity (41). Drug-mediated blockade of EGFR leads to growth arrest and apoptosis in EGFR-dependent cells for survival by inhibiting downstream pathways, including mitogen-activated protein kinase pathway, MAPK for Abbreviation, phosphatidylinositol 3-kinase-Protein Kinase B pathway, PI3K-Akt for Abbreviation. And there are two pathways involving the stress-activated protein kinase pathway, one is protein kinase C, and the other is Janus kinase-signal transducer and activator of transcription, Jak–STAT for Abbreviation (42). Some skin AEs are considered to be triggered by impact of EGFR on basal keratinocytes. Suppression of signaling pathways mediated by EGFR exerts multiple effects on keratinocytes. It results in growth arrest and apoptosis induction, decreasing cell migration, enhancing cell attachment and differentiation, and triggering inflammation (43). ERK 1 (extracellular signal-regulated kinase 1) and ERK 2 were found to mediate releasing cytokine of epithelial cells (41). This may coordinate the recruiting and activating of leukocyte, including neutrophils, lymphocytes, and monocytes, With the increased release of effector cytokines, chemokine production is amplified and leukocyte recruitment is enhanced, triggering the occurrence of papulopustular rash and paronychia as a consequence (44). Collectively, the aforementioned studies provide evidence supporting the pathogenesis of skin rash and acneiform dermatitis.

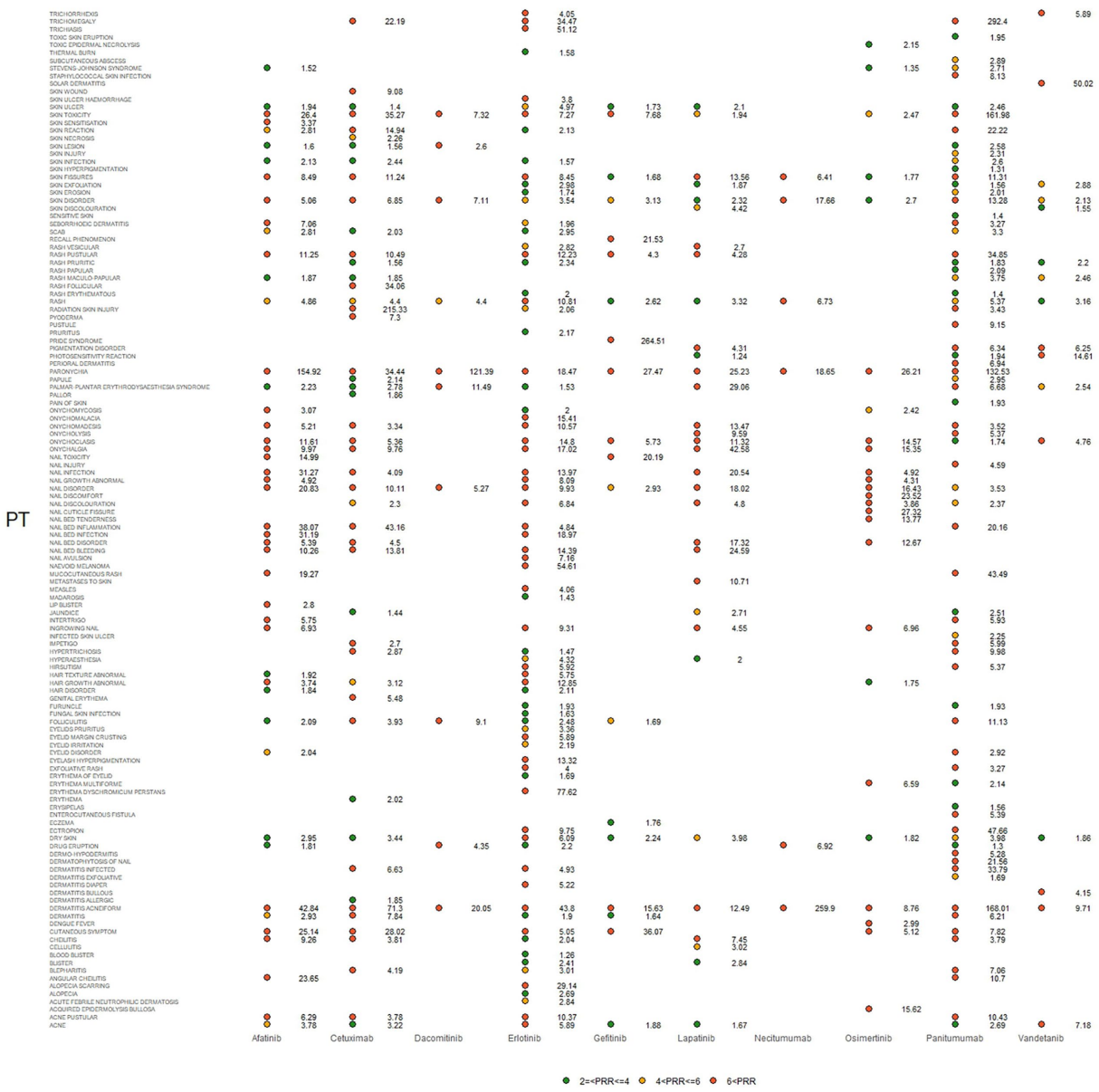

FIGURE 4
Signals were observed for epidermal growth factor inhibitor (EGFRI) drugs in relation to detailed adverse events (AEs) associated with dermal toxicity. PT, preferred term; PRR not less than 2 was deemed a signal.

Analyzing spontaneous reporting systems is a useful way to identify potential signals, and the FAERS database is one of the largest sources of data. However, our study currently has the following limitations: First, the accurate assessment of an event can be influenced by the variability in information completeness across different reports. And FAERS post-marketing data are spontaneous reports and cannot fully reflect the incidence of adverse events. Second, filling in the information in the FAERS database is affected by the patient's subjective wishes. For example, the current condition is relatively mild, and some patients may not choose to report. In addition, due to the lack of follow-up/censored data, only the correlation between EGFRI and cutaneous adverse events can be determined, and it is difficult to establish causality. Finally, only cutaneous toxicities have been explored, the link of EGRFI to other organ systems has not been explored furthermore. In the future, we will compare other system organ class AE signals of EGFRIs to strengthen the study.

5 Conclusion

The correlation between EGFRI and skin AEs was thoroughly evaluated using the FAERS database and data mining methods in this study. Overall, significant associations were detected between EGFRIs and cutaneous AEs, with relatively notable signals for several of the EGFRI drugs. Part of the results align with prior research. Rash and acneiform dermatitis exhibited an association with all drugs, and paronychia was associated with most drugs

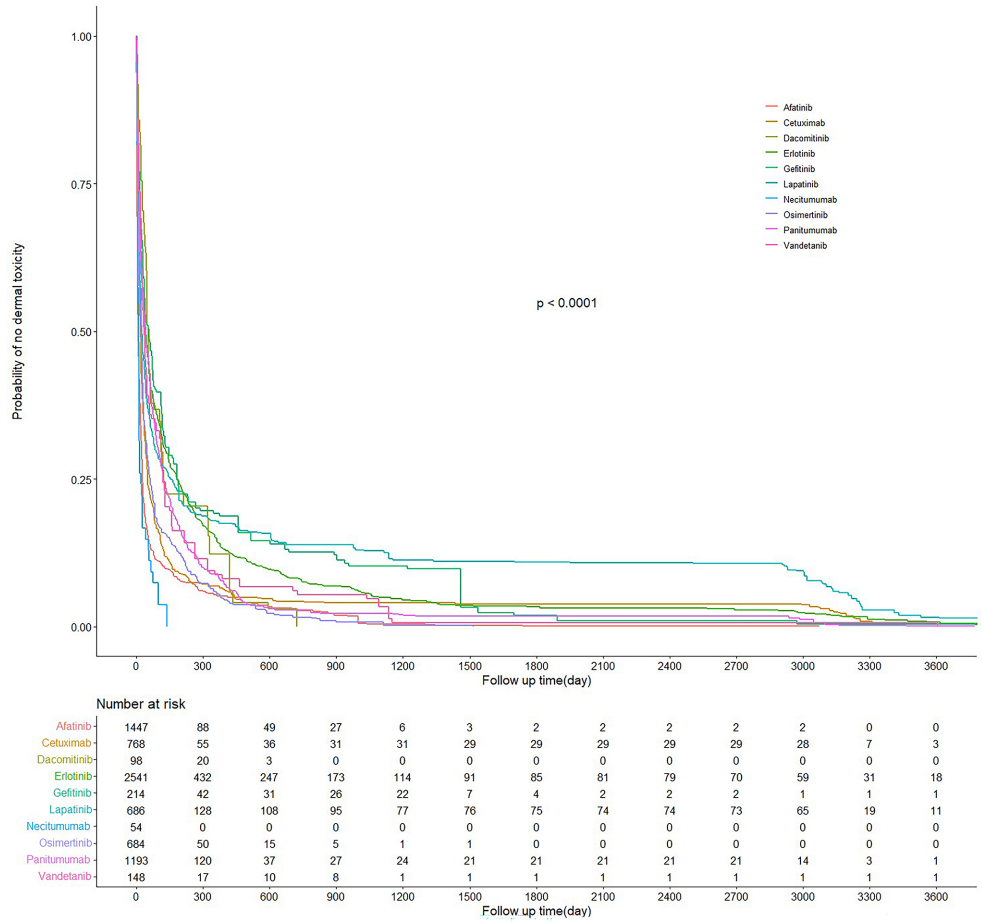

FIGURE 5
AE reports of EGFRI-induced dermatologic toxicities in Kaplan–Meier curves and risk tables.

TABLE 6 The occurrence time of skin AEs for EGFRIs.

	Q1	Median	Q3	IQR
Afatinib	4	9	28	24
Cetuximab	7	19	50	43
Dacomitinib	27	54	123	96
Erlotinib	11	42	186	175
Gefitinib	14	57	182	168
Lapatinib	7	24	154	147
Necitumumab	5	9	16	11
Osimertinib	7	22	64	57
Panitumumab	13	42	125	112
Vandetanib	13	38	119	106

Q1 refer to the first quartile time, Q3 refer to the third quartile time, all values are in days.

(except vandetanib). As a clinician, when using EGFRIs clinically, it is necessary to educate and empower patients, especially elderly patients, to prevent and report toxicity in a timely manner, pay attention to the development of cutaneous toxicity AE and intervene when necessary. In particular, rash, an adverse reaction that may be underrecognized and undertreated, should be discussed with the patient about how the rash affects quality of life and how to manage it appropriately. These more common adverse reactions require communication and evaluation with the patient. To determine whether it is necessary to stop the drug or take other measures. In

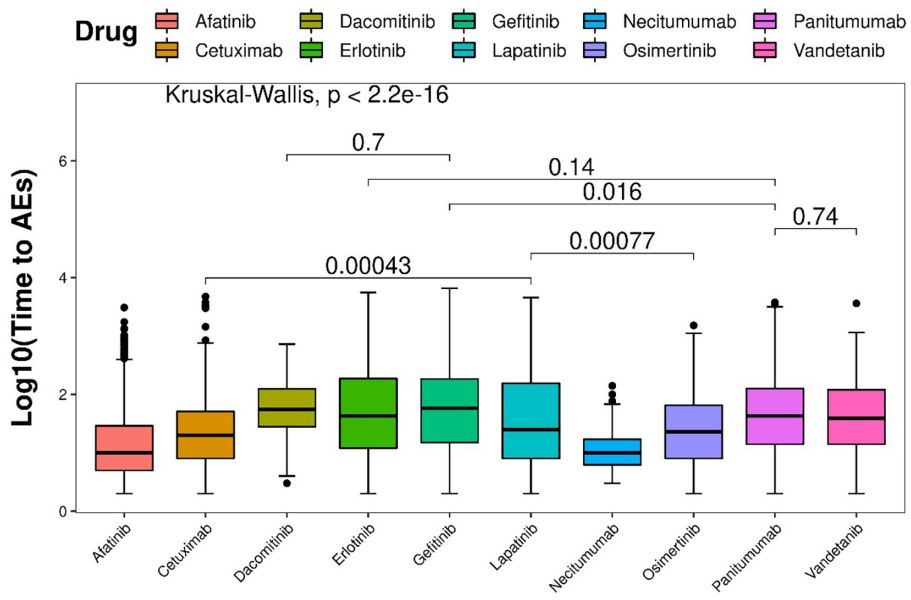

FIGURE 6
Time interval disparities analysis between durations of EGFRIs-related dermatologic AEs.

addition to rash, other skin-related AEs should be paid more attention to. Although there are fewer newly discovered signal cases, they still need to be paid attention to. Customizing a specific treatment plan for each patient is essential due to the variations in the onset and duration of each EGFRI drug, as well as the differences in AEs observed across different various groups.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

HD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. QJ: Writing – original draft, Data curation, Formal analysis. XJ: Writing – original draft, Data curation, Formal analysis. GQ: Software, Visualization, Writing – original draft. DZ: Writing – review & editing. ZL: Writing – review & editing, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1283807/full#supplementary-material

References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Mol Cell Biol*. (2001) 2:127–37. doi: 10.1038/35052073

2. Cohen SJC. The epidermal growth factor (EGF). *Cancer*. (1983) 51:1787–91. doi: 10.1002/1097-0142(19830515)51:10<1787::AID-CNCR2820511004>3.0.CO;2-A

3. Thompson D, Gill GN. The EGF receptor: structure, regulation and potential role in malignancy. *Cancer Surv*. (1985) 4:767–88.

4. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J*. (2000) 19:3159–67. doi: 10.1093/emboj/19.13.3159

5. Peréz-Soler R, Saltz L. Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? *JCO*. (2005) 23:5235–46. doi: 10.1200/JCO.2005.00.6916

6. Reichert JM, Dhimolea E. The future of antibodies as cancer drugs. *Drug Discov Today*. (2012) 17:954–63. doi: 10.1016/j.drudis.2012.04.006

7. Peng Y, Li Q, Zhang J, Shen W, Zhang X, Sun C, et al. Update review of skin adverse events during treatment of lung cancer and colorectal carcinoma with epidermal growth receptor factor inhibitors. *BST*. (2018) 12:537–52. doi: 10.5582/bst.2018.01246

8. Zhou Y, Zhang Y, Zou H, Cai N, Chen X, Xu L, et al. The multi-targeted tyrosine kinase inhibitor vandetanib plays a bifunctional role in non-small cell lung cancer cells. *Sci Rep*. (2015) 5:8629. doi: 10.1038/srep08629

9. GJJOCO G. Epidermal growth factor receptor inhibitors in the treatment of non–small-cell lung cancer. *J Clin Oncol*. (2005) 23:3235–42. doi: 10.1200/JCO.2005.08.409

10. Bokemeyer C, Bondarenko I, Hartmann J, De Braud F, Schuch G, Zubel A, et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol*. (2011) 22:1535–46. doi: 10.1093/annonc/mdq632

11. Awada A, Saliba W, Bozovic-Spasojevic I. Lapatinib ditosylate: expanding therapeutic options for receptor tyrosine-protein kinase erbB-2-positive breast cancer. *Drugs Today (Barc)*. (2011) 47:335–45. doi: 10.1358/dot.2011.47.5.1584110

12. Liebner DA, Shah MH. Thyroid cancer: pathogenesis and targeted therapy. *Ther Adv Endocrinol Metab*. (2011) 2:173–95. doi: 10.1177/2042018811419889

13. Willett CG, Duda DG, Czito BG, Bendell JC, Clark JW, Jain RKJO. Targeted therapy in rectal cancer. *Oncology (Williston Park)*. (2007) 21:1055.

14. Mok TS, Wu Y-L, Thongprasert S, Yang C-H, Chu D-T, Saijo N, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. *N Engl J Med Overseas Ed*. (2009) 361:947–57. doi: 10.1056/NEJMoa0810699

15. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: head and neck cancers. (2018).

16. Milanezi F, Carvalho S, Schmitt FC. EGFR/HER2 in breast cancer: a biological approach for molecular diagnosis and therapy. *Expert Rev Mol Diagn*. (2008) 8:417–34. doi: 10.1586/14737159.8.4.417

17. Huang A, Shen Q, Yu X, Wang H, Shi C, Han B, et al. Efficacy, safety and prognostic factors analysis of first-line icotinib treatment in advanced non-small cell lung cancer patients with mutated EGFR. *TCR*. (2018) 7:600–8. doi: 10.21037/tcr.2018.05.30

18. Hofheinz R-D, Segaert S, Safont MJ, Demonty G, Prenen H. Management of adverse events during treatment of gastrointestinal cancers with epidermal growth factor inhibitors. *Crit Rev Oncol Hematol*. (2017) 114:102–13. doi: 10.1016/j.critrevonc.2017.03.032

19. Patil VM, Noronha V, Joshi A, Choughule AB, Bhattacharjee A, Kumar R, et al. Phase III study of gefitinib or pemetrexed with carboplatin in EGFR-mutated advanced lung adenocarcinoma. *ESMO Open*. (2017) 2:e000168. doi: 10.1136/esmoopen-2017-000168

20. Anderson KR, Chambers CR, Lam N, Yau PS, Cusano F, Savoie ML, et al. Medication adherence among adults prescribed imatinib, dasatinib, or nilotinib for the treatment of chronic myeloid leukemia. *J Oncol Pharm Pract*. (2015) 21:19–25. doi: 10.1177/1078155213520261

21. Hauben M, Bate A. Decision support methods for the detection of adverse events in post-marketing data. *Drug Discov Today*. (2009) 14:343–57. doi: 10.1016/j.drudis.2008.12.012

22. Norén GN, Hopstadius J, Bate A. Shrinkage observed-to-expected ratios for robust and transparent large-scale pattern discovery. *Stat Methods Med Res*. (2013) 22:57–69. doi: 10.1177/0962280211403604

23. Evans SJ, Waller PC, Davis SJP. Use of proportional reporting ratios (PRRs) for signal generation from spontaneous adverse drug reaction reports. *Pharmacoepidemiol Drug Saf*. (2001) 10:483–6. doi: 10.1002/pds.677

24. Stricker BHC, Tijssen JGP. Serum sickness-like reactions to cefaclor. *J Clin Epidemiol*. (1992) 45:1177–84. doi: 10.1016/0895-4356(92)90158-J

25. Bate A, Evans SJP. Quantitative signal detection using spontaneous ADR reporting. *Pharmacoepidemiol Drug Saf*. (2009) 18:427–36. doi: 10.1002/pds.1742

26. Xie X, Wang X, Wu S, Yang H, Liu J, Chen H, et al. Fatal toxic effects related to EGFR tyrosine kinase inhibitors based on 53 cohorts with 9,569 participants. *J Thorac Dis*. (2020) 12:4057–69. doi: 10.21037/jtd-19-4000A

27. Kunimasa K, Kamada R, Oka T, Oboshi M, Kimura M, Inoue T, et al. Cardiac adverse events in EGFR-mutated non-small cell lung cancer treated with Osimertinib. *JACC CardioOncology*. (2020) 2:1–10. doi: 10.1016/j.jaccao.2020.02.003

28. Wacker B, Nagrani T, Weinberg J, Witt K, Clark G, Cagnoni PJJCCR. Correlation between development of rash and efficacy in patients treated with the epidermal growth factor receptor tyrosine kinase inhibitor Erlotinib in two large phase III studies. *Clin Cancer Res*. (2007) 13:3913–21. doi: 10.1158/1078-0432.CCR-06-2610

29. Lacouture M, Laabs S, Koehler M, Sweetman R, Preston A, di Leo A, et al. Analysis of dermatologic events in patients with cancer treated with lapatinib. *Breast Cancer Res Treat*. (2009) 114:485–93. doi: 10.1007/s10549-008-0020-7

30. Yang X-D, Jia X-C, Corvalan JR, Wang P. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. *Crit Rev Oncol Hematol*. (2001) 38:17–23. doi: 10.1016/S1040-8428(00)00134-7

31. Malik I, Hecht JR, Patnaik A, Venook A, Berlin J, Croghan G, et al. Safety and efficacy of panitumumab monotherapy in patients with metastatic colorectal cancer (mCRC). *J Clin Oncol*. (2005) 23:3520. doi: 10.1200/jco.2005.23.16_suppl.3520

32. Wheatley-Price P, Ding K, Seymour L, Clark GM, Shepherd FA. Erlotinib for advanced non–small-cell lung cancer in the elderly: an analysis of the National Cancer Institute of Canada clinical trials group study BR.21. *J Clin Oncol*. (2008) 26:2350–7. doi: 10.1200/JCO.2007.15.2280

33. Li J, Zhao M, He P, Hidalgo M, Baker SDJCCR. Differential metabolism of Gefitinib and Erlotinib by human cytochrome P450 enzymes. *Clin Cancer Res*. (2007) 13:3731–7. doi: 10.1158/1078-0432.CCR-07-0088

34. Ling J, Johnson KA, Miao Z, Rakhit A, Pantze MP, Hamilton M, et al. Metabolism and excretion of ERLOTINIB, A small molecule inhibitor of epidermal growth factor receptor tyrosine kinase, in healthy male volunteers. *Drug Metab Dispos*. (2006) 34:420–6. doi: 10.1124/dmd.105.007765

35. Sotaniemi EA, Arranto AJ, Pelkonen O, Pasanen M. Age and cytochrome P450-linked drug metabolism in humans: an analysis of 226 subjects with equal histopathologic conditions*. *Clin Pharmacol Ther*. (1997) 61:331–9. doi: 10.1016/S0009-9236(97)90166-1

36. Cotreau MM, von Moltke LL, Greenblatt DJ. The influence of age and sex on the clearance of cytochrome P450 3A substrates. *Clin Pharmacokinet*. (2005) 44:33–60. doi: 10.2165/00003088-200544010-00002

37. Shi Y, Au JS-K, Thongprasert S, Srinivasan S, Tsai C-M, Khoa MT, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non–small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol*. (2014) 9:154–62. doi: 10.1097/JTO.0000000000000033

38. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J Clin Oncol*. (2003) 21:2787–99. doi: 10.1200/JCO.2003.01.504

39. RJCLC P-S. Rash as a surrogate marker for efficacy of epidermal growth factor receptor inhibitors in lung cancer. *Clin Lung Cancer*. (2006) 8:S7–S14. doi: 10.3816/CLC.2006.s.008

40. Pastore S, Mascia F, Mariotti F, Dattilo C, Mariani V, Girolomoni G. ERK1/2 regulates epidermal chemokine expression and skin inflammation. *J Immunol*. (2005) 174:5047–56. doi: 10.4049/jimmunol.174.8.5047

41. Tanaka T, Matsuoka M, Sutani A, Gemma A, Maemondo M, Inoue A, et al. Frequency of and variables associated with the EGFR mutation and its subtypes. *Int J Cancer Suppl*. (2010) 126:651–5. doi: 10.1002/ijc.24746

42. Kari C, Chan TORocha de Quadros M, UJCR R. Targeting the epidermal growth factor receptor in cancer: apoptosis takes center stage. *Cancer Res*. (2003) 63:1–5.

43. Vallböhmer D, Zhang W, Gordon M, Yang DY, Yun J, Press OA, et al. Molecular determinants of Cetuximab efficacy. *J Clin Oncol*. (2005) 23:3536–44. doi: 10.1200/JCO.2005.09.100

44. Pérez-Soler R, Delord JP, Halpern A, Kelly K, Krueger J, Sureda BM, et al. HER1/EGFR inhibitor-associated rash: future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum. *Oncologist*. (2005) 10:345–56. doi: 10.1634/theoncologist.10-5-345

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Advancements in elucidating the pathogenesis of actinic keratosis: present state and future prospects

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Solar keratosis, also known as actinic keratosis (AK), is becoming increasingly prevalent. It is a benign tumor that develops in the epidermis. Individuals with AK typically exhibit irregular, red, scaly bumps or patches as a result of prolonged exposure to UV rays. These growths primarily appear on sun-exposed areas of the skin such as the face, scalp, and hands. Presently, dermatologists are actively studying AK due to its rising incidence rate in the United States. However, the underlying causes of AK remain poorly understood. Previous research has indicated that the onset of AK involves various mechanisms including UV ray-induced inflammation, oxidative stress, complex mutagenesis, resulting immunosuppression, inhibited apoptosis, dysregulated cell cycle, altered cell proliferation, tissue remodeling, and human papillomavirus (HPV) infection. AK can develop in three ways: spontaneous regression, persistence, or progression into invasive cutaneous squamous cell carcinoma (cSCC). Multiple risk factors and diverse signaling pathways collectively contribute to its complex pathogenesis. To mitigate the risk of cancerous changes associated with long-term UV radiation exposure, prompt identification, management, and prevention of AK are crucial. The objective of this review is to elucidate the primary mechanisms underlying AK malignancy and identify potential treatment targets for dermatologists in clinical settings.

KEYWORDS

actinic keratosis, mechanisms, malignant transformation, human papillomavirus, UV

1 Introduction

AK, is a harmless growth within the epidermis that is becoming more common nowadays. The main presentation is characterized by atypical epidermal keratinocyte proliferation and chronic UV radiation resulting in red, scaly papules or plaques. It is well recorded that AK is frequently present on areas of skin that are exposed to the sun, including the face, scalp that is balding, and the back of the hands, particularly in older males with fair skin (1). AK is among the most frequently assessed skin disorders by dermatologists, with an estimated incidence of nearly 40 million and an annual cost exceeding $1 billion (USD) in the USA in 2004. The causes of AK are still unknown, and less attention is given to AK compared to other types of skin cancer (2). Previous studies have shown that AK can develop in three ways: spontaneous resolution, persistence, or progression to invasive cSCC. Its complex pathogenesis

involves multiple risk factors and diverse signaling pathways. Therefore, it is crucial to promptly diagnose, treat, and prevent AK in order to reduce the risk of developing cSCC due to chronic UV radiation exposure (2). Extensive research has been conducted to investigate various mechanisms and pathways associated with AK signaling. The purpose of this assessment is to elucidate the seven primary processes of AK leading to cancerous transformation. Its aim is to mitigate the risk of malignant transformation and provide clinical dermatologists with potential treatment objectives.

The objective of this analysis was to examine recent research and gain a comprehensive understanding of the main causes of AK. Furthermore, the review highlighted the limitations of previously conducted studies, offering valuable insights for future research directions.

2 UV radiation exposure

The primary cause of AK is the accumulation of excessive ultraviolet radiation from the sun. This excessive UV radiation can disrupt complex regulatory pathways involved in cell growth and differentiation, leading to various pathological alterations in epidermal keratinocytes. AK is formed when dysplastic intra-epidermal keratinocytes proliferate, and this proliferation is enhanced by factors such as DNA damage, inflammation, immunosuppression, and mutagenesis. Additionally, exposure to UV radiation stimulates the production of arachidonic acid, pro-inflammatory cytokines, adhesion molecules, and mediators derived from mast cells (1, 3, 4). In addition, being exposed to UV radiation can function as a promoter of tumors, starting events that lead to cancer, resulting in changes to genes, and even hastening the advancement of AK to cSCC (5). AK seems to exhibit an intermediate level of mutational burden compared to both normal skin that is habitually exposed to sunlight (photodamaged skin) and cSCC (6). The alteration of UV is not related just to epidermal keratinocytes but also to fibroblasts, the suppression of the Notch effector CSL (also referred to as RBP-Jj) in dermal fibroblasts alone is adequate to trigger the activation of cancer-associated fibroblasts (CAFs) and subsequently lead to the development of tumors derived from keratinocytes. This phenomenon has been observed in stromal fibroblasts present in premalignant AK lesions as well as *in situ* SCCs (7). Numerous studies have suggested that canonical DNA bases poorly absorb UVA radiation (315–400 nm), leading to indirect DNA damage. Moreover, exposure to UVA radiation results in the production of reactive oxygen species (ROS) like superoxide anions, hydroxyl radicals, and hydrogen peroxide, which initiate oxidative harm in nucleic acids, membrane lipids, and proteins. Abnormal cell growth can be caused by the impairment of regular pathways for transmitting cellular signals. Additionally, UVA radiation causes the creation of 8-hydroxyguanine adducts, resulting in characteristic changes from thymine (T) to guanine (G) known as signature transitions (4). On the other hand, DNA can easily absorb UVB radiation (280–315 nm) and result in direct damage to DNA. Recent research has shown that UVB radiation causes cytosine-containing cyclobutane pyrimidine dimers and pyrimidine-pyrimidone 6–4 photoproducts, leading to C->T and CC->TT signature transitions that significantly interfere with normal replication and transcription processes. In a study conducted on mice, both continuous and intermittent regimens of chronic UVB treatment resulted in the development of skin tumors in all cases, demonstrating a 100% incidence rate. While the progression of this process was delayed upon discontinuation of chronic UVB exposure intermittently, it was not entirely prevented. This suggests that prolonged avoidance of UVB exposure merely postpones the timeline of tumor development (8). Moreover, c-Jun N-terminal Kinases (JNKs) and p38 Mitogen-Activated Protein Kinases (MAPKs) are activated by environmental stresses, pro-inflammatory cytokines, DNA damage, and oxidative stress, leading to the initiation of various intracellular signaling pathways such as stress adaptation, proliferation, differentiation, and apoptosis. Additionally, p38 MAPK serves as the precursor kinase for Mitogen and Stress Activated protein Kinase (MSK1) and phosphorylated H2AX (γ-H2AX), both of which play a role in the development of UVB-induced skin cancer (4). UV damage hastens the accumulation of mutations. With minimal damage, the skin's appearance typically remains normal for most individuals. However, as damage progresses, individuals with fair skin typically demonstrate heightened sensitivity to ultraviolet radiation due to lower melanin content in their skin. Consequently, when exposed to sunlight, fair-skinned individuals are more prone to photodamage, thereby elevating the risk of developing AK. The rate of malignant transformation to SCC per individual AK lesion per year has been estimated to range from 0.025 to 20%, usually falling below 1%. However, up to 60% of SCCs originate from pre-existing AKs, justifying the need for therapy (10, 11). The events mentioned above concurrently contribute to the acceleration of AK's progression to cSCC (Table 1).

3 Inflammation

The development of squamous cell carcinoma is strongly associated with chronic inflammation. This inflammation can be caused by inflammatory diseases such as AK resulting from exposure to ultraviolet radiation. The inflammatory state activates signals like nuclear factor kappa B (NF-kB) and mitogen-activated protein kinases (MAPKs), which encourage tumor growth. It also leads to the release of pro-inflammatory cytokines, prostaglandins, and reactive oxygen species (ROS). Inflammation and cancer development are closely linked to pathological processes influenced by inflammasomes, autophagy, and sirtuins (12). Recent research suggests that up to 25% of identified tumors have a significant inflammatory component, highlighting the substantial impact of persistent inflammation on the likelihood of acquiring AK (12).

The probability of developing skin tumors is higher due to chronic inflammation, which can occur through two pathways. The first pathway is caused by exposure to UV light and its associated activities. The second pathway is intrinsic and is triggered by genetic changes, including mutations in oncogenes (such as RAS oncogenes), tumor suppressor genes (such as adenomatosis polyposis coli (APC) and TP53), and DNA repair genes (such as MSH-2, MSH-6, and PMS-2). These genetic mutations can lead to cell transformation and the independent proliferation of transformed cells. Moreover, inherent imperfections can potentially cause changes in the immune system, resulting in the generation of substances that cause inflammation and contribute to the development of an inflammatory environment within tumors. Unfortunately, this can also accelerate the progression of the disease (12). Additionally, research has found that *Staphylococcus aureus* (*S. aureus*) could potentially be involved in the onset of AK and

TABLE 1 Common mutated genes of AK.

Genes	Molecular function	Biological process	Expression	References
TP53	Activator, DNA-binding, Repressor	Apoptosis, Biological rhythms, Cell cycle, Host-virus interaction, Necrosis, Transcription, Transcription regulation	Up	Jacobs et al. (12)
MYC	Activator, DNA-binding	Transcription, Transcription regulation	Up	Toll et al. (13)
NOTCH	Activator, Developmental protein, Receptor	Angiogenesis, Differentiation, Notch signaling pathway, Transcription, Transcription regulation	Up	South et al. (14)
RAS	Cell proliferation, Cell differentiation	Adenylate cyclase-activating G protein-coupled, Receptor signaling pathway, Positive regulation of adenylate cyclase activity, Protein localization to bud neck	Up	Corchado-Cobos et al. (15)
RB1	Chromatin regulator, DNA-binding, Repressor	Cell cycle, Host-virus interaction, Transcription, Transcription regulation	Up	Murao et al. (16)
CDKN2A	DNA-binding	Apoptosis, Cell cycle, rRNA processing, Transcription, Transcription regulation, Ubl conjugation pathway	Up	Pickering et al. (17)
FBXW7	Ubiquitination degradation	Biological rhythms, Host-virus interaction, Ubl conjugation pathway	Up	Kim et al. (18)
PIK3CA	Kinase, Serine/threonine-protein kinase, Transferase	Angiogenesis, Lipid metabolism, Phagocytosis	Up	Kim et al. (18)
ASXL1	Chromatin regulator, Repressor	Transcription, Transcription regulation, Ubl conjugation pathway	Up	Kim et al. (18)
FGFR3	Kinase, Receptor, Transferase, Tyrosine-protein kinase	Apoptosis	Up	Kim et al. (18)
EGFR	Developmental protein, Host cell receptor for virus entry, Kinase, Receptor, Transferase, Tyrosine-protein kinase	Host-virus interaction	Up	Murao et al. (16)
EZH2	Chromatin regulator, Methyltransferase, Repressor, Transferase	Biological rhythms, Transcription, Transcription regulation	Up	Kim et al. (18)
IRF4	Activator, DNA-binding	Transcription, Transcription regulation	Up	Jacobs et al. (12)
MC1R	G-protein coupled receptor, Receptor, Transducer	Apoptosis, Notch signaling pathway	Up	Jacobs et al. (12)
TYR	Monooxygenase, Oxidoreductase, Tumor antigen	Melanin biosynthesis	Up	Jacobs et al. (12)
APC	Signal transduction	Wnt signaling pathway	Down	Wang et al. (19)
MSH2	DNA-binding	DNA damage, DNA repair	Down	Sun et al. (20)
MLH1	DNA-binding	Cell cycle, DNA damage, DNA repair	Down	Kim et al. (18)
CDH1	cell–cell adhesions, mobility and proliferation	Cell adhesion	Down	Murao et al. (16)
TGF-b	cell proliferation	Transcription, Transcription regulation	Down	Thomson et al. (21)
SMAD2	DNA-binding	Transcription, Transcription regulation	Down	Xu et al. (22)
SMAD4	DNA-binding	Transcription, Transcription regulation	Down	Xu et al. (22)
CHK1	Kinase, Serine/threonine-protein kinase, Transferase	Cell cycle, DNA damage, DNA repair	–	Ming et al. (23)
KLF4	Activator, DNA-binding	Transcription, Transcription regulation	–	Lu et al. (24)
PTEN	Hydrolase, Protein phosphatase	Apoptosis, Lipid metabolism, Neurogenesis	–	Ming et al. (23)

the progression from AK to SCC by inducing chronic inflammation. This inflammatory response may involve the production of nitric oxide and cytokines, which contribute to the process of carcinogenesis (25). Moreover, it has been demonstrated that the staphylococcal alpha-toxin can stimulate various cytokines and NF-kB. This provides additional evidence to support the hypothesis that *S. aureus* plays a causative role in the initiation of AK and its progression to SCC (26).

4 Oxidative stress

Increasing evidence suggests that oxidative stress is a crucial factor in the formation of skin cancer due to sunlight exposure (27). Reactive nitrogen and oxygen species (RNS and ROS) are produced during various pathological processes, such as DNA damage and lipid oxidation, and are considered significant contributors to the development of tumors in AK and other related conditions (1). Oxidative stress, caused by a weakened antioxidant defense system, plays a role in skin cancer-related aging and cancer formation (28). Different types of tumors generate large amounts of ROS, both inside and outside cells. The *in vivo* generation of reactive ROS can promote aggressive cancer cells, hinder anti-proteases, damage nearby tissues, and encourage tumor heterogeneity, invasion, and metastasis. As a result, malignant tumors maintain higher basal levels of reactive oxygen species compared to normal cells, perpetuating a harmful cycle. It is worth noting that while elevated levels of ROS may lead to oxidative stress and cellular demise, reduced levels of superoxide and H_2O_2 can facilitate the G1 → S cell cycle progression in various cellular models. The pathophysiological implications of extracellular ROS should also be considered. Superoxide dismutases (SODs) have been reported to play a crucial role as the primary defense mechanism against injury caused by ROS. These enzymes facilitate the dismutation of the superoxide anion free radical (O_2-) by catalyzing its conversion into molecular oxygen and H_2O_2. This enzymatic action effectively reduces the levels of O_2-, mitigating cellular damage associated with excessive concentrations of this radical (29). The malignant transformation of AK is highly correlated with an increased level of oxidative status and a significant quantity of ROS (Table 2).

5 Mutagenesis

AK shares similarities with cSCC at the genomic level, exhibiting mutations in 44 driver genes. These genes include TP53, NOTCH1, NOTCH2, FAT1, and KMT2C, among which TP53 is the most commonly mutated gene (30). Other genes that are mutated include

TABLE 2 Epidemiological Literature on AK and cSCC: References from the Past Decade.

Titles	Authors	Journal	DOI	Pub_Date
Impact of COVID-19 Pandemic on Cutaneous Squamous Cell Carcinoma: A Single-Centre Study of Epidemiologic, Clinic and Histopathological Factors	Díaz-Calvillo P et al.	Actas Dermosifiliogr	10.1016/j.ad.2024.01.004	2024
Impact of COVID-19 Pandemic on Cutaneous Squamous Cell Carcinoma: A Single-Centre Study of Epidemiologic, Clinic and Histopathological Factors	Díaz-Calvillo P et al.	Actas Dermosifiliogr	10.1016/j.ad.2023.10.003	2023
The Global Epidemiology of Actinic Keratosis in the General Population: A Systematic Review and Meta-Analysis	George CD; et al.	Br J Dermatol	10.1093/bjd/ljad371	2023
Comparison of the clinical characteristics of benign and malignant eyelid lesions: an analysis of 1423 eyelid lesions, compared between ophthalmology department and plastics department	Levinkron O; et al.	Graefes Arch Clin Exp Ophthalmol	10.1007/s00417-023-06244-5	2023
Alcohol and Health Outcomes: An Umbrella Review of Meta-Analyses Base on Prospective Cohort Studies	Zhong L; et al.	Front Public Health	10.3389/fpubh.2022.859947	2022
Validation of actinic keratosis diagnosis and treatment codes among veterans living with HIV	Supapannachart KJ; et al.	Pharmacoepidemiol Drug Saf	10.1002/pds.5430	2022
Incidence and Prevalence of Skin Cancers in South Korea from 2008 to 2016: A Nation-Wide Population Based Study	Park K; et al.	Ann Dermatol	10.5021/ad.2022.34.2.105	2022
Incidence of Multiple vs. First Cutaneous Squamous Cell Carcinoma on a Nationwide Scale and Estimation of Future Incidences of Cutaneous Squamous Cell Carcinoma	Tokez S; et al.	JAMA Dermatol	10.1001/jamadermatol.2020.3677	2020
Nationwide Incidence of Metastatic Cutaneous Squamous Cell Carcinoma in England	Venables ZC; et al.	JAMA Dermatol	10.1001/jamadermatol.2018.4219	2019
Association of Vitamin A Intake With Cutaneous Squamous Cell Carcinoma Risk in the United States	Kim J; et al.	JAMA Dermatol	10.1001/jamadermatol.2019.1937	2019
Prognostic factors for parotid metastasis of cutaneous squamous cell carcinoma of the head and neck	Bobin C; et al.	Eur Ann Otorhinolaryngol Head Neck Dis	10.1016/j.anorl.2017.09.006	2018
Current perspective on actinic keratosis: a review	Siegel J; et al.	Br J Dermatol	10.1111/bjd.14852.	2017
Incidence, Mortality, and Trends of Nonmelanoma Skin Cancer in Germany	Leiter U; et al.	J Invest Dermatol	10.1016/j.jid.2017.04.020.	2017
Human polyomaviruses and incidence of cutaneous squamous cell carcinoma in the New Hampshire skin cancer study	Gossai A; et al.	Cancer Med	10.1002/cam4.674	2016
Aspirin and nonsteroidal anti-inflammatory drugs can prevent cutaneous squamous cell carcinoma: a systematic review and meta-analysis	Muranushi C; et al.	J Invest Dermatol	10.1038/jid.2014.531	2015
Epidemiology of actinic keratoses	Green AC	Curr Probl Dermatol	10.1159/000366525.	2015
Clinical characteristics of patients with cutaneous melanoma according to variants in the melanocortin 1 receptor gene	Pe?a-Vilabelda MM; et al.	Actas Dermosifiliogr	10.1016/j.ad.2013.10.001	2014
Cutaneous squamous cell carcinoma and human papillomavirus: is there an association?	Aldabagh B; et al.	Dermatol Surg	10.1111/j.1524-4725.2012.02558.x	2013
Sunscreen use on the dorsal hands at the beach	Warren DB; et al.	J Skin Cancer	10.1155/2013/269583	2013
The relevance of the vitamin D endocrine system (VDES) for tumorigenesis, prevention, and treatment of non-melanoma skin cancer (NMSC): Present concepts and future perspectives	Reichrath J; et al.	Dermatoendocrinol	10.4161/derm.24156	2013
Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012	Pritesh S; et al.	J Am Acad Dermatol	10.1016/j.jaad.2012.11.037	2013
The natural history of actinic keratosis: a systematic review	R N Werner; et al.	Br J Dermatol	10.1111/bjd.12420	2013
Smoking and the risk of nonmelanoma skin cancer: systematic review and meta-analysis	Leonardi-Bee J; et al.	Arch Dermatol	10.1001/archdermatol.2012.1374	2012
Epidemiologic study of skin diseases among immigrants in Alicante, Spain	Albares MP; et al.	Actas Dermosifiliogr	10.1016/j.ad.2011.07.008	2012
Supplement use and risk of cutaneous squamous cell carcinoma	Asgari MM; et al.	J Am Acad Dermatol	10.1016/j.jaad.2010.09.009	2011
Association of tea consumption and cutaneous squamous cell carcinoma	Asgari MM; et al.	Nutr Cancer	10.1080/01635581.2011.523496	2011
Potential risk factors for cutaneous squamous cell carcinoma include oral contraceptives: results of a nested case–control study	Asgari MM; et al.	Int J Environ Res Public Health	10.3390/ijerph7020427	2010
Occupational exposure to non-artificial UV-light and non-melanocytic skin cancer - a systematic review concerning a new occupational disease	Schmitt J; et al.	J Dtsch Dermatol Ges	10.1111/j.1610-0387.2009.07260.x	2010
Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals	Asgari MM; et al.	J Invest Dermatol	10.1038/sj.jid.5701227	2008
Guidelines for the management of squamous cell carcinoma in organ transplant recipients	Stasko T; et al.	Dermatol Surg	10.1111/j.1524-4725.2004.30150.x	2004
Presence of human papillomavirus DNA in plucked eyebrow hairs is associated with a history of cutaneous squamous cell carcinoma	Struijk L; et al.	J Invest Dermatol	10.1046/j.1523-1747.2003.12632.x	2003

the ras genes, c-myc proto-oncogenes, p16INK4a tumor suppressor genes, and genes associated with telomerase activity. However, due to the complexity of these genetic alterations, only a few of them have been identified in current research. The following genes are some of the typical genes among the mutated gene family.

5.1 TP53

Skin cancer is frequently associated with genetic abnormalities in the TP53 gene, such as AK (31, 32). TP53 mutations are found in more than 50% of human cancers, including keratinocytic skin cancers. These mutations usually manifest as a CC- > TT base change and are thought to arise during the early stages of long-term sun-induced skin cancer development. To indirectly indicate mutations, TP53 expression has been detected through immunohistochemical methods in various studies (33, 34). Khorshid et al. more than 50% of tumor cells expressed TP53, which was associated with mutations (34). Karagece found TP53 expression in all AK cases, including non-dysplastic areas on H&E slides. The collective findings suggest that TP53 plays a critical role in the early stages of skin cancer development, which is likely triggered by prolonged exposure to UV radiation (35).

5.2 MYC

The MYC gene family, consisting of MYC (also known as C-Myc), MYCN (N-Myc), and MYCL1 (LMYC), plays a significant role in promoting epidermal differentiation, cell proliferation, apoptosis, and the development of specific human cancers. One common cytogenetic abnormality observed in the progression from AK to cSCC is the amplification of the MYC gene, which is located in the 8q24 chromosome band. Multiple lines of evidence suggest that MYC may contribute to the lack of differentiation and accelerate the progression from low-grade AK to advanced stages of cSCC. Mutations in the MYC gene that affect DNA replication can lead to a mutator phenotype, triggering a process that enhances proliferation advantage (31).

In AK, MYC amplification may lead to further genomic rearrangements. As a driver gene, MYC can contribute to genomic instability (32). MYC can play a critical role in the transition from a benign pre-cancerous lesion to its malignant form when it carries genetic abnormalities. MYC numerical abnormalities are more common in advanced and undifferentiated stages of the disease, suggesting its involvement in the development of a more aggressive phenotype (31).

5.3 TSG

Emerging evidence indicates that the Tumor Suppressor Gene (TSG) plays a vital role in various cellular processes, such as DNA damage repair, cell division inhibition, apoptosis induction, and metastasis suppression. The TSG family encompasses several members, including TP53, p16, p14, APC, MSH2, among others. The inactivation or loss of TSG function is a crucial factor in promoting tumor development (36, 37). Previous studies have suggested that a single copy of TSG can regulate cell growth, while complete inactivation or loss of both alleles is required for tumor formation (17, 36). Additionally, recent findings propose that tumorigenesis is also influenced by the functional deactivation of TSGs through cellular mechanisms such as transcriptional regulation, abnormal cellular localization, and proteasomal degradation (31).

The significance of TSG in the development of skin cancer through exposure to UV radiation has been well-documented. This is evident from the high prevalence of TP53 mutations observed in sun-exposed skin over prolonged periods, as well as in AK and cSCC. Additionally, the disruption of the TSG cluster comprising p14ARF, p15INK4b, and p16INK4a on chromosome 9p21 has been found to promote carcinogenesis. In addition, the skin surrounding AK lesions, which is exposed to sunlight and appears healthy in terms of morphology, showed changes in the expression of p14ARF, p15INK4b, p16INK4a, and TP53 mutations, as documented in Kanellou et al. (31).

5.4 The genes IRF4, MC1R and TYR

Mutations in the IRF4 gene, located on chromosome 6p25.3, have a significant impact on melanin synthesis and the host immune response. Down-regulation of IRF4 leads to reduced expression of TYR, a key enzyme in melanin production. Moreover, it adversely affects the toll-like receptor signaling pathway, which is responsible for triggering adaptive immunity. This suggests that a decrease in IRF4 expression may increase vulnerability to AK by weakening the body's ability to combat abnormal keratinocytes and melanocytes. MC1R, located on chromosome 16q24.3, is a crucial pigmentation gene that regulates eumelanin synthesis by encoding the melanocortin 1 receptor. The rs1805008(T) SNP limits MC1R's ability to bind with its ligand, resulting in limited melanin synthesis. Melanocytes with loss of function MC1R exhibit reduced DNA repair function after UV exposure, a known cause of skin cancer. This likely contributes to the effects of MC1R on AK, which are independent of pigmentation. TYR, located on chromosome 11q14.3, is responsible for producing the essential enzyme tyrosinase, which plays a crucial role in melanin synthesis. Genetic variations in the TYR gene can lead to reduced enzyme activity, resulting in a lightly pigmented appearance. The risk of developing AK may be higher due to a specific type of genetic variation in the TYR gene, which weakens the immune response to melanocytes. This genetic variation is strongly associated with the aforementioned lightly pigmented phenotype and is identified by the rs1393350(A) single nucleotide polymorphism.

A recent investigation has revealed that the genes IRF4, MC1R, and TYR may have multiple effects, impacting both pigmentation and oncogenic functions. This dual impact may increase the risk of AK. The study also discovered a significant correlation between SNPs and the genes IRF4 locus, SLC45A2, HERC2, MC1R, and TYR. AK is significantly influenced by independent risk factors such as sex, age, and the genes IRF4, MC1R, and TYR. Age and gender were responsible for the majority of AK variance (15%), while the three significant SNPs IRF4, MC1R, and TYR collectively accounted for 2.6%. These findings align with those commonly observed in genome-wide association studies of complex human systems (38–40).

Single-cell RNA sequencing (scRNA-seq) technology provides a powerful method to investigate changes in gene expression at the

individual cell level. In a recent scRNA-seq study, researchers identified a group of important candidate genes that may be associated with the development and progression of AK. The study revealed a significant increase in the expression of acetaldehyde dehydrogenase 3A1 (ALDH3A1) and insulin-like growth factor binding protein 2 (IGFBP2) in AK tissues, specifically in epidermal keratinocytes. Interestingly, neither Squamous Cell Carcinoma *in situ* (SCCIS) nor cSCC showed a significant upregulation of ALDH3A1 and IGFBP2, and ALDH3A1 was even found to be downregulated in cSCC. This suggests that ALDH3A1 and IGFBP2 play a distinct role in skin precancerous lesions. The increased expression of ALDH3A1 and IGFBP2, particularly in basal cells, is likely to contribute to the development of AK and may act as key driver genes in the transition from photoaged skin to AK (41).

6 Immunosuppression

The immune cells responsible for suppressing the immune response, known as T regulatory cells (Tregs), play a crucial role in the progression of tumors by inhibiting the immune system's ability to fight tumor cells. The expression of the Foxp3 transcription factor by these cells is highly correlated with the transition from AK to cSCC. Specifically, Tregs producing interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) hinder the activation of CD4 T cells and dendritic cells, promote growth, and produce various cytokines.

The initial events in UV-induced immunosuppression include the release of platelet-activating factor (PAF) and the conversion of the photoreceptor trans-urocanic acid (tUCA) to the immunosuppressive cis-urocanic acid (cUCA). During UV-induced oxidative stress, PAF receptors activate cytokine transcripts by generating PAF, a phospholipid. PAF and cUCA not only regulate immunosuppression but also impact DNA damage by inhibiting nucleotide excision repair and facilitating the creation of 8-oxo-deoxyguanosine. Additionally, PAF and cUCA promote the production of reactive oxygen species (ROS), which links genetic damage, DNA repair, and immunosuppression.

To summarize, the development of cSCC from AK is highly associated with the rise in Tregs, whereas the release of PAF and the conversion of tUCA to cUCA are two initial occurrences in UV-induced immunosuppression (Table 3).

7 Impaired apoptosis

Apoptosis is crucial in regulating skin development, homeostasis, and carcinogenesis by balancing epidermal proliferation and removing mutated or potentially cancerous cells. Exposure to UV radiation can lead to the death of skin cells and the development of cancerous growths, as stated in reference (49). Furthermore, UV radiation can cause damage to the DNA of keratinocytes, leading to harmful effects that are not yet fully understood in the process of apoptosis. This is due to the appearance of molecules that either promote or hinder apoptosis (16). The processes described are heavily influenced by the TP53 gene, which functions as a tumor suppressor. TP53 plays a crucial role in activating apoptosis and facilitating cell cycle arrest. Activation of TP53-related genes leads to delayed cell cycle progression, DNA repair, and apoptosis. It also initiates mechanisms for the removal of DNA damage in response to UV-induced damage (49). As mentioned before, the TP53 molecule that encourages programmed cell death is heavily involved in the development of skin cancer and also hinders apoptosis in cells that have DNA damage. Studies suggest that other molecules, such as Human TNF-related apoptosis inducing ligand (TRAIL) and Fas-ligand (FasL), which promote apoptosis, can bypass the immune system (16).

Keratinocytes trigger the process of apoptosis through intrinsic and extrinsic pathways that are regulated by various factors such as MAPKs, JNK, p38, and p53. These factors may be influenced by both environmental and constitutional factors. Apoptosis resistance may occur if there is any deregulation in the critical steps of apoptotic pathways. The deregulation of proteins like Bcl-2, death receptors, and death ligands is often caused by processes such as TP53 inactivation, EGFR overexpression, COX-2 overexpression, and MAPKs overexpression (43).

8 HPV infection

In recent years, there has been growing evidence that HPV plays a significant role in the development of AK and cSCC, along with chronic UV irradiation, immunosuppression, and genetic predispositions. A cross-sectional investigation using skin swabs found a correlation between the presence of AK and HPV species 1 and 2 from the Betapapillomavirus genus. In fact, individuals with AK or cSCC, or AK alone, had a higher number of HPV types per sample compared to healthy participants (44). Besides, four novel human betapapillomaviruses of species 2 designated HPV-107, −110 and −111, and FA75[KI88-03], preferentially found in AK (45). The selective detection of HPV DNA at sites exposed to sunlight could stem from enhanced promoter activity following UV irradiation, coupled with a reduction in apoptosis (46). In particular, the suppression of apoptosis in response to UV-induced damage by the E6 protein from various cutaneous HPV types might significantly contribute to giving genetically damaged keratinocytes a survival edge, leading to the development of AK and cSCC (47). Epidermodysplasia verruciformis-associated HPVs (EV-HPVs) might also play a crucial role in the emergence of AK, as indicated by serological studies, and are implicated in the pathogenesis of SCC (48). In EV-associated cSCCs, a variety of betaHPV types, notably HPV5 and HPV8, are identified. These types are also associated with the onset of actinic keratoses and cSCC in individuals from the general population (49–52). In addition, Bolatti et al. observed a greater prevalence of HPV and higher viral loads in AK compared to cSCC. They also identified a higher prevalence of gammaHPV in AK when compared to betaHPV and alphaHPV types. As a result, it appears challenging to specifically designate high-risk cutaneous HPV types, suggesting that multiple cutaneous HPV types may contribute to tumorigenesis (53). Interestingly, a case study employing the off-label use of the 9-valent HPV vaccine for the management of AK demonstrated regression of AK lesions starting within months of the initial injection. This resulted in the clearance of thousands of lesions even before completing the entire vaccination protocol (54).

9 Summary and perspectives on AK studies

There are three possible outcomes for AK: spontaneous disappearance, persistence, or progression to invasive cSCC (11, 14, 55). However, accurately predicting the development of AK lesions is challenging due to current limitations in diagnosis (2). The natural

TABLE 3 Mechanistic Insights into AK Progression to cSCC: References from the Past Decade.

Title	Authors	Journal	DOI	Pub_Date
Single-cell sequencing highlights heterogeneity and malignant progression in actinic keratosis and cutaneous squamous cell carcinoma	Zou DD; et al.	Elife	10.7554/eLife.85270	2023
Targeting *Staphylococcus aureus* dominated skin dysbiosis in actinic keratosis to prevent the onset of cutaneous squamous cell carcinoma: Outlook for future therapies?	Bromfield JI; et al.	Front Oncol	10.3389/fonc.2023.1091379	2023
Driver gene combinations dictate cutaneous squamous cell carcinoma disease continuum progression	Bailey P; et al.	Nat Commun	10.1038/s41467-023-40822-9	2023
Genetic Studies of Actinic Keratosis Development: Where Are We Now?	Lee YB; et al.	Ann Dermatol	10.5021/ad.23.072	2023
Non-Melanoma Skin Cancer and Vitamin D: The Lost Sunlight "Paradox and the Oxidative Stress Explanation"	Karampinis E; et al.	Antioxidants (Basel)	10.3390/antiox12051107	2023
Significant Biomarkers Identification Associated with Cutaneous Squamous Cell Carcinoma Progression	Qiu CG; et al.	Int J Gen Med	10.2147/IJGM.S357022	2022
Skin Cancer-Associated *S. aureus* Strains Can Induce DNA Damage in Human Keratinocytes by Downregulating DNA Repair and Promoting Oxidative Stress	Krueger A; et al.	Cancers (Basel)	10.3390/cancers14092143	2022
Inhibition of Cell Proliferation and Cell Viability by Sinecatechins in Cutaneous SCC Cells Is Related to an Imbalance of ROS and Loss of Mitochondrial Membrane Potential	Zhu J; et al.	Antioxidants (Basel)	10.3390/antiox11071416	2022
Cutaneous Squamous Cell Carcinoma: From Pathophysiology to Novel Therapeutic Approaches	Fania L; et al.	Biomedicines	10.3390/biomedicines9020171	2021
Telomeres and Telomerase in Cutaneous Squamous Cell Carcinoma	Ventura A; et al.	Int J Mol Sci	10.3390/ijms20061333	2019
Neoantigen Fitness Model Predicts Lower Immune Recognition of Cutaneous Squamous Cell Carcinomas Than Actinic Keratoses	Borden ES; et al.	Front Immunol	10.3389/fimmu.2019.02799	2019
The Role of Human Papillomaviruses and Polyomaviruses in BRAF-Inhibitor Induced Cutaneous Squamous Cell Carcinoma and Benign Squamoproliferative Lesions	Purdie KJ; et al.	Front Microbiol	10.3389/fmicb.2018.01806	2018
Immune consequences induced by photodynamic therapy in non-melanoma skin cancers: a review	Yu X; et al.	Environ Sci Pollut Res Int	10.1007/s11356-018-2426-z	2018
A review of BF-200 ALA for the photodynamic treatment of mild-to-moderate actinic keratosis	Reinhold U	Future Oncol	10.2217/fon-2017-0247	2017
MiR-204 silencing in intraepithelial to invasive cutaneous squamous cell carcinoma progression	Toll A; et al.	Mol Cancer	10.1186/s12943-016-0537-z	2016
Gene expression profiling of the leading edge of cutaneous squamous cell carcinoma: IL-24-driven MMP-7	Mitsui H; et al.	J Invest Dermatol	10.1038/jid.2013.494	2014
The role of apoptosis in therapy and prophylaxis of epithelial tumors by nonsteroidal anti-inflammatory drugs (NSAIDs)	Fecker LF; et al.	Br J Dermatol	10.1111/j.1365-2133.2007.07856.x	2007
Analysis of promoter hypermethylation of death-associated protein kinase and p16 tumor suppressor genes in actinic keratoses and squamous cell carcinomas of the skin	Tyler LN; et al.	Mod Pathol	10.1097/01.MP.0000077516.90063.7D	2003

history of AK typically involves high turnover rates, with many lesions developing, regressing, and recurring over time (14). Research indicates that thicker AK lesions have a higher likelihood of progressing to cSCC (56). Several risk factors contribute to this progression. The most significant constitutional risk factors for AK include old age, male gender, fair skin, immunosuppression, and a previous history of AK. Additionally, chronic sun exposure is the most significant environmental factor contributing to AK (13, 57–60). Individuals with HPV infection or chronic lymphocytic leukemia who have undergone solid-organ transplantation are at a greater risk of developing cSCC compared to the general population (61–66). In summary, AK can progress to cSCC and serves as a pre-cancerous lesion (49). The development of cSCC involves molecular pathways, including genomic instability caused by TP53 mutations induced by UV radiation (67).

Compared to other types of solid tumors, the development of cSCC involves multiple genetic mutations, which may have potential therapeutic implications (68). Additional genetic alterations occur in tumor suppressor genes such as CDKN2A and NOTCH (69), as well as in oncogenes such as RAS (68). The accumulation of these gene mutations activates various signaling pathways, including NF-kB, MAPK, and PI3K/AKT/mTOR pathways (70, 71), leading to the overexpression of the epidermal growth factor receptor (EGFR). A recent study found no significant correlation between numerical gains in EGFR and tumor depth or size. However, the study suggests that EGFR numerical aberrations occur during the early stages of cancer development. Currently, there is no available literature assessing the predictive role of EGFR cytogenetic aberrations in the treatment of metastatic or recurrent SCC with tyrosine kinase inhibitors. Furthermore, the effectiveness of anti-EGFR drugs in treating AK remains unexplored. Nevertheless, reports indicate that patients undergoing treatment with erlotinib experience inflammatory flare-up reactions resulting in partial destruction of AK. In summary, a significant proportion of *in situ* SCC already exhibit EGFR numerical gains, but these alterations do not appear to contribute to the progression from low-grade SCC to more aggressive phenotypes (71). Studies have revealed that several signaling pathways, which are activated in cSCC, exhibit pre-existing activation in AK, thereby supporting AK as precursor lesions of cSCC.

Through comprehensive genome-wide SNP microarray and expression microarray analyses, pathways such as NF-kB1 and the tumor necrosis factor pathways have been identified. It is noteworthy that both NF-kB1 and the tumor necrosis factor pathways are classic proinflammatory signaling pathways. Another investigation sheds

light on the involvement of the MAPK pathway and apoptosis-related genes in the pathogenesis of cSCC and AK. These findings underscore the participation of pathways related to cell cycle regulation, apoptosis, inflammation, and epidermal differentiation in the development and progression of cSCC from AK (15, 19). A recent study discovered dysregulation of TGFβ signaling that varies depending on the progression stage, ranging from normal skin to AK to cSCC. One group of TGFβ-associated genes consistently showed increased activity throughout the progression, while another group exhibited decreased activity. These findings indicate the potential involvement of TGFβ signaling in the transition from AK to cSCC (20). The study of signaling pathways can offer potential targets for future treatments of AK and cSCC. Furthermore, it has been observed that epigenetic alterations may occur during the progression of AK and cSCC. Several studies have investigated the use of DNA methylation arrays to assess AK (21–35). It has been proposed that during the transition from normal skin to AK and cSCC, there may be an increase in E-cadherin promoter hypermethylation (35). Furthermore, the malignant potential of AK has been highlighted by observing AK methylomes exhibiting typical cancer-related characteristics, including CpG island promoter hypermethylation and hypomethylation of lamina-associated domains (21). DNA methylation signature could discriminate different stages of disease ranging from premalignant AK to low-risk invasive and high-risk non-metastatic and metastatic CSCC in the future.

Genetic alterations, such as TP53 mutations, ras gene mutations, c-myc proto-oncogene mutations, p16INK4a tumor suppressor gene mutations, and telomerase activity, are closely associated with the development of AK. The progression of AK lesions is difficult to predict, but thicker lesions are more likely to progress into cSCC (3). Environmental and constitutional risk factors for AK include chronic sun exposure, advanced age, male gender, fair skin, immunosuppression, previous history of AK, and HPV infection. EGFR overexpression is linked to various pathways, including NF-kB, MAPK, and PI3K/AKT/mTOR. These pathways can be activated by UV-induced TP53 mutations, CDKN2A and NOTCH alterations, and RAS mutations (72–78).

This review has certain limitations that should be considered. Firstly, the current research on the pathogenesis of AK is not comprehensive enough. Secondly, our findings were drawn from existing literature and evaluations, underscoring the imperative for additional enhancements in assessment methods within the field to attain a more comprehensive understanding of the pathogenesis of AK. However, we hypothesize that AK has the potential to progress to cSCC, and timely intervention in the signaling pathways could lead to successful treatment. It is crucial to protect the skin from sunburn damage, as UV radiation is the primary cause of AK. Technological advancements have facilitated the identification of more genes associated with AK, offering potential targets for treatment. We speculate that with research providing deeper understanding of AK pathogenesis, it could be diagnosed more accurately in the future and treated with more effective medications.

10 Conclusion

In conclusion, AK is a skin disorder that is increasingly prevalent and has the potential to progress to cSCC. The development of AK involves various intricate mechanisms, which offer potential avenues for treatment. Timely diagnosis, treatment, and prevention of AK are of utmost importance. Further research is needed to enhance our comprehensive understanding of this disease.

Author contributions

ZW: Writing – original draft, Writing – review & editing. XW: Conceptualization, Writing – original draft. YS: Data curation, Writing – original draft. SW: Formal analysis, Writing – review & editing. YD: Software, Writing – original draft. GY: Methodology, Project administration, Writing – original draft. JC: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Berman B, Cockerell CJ. Pathobiology of actinic keratosis: ultraviolet-dependent keratinocyte proliferation. *J Am Acad Dermatol*. (2013) 68:S10–9. doi: 10.1016/j.jaad.2012.09.053

2. Grada A, Feldman SR, Bragazzi NL, Damiani G. Patient-reported outcomes of topical therapies in actinic keratosis: a systematic review. *Dermatol Ther*. (2021) 34:e14833. doi: 10.1111/dth.14833

3. Marques E, Chen TM. *Actinic keratosis*. Treasure Island, FL: StatPearls Publishing (2023).

4. Sample A, He YY. Mechanisms and prevention of UV-induced melanoma. *Photodermatol Photoimmunol Photomed*. (2018) 34:13–24. doi: 10.1111/phpp.12329

5. Padilla RS, Sebastian S, Jiang Z, Nindl I, Larson R. Gene expression patterns of normal human skin, actinic keratosis, and squamous cell carcinoma: a spectrum of disease progression. *Arch Dermatol*. (2010) 146:288–93. doi: 10.1001/archdermatol.2009.378

6. Albibas AA, Rose-Zerilli MJJ, Lai C, Pengelly RJ, Lockett GA, Theaker J, et al. Subclonal evolution of cancer-related gene mutations in p 53 immunopositive patches in human skin. *J Invest Dermatol*. (2018) 138:189–98. doi: 10.1016/j.jid.2017.07.844

7. Figueras Nart I, Cerio R, Dirschka T, Dréno B, Lear JT, Pellacani G, et al. Defining the actinic keratosis field: a literature review and discussion. *J Eur Acad Dermatol Venereol*. (2018) 32:544–63. doi: 10.1111/jdv.14652

8. Melnikova VO, Pacifico A, Chimenti S, Peris K, Ananthaswamy HN. Fate of UVB-induced p53 mutations in SKH-hr1 mouse skin after discontinuation of irradiation: relationship to skin cancer development. *Oncogene*. (2005) 24:7055–63. doi: 10.1038/sj.onc.1208863

9. Siegel JA, Korgavkar K, Weinstock MA. Current perspective on actinic keratosis: a review. *Br J Dermatol*. (2016) 177:350–8. doi: 10.1111/bjd.14852

10. Criscione VD, Weinstock MA, Naylor MF, Luque C, Eide MJ, Bingham SF, et al. Actinic keratoses: natural history and risk of malignant transformation in the veterans affairs topical tretinoin chemoprevention trial. *Cancer*. (2009) 115:2523–30. doi: 10.1002/cncr.24284

11. Dréno B, Amici J, Basset-Seguin N, Cribier B, Claudel JP, Richard MA, et al. Management of actinic keratosis: a practical report and treatment algorithm fromAKTeamTMexpert clinicians. *J Eur Acad Dermatol Venereol*. (2014) 28:1141–9. doi: 10.1111/jdv.12434

12. Jacobs LC, Liu F, Pardo LM, Hofman A, Uitterlinden AG, Kayser M, et al. IRF4, MC1R and TYR genes are risk factors for actinic keratosis independent of skin color. *Hum Mol Genet*. (2015) 24:3296–303. doi: 10.1093/hmg/ddv076

13. Toll A, Salgado R, Yébenes M, Martín-Ezquerra G, Gilaberte M, Baró T, et al. MYC gene numerical aberrations in actinic keratosis and cutaneous squamous cell carcinoma. *Br J Dermatol*. (2009) 161:1112–8. doi: 10.1111/j.1365-2133.2009.09351.x

14. South AP, Purdie KJ, Watt SA, Haldenby S, den Breems NY, Dimon M, et al. NOTCH1 mutations occur early during cutaneous squamous cell carcinogenesis. *J Invest Dermatol*. (2014) 134:2630–8. doi: 10.1038/jid.2014.154

15. Corchado-Cobos R, García-Sancha N, González-Sarmiento R, Pérez-Losada J, Cañueto J. Cutaneous Squamous Cell Carcinoma: From Biology to Therapy. *Int J Mol Sci*. (2020) 21:2956. doi: 10.3390/ijms21082956

16. Murao K, Kubo Y, Ohtani N, Hara E, Arase S. Epigenetic abnormalities in cutaneous squamous cell carcinomas: Frequent inactivation of the RB1/p 16 and p 53 pathways. *Br J Dermatol*. (2006) 155:999–1005. doi: 10.1111/j.1365-2133.2006.07487.x

17. Pickering CR, Zhou JH, Lee JJ, Drummond JA, Peng SA, Saade RE, et al. Mutational landscape of aggressive cutaneous squamous cell carcinoma. *Clin Cancer Res*. (2014) 20:6582–92. doi: 10.1158/1078-0432.CCR-14-1768

18. Kim Y-S, Shin S, Jung S-H, Park YM, Park GS, Lee SH, et al. Genomic progression of precancerous actinic keratosis to squamous cell carcinoma. *J Invest Dermatol*. (2022) 142:528–538.e8. doi: 10.1016/j.jid.2021.07.172

19. Wang LH, Wu CF, Rajasekaran N, Shin YK. Loss of Tumor Suppressor Gene Function in Human Cancer: An Overview. *Cell Physiol Biochem*. (2018) 51:2647–93. doi: 10.1099/vir.0.2008/001925-0

20. Sun W, Yang J. Functional mechanisms for human tumor suppressors. *J Cancer*. (2010) 1:136–40. doi: 10.7150/jca.1.136

21. Thomson J, Bewicke-Copley F, Anene CA, Gulati A, Nagano A, Purdie K, et al. The genomic landscape of actinic keratosis. *J Invest Dermatol*. (2021) 141:1664–1674.e7. doi: 10.1016/j.jid.2020.12.024

22. Xu D, Yuan R, Gu H, Liu T, Tu Y, Yang Z, et al. The effect of ultraviolet radiation on the transforming growth factor beta 1/Smads pathway and p 53 in actinic keratosis and normal skin. *Arch Dermatol Res*. (2013) 305:777–86. doi: 10.1007/s00403-013-1361-6

23. Ming M, Feng L, Shea CR, Soltani K, Zhao B, Han W, et al. PTEN positively regulates UVB-induced DNA damage repair. *Cancer Res*. (2011) 71:5287–95. doi: 10.1158/0008-5472.CAN-10-4614

24. Lu J, Goldstein KM, Chen P, Huang S, Gelbert LM, Nagpal S. Transcriptional profiling of keratinocytes reveals a vitamin D-regulated epidermal differentiation network. *J Invest Dermatol*. (2005) 124:778–85. doi: 10.1111/j.0022-202X.2005.23641.x

25. Talero E, García-Mauriño S, Ávila-Román J, Rodríguez-Luna A, Alcaide A, Motilva V. Bioactive compounds isolated from microalgae in chronic inflammation and cancer. *Mar Drugs*. (2015) 13:6152–209. doi: 10.3390/md13106152

26. Thomas-Ahner JM, Wulff BC, Tober KL, Kusewitt DF, Riggenbach JA, Oberyszyn TM. Gender differences in UVB-induced skin carcinogenesis, inflammation, and DNA damage. *Cancer Res*. (2007) 67:3468–74. doi: 10.1158/0008-5472.CAN-06-3798

27. Sinclair R, Baker C, Spelman L, Supranowicz M, MacMahon B. A review of actinic keratosis, skin field cancerisation and the efficacy of topical therapies. *Australas J Dermatol*. (2021) 62:119–23. doi: 10.1111/ajd.13447

28. Hameetman L, Commandeur S, Bavinck JN, Wisgerhof HC, de Gruijl FR, Willemze R, et al. Molecular profiling of cutaneous squamous cell carcinomas and actinic keratoses from organ transplant recipients. *BMC Cancer*. (2013) 13:58. doi: 10.1186/1471-2407-13-58

29. Massari LP, Kastelan M, Gruber F. Epidermal malignant tumors: pathogenesis, influence of UV light and apoptosis. *Coll Antropol*. (2007) 31:83–5. doi: 10.3390/antiox12091675

30. Vattemi E, Claudio PP. Tumor suppressor genes as cancer therapeutics. *Drug News Perspect*. (2007) 20:511–20. doi: 10.1016/j.jid.2021.02.761

31. Kanellou P, Zaravinos A, Zioga M, Stratigos A, Baritaki S, Soufla G, et al. Genomic instability, mutations and expression analysis of the tumour suppressor genes p14ARF, p15INK4b, p16INK4a and p53 in actinic keratosis. *Cancer Lett*. (2008) 264:145–61. doi: 10.1016/j.canlet.2008.01.042

32. Lambert SR, Mladkova N, Gulati A, Hamoudi R, Purdie K, Cerio R, et al. Key differences identified between actinic keratosis and cutaneous squamous cell carcinoma by transcriptome profiling. *Br J Cancer*. (2014) 110:520–9. doi: 10.1038/bjc.2013.760

33. Rodríguez-Paredes M, Bormann F, Raddatz G, Gutekunst J, Lucena-Porcel C, Köhler F, et al. Methylation profiling identifies two subclasses of squamous cell carcinoma related to distinct cells of origin. *Nat Commun*. (2018) 9:577. doi: 10.1038/s41467-018-03025-1

34. Hervás-Marín D, Higgins F, Sanmartín O, López-Guerrero JA, Bañó MC, Igual JC, et al. Genome wide DNA methylation profiling identifies specific epigenetic features in high-risk cutaneous squamous cell carcinoma. *PLoS One*. (2019) 14:e0223341. doi: 10.1371/journal.pone.0223341

35. Chiles MC, Ai L, Zuo C, Fan CY, Smoller BR. E-cadherin promoter hypermethylation in preneoplastic and neoplastic skin lesions. *Mod Pathol*. (2003) 16:1014–8. doi: 10.1097/01.MP.0000089779.35435.9D

36. Wood DLA, Lachner N, Tan JM, Tang S, Angel N, Laino A, et al. A natural history of actinic keratosis and cutaneous squamous cell carcinoma microbiomes. *mBio*. (2018) 9:e01432-18. doi: 10.1128/mBio.01432-18

37. Dragneva Y, Anuradha CD, Valeva A, Hoffmann A, Bhakdi S, Husmann M. Subcytocidal Attack by Staphylococcal Alpha-Toxin Activates NF-κB and Induces Interleukin-8 Production. *Infect Immun*. (2001) 69:2630–5. doi: 10.1128/IAI.69.4.2630-2635.2001

38. Narendhirakannan RT, Hannah AC. Oxidative Stress and Skin Cancer: An Overview. Indian. *J Clin Biochem*. (2013) 28:110–5. doi: 10.1007/s12291-012-0278-8

39. Sander CS, Chang H, Hamm F, Elsner P, Thiele JJ. Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. *Int J Dermatol*. (2004) 43:326–35. doi: 10.1111/j.1365-4632.2004.02222.x

40. Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q. The Applications and Mechanisms of Superoxide Dismutase in Medicine, Food, and Cosmetics. *Antioxidants*. (2023) 12:1675. doi: 10.3390/antiox12091675

41. Hedberg M, Seykora JT. Clarifying Progress on the Genomic Landscape of Actinic Keratosis. *J Invest Dermatol*. (2021) 141:1622–4. doi: 10.1016/j.jid.2021.02.761

42. Boukamp P. Non-melanoma skin cancer: what drives tumor development and progression? *Carcinogenesis*. (2005) 26:1657–67. doi: 10.1093/carcin/bgi123

43. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p 53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res*. (1994) 54:4855–78.

44. Khorshid SM, Glover MT, Churchill L, McGregor JM, Proby CM. p53 immunoreactivity in non-melanoma skin cancer from immunosuppressed and immunocompetent individuals: a comparative study of 246 tumours. *J Cutan Pathol*. (1996) 23:229–33. doi: 10.1111/j.1600-0560.1996.tb01471.x

45. Karagece Yalçin U, Seçkın S. The expression of p 53 and COX-2 in basal cell carcinoma, squamous cell carcinoma, and actinic keratosis cases. *Turk Patoloji Derg*. (2012) 28:119–27. doi: 10.5146/tjpath.2012.01110

46. Global Lipids Genetics Consortium. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. (2013) 45:1274–83. doi: 10.1038/ng.2797

47. Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature*. (2010) 467:832–8. doi: 10.1038/nature09410

48. Zou DD, Sun YZ, Li XJ, Wu WJ, Xu D, He YT, et al. Single-cell sequencing highlights heterogeneity and malignant progression in actinic keratosis and cutaneous squamous cell carcinoma. *eLife*. (2023) 12:e85270. doi: 10.7554/eLife.85270

49. Apoptoza TD. UV-zracenje, prekanceroze itumorikoze. Apoptosis, UV-radiation, precancerosis and skin tumors. *Acta Med Croatica*. (2009) 63:53–8. doi: 10.1128/jvi.01003-18

50. Rodust PM, Stockfleth E, Ulrich C, Leverkus M, Eberle J. UV-induced squamous cell carcinoma - a role for antiapoptotic signalling pathways. *Br J Dermatol*. (2009) 161:107–15. doi: 10.1111/j.1365-2133.2009.09458.x

51. Alotaibi L, Provost N, Gagnon S, Franco EL, Coutlée F. Diversity of cutaneous human papillomavirus types in individuals with and without skin lesion. *J Clin Virol*. (2006) 36:133–40. doi: 10.1016/j.jcv.2006.02.007

52. Vasiljević N, Hazard K, Dillner J, Forslund O. Four novel human betapapillomaviruses of species 2 preferentially found in actinic keratosis. *J Gen Virol*. (2008) 89:2467–74. doi: 10.1099/vir.0.2008/001925-0

53. Jackson S, Storey A. E6 proteins from diverse cutaneous HPV types inhibit apoptosis in response to UV damage. *Oncogene*. (2000) 19:592–8. doi: 10.1038/sj.onc.1203339

54. Bouwes Bavinck JN, Feltkamp M, Struijk L, ter Schegget J. Human papillomavirus infection and skin cancer risk in organ transplant recipients. *J Investig Dermatol Symp Proc*. (2001) 6:207–11. doi: 10.1046/j.0022-202x.2001.00048.x

55. Bouwes Bavinck JN, Stark S, Petridis AK, Marugg ME, ter Schegget J, Westendorp RGJ, et al. The presence of antibodies against virus-like particles of epidermodysplasia verruciformis-associated humanpapillomavirus type 8 in patients with actinic keratoses. *Br J Dermatol*. (2000) 142:103–9. doi: 10.1046/j.1365-2133.2000.03248.x

56. Bouwes Bavinck JN, Feltkamp MCW, Green AC, Fiocco M, Euvrard S, Harwood CA, et al. Human papillomavirus and posttransplantation cutaneous squamous cell carcinoma: a multicenter, prospective cohort study. *Am J Transplant*. (2018) 18:1220–30. doi: 10.1111/ajt.14537

57. Hasche D, Vinzon SE, Rosl F. Cutaneous papillomaviruses and non-melanoma skin cancer: causal agents or innocent bystanders? *Front Microbiol*. (2018) 9:874. doi: 10.1016/j.jaad.2017.08.059

58. Rollison DE, Viarisio D, Amorrortu RP, Gheit T, Tommasino M. An emerging issue in oncogenic virology: the role of beta HPV types in development of cutaneous squamous cell carcinoma. *J Virol*. (2019) 93:e01003-18. doi: 10.1128/JVI.01003-18

59. Tommasino M. HPV and skin carcinogenesis. *Papillomavirus Res*. (2019) 7:129–31. doi: 10.1016/j.pvr.2019.04.003

60. Bolatti EM, Hošnjak L, Chouhy D, Re-Louhau MF, Casal PE, Bottai H, et al. High prevalence of Gammapapillomaviruses (Gamma-PVs) in pre-malignant cutaneous lesions of immunocompetent individuals using a new broad-spectrum primer system, and identification of HPV210, a novel Gamma-PV type. *Virology*. (2018) 525:182–91. doi: 10.1016/j.virol.2018.09.006

61. Wenande E, Bech-Thomsen N, Haedersdal M. Reduction in actinic keratoses following 9-valent human papilloma virus vaccination. *Dermatol Ther*. (2020) 33:13454.

62. Gupta AK, Paquet M, Villanueva E, Brintnell WCochrane Skin Group. Interventions for actinic keratoses. *Cochrane Database Syst Rev*. (2012) 12:CD004415. doi: 10.1002/14651858.CD004415.pub2

63. Schmitt JV, Miot HA. Actinic keratosis: a clinical and epidemiological revision. *An Bras Dermatol*. (2012) 87:425–34. doi: 10.1590/S0365-05962012000300012

64. Que SKT, Zwald FO, Schmults CD. Cutaneous squamous cell carcinoma: Incidence, risk factors, diagnosis, and staging. *J Am Acad Dermatol*. (2018) 78:237–47. doi: 10.1067/mjd.2002.125579

65. Garcovich S, Colloca G, Sollena P, Andrea B, Balducci L, Cho WC, et al. Skin Cancer Epidemics in the Elderly as An Emerging Issue in Geriatric Oncology. *Aging Dis*. (2017) 8:643–61. doi: 10.14336/AD.2017.0503

66. Oberyszyn TM. Non-melanoma skin cancer: importance of gender, immunosuppressive status and vitamin D. *Cancer Lett*. (2008) 261:127–36. doi: 10.1016/j.canlet.2008.01.009

67. Gloster HM Jr, Neal K. Skin cancer in skin of color. *J Am Acad Dermatol*. (2006) 55:741–60. doi: 10.1016/j.jaad.2005.08.063

68. de Oliveira ECV, da Motta VRV, Pantoja PC, Ilha CSO, Magalhães RF, Galadari H, et al. Actinic keratosis - review for clinical practice. *Int J Dermatol*. (2019) 58:400–7. doi: 10.1111/ijd.14147

69. Kivisaari A, Kähäri VM. Squamous cell carcinoma of the skin: Emerging need for novel biomarkers. *World J Clin Oncol*. (2013) 4:85–90. doi: 10.5306/wjco.v4.i4.85

70. Berg D, Otley CC. Skin cancer in organ transplant recipients: Epidemiology, pathogenesis, and management. *J Am Acad Dermatol*. (2002) 47:1–17. doi: 10.1158/1078-0432.ccr-14-1768

71. Lindelöf B, Sigurgeirsson B, Gäbel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. *Br J Dermatol*. (2000) 143:513–9.

72. Mehrany K, Weenig RH, Pittelkow MR, Roenigk RK, Otley CC. High recurrence rates of squamous cell carcinoma after Mohs' surgery in patients with chronic lymphocytic leukemia. *Dermatologic Surg*. (2005) 31:38–42. doi: 10.1097/00042728-200501000-00008

73. Dang C, Koehler A, Forschner T, Sehr P, Michael K, Pawlita M, et al. E6/E7 expression of human papillomavirus types in cutaneous squamous cell dysplasia and carcinoma in immunosuppressed organ transplant recipients. *Br J Dermatol*. (2006) 155:129–36. doi: 10.1111/j.1365-2133.2006.07378.x

74. Werner RN, Sammain A, Erdmann R, Hartmann V, Stockfleth E, Nast A. The natural history of actinic keratosis: A systematic review. *Br J Dermatol*. (2013) 169:502–18. doi: 10.1111/bjd.12420

75. Cañueto J, Cardeñoso E, García JL, Santos-Briz Á, Castellanos-Martín A, Fernández-López E, et al. Epidermal growth factor receptor expression is associated with poor outcome in cutaneous squamous cell carcinoma. *Br J Dermatol*. (2017) 176:1279–87. doi: 10.1111/bjd.14936

76. Toll A, Salgado R, Yébenes M, Martín-Ezquerra G, Gilaberte M, Baró T, et al. Epidermal growth factor receptor gene numerical aberrations are frequent events in actinic keratoses and invasive cutaneous squamous cell carcinomas. *Exp Dermatol*. (2010) 19:151–3. doi: 10.1111/j.1600-0625.2009.01028.x

77. Brown VL, Harwood CA, Crook T, Cronin JG, Kelsell DP, Proby CM. p16INK4a and p14ARF tumor suppressor genes are commonly inactivated in cutaneous squamous cell carcinoma. *J Invest Dermatol*. (2004) 122:1284–92. doi: 10.1111/j.0022-202X.2004.22501.x

78. Ratushny V, Gober MD, Hick R, Ridky TW, Seykora JT. From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. *J Clin Invest*. (2012) 122:464–72. doi: 10.1172/JCI57415

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Interplay of cytokines in the pathophysiology of atopic dermatitis: insights from Murin models and human

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The pathogenesis of atopic dermatitis (AD) is understood to be crucially influenced by three main factors: dysregulation of the immune response, barrier dysfunction, and pruritus. In the lesional skin of AD, various innate immune cells, including Th2 cells, type 2 innate lymphoid cells (ILC2s), and basophils, produce Th2 cytokines [interleukin (IL)-4, IL-5, IL-13, IL-31]. Alarmins such as TSLP, IL-25, and IL-33 are also produced by epidermal keratinocytes, amplifying type 2 inflammation. In the chronic phase, not only Th2 cells but also Th22 and Th17 cells increase in number, leading to suppression of filaggrin expression by IL-4, IL-13, and IL-22, which further deteriorates the epidermal barrier function. Dupilumab, which targets IL-4 and IL-13, has shown efficacy in treating moderate to severe AD. Nemolizumab, targeting IL-31RA, effectively reduces pruritus in AD patients. In addition, clinical trials with fezakinumab, targeting IL-22, have demonstrated promising results, particularly in severe AD cases. Conversely, in murine models of AD, several cytokines, initially regarded as promising therapeutic targets, have not demonstrated sufficient efficacy in clinical trials. IL-33 has been identified as a potent activator of immune cells, exacerbating AD in murine models and correlating with disease severity in human patients. However, treatments targeting IL-33 have not shown sufficient efficacy in clinical trials. Similarly, thymic stromal lymphopoietin (TSLP), integral to type 2 immune responses, induces dermatitis in animal models and is elevated in human AD, yet clinical treatments like tezepelumab exhibit limited efficacy. Therapies targeting IL-1α, IL-5, and IL-17 also failed to achieve sufficient efficacy in clinical trials. It has become clear that for treating AD, IL-4, IL-13, and IL-31 are relevant therapeutic targets during the acute phase, while IL-22 emerges as a target in more severe cases. This delineation underscores the necessity of considering distinct pathophysiological aspects and therapeutic targets in AD between mouse models and humans. Consequently, this review delineates the distinct roles of cytokines in the pathogenesis of AD, juxtaposing their significance in human AD from clinical trials against insights gleaned from AD mouse models. This approach will improve our understanding of interspecies variation and facilitate a deeper insight into the pathogenesis of AD in humans.

KEYWORDS

AD, cytokines, clinical trials, Th2, Th22

1 Introduction

Atopic dermatitis (AD) is a chronic, relapsing inflammatory dermatosis characterized by pruritic, erythematous, and edematous lesions. Predominantly manifesting in early childhood, the condition exhibits a variable incidence across different ages. The clinical presentation of AD is marked by episodic exacerbations and remissions, with affected individuals frequently presenting with xerosis, which exacerbates the itch-scratch cycle (1). The pathogenesis of AD is currently understood to be crucially influenced by three main factors: dysregulation of the immune response, barrier dysfunction, and pruritus (2). The underlying immune dysregulation in AD is characterized by an overactive T helper cell type (Th) 2 response. Additionally, mutations in the filaggrin gene, which plays a vital role in skin barrier function, are a primary cause of barrier dysfunction (3). Approximately 10–30% of AD patients exhibit mutations in the filaggrin gene (4). A meta-analysis of a genome-wide association study (GWAS) and GWAS revealed 31 loci associated with AD, including four loci with secondary independent signals (5). Several AD risk loci supported existing findings, including the role of skin barrier and type 2 inflammation in AD pathogenesis. In addition, it notes the identification of rare protein-coding variations contributing to AD heritability, including in genes such as interleukin (IL) 4 receptor (R), IL13, Janus kinase (JAK)1, JAK2, and TYK2, plus novel candidate genes.

In the lesional skin of AD, various innate immune cells, including Th2 cells, type 2 innate lymphoid cells (ILC2s), and basophils, produce Th2 cytokines (IL-4, IL-5, IL-13, IL-31). Alarmins such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 are also produced by epidermal keratinocytes, amplifying type 2 inflammation (6). These cytokines further decrease the expression of barrier-associated proteins such as filaggrin, loricrin, and involucrin, leading to impaired barrier function (7).

Pruritus is one of the most prominent features of AD, although the mechanisms underlying AD-associated pruritus are not as well understood as those for barrier dysfunction and immune activation. Recently, several cytokines, including TSLP, IL-4/13, and IL-31, have been reported to be involved in AD-associated pruritus, and biological agents targeting these cytokines have been shown to improve pruritus in AD patients (8).

While numerous mouse models have been utilized to study AD and have significantly contributed to our understanding of the disease, it is important to note that the roles of various cytokines may not be completely identical between mice and humans. We searched PubMed and ClinicalTrials.gov from database inception to September 2023. Searches were adapted for each database, using keywords that included a combination of terms related to atopic dermatitis and clinical trial. We targeted biological agents undergoing phase 2 trials for AD. We searched for papers involving cytokines targeted by biological agents and relevant to the pathophysiology of AD, dividing the papers into mouse and human data (**Table 1**). In this review, we discuss the role of cytokines in the pathophysiology of separately for humans and mice and summarize their effects in clinical trials (**Figure 1**).

2 Non-lesional skin

The barrier dysfunction that occurs in the early stages of AD facilitates the penetration of allergens into the skin, and damaged keratinocytes produce cytokines such as TSLP, IL-25, and IL-33. These cytokines activate Th2 cells and ILC2s, leading to the production of Th2 cytokines (9). Additionally, TSLP matures Langerhans cells (LCs) in the epidermis and induces Th2 cells (10).

Thus, in AD, subclinical inflammation is present even in the non-lesional skin at the initial stages of the disease, characterized by an increased expression of Th2 cytokines (IL-4, IL-13, IL-31, TSLP, IL-5) mediated by Th2 cells and other immune cells.

A decrease in the diversity of the microbial community on the epidermis and a relative increase in *Staphylococcus aureus* (*S. aureus*) have also been observed, which further amplify the Th2 immune response (11). This complex interplay of factors contributes to the exacerbation of AD symptoms and the perpetuation of the inflammatory state (12).

2.1 IL-33

IL-33, a member of the IL-1 family, is a multifaceted cytokine that affects various cell types including Th2 cells, mast cells, basophils, eosinophils, macrophages, dendritic cells (DCs), and ILC2s. It is primarily produced by epithelial cells, fibroblasts, and endothelial cells. IL-33 binds to a heterodimeric receptor consisting of ST2 (also known as IL-1RL1) and the IL-1 Receptor Accessory Protein (IL-1RAcP), leading to the activation of the NF-kB and mitogen-activated protein kinases (MAPK) (Extracellular signal-Regulated Kinase (ERK), p38, c-Jun N-terminal kinase (JNK)) signaling pathways (13). Full-length IL-33, released from epithelial cells, is cleaved into its active form by various proteases and allergens (14). The IL-33 receptor, ST2, is expressed on immune cells such as Th2 cells, mast cells, eosinophils, and basophils. Consequently, IL-33 acts as a sensitive sensor to protease allergens, promoting the proliferation, activation, and recruitment of Th2 cells (15).

2.1.1 Roles in mouse AD model

In a mouse AD model, IL-33 overexpression in the skin of IL-33 Transgenic (Tg) mice spontaneously activated ILC2s and induced a pruritic dermatitis like AD, suggesting that IL-33 is involved in the onset of AD (16). The inflammation induced by IL-33 in this model depends on a natural immune response mediated by ILC2s in coordination with basophils (17). Treatment with αIL-33 Antibody (Ab) in a Dinitrochlorobenzene (DNCB)-induced AD mouse model improved AD-like symptoms, reduced eosinophil and mast cell infiltration, and decreased serum Immunoglobulin E (IgE) levels (18). Additionally, propionate, a metabolic product of sebum, suppressed skin inflammation in a calcipotriol (MC903), a calcium analog of vitamin D3, -induced AD-like dermatitis mouse model by inhibiting IL-33 production in keratinocytes (19).

2.1.2 Roles in human AD

In human AD, serum IL-33 levels correlate with clinical severity (20), and expressions of IL-33 and its receptor components, ST2 and IL-1RAcP, are increased in lesional skin. Skin lesions

TABLE 1 Clinical trials and their outcomes in atopic dermatitis treatment.

Targeted cytokines	Agent	Mechanism	Description	Number of patient (Age)	Endpoint	Intervention arm	Results	ClinicalTrials. identifier	Developmen status	References
IL-33	Etokimab	Anti-IL-33	Monoclonal	302 (18 to 75 Years)	Week 16	Etokimab 600 mg/300 mg SC Q4W	Percent change in EASI Score: −44.56 (7.811)/−47.40 (6.091)/−55.70 (6.206)/−41.63 (6.707)/−49.38 (7.124)	NCT03533751	Phase 2 completed	(25)
						Etokimab 300 mg/150 mg SC Q4W				
						Etokimab 300 mg/150 mg SC Q8W				
						Etokimab 20 mg SC Q4W				
						Placebo				
IL-33	Astegolimab	Anti-IL-33		65 (18 to 75 Years)	Week 16	A loading dose of 245 mg SC MSTT1041A, followed by 490 mg of SC MSTT1041A every 4 weeks (Q4W)	Percent change of total EASI score: −58.24/−51.47	NCT03747575	Phase 2 completed	(26)
						Placebo				
TSLP	Tezepelumab	Anti-TSLP	Human monoclonal	251 (18 to 75 Years)	Week 16	Tezepelumab 420 mg Q2W	Number of participants with IGA score of 0 or 1: 5(7.2%)/2(3.2%)/4(6.5%)/2(3.2%)	NCT03809663	Phase 2 completed	(44)
						Tezepelumab 280 mg Q2W				
						Tezepelumab 210 mg Q4W				
						Placebo				
IL-1α	Bermekimab	Anti-IL-1α	Humanized monoclonal	6 (18 to 65 Years)	Week 16	Bermekimab 1200 mg IV	Percentage of participants with EASI-75: 0/0/0	NCT04990440	Phase 2 completed	
						Bermekimab 800 mg IV				
						Placebo				

(Continued)

10.3389/fmed.2024.1342176

TABLE 1 (Continued)

Targeted cytokines	Agent	Mechanism	Description	Number of patient (Age)	Endpoint	Intervention arm	Results	ClinicalTrials. identifier	Developmen status	References
IL-5	Mepolizumab	Anti- IL-5	Humanized monoclonal	34 (18 to 70 ears)	Week 16	Mepolizumab 100 mg SC	Number of participants with IGA score of 0 or 1 and at Least a 2- Grade Improvement: 2(11.1%)/0(0%)	NCT03055195	Phase 2 completed	(64)
						Placebo				
IL-4	Dupilumab	Anti-IL-4 receptor α	Human monoclonal	838 (18 Years and older)	Week 12	Dupilumab 300 mg + Oral placebo	IGA 0 or 1 and reduction (> =) 2 points rate: 36.5/48.4/36.6/14.0	NCT03720470	Phase 3 completed	(86)
						PF-04965842 200 mg + Placebo injection	EASI response > = 75 percent (%) improvement rate: 58.1/70.3/58.7/27.1			
						PF-04965842 100 mg + Placebo injection				
						Placebo				
				740 (18 Years and older)	Week 16	Dupilumab qw + Topical corticosteroids	IGA 0 or 1 and reduction (> =) 2 points rate: 39/39/12	NCT02260986	Phase 3 completed	(87)
						Dupilumab q2w + Topical corticosteroids	EASI response > = 75 percent (%) improvement rate: 64/69/23			
						Placebo + Topical corticosteroids				
				251 (12 to 17 Years)	Week 16	Dupilumab 200 or 300 mg Q2W	IGA 0 or 1 and reduction (> =) 2 points rate: 24.4/17.9/2.4	NCT03054428	Phase 3 completed	(88)
						Dupilumab 300 mg Q4W	EASI response > = 75 percent (%) improvement rate: 41.5/38.1/8.2			
						Placebo				
				294 (12 to 17 Years)	Week 52		Proportion of patients achieving IGA 0/1 (%): 42.7	NCT02612454	Phase 3 completed	(89)

(Continued)

10.3389/fmed.2024.1342176

TABLE 1 (Continued)

Targeted cytokines	Agent	Mechanism	Description	Number of patient (Age)	Endpoint	Intervention arm	Results	ClinicalTrials. identifier	Developmen status	References
							Proportion of patients achieving EASI 75 (%): 81.2			
							Mean % change in EASI from PSBL: −83.5			
							Mean % change in SCORAD from PSBL: −65.0			
				367 (6 to 11 Years)	Week 16	Dupilumab q2w + Topical corticosteroids	IGA 0 or 1 and reduction (> =) 2 points rate: :32/33/11	NCT03345914	Phase 3 completed	(90)
						Dupilumab q4w + Topical corticosteroids	EASI response > = 75 percent (%) improvement rate: 67.2/69.7/26.8			
						Placebo + Topical corticosteroids				
				202 (6 Months to 5 Years)	Week 16	Dupilumab q4w + Topical corticosteroids	IGA 0 or 1 and reduction (> =) 2 points rate: 28/4	NCT03346434	Phase 3 completed	(91)
						Placebo + Topical corticosteroids	EASI response > = 75 percent (%) Improvement rate: 53/11			
IL-13	Lebrikizumab	Anti-IL-13	Humanized monoclonal	212 (18 to 75 Years)	Week 12	Lebrikizumab + TCS	IGA 0 or 1 and reduction (> =) 2 points rate: :33/19	NCT02340234	Phase 2 completed	(93)
						Placebo + TCS	EASI response > = 50 percent (%) improvement rate: 82/52			
				280 (18 Years and older)	Week 16	Lebrikizumab 250 mg q2w	IGA 0 or 1 and reduction (> =) 2 points rate: :44.6/33.7/26.6/15.3	NCT03443024	Phase 2 completed	(96)
						Lebrikizumab 250 mg q4w	EASI response > = 75 percent (%) improvement rate: 60.6/56.1/43.3/24.3			

(Continued)

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TABLE 1 (Continued)

Targeted cytokines	Agent	Mechanism	Description	Number of patient (Age)	Endpoint	Intervention arm	Results	ClinicalTrials. identifier	Developmen status	References
						Lebrikizumab 125 mg q4w				
						Placebo q2w				
				206 (12 to 17 Years)	Week 52	Lebrikizumab 500 mg loading doses at baseline and Week 2, followed by 250 mg Q2W	IGA 0 or 1 and reduction (> =) 2 points rate: 62.6	NCT04250350	Phase 3 completed	(97)
							EASI response > = 75 percent (%) improvement rate: 81.9			
							EASI response > = 50 percent (%) improvement rate: 94.4			
IL-13	Tralokinumab	Anti-IL-13	Human monoclonal	130 (12 Years and older)	Week 16	Tralokinumab + TCS	IGA 0 or 1 and reduction (> =) 2 points rate: 27/12	NCT05194540	Phase 3 completed	(93)
						Placebo + TCS	EASI response > = 50 percent (%) improvement rate: 73/52			
				794 (18 Years and older)	Week 16	Tralokinumab 300 mg q2w	IGA 0 or 1 and reduction (> =) 2 points rate: 15-8/7-1 (ECZTRA1), 22-2/10-9 (ECZTRA2)	NCT03131648 (ECZTRA1), NCT03160885 (ECZTRA2)	Phase 3 completed	(94)
						Placebo q2w	EASI response > = 75 percent (%) improvement rate: 25-0/12-7 (ECZTRA1), 33-2/11–4 (ECZTRA2)			
				301 (12 to 17 Years)	Week 16	Tralokinumab 300 mg q2w	IGA 0 or 1 and reduction (> =) 2 points rate: 17. 5/21.4/4.3	NCT03526861	Phase 3 completed	(95)
						Tralokinumab 150 mg q2w	EASI response > = 75 percent (%) improvement rate: 27.8/8.6/6.4			
						Placebo q2w				

(Continued)

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TABLE 1 (Continued)

Targeted cytokines	Agent	Mechanism	Description	Number of patient (Age)	Endpoint	Intervention arm	Results	ClinicalTrials. identifier	Developmen status	References
IL-31	Nemolizumab	Anti- IL-31 receptor	Humanized monoclonal	215 (12 Years and older)	Week 16	Nemolizumab 60 mg q4w	Change in VAS score for pruritus to Week 16: −42.8/−21.4	NCT03985943	Phase 3 completed	(117)
						Placebo q4w	Change in VAS score for pruritus to Day 15: −30.4/−11.1			
							Change in EASI score : −45.9/−33.2			
IL-22	Fezakinumab	Anti- IL-22	Monoclonal	60 (18 to 75 Years)	Week 12	ILV-094	Percentage change in SCORAD: −18.8/−11.7	NCT01941537	Phase 2 completed	(135)
						Placebo				
IL-17	Secukinumab, MOR106	Anti-IL-17A, Anti-IL-17C	Monoclonal	41 (18 Years and older)	Week 16	Secukinumab (300 mg) via	Fold-change in Epidermal thickness of Lesional skin: 1.18/1.15 (for Extrinsic AD)	NCT02594098	Phase 2 completed	(1, 51, 152)
						Placebo	Fold-change in Epidermal Thickness of Lesional skin: −1/1.5 (for Intrinsic AD)			
IL-23p19	Risankizumab	Anti-IL-23A	Humanized monoclonal	172 (12 Years and older)	Week 16	Risankizumab 300 mg	Percentage of participants achieving at least EASI-75: 21.7/24.6/11.8	NCT03706040	Phase 2 completed	(165)
						Risankizumab 150 mg				
						Placebo				
IL-12/23	Ustekinumab	Anti-IL-12 and -IL-23	Human monoclonal	79 (20 to 65 Years)	Week 12	Ustekinumab 90 mg	Percent Change in EASI score: −39.39/−38.62/−37.54	NCT01945086	Phase 2 completed	
						Ustekinumab 45 mg				
						Placebo				

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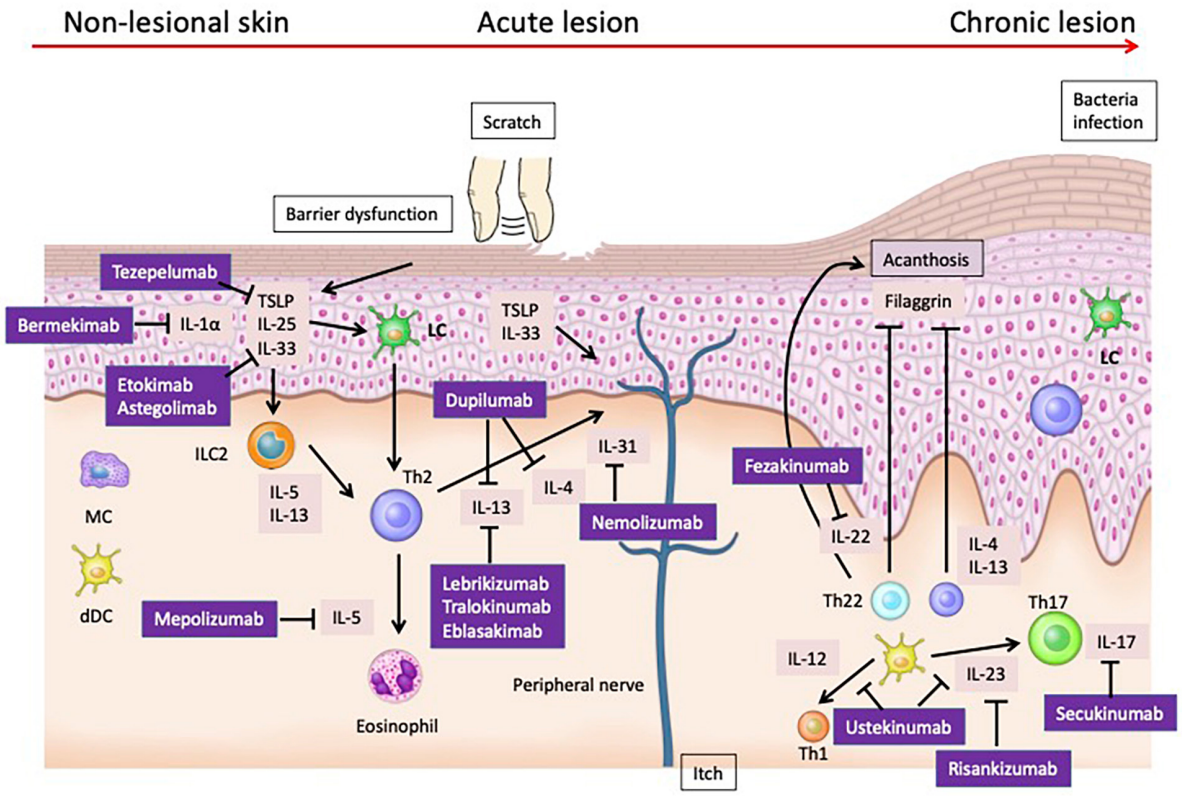

FIGURE 1
Pathogenesis and Therapeutic Targets of Atopic Dermatitis: Insights from Mouse Models and Human Specimens. The barrier dysfunction that occurs in the early stages of AD facilitates the penetration of allergens into the skin, and damaged keratinocytes produce cytokines such as TSLP, IL-25, and IL-33. These cytokines activate Th2 cells and ILC2s, leading to the production of Th2 cytokines. Additionally, TSLP matures Langerhans cells (LCs) in the epidermis and induces Th2 cells. During the acute phase, the skin barrier continues to deteriorate further. Damaged keratinocytes release various chemokines such as CCL17/thymus and activation-regulated chemokine and CCL22/macrophage-derived chemokine, as well as cytokines like TSLP, IL-1β, IL-25, and IL-33. These mediators activate ILC2 and Th2 cells at the lesion site. ILC2 cells produce IL-5 and IL-13, while Th2 cells produce IL-4, IL-13, IL-31, and IL-5. In the chronic phase, not only Th2 cells but also Th22 and Th17 cells increase in number, leading to suppression of filaggrin expression by IL-4, IL-13, and IL-22, further deteriorating the epidermal barrier function. Additionally, IL-4 and IL-13 suppress the production of AMPs, weakening the barrier function against microbes. Th17 and Th22 cells also produce IL-22, which induces epidermal thickening.

from AD patients, after application of house dust mite (HDM) or staphylococcal enterotoxin B (SEB), also showed increased expressions of IL-33 and ST2 (21). Pathogenic factors from *S. aureus* directly promote IL-33 production from human keratinocytes and destroy skin barrier functions, suggesting that these factors from S. aureus adhering to the skin may initiate type 2 inflammation via IL-33 in AD (22). *In vitro* stimulation of human basophils with IL-33 induces the production of IL-4 and IL-13 (23). IL-33 also activates human eosinophils, promoting their adhesion and survival (24, 25).

Despite these findings suggesting the involvement of IL-33 in the pathophysiology of human AD, clinical trials have yet to prove the efficacy of anti-IL-33 antibodies. In a phase IIa trial, a single systemic dose of etokimab, an IgG1 monoclonal antibody against IL-33, provided rapid and sustained clinical benefits in 12 adult patients with moderate to severe AD (26). However, a subsequent phase II placebo-controlled trial with 302 participants did not show the efficacy of etokimab compared to the placebo at 16 weeks, based on the Eczema Area and Severity Index (EASI) change rate. Additionally, a fully human IgG2 monoclonal antibody, astegolimab, did not show significant differences compared to placebo in a phase II trial (27). A randomized placebo-controlled phase II trial of astegolimab in adults with moderate to severe AD also failed to show efficacy (28). These results suggest that IL-33 may play a limited role in human AD compared to mouse models.

2.2 TSLP

Thymic stromal lymphopoietin binds to a heterodimeric receptor composed of the TSLP receptor (TSLPR) chain closely related to the IL-7 receptor (IL-7R) α chain and the common receptor γ chain (γc), exhibiting biological activity across a wide range of cell types. While TSLPR alone has a low affinity for TSLP, the affinity significantly increases when TSLPR and IL-7Rα bind together, forming a high-affinity binding site for TSLP and inducing signal transduction (29). TSLP is extensively studied as a master regulator of type 2 immune responses occurring at barrier surfaces such as skin, lungs, and intestines. TSLP produced by epithelial cells activates DCs expressing the TSLPR, leading to the induction of functional Th2 cells (30). Furthermore, in both acquired and innate immunity, basophils and ILC2s play crucial roles downstream of TSLP (31).

2.2.1 Roles in mouse AD model

In mouse models of AD, topical application of MC903 induces TSLP expression in epidermal keratinocytes, triggering AD-like dermatitis (32). Overexpression of TSLP in skin-specific manners results in a phenotype resembling AD, including infiltration of inflammatory cells in the dermis, development of eczematous lesions, a dramatic increase in Th2 CD4+ T cells expressing skin-homing receptors, and elevated serum IgE levels (33). LCs acting as antigen-presenting cells in the epidermal signaling pathway via TSLP-TSLPR play a crucial role in inducing Th2 immune responses in Ovalbumin (OVA)-induced mouse AD models (10). TSLP promotes peripheral basophil proliferation, and basophils expressing TSLPR restore Th2 immunity in mice (34). In the aforementioned MC903-induced mouse AD model, ILC2s play a significant role in inflammation onset (35). Additionally, IL-13 induces AD through a TSLP-dependent mechanism (36). Concerning pruritus in AD, injecting TSLP into the skin of mouse cheeks triggers scratching behavior dependent on IL-7Rα and primary afferent neurons. This response is due to the direct expression of TSLPR on primary afferent sensory neurons, requiring the Transient receptor potential cation channel subfamily A member 1 (TRPA1) ion channel for TSLPR activation (37). These data identify TSLP as a novel endogenous pruritogen, suggesting that keratinocyte-derived TSLP could be a therapeutic target for pruritus in AD. Overall, an increase in TSLP levels is known to be involved in the enhancement of Th2 immune responses (38).

2.2.2 Roles in human AD

In humans with AD, TSLP serum levels are significantly higher in both children and adults compared to healthy individuals (39, 40). TSLP is expressed in keratinocytes at acute and chronic AD lesion sites but not in non-lesion skin of AD patients, lesion sites of patients with nickel-induced allergic contact dermatitis (ACD), or cutaneous lupus erythematosus (41). In AD patients, circulating CD4+ T cells express higher levels of TSLPR compared to healthy individuals, and the levels of circulating TSLPR+ CD4+ T cells correlate with serum Thymus and activation-regulated chemokine/chemokine ligand 17 (TARC/CCL17) and IgE levels, as well as eosinophil counts (42). When inflammation in AD is exacerbated, *S. aureus* produces proteases and invades the dermis of AD patients, leading to increased production of type 2 cytokines such as TSLP, IL-4, and IL-13 (43). The cell wall components of *S. aureus* also signal through toll-like-receptor 2/6, inducing TSLP production in keratinocytes (44). These findings suggest that TSLP is involved in the pathogenesis of AD.

In clinical trials, tezepelumab, an anti-TSLP monoclonal antibody, demonstrated good safety and tolerability profiles, with linear pharmacokinetics in both healthy individuals and AD subjects (45). However, in a Phase II trial comparing tezepelumab and topical corticosteroids (TCS) combination therapy to placebo and TCS, although there were numerical improvements in the proportion of patients achieving EASI50 at week 12 and exploratory endpoints, and further improvement at week 16, no significant difference was observed (NCT03809663) (46). While tezepelumab has proven efficacy in asthma, its effects in AD were insufficient. These results suggest that targeting TSLP for the treatment of AD in humans may have limited potential.

2.3 IL-1α

The IL-1 family plays a crucial role in the proper functioning and control of the innate immune system, connecting innate and adaptive immune responses (47). This complex family consists of several cytokines, receptors, and co-receptors, all working together in balance to maintain homeostasis (47). Dysregulation of these processes can lead to tissue inflammation and contribute to the pathogenesis of common inflammatory skin diseases such as psoriasis, pustular sweat gland inflammation, and AD (47).

The IL-1 family of cytokines comprises 11 cytokine members, with 7 agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ) and 4 antagonists [IL-1 receptor antagonist (Ra), IL-36Ra, IL-37, IL-38] (48). Based on their structural and functional characteristics, these cytokines are further classified into four subfamilies: IL-1, IL-18, IL-33, and IL-36. Both IL-1α and IL-1β are pro-inflammatory cytokines. IL-1α is constitutively or inducible expressed in hematopoietic immune cells and other cell types such as intestinal epithelial cells and skin keratinocytes (49). IL-1α expression can be induced by inflammatory stimuli, leading to binding to IL-1R1 and the subsequent expression of inflammatory genes targeting type 1 or 17 immune responses. This results in the recruitment and activation of T cells, DCs, neutrophils, and monocytes/macrophages, further releasing inflammatory cytokines and chemokines, forming a self-amplifying inflammatory loop (50). On the other hand, IL-1β is primarily circulating, and its expression is inducible only in monocytes, macrophages, and DCs (50). The antagonist IL-1Ra competes with IL-1α and IL-1β for binding to the IL-1R1 receptor, exerting an anti-inflammatory effect (51).

2.3.1 Roles in mouse AD model

In a study exploring the anti-inflammatory effects of topical Tetracycline (TET) on AD in a mouse model, TET was found to suppress the expression of inflammatory cytokines, including IL-1β, in skin lesions. High levels of these cytokines were observed in the AD group, indicating a role for IL-1β in the inflammatory process of AD (52). Another study showed that skin and keratinocytes from mice with filaggrin deficiency had upregulated expression of IL-1β and IL-1RA mRNA (53).

2.3.2 Roles in human AD

Bermekimab, an inhibitor of IL-1α, showed promising results in a Phase II open-label trial with hidradenitis suppurativa (HS) patients, demonstrating a significant reduction in inflammatory lesions even after anti-tumor necrosis factor (TNF) therapy failure without severe drug-related adverse events (51, 54, 55). Contrasting efficacy has been reported for Canakinumab, a human monoclonal antibody targeting IL-1β, in case reports of HS (56, 57). While effective in severe cases of pustular psoriasis (58), it was reported ineffective in two patients with severe palmoplantar pustulosis (59).

From the above, it can be inferred that the IL-1 family is involved in the pathogenesis of inflammatory skin diseases, including AD. However, two Phase II trials on Bermekimab, an anti-IL-1αAb, for moderate to severe adult AD patients (NCT04990440 and NCT04021862) were discontinued due to lack of efficacy, suggesting a limited role of IL-1α in human AD.

3 Acute lesion

During the acute phase, the skin barrier continues to deteriorate further. Damaged keratinocytes release various chemokines such as CCL17/thymus and activation-regulated chemokine and CCL22/macrophage-derived chemokine, as well as cytokines like TSLP, IL-1β, IL-25, and IL-33 (60). These mediators activate ILC2 and Th2 cells at the lesion site. ILC2 cells produce IL-5 and IL-13 (61), while Th2 cells produce IL-4, IL-13, IL-31, and IL-5.

At the site of the lesion, there is an infiltration of CD4+ cells and an increase in the number of DCs, including LCs. DCs extend their dendritic processes beyond tight junctions to capture antigens. Furthermore, IL-4 and IL-13 promote IgE class-switching in B cells. In addition to these processes, various chemokines produced by keratinocytes at the inflammation site are involved in recruiting immune cells to the lesion. For example, eosinophils are activated by IL-5.

Regarding pruritus, IL-4 and IL-13 are suggested to act on IL-4Ra expressed on peripheral nerves, transmitting chronic pruritus through the Janus kinase (JAK) 1 signaling pathway. IL-31 acts on IL-31R expressed on peripheral nerves, eliciting pruritus (60). IL-31 is considered a primary cause of pruritus in AD. TSLP induces the expression of CD134 (OX40) ligand on DCs, which binds to OX40 on T cells, further stimulating the production of IL-4, IL-13, IL-5, and the pruritus-specific cytokine, IL-31.

3.1 IL-5

IL-5 plays a critical role in the development, survival, and proliferation of eosinophils (62). The primary producers of IL-5 are Th2 cells and ILC2, but mast cells, eosinophils, basophils, epithelial cells, and smooth muscle cells also produce IL-5 (62). IL-5 binds to a heterodimeric receptor composed of IL-5R subunit α (IL-5Rα) and the common subunit β (βc) (62). The βc subunit is also associated with IL-3Rα and the granulocyte-macrophage colony-stimulating factor (GM-CSF) Rα. In concert with IL-3 and GM-CSF, IL-5 promotes the proliferation, differentiation, and activation of eosinophils (62).

3.1.1 Roles in mouse AD model

In transgenic mice overexpressing IL-5, specifically in keratinocytes, there is an infiltration of eosinophils in the epidermis, displaying an AD-like phenotype (63). Additionally, these mice show a significant increase in the number of sensory neurons in the epidermis, suggesting a potential involvement of IL-5 in the branching of nerve cells in AD (63).

3.1.2 Roles in human AD

In humans, stimulation of peripheral blood mononuclear cells from children with AD using house dust mite extract resulted in IL-5 production correlating with the severity of AD (64). Moreover, infusion of the anti-IL-5Ab mepolizumab in AD patients significantly reduced eosinophil infiltration at the allergen injection site in the skin after 6 and 48 h and significantly reduced the number of tenascin-immunoreactive cells, a marker for repair and remodeling, after 48 h (65). These results suggest that IL-5 plays a significant role in the development of AD. However, mepolizumab did not demonstrate sufficient efficacy in AD patients (NCT03055195) (66), while it showed significant efficacy in specific subtypes of asthma patients (67, 68), suggesting that the role of eosinophils in the pathogenesis may differ between AD and asthma.

3.2 IL-4/13

IL-4 and IL-13 are representative cytokines of type 2 inflammatory responses and share many common functions. IL-4 is involved in Th2 differentiation and controls lymphocyte functions such as IgE synthesis in B cells. On the other hand, IL-13 is an effector cytokine that controls the construction of smooth muscle cells and mucus production in the airway epithelium in allergic asthma (69). Th2 cells, mast cells, eosinophils, and basophils all produce both IL-4 and IL-13 (70). ILC2s can produce IL-4, but generally at lower levels compared to their robust production of IL-13 (71).

IL-4 binds to either type 1 IL-4 receptor (IL-4R) or type 2 IL-4R. Type 1 IL-4R consists of IL-4Rα subunit and the common γ subunit of cytokine receptors. Type 2 IL-4R, on the other hand, consists of IL-4Rα and IL-13Rα1 chain (70). Therefore, type 2 IL-4R also functions as IL-13R. Hematopoietic/immune cells mainly express type 1 IL-4R, while type 2 IL-4R/IL-13R is ubiquitously expressed in non-hematopoietic cells and tissue-resident cells. Myeloid cells can express either type 1 or type 2 IL-4R. Because of the different distribution of IL-4R/IL-13R, IL-4 mainly functions in hematopoietic/immune cells, whereas IL-13 functions in non-hematopoietic cells and tissue-resident cells.

In the pathogenesis of AD, IL-4 and IL-13 are involved in (i) chemokine production, (ii) barrier function, (iii) pruritus, (iv) antimicrobial peptide (AMP) production, and (v) fibrosis. In terms of chemokine production, IL-4/IL-13 can induce various chemokines such as TARC/CCL17, CCL5, eotaxin-1/CCL11, and eotaxin-3/CCL26 either alone or in combination with other cytokines such as TNF-α or Interferon (IFN)-γ. These chemokines are highly expressed in the lesional skin of AD (72), and they recruit inflammatory cells such as T cells, eosinophils, and basophils to the skin lesions. In terms of barrier function, either IL-4 or IL-13 reduces the expression of barrier-associated molecules such as filaggrin, loricrin, and involucrin, leading to disruption of tight junctions and impaired ceramide production in the skin (73). IL-13 may directly or indirectly increase collagen deposition and fibrotic tissue remodeling (74), which is clinically observed in lichenified lesions of chronic AD.

3.2.1 Roles in mouse AD model

Various genetically modified mice have demonstrated the importance of IL-4 or IL-13 in the development of AD. For example, mice overexpressing IL-4 or IL-13 in keratinocytes exhibit xerosis and pruritic dermatitis, major characteristics of human AD, accompanied by a type 2 immune response (75–77). IL-4 plays a crucial role in the control of epidermal homeostasis and the natural barrier function (78). In IL-4 transgenic mice, hundreds of dysregulated factors have been identified before and after the onset of skin lesions, with a significant increase in the expression of factors such as C-X-C motif chemokine ligand 5 (CXCL5), IL-1β, IL-24, IL-6, oncostatin M (OSM), prostaglandin-endoperoxide synthase 2 (PTGS2), Formyl Peptide Receptor 1

(FPR1), and Regenerating Islet-Derived Protein 3 Gamma (REG3γ) (79). Moreover, IL-4 and/or IL-13 have been proven to directly induce scratching behavior in mice (80).

3.2.2 Roles in human AD

IL-4 and IL-13 inhibit the production of AMPs, human β-defensin (HBD)-2, and HBD-3 (81). This aligns with findings that AD patients exhibit lower expression levels of AMPs, such as cathelicidin (LL-37) and HBD-2 (82). These findings partially explain why AD patients are more susceptible to skin infections (83). IL-13 induces the expression of matrix metalloproteinase (MMP)-9 in human keratinocytes, acting on collagen IV in the basement membrane to promote cell movement and tissue remodeling (84, 85). In contrast, IL-13 downregulates MMP-13 expression in human fibroblasts, potentially leading to reduced collagen degradation and fibrosis observed in the thickened dermis of chronic lichenified AD lesions (86). Additionally, both mouse and human primary sensory neurons express receptors for IL-4 and IL-13 (87). Neurons pre-treated with IL-4 and IL-13 respond to sub-threshold concentrations of histamine and IL-31 (87).

Dupilumab, developed based on these findings, is a fully human monoclonal antibody against IL-4Rα. It binds to the IL-4Rα subunit of both type I and type II receptors, inhibiting both IL-4 and IL-13 mediated signaling pathways. It has shown significant efficacy in moderate to severe AD patients (88). The effectiveness of dupilumab has been demonstrated in studies involving adults and adolescents with AD (89, 90). Long-term administration in adolescents maintained effectiveness and showed a tolerable safety profile, highlighting the importance of continuous treatment for sustained efficacy (91). The q2w dosing regimen was found to be optimal for this age group (91). Dupilumab administration in younger AD patients (6 months to 11 years old) was effective and well-tolerated, with a safety profile consistent with that in older children and adults (92, 93).

A meta-analysis of 22 studies involving 3303 AD patients reported significant improvements in EASI scores and a high tolerance for dupilumab treatment, confirming its effectiveness in treating AD (94). Adverse events, such as conjunctivitis, were observed, but the treatment was generally well-tolerated (94). A 52-week retrospective study examined patients with moderate-to-severe atopic AD treated with dupilumab at labeled dosage (95). Patients were split into Group A (patients with significant comorbidities) and Group B (patients without significant comorbidities). Disease severity was measured using EASI, Pruritus-Numerical Rating Scale (P-NRS), and Dermatology Life Quality Index (DLQI) at baseline and weeks 4, 16, 24, and 52. The study included 263 patients, with 25 in Group A and 238 in Group B. Significant reductions in EASI, DLQI, and P-NRS were observed in both groups at each follow-up visit ($p < 0.0001$), with no notable differences between the groups. Safety outcomes were similar between the two groups. Serious side effects were not collected, and the main side effect was injection site reactions for both groups (Group A: 3, 12.0%; Group B: 41, 17.22%), followed by conjunctivitis (Group A: 2, 8.0%; Group B: 21, 11.34%). Another retrospective study demonstrated dupilumab's effectiveness in treating adults with moderate to AD and chronic rhinosinusitis with nasal polyps (96). Using various measures, including EASI and 22-item Sino-Nasal Outcome Test, they observed significant improvements in both conditions at weeks 16 and 24. The long-term effectiveness and safety of dupilumab have been evaluated in patients with AD who also have comorbidities such as malignancy, severe renal insufficiency requiring dialysis, hepatitis B or C, AIDS, Parkinson's disease, multiple sclerosis, or undergoing organ transplant (95). Based on this, targeted therapy against IL-4 and IL-13 is considered to have very high safety.

Other biological agents targeting the IL-13 signaling pathway, such as lebrikizumab and tralokinumab, have also demonstrated significant efficacy in adult AD patients (97). Tralokinumab has shown long-term efficacy and tolerability in adults and good tolerability in adolescents (98), supporting its value as a therapeutic for moderate to severe young AD (99). Lebrikizumab demonstrated rapid, dose-dependent effectiveness across a broad range of clinical symptoms in adults with AD and maintained a favorable safety profile in adolescents (100), significantly improving AD symptoms and quality of life (101).

IL-13 is considered a major mediator involved in the inflammation, epidermal barrier dysfunction, and pruritus associated with AD. Selective IL-13 inhibitors such as tralokinumab, lebrikizumab, and eblasakimab have shown promising efficacy in the treatment of moderate to severe AD (102). While their safety profiles are generally favorable, there is a heightened risk of conjunctivitis, necessitating monitoring (102). These findings collectively affirm the pivotal role of IL-4/13 in human AD.

3.3 IL-31

IL-31 is a member of the IL-6 cytokine family, predominantly produced by activated CD4+ T cells, particularly activated Th2 cells, as well as mast cells, macrophages, and DCs (103–107). The expression of IL-31 mRNA has been reported in various human tissues, including testes, bone marrow, skeletal muscle, and kidneys (103). The receptor for IL-31 is a heterodimer composed of IL-31 receptor A (IL-31RA) and OSM receptor (OSMR) (103). IL-31RA mRNA expression is observed in various tissues and cells, including testes, bone marrow, skin, dorsal root ganglia, activated monocytes, macrophages, DCs, eosinophils, basophils, and keratinocytes, while OSMR mRNA is broadly expressed in many tissues (107–109).

3.3.1 Roles in mouse AD model

In the mouse AD model, IL-31 is implicated in skin pruritus as evidenced in the Fluorescein Isothiocyanate (FITC) and Dinitrofluorobenzene (DNFB)-induced contact dermatitis model, though it does not appear to be involved in inducing local skin inflammation (110). Moreover, repeated administration of IL-31 also increased the expression of IL-31RA and OSMR β in dorsal root ganglia, suggesting an upregulation of IL-31RA expression in dorsal root ganglion (DRG) neuron cell bodies by IL-31 (111). This correlation is further supported by enhanced scratching behavior observed upon continuous subcutaneous injection of IL-31 in mice (111). Additionally, a single dose of IL-31 in mice induced strong pruritus upon skin and intrathecal injection, with concentrations significantly increased in the skin of mice with atopic-like dermatitis, leading to persistent scratching behavior (19). This implication of IL-31 in pruritus and the promotion of

scratching behavior was further corroborated in Nishiki-nezumi Cinnamon/Nagoya (NC/Nga) mice with dermatological lesions, serving as a model for AD (112, 113). Furthermore, transgenic mice overexpressing IL-31 developed severe pruritus and skin lesions, suggesting a role for IL-31 in allergic dermatitis (103). In a contrasting observation, IL-31RA knockout mice showed increased OSM-induced cytokine levels during airway sensitization and challenge (114). Finally, the administration of anti-IL-31 antibodies was found to improve scratching behavior in NC/Nga mice (115).

3.3.2 Roles in human AD

IL-31, through the phosphorylation of signal transduction and activator of transcription (STAT)-1 and STAT-5, induces pro-inflammatory effects in activated human macrophages (116). The activation of ERK1/2 by IL-31 contributes to the underlying mechanism of Th1 cytokine IL-12 suppression in macrophages (116). Although IL-31 activates STAT-3 phosphorylation and enhances C-C motif chemokine 2 (CCL2) secretion in human primary keratinocytes, this phenomenon is not observed in AD keratinocytes with low TLR-2 expression, suggesting a potential link between the functional change of IL-31 and skin inflammation (117). Additionally, IL-31 is activated when the IL-31Rα receptor chain in primary human CD1c+ and monocyte-derived DCs is upregulated by IFN-γ stimulation, leading to a dose-dependent release of inflammatory mediators such as TNF-α, IL-6, CXCL8, CCL2, CCL5, and CCL22, causing skin inflammation (109). IL-31 is also present in eccrine sweat and activates keratinocytes to produce the inflammatory cytokine CCL2 (118). Human dorsal root ganglion neurons, many of which co-express TRP, Subfamily V, Member 1 (TRPV1), also express IL-31RA (19). Blocking TRPV1 *in vivo* interrupts IL-31 signaling (19). An increase in skin IL-31 may be associated with pruritus in diabetes mellitus (DM), and ongoing clinical trials aim to evaluate the systemic treatment effects on IL-31 and pruritus in DM (119). Staphylococcal superantigens have been shown to rapidly induce IL-31 expression in atopic patients (120), and administration of Fexofenadine significantly reduces serum IL-31 levels in AD patients (47).

Nemolizumab, developed based on these findings, is a humanized monoclonal antibody against the IL-31 receptor A (IL-31RA), administered subcutaneously, involved in pruritus and inflammation in AD. In a 16-week double-blind Phase III trial, Japanese AD patients with moderate to severe pruritus, insufficiently controlled by topical agents, were treated with subcutaneous nemolizumab in addition to topical agents. The results showed a reduction in pruritus compared to placebo plus topical agents (NCT03985943) (121). Further investigation into the long-term efficacy and safety of nemolizumab revealed continuous improvement in pruritus, AD signs, and QoL for up to 68 weeks when combined with topical agents, with a favorable safety profile (122). In another clinical trial with AD patients, nemolizumab rapidly and sustainably improved skin signs of inflammation and pruritus, with maximum effects observed at 30 mg, and the safety profile of nemolizumab was within an acceptable range. Nemolizumab significantly and rapidly improved inflammation, pruritus, and sleep in patients with a baseline EASI ≥16 (NCT03100344) (123, 124). Moreover, oral JAK inhibitors, which are also under development, have shown very promising effects on chronic pruritus through the Janus kinase 1/2 signaling pathway, a pathway involved with IL-4, IL-13, and IL-31 (125).

In summary, IL-31 plays a crucial role in the pathophysiology of human AD, particularly in its role in pruritus, highlighting its importance in the condition.

4 Chronic lesion

In the chronic phase, not only Th2 cells but also Th22 and Th17 cells increase in number, leading to suppression of filaggrin expression by IL-4, IL-13, and IL-22, which further deteriorates the epidermal barrier function. Additionally, IL-4 and IL-13 suppress the production of AMPs, weakening the barrier function against microbes. Th17 and Th22 cells also produce IL-22, which induces epidermal thickening.

4.1 IL-22

IL-22 is a cytokine produced by adaptive Th17 and Th22 cells, natural lymphocytes including γδT cells and type 3 innate lymphoid cells (ILC3), as well as myeloid cells including neutrophils. It belongs to the IL-10 family of cytokines (126). IL-22 is known to induce keratinocyte proliferation, and its serum levels are elevated in AD, with Th22 cells infiltrating the skin lesions of AD (127, 128). Additionally, the IL-22 receptor (IL-22R) is expressed on epithelial cells, including keratinocytes, but not on immune cells (129), suggesting that IL-22 signaling plays a crucial role in barrier function (130).

4.1.1 Roles in mouse AD model

In mouse AD models, *in vivo* injection of IL-22 into the skin induces keratinocyte proliferation and epidermal thickening (131). When antigen is applied to the skin of mice subjected to tape stripping, an alternative to scratching, an IL-22 response that promotes epidermal hyperplasia and keratinocyte proliferation is induced (132).

4.1.2 Roles in human AD

In human AD, there is a significant increase in IL22 mRNA expression and IL-22-producing T cells in the skin lesions (133–135). Serum IL-22 levels are also elevated in AD patients (127, 128). *In vitro* application of IL-22 to keratinocytes results in proliferation, and reconstituted human epidermis in a 3D matrix thickens (136, 137). In a clinical trial involving *in vivo* administration of fezakinumab to patients with moderate to severe AD, the IL-22 high-expression group showed much stronger improvement in transcriptomics mean values compared to the IL-22 high-expression placebo group and the IL-22 low-expression group (138).

From the above, it is suggested that IL-22 is involved in the pathophysiology of AD. In clinical trials, fezakinumab has demonstrated efficacy in adult patients with moderate to severe AD, with good tolerability (NCT01941537) (139). Specifically, larger and more significant differences were observed in the severe AD patient group.

In conclusion, IL-22 plays a crucial role in the pathophysiology of human AD, particularly in moderate to severe AD, demonstrating its significance in this condition.

4.2 IL-17A/25

IL-17A is pivotal for skin immunity, especially in inflammatory skin conditions like psoriasis, and it plays a vital role in the body's defense against microbial pathogens. This cytokine is produced predominantly by Th17 cells, a unique lineage of proinflammatory T helper cells crucial for both autoimmune diseases and the regulation of innate immunity in epithelial cells, including keratinocytes, which form most skin cells. IL-17A, along with other cytokines secreted by Th17 cells, boosts the production of AMPs by human keratinocytes, strengthening the skin's defense mechanisms against microbial invaders (140).

IL-25, also known as IL-17E, is a member of the IL-17 cytokine family. It is primarily produced by epithelial cells like keratinocytes but is also known to be produced by other immune cells such as T cells, DC, and ILC2 (141). IL-25 binds to a heterodimeric receptor composed of IL-25R and IL-17 receptor B (IL-17RB), also known as IL-17RA. The cellular targets of IL-25 include a variety of cells, such as T cells, ILC2, myeloid cell populations, invariant natural killer T (NKT) cells, fibroblasts, epithelial cells, endothelial cells, and mesenchymal cells (141).

4.2.1 Roles in mouse AD model

In murine models of AD, IL-17A has been found to mediate Th2-type immune responses, positioning IL-17A signaling as a potential therapeutic target in AD (142). Studies using mice have demonstrated that both IL-25 and IL-33 are crucial for the development of allergic dermatitis through the regulation of ILC2 (143). However, while skin-associated ILC2 responses and AD-like dermatitis in a murine AD model are critically dependent on TSLP signaling, they are not dependent on IL-25 signaling (35). This suggests that IL-25 derived from Th2 T cells could amplify allergic-type inflammatory responses by acting on other cell types (144). In murine models, the depletion of a specific type of neonatal-derived γδT cell from birth resulted in a spontaneous and pervasive form of AD that displayed many key features of human AD (145). In the Flaky tail murine model of AD-like dermatitis, IL-17A has been proven to be involved in the activation of macrophages that are in the process of adopting heterogeneous profiles of both M1 and M2 states in the skin (146). Lastly, IL-17A mediates Th2-type immune responses in murine models of AD (142).

This comprehensive analysis underscores the multifaceted role of IL-17A and IL-25 in skin immunity and AD, highlighting their potential as targets for therapeutic intervention.

4.2.2 Roles in human AD

In the context of AD, studies have demonstrated a marked increase in IL-17A levels in the serum of both adults and infants diagnosed with the condition. Importantly, this elevation in IL-17A levels has been found to correlate positively with the severity of the disease (147), suggesting a potential role in disease progression. Furthermore, a study has revealed that the interplay between specific genetic factors, such as the coexistence of the GG genotype of IL-17A rs2275913 and a mutation in the filaggrin gene (2282del4), can significantly heighten the risk of AD, highlighting the complexity of IL-17A's role in AD and underscoring the need for further research to fully elucidate its mechanisms of action and potential as a therapeutic target (148).

Within the skin affected by AD, there has been observed an upregulation of both IL-25 and its receptor IL-17RB (149). This finding implies a potential involvement of the IL-25 signaling pathway in the pathogenesis of AD, though the precise impact of IL-25 on the skin barrier remains largely undefined. *In vitro* experiments have produced mixed results; for instance, IL-25 was found to decrease the expression of filaggrin mRNA in human keratinocytes cultured under high calcium conditions (149, 150), but this effect was not observed under other conditions (151). Furthermore, activated human eosinophils and basophils have been shown to produce IL-25 *in vitro* (149, 152). IL-25 has also been implicated in the pathophysiology of pruritus, a hallmark symptom of AD, through its ability to increase the expression of the pruritogenic substance endothelin-1 in cultured keratinocytes from both mice and humans, via the ERK1/2 or JNK pathways (153). Additionally, the administration of a specific probiotic strain, Lactobacillus plantarum IS-10506, resulted in the suppression of IL-4 and IL-17, accompanied by an alleviation of AD symptoms (154), further supporting the potential involvement of IL-17 in the pathology of AD.

However, it is crucial to acknowledge that the role of IL-17A in AD is intricate and multifaceted. Clinical trials with therapeutics targeting IL-17 pathways, such as MOR106 (Anti-IL-17C) and Secukinumab, have not demonstrated sufficient efficacy in AD patients (NCT02594098) (155, 156). Based on the preceding discussion, it can be inferred that the therapeutic targeting of IL-17 in human AD appears to offer constrained possibilities.

4.3 IL-23p19, IL-12/23p40

IL-23 is a cytokine belonging to the IL-12 family, uniquely composed of a specific p19 subunit and a shared p40 subunit with IL-12 (157). This cytokine is produced by various cells, including epidermal LCs, DCs, macrophages, and keratinocytes (158–160). IL-23 receptor (IL-23R) expression is found on several immune cells such as LC, DC, NK cells, NKT cells, γδT cells, and Th17 cells (161–163). IL-23 plays a critical role in promoting the polarization of Th17 cells (164, 165) and is essential for inducing the expression of IL-22 (131, 166).

4.3.1 Roles in mouse AD model

In the context of mouse AD models, IL-23 released from keratinocytes in response to endogenous TLR4 ligands upregulates endogenous IL-23 production in skin DC, which selectively express IL-23R. This, in turn, drives the IL-22 response in naïve CD4+ T cells, leading to epidermal thickening (132).

4.3.2 Roles in human AD

In human AD, an upregulation of the Th17/IL-23 axis has been demonstrated (142, 167). IL-23 is released in human skin after scratching and polarizes human skin DC to drive the IL-22 response (132). Risankizumab, an antibody that binds to the p19 subunit of IL-23, inhibiting its action (168), has been approved for the treatment of moderate to severe plaque psoriasis, active psoriatic arthritis, and moderate to severe active Crohn's disease in adults.

Despite these findings, IL-23, and by extension the IL-17/23 axis, appears to be an insufficient therapeutic target for AD. This

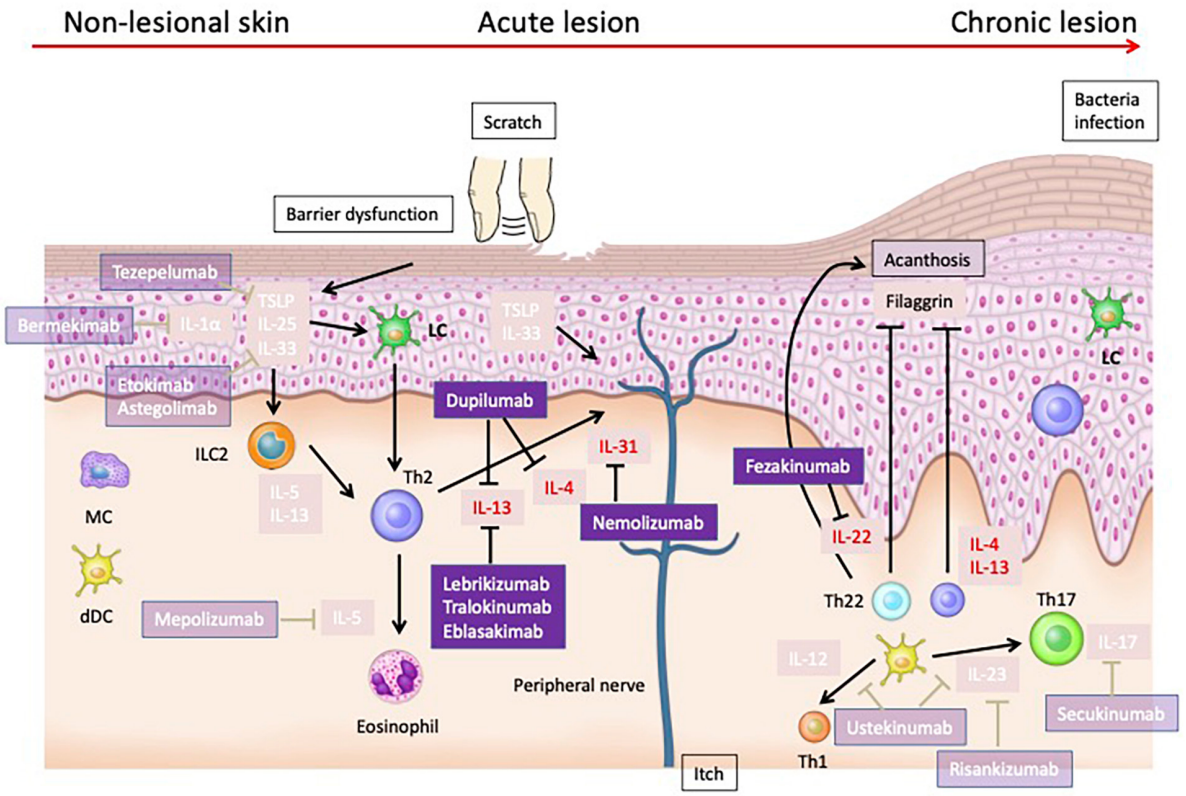

FIGURE 2
Cytokines that have been validated as therapeutic targets in human atopic dermatitis. Dupilumab, which targets IL-4 and IL-13, has shown efficacy in treating moderate to severe AD. Nemolizumab, targeting IL-31RA, effectively reduces pruritus in AD patients. Fezakinumab, targeting IL-22, have demonstrated promising results, particularly in severe AD cases. IL-33 has been identified as a potent activator of immune cells, exacerbating AD in murine models and correlating with disease severity in human patients. However, treatments targeting IL-33 have not shown sufficient efficacy in clinical trials. Similarly, TSLP, integral to type 2 immune responses, induces dermatitis in animal models and is elevated in human AD, yet clinical treatments like tezepelumab exhibit limited efficacy. Therapies targeting IL-1α, IL-5, and IL-17 also failed to achieve sufficient efficacy in clinical trials.

is supported by clinical trials conducted with risankizumab (an anti-IL-23A Ab) in AD patients aged 12 and older (NCT03706040) (169), as well as with ustekinumab (an anti-IL-12/23p40 Ab) in adult AD patients (NCT01945086), both of which did not demonstrate efficacy. These results suggest that while IL-23 is implicated in the pathophysiology of inflammatory diseases, including AD, targeting the IL-17/23 axis may not be an adequate strategy for AD treatment.

5 Discussion and conclusion

The pathogenesis of AD is complex and multifactorial, involving immune response dysregulation, compromised barrier function, and pruritus. Cytokines are pivotal in this process, with Th2, Th22, and Th17 cells contributing to the disease's progression. IL-4 and IL-13, both Th2 cytokines, are key players in atopic inflammation, exacerbating epidermal barrier dysfunction, pruritus, and promoting type 2 immune deviation (170). IL-31, a pruritogenic cytokine, is produced by type 2 T cells and amplifies the IL-31-mediated sensory nerve signal (170). IL-22, produced by Th22 cells, mediates keratinocyte proliferation, epidermal hyperplasia, and antimicrobial protein production, and is implicated in the pathogenesis of atopic dermatitis (171). The efficacy of targeted treatments such as Dupilumab, Nemolizumab, and Fezakinumab in clinical trials underscores the importance of IL-4, IL-13, IL-31, and IL-22 as therapeutic targets in both acute and severe phases of AD. However, challenges remain, as treatments targeting IL-33, TSLP, IL-1α, IL-5, and IL-17 have shown limited success in clinical trials (Figure 2). This disparity between therapeutic effectiveness in murine models and human patients highlights the need for a nuanced understanding of AD's pathophysiology. JAK inhibitors have shown promising results in the treatment of AD, with improvements in objective and subjective scoring indices observed in patients receiving both topical and oral formulations (172, 173). They have been associated with higher rates of achieving EASI75, Investigator's Global Assessment response, and pruritus numerical rating scale response (173). However, they also carry a higher risk of treatment-emergent adverse events (173). Upadacitinib and abrocitinib, both selective JAK1 inhibitors, have been identified as effective and well-tolerated agents for moderate-to-severe atopic dermatitis (174). Despite these positive findings, further research is needed to establish the long-term efficacy and safety of JAK inhibitors in atopic dermatitis (175). The strength of this paper lies in its comprehensive examination of the roles of various cytokines in AD, taking into account unpublished negative trial data from the ClinicalTrials.gov

database. A limitation, however, is the inability to conclusively determine whether the ineffectiveness of certain cytokine targets in trials is due to the drug itself or the unsuitability of the cytokine as a therapeutic target for AD.

Future treatment strategies must consider the differences between mouse model analyses and clinical trials differences to effectively address the diverse manifestations of AD.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Ständer S. Atopic dermatitis. *N Engl J Med.* (2021) 384:1136–43.

2. Kabashima K. New concept of the pathogenesis of atopic dermatitis: Interplay among the barrier, allergy, and pruritus as a trinity. *J Dermatol Sci.* (2013) 70:3–11. doi: 10.1016/j.jdermsci.2013.02.001

3. Palmer CNA, Irvine A, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee S, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet.* (2006) 38:441–6. doi: 10.1038/ng1767

4. Rodriguez E, Baurecht H, Herberich E, Wagenpfeil S, Brown S, Cordell H, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: Robust risk factors in atopic disease. *J Allergy Clin Immunol.* (2009) 123:1361–70.e7. doi: 10.1016/j.jaci.2009.03.036

5. Brown SJ. What have we learned from GWAS for atopic dermatitis? *J Invest Dermatol.* (2021) 141:19–22.

6. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet.* (2020) 396:345–60.

7. Beck LA, Cork M, Amagai M, De Benedetto A, Kabashima K, Hamilton J, et al. Type 2 inflammation contributes to skin barrier dysfunction in atopic dermatitis. *JID Innov.* (2022) 2:100131.

8. Puar N, Chovatiya R, Paller AS. New treatments in atopic dermatitis. *Ann Allergy Asthma Immunol.* (2021) 126:21–31.

9. Hasegawa T, Oka T, Demehri S. Alarmin cytokines as central regulators of cutaneous immunity. *Front Immunol.* (2022) 13:876515. doi: 10.3389/fimmu.2022.876515

10. Nakajima S, Igyártó B, Honda T, Egawa G, Otsuka A, Hara-Chikuma M, et al. Langerhans cells are critical in epicutaneous sensitization with protein antigen via thymic stromal lymphopoietin receptor signaling. *J Allergy Clin Immunol.* (2012) 129:1048–55.e6. doi: 10.1016/j.jaci.2012.01.063

11. Kong HH, Oh J, Deming C, Conlan S, Grice E, Beatson M, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.* (2012) 22:850–9. doi: 10.1101/gr.131029.111

12. Nakatsuji T, Chen T, Narala S, Chun K, Two A, Yun T, et al. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci Transl Med.* (2017) 9:eaah4680. doi: 10.1126/scitranslmed.aah4680

13. Liew FY, Girard J-P, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* (2016) 16:676–89.

14. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: The new kid in the IL-1 family. *Nat Rev Immunol.* (2010) 10:103–10. doi: 10.1038/nri2692

15. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan T, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* (2005) 23:479–90. doi: 10.1016/j.immuni.2005.09.015

16. Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, et al. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci USA.* (2013) 110:13921–6.

17. Imai Y, Yasuda K, Nagai M, Kusakabe M, Kubo M, Nakanishi K, et al. IL-33-induced atopic dermatitis-like inflammation in mice is mediated by group 2 innate lymphoid cells in concert with basophils. *J Invest Dermatol.* (2019) 139: 2185–94.e3. doi: 10.1016/j.jid.2019.04.016

18. Peng G, Mu Z, Cui L, Liu P, Wang Y, Wu W, et al. Anti-IL-33 antibody has a therapeutic effect in an atopic dermatitis murine model induced by 2, 4-dinitrochlorobenzene. *Inflammation.* (2018) 41:154–63. doi: 10.1007/s10753-017-0673-7

19. Cevikbas F, Wang X, Akiyama T, Kempkes C, Savinko T, Antal A, et al. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. *J Allergy Clin Immunol.* (2014) 133:448–60. doi: 10.1016/j.jaci.2013.10.048

20. Tamagawa-Mineoka R, Okuzawa Y, Masuda K, Katoh N. Increased serum levels of interleukin 33 in patients with atopic dermatitis. *J Am Acad Dermatol.* (2014) 70:882–8.

21. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimäki S, et al. IL-33 and ST2 in atopic dermatitis: Expression profiles and modulation by triggering factors. *J Invest Dermatol.* (2012) 132:1392–400. doi: 10.1038/jid.2011.446

22. Al Kindi A, Williams H, Matsuda K, Alkahtani A, Saville C, Bennett H, et al. *Staphylococcus aureus* second immunoglobulin-binding protein drives atopic dermatitis via IL-33. *J Allergy Clin Immunol.* (2021) 147:1354–1368.e3. doi: 10.1016/j.jaci.2020.09.023

23. Suzukawa M, Iikura M, Koketsu R, Nagase H, Tamura C, Komiya A, et al. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol.* (2008) 181:5981–9.

24. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol.* (2008) 121:1484–90. doi: 10.1016/j.jaci.2008.04.005

25. Suzukawa M, Koketsu R, Iikura M, Nakae S, Matsumoto K, Nagase H, et al. Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest.* (2008) 88:1245–53. doi: 10.1038/labinvest.2008.82

26. Chen Y-L, Gutowska-Owsiak D, Hardman C, Westmoreland M, MacKenzie T, Cifuentes L, et al. Proof-of-concept clinical trial of etokimab shows a key role for IL-33 in atopic dermatitis pathogenesis. *Sci Transl Med.* (2019) 11:eaax2945. doi: 10.1126/scitranslmed.aax2945

27. Maurer M, Cheung D, Theess W, Yang X, Dolton M, Guttman A, et al. Phase 2 randomized clinical trial of astegolimab in patients with moderate to severe atopic dermatitis. *J Allergy Clin Immunol.* (2022) 150:1517–24. doi: 10.1016/j.jaci.2022.08.015

28. Laquer V, Parra V, Lacour J, Takahashi H, Knorr J, Okragly A, et al. Interleukin-33 antibody failed to demonstrate benefit in a phase II, double-blind, randomized, placebo-controlled study in adult patients with moderate-to-severe atopic dermatitis. *Br J Dermatol.* (2022) 187:599–602. doi: 10.1111/bjd.21631

29. He R, Geha RS. Thymic stromal lymphopoietin. *Ann N Y Acad Sci.* (2010) 1183:13–24.

30. Roan F, Obata-Ninomiya K, Ziegler SF. Epithelial cell-derived cytokines: More than just signaling the alarm. *J Clin Invest.* (2019) 129:1441–51. doi: 10.1172/JCI124606

31. Nakashima C, Otsuka A, Kabashima K. Recent advancement in the mechanism of basophil activation. *J Dermatol Sci.* (2018) 91:3–8.

32. Li M, Hener P, Zhang Z, Kato S, Metzger D, Chambon P. Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. *Proc Natl Acad Sci USA.* (2006) 103:11736–41.

33. Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, et al. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J Exp Med.* (2005) 202:541–9.

34. Siracusa MC, Saenz S, Hill D, Kim B, Headley M, Doering T, et al. TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature.* (2011) 477:229–33. doi: 10.1038/nature10329

35. Kim BS, Siracusa M, Saenz S, Noti M, Monticelli L, Sonnenberg G, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med.* (2013) 5:170ra16. doi: 10.1126/scitranslmed.3005374

36. Zhu Z, Oh M-H, Yu J, Liu YJ, Zheng T. The Role of TSLP in IL-13-induced atopic march. *Sci Rep.* (2011) 1:23. doi: 10.1038/srep00023

37. Wilson SR, Thé L, Batia L, Beattie K, Katibah G, McClain S, et al. The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. *Cell.* (2013) 155:285–95.

38. Bromley SK, Larson RP, Ziegler SF, Luster AD. IL-23 induces atopic dermatitis-like inflammation instead of psoriasis-like inflammation in CCR2-deficient mice. *PLoS One.* (2013) 8:e58196. doi: 10.1371/journal.pone.0058196

39. Nygaard U, Hvid M, Johansen C, Buchner M, Fölster-Holst R, Deleuran M, et al. TSLP, IL-31, IL-33 and sST2 are new biomarkers in endophenotypic profiling of adult and childhood atopic dermatitis. *J Eur Acad Dermatol Venereol.* (2016) 30:1930–8. doi: 10.1111/jdv.13679

40. Lee EB, Kim K, Hong J, Jee H, Sohn M, Kim K. Increased serum thymic stromal lymphopoietin in children with atopic dermatitis. *Pediatr Allergy Immunol.* (2010) 21:e457–60.

41. Soumelis V, Reche P, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol.* (2002) 3:673–80.

42. Tatsuno K, Fujiyama T, Yamaguchi H, Waki M, Tokura YTSLP. Directly interacts with skin-homing Th2 cells highly expressing its receptor to enhance il-4 production in atopic dermatitis. *J Invest Dermatol.* (2015) 135:3017–24. doi: 10.1038/jid.2015.318

43. Nakatsuji T, Chen T, Two A, Chun K, Narala S, Geha R, et al. *Staphylococcus aureus* exploits epidermal barrier defects in atopic dermatitis to trigger cytokine expression. *J Invest Dermatol.* (2016) 136:2192–200. doi: 10.1016/j.jid.2016.05.127

44. Vu AT, Baba T, Chen X, Le T, Kinoshita H, Xie Y, et al. *Staphylococcus aureus* membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway. *J Allergy Clin Immunol.* (2010) 126:985–93. doi: 10.1016/j.jaci.2010.09.002

45. Parnes JR, Sullivan JT, Chen L, Dias C. Pharmacokinetics, safety, and tolerability of tezepelumab (AMG 157) in healthy and atopic dermatitis adult subjects. *Clin Pharmacol Ther.* (2019) 106:441–9. doi: 10.1002/cpt.1401

46. Simpson EL, Parnes J, She D, Crouch S, Rees W, Mo M, et al. Tezepelumab, an anti-thymic stromal lymphopoietin monoclonal antibody, in the treatment of moderate to severe atopic dermatitis: A randomized phase 2a clinical trial. *J Am Acad Dermatol.* (2019) 80:1013–21. doi: 10.1016/j.jaad.2018.11.059

47. Iznardo H, Puig L. IL-1 family cytokines in inflammatory dermatoses: Pathogenetic role and potential therapeutic implications. *Int J Mol Sci.* (2022) 23:9479. doi: 10.3390/ijms23169479

48. Boutet M-A, Nerviani A, Pitzalis C. IL-36, IL-37, and IL-38 cytokines in skin and joint inflammation: A comprehensive review of their therapeutic potential. *Int J Mol Sci.* (2019) 20:1257. doi: 10.3390/ijms20061257

49. Di Paolo NC, Shayakhmetov DM. Interleukin 1α and the inflammatory process. *Nat Immunol.* (2016) 17:906–13.

50. Mantovani A, Dinarello CA, Molgora M, Garlanda C. Interleukin-1 and related cytokines in the regulation of inflammation and immunity. *Immunity.* (2019) 50:778–95. doi: 10.1016/j.immuni.2019.03.012

51. Højen JF, Kristensen M, McKee A, Wade M, Azam T, Lunding L, et al. IL-1R3 blockade broadly attenuates the functions of six members of the IL-1 family, revealing their contribution to models of disease. *Nat Immunol.* (2019) 20:1138–49. doi: 10.1038/s41590-019-0467-1

52. Liu X-J, Mu Z-L, Zhao Y, Zhang J-Z. Topical tetracycline improves MC903-induced atopic dermatitis in mice through inhibition of inflammatory cytokines and thymic stromal lymphopoietin expression. *Chin Med J.* (2016) 129:1483–90. doi: 10.4103/0366-6999.183427

53. Kezic S, O'Regan G, Lutter R, Jakasa I, Koster E, Saunders S, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol.* (2012) 129: 1031–9.e1. doi: 10.1016/j.jaci.2011.12.989

54. Kanni T, Argyropoulou M, Spyridopoulos T, Pistiki A, Stecher M, Dinarello C, et al. MABp1 targeting IL-1α for moderate to severe hidradenitis suppurativa not eligible for adalimumab: A randomized study. *J Invest Dermatol.* (2018) 138:795–801.

55. Kanni T, Argyropoulou M, Dinarello CA, Simard J, Giamarellos-Bourboulis EJ. MABp1 targeting interleukin-1α in hidradenitis suppurativa ineligible for adalimumab treatment: Results of the open-label extension period. *Clin Exp Dermatol.* (2021) 46:162–3.

56. Sun NZ, Ro T, Jolly P, Sayed CJ. Non-response to interleukin-1 antagonist canakinumab in two patients with refractory pyoderma gangrenosum and hidradenitis suppurativa. *J Clin Aesthet Dermatol.* (2017) 10:36–8.

57. Houriet C, Seyed Jafari S, Thomi R, Schlapbach C, Borradori L, Yawalkar N, et al. Canakinumab for severe hidradenitis suppurativa: Preliminary experience in 2 cases. *JAMA Dermatol.* (2017) 153:1195–7. doi: 10.1001/jamadermatol.2017.2392

58. Skendros P, Papagoras C, Lefaki I, Giatromanolaki A, Kotsianidis I, Speletas M, et al. Successful response in a case of severe pustular psoriasis after interleukin-1β inhibition. *Br J Dermatol.* (2017) 176:212–5.

59. Mansouri B, Kivelevitch D, Campa M, Menter A. Palmoplantar pustular psoriasis unresponsive to the interleukin-1β antagonist canakinumab. *Clin Exp Dermatol.* (2016) 41:324–6.

60. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. *Nat Rev Dis Prim.* (2018) 4:1.

61. Otsuka A, Nomura T, Rerknimitr P, Seidel J, Honda T, Kabashima K. The interplay between genetic and environmental factors in the pathogenesis of atopic dermatitis. *Immunol Rev.* (2017) 278:246–62.

62. Dougan M, Dranoff G, Dougan SK. GM-CSF, IL-3, and IL-5 family of cytokines: Regulators of inflammation. *Immunity.* (2019) 50:796–811. doi: 10.1016/j.immuni.2019.03.022

63. Foster EL, Simpson E, Fredrikson L, Lee J, Lee N, Fryer A, et al. Eosinophils increase neuron branching in human and murine skin and in vitro. *PLoS One.* (2011) 6:e22029. doi: 10.1371/journal.pone.0022029

64. Kimura M, Tsuruta S, Yoshida T. Correlation of house dust mite-specific lymphocyte proliferation with IL-5 production, eosinophilia, and the severity of symptoms in infants with atopic dermatitis. *J Allergy Clin Immunol.* (1998) 101:84–9. doi: 10.1016/S0091-6749(98)70197-6

65. Phipps S, Flood-Page P, Menzies-Gow A, Ong YE, Kay AB. Intravenous anti-IL-5 monoclonal antibody reduces eosinophils and tenascin deposition in allergen-challenged human atopic skin. *J Invest Dermatol.* (2004) 122:1406–12. doi: 10.1111/j.0022-202X.2004.22619.x

66. Oldhoff JM, Darsow U, Werfel T, Katzer K, Wulf A, Laifaoui J, et al. Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. *Allergy.* (2005) 60:693–6. doi: 10.1111/j.1398-9995.2005.00791.x

67. Bel EH, Wenzel S, Thompson P, Prazma C, Keene O, Yancey S, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med.* (2014) 371:1189–97.

68. Ortega HG, Liu M, Pavord I, Brusselle G, FitzGerald J, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med.* (2014) 371:1198–207.

69. Akdis CA, Arkwright P, Brüggen M, Busse W, Gadina M, Guttman-Yassky E, et al. Type 2 immunity in the skin and lungs. *Allergy.* (2020) 75:1582–605.

70. Junttila IS. Tuning the cytokine responses: An update on interleukin (IL)-4 and IL-13 receptor complexes. *Front Immunol.* (2018) 9:888. doi: 10.3389/fimmu.2018.00888

71. Ebihara T, Tatematsu M, Fuchimukai A, Yamada T, Yamagata K, Takasuga S, et al. Trained innate lymphoid cells in allergic diseases. *Allergol Int.* (2021) 70:174–80.

72. Chieosilapatham P, Kiatsurayanon C, Umehara Y, Trujillo-Paez J, Peng G, Yue H, et al. Keratinocytes: Innate immune cells in atopic dermatitis. *Clin Exp Immunol.* (2021) 204:296–309.

73. Furue M. Regulation of filaggrin, loricrin, and involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: Pathogenic implications in atopic dermatitis. *Int J Mol Sci.* (2020) 21:5382. doi: 10.3390/ijms21155382

74. McCormick SM, Heller NM. Commentary: IL-4 and IL-13 receptors and signaling. *Cytokine.* (2015) 75:38–50.

75. Chan LS, Robinson N, Xu L. Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: An experimental

animal model to study atopic dermatitis. *J Invest Dermatol.* (2001) 117:977–83. doi: 10.1046/j.0022-202x.2001.01484.x

76. Chen L, Martinez O, Overbergh L, Mathieu C, Prabhakar B, Chan L. Early up-regulation of Th2 cytokines and late surge of Th1 cytokines in an atopic dermatitis model. *Clin Exp Immunol.* (2004) 138:375–87. doi: 10.1111/j.1365-2249.2004.02649.x

77. Zheng T, Oh M, Oh S, Schroeder J, Glick A, Zhu Z. Transgenic expression of interleukin-13 in the skin induces a pruritic dermatitis and skin remodeling. *J Invest Dermatol.* (2009) 129:742–51. doi: 10.1038/jid.2008.295

78. Sehra S, Yao Y, Howell M, Nguyen E, Kansas G, Leung D, et al. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J Immunol.* (2010) 184:3186–90.

79. Bao L, Zhang H, Mohan GC, Shen K, Chan LS. Differential expression of inflammation-related genes in IL-4 transgenic mice before and after the onset of atopic dermatitis skin lesions. *Mol Cell Probes.* (2016) 30:30–8. doi: 10.1016/j.mcp.2015.11.001

80. Campion M, Smith L, Gatault S, Métais C, Buddenkotte J, Steinhoff M. Interleukin-4 and interleukin-13 evoke scratching behaviour in mice. *Exp Dermatol.* (2019) 28:1501–4. doi: 10.1111/exd.14034

81. Nomura I, Goleva E, Howell M, Hamid Q, Ong P, Hall C, et al. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *J Immunol.* (2003) 171:3262–9. doi: 10.4049/jimmunol.171.6.3262

82. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med.* (2002) 347:1151–60.

83. Wang V, Boguniewicz J, Boguniewicz M, Ong PY. The infectious complications of atopic dermatitis. *Ann Allergy Asthma Immunol.* (2021) 126:3–12.

84. Purwar R, Kraus M, Werfel T, Wittmann M. Modulation of keratinocyte-derived MMP-9 by IL-13: A possible role for the pathogenesis of epidermal inflammation. *J Invest Dermatol.* (2008) 128:59–66. doi: 10.1038/sj.jid.5700940

85. Shin JW, Choi Y, Choi H, Na J, Kim K, Park I, et al. Defective basement membrane in atopic dermatitis and possible role of IL-13. *J Eur Acad Dermatol Venereol.* (2015) 29:2060–2. doi: 10.1111/jdv.12596

86. Moriya C, Jinnin M, Yamane K, Maruo K, Muchemwa F, Igata T, et al. Expression of matrix metalloproteinase-13 is controlled by IL-13 via PI3K/Akt3 and PKC-δ in normal human dermal fibroblasts. *J Invest Dermatol.* (2011) 131:655–61. doi: 10.1038/jid.2010.361

87. Oetjen LK, Mack M, Feng J, Whelan T, Niu H, Guo C, et al. Sensory neurons co-opt classical immune signaling pathways to mediate chronic itch. *Cell.* (2017) 171:217–28.e13. doi: 10.1016/j.cell.2017.08.006

88. Gooderham MJ, Hong HC-H, Eshtiaghi P, Papp KA. Dupilumab: A review of its use in the treatment of atopic dermatitis. *J Am Acad Dermatol.* (2018) 78:S28–36.

89. Blauvelt A, de Bruin-Weller M, Gooderham M, Cather J, Weisman J, Pariser D, et al. Long-term management of moderate-to-severe atopic dermatitis with dupilumab and concomitant topical corticosteroids (LIBERTY AD CHRONOS): A 1-year, randomised, double-blinded, placebo-controlled, phase 3 trial. *Lancet.* (2017) 389:2287–303. doi: 10.1016/S0140-6736(17)31191-1

90. Simpson EL, Paller A, Siegfried E, Boguniewicz M, Sher L, Gooderham M, et al. Efficacy and safety of dupilumab in adolescents with uncontrolled moderate to severe atopic dermatitis: A phase 3 randomized clinical trial. *JAMA Dermatol.* (2020) 156:44–56.

91. Blauvelt A, Guttman-Yassky E, Paller A, Simpson E, Cork M, Weisman J, et al. Long-term efficacy and safety of dupilumab in adolescents with moderate-to-severe atopic dermatitis: Results through week 52 from a phase III open-label extension trial (LIBERTY AD PED-OLE). *Am J Clin Dermatol.* (2022) 23:365–83. doi: 10.1007/s40257-022-00683-2

92. Paller AS, Siegfried E, Thaçi D, Wollenberg A, Cork M, Arkwright P, et al. Efficacy and safety of dupilumab with concomitant topical corticosteroids in children 6 to 11 years old with severe atopic dermatitis: A randomized, double-blinded, placebo-controlled phase 3 trial. *J Am Acad Dermatol.* (2020) 83:1282–93. doi: 10.1016/j.jaad.2020.06.054

93. Paller AS, Simpson E, Siegfried E, Cork M, Wollenberg A, Arkwright P, et al. Dupilumab in children aged 6 months to younger than 6 years with uncontrolled atopic dermatitis: A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* (2022) 400:908–19. doi: 10.1016/S0140-6736(22)01539-2

94. Halling A-S, Loft N, Silverberg JI, Guttman-Yassky E, Thyssen JP. Real-world evidence of dupilumab efficacy and risk of adverse events: A systematic review and meta-analysis. *J Am Acad Dermatol.* (2021) 84:139–47. doi: 10.1016/j.jaad.2020.08.051

95. Patruno C, Potestio L, Scalvenzi M, Battista T, Raia F, Picone V, et al. Dupilumab for the treatment of adult atopic dermatitis in special populations. *J Dermatol Treat.* (2022) 33:3028–33.

96. Napolitano M, Maffei M, Patruno C, Leone C, Di Guida A, Potestio L, et al. Dupilumab effectiveness for the treatment of patients with concomitant atopic dermatitis and chronic rhinosinusitis with nasal polyposis. *Dermatol Ther.* (2021) 34:e15120. doi: 10.1111/dth.15120

97. Bieber T. Interleukin-13: Targeting an underestimated cytokine in atopic dermatitis. *Allergy.* (2020) 75:54–62. doi: 10.1111/all.13954

98. Wollenberg A, Blauvelt A, Guttman-Yassky E, Worm M, Lynde C, Lacour J, et al. Tralokinumab for moderate-to-severe atopic dermatitis: Results from two 52-week, randomized, double-blind, multicentre, placebo-controlled phase III trials (ECZTRA 1 and ECZTRA 2). *Br J Dermatol.* (2021) 184:437–49. doi: 10.1111/bjd.19574

99. Paller AS, Flohr C, Cork M, Bewley A, Blauvelt A, Hong H, et al. Efficacy and safety of tralokinumab in adolescents with moderate to severe atopic dermatitis: The phase 3 ECZTRA 6 randomized clinical trial. *JAMA Dermatol.* (2023) 159:596–605. doi: 10.1001/jamadermatol.2023.0627

100. Guttman-Yassky E, Blauvelt A, Eichenfield L, Paller A, Armstrong A, Drew J, et al. Efficacy and safety of lebrikizumab, a high-affinity interleukin 13 inhibitor, in adults with moderate to severe atopic dermatitis: A phase 2b randomized clinical trial. *JAMA Dermatol.* (2020) 156:411–20. doi: 10.1001/jamadermatol.2020.0079

101. Paller AS, Flohr C, Eichenfield L, Irvine A, Weisman J, Soung J, et al. Safety and efficacy of lebrikizumab in adolescent patients with moderate-to-severe atopic dermatitis: A 52-week, open-label, phase 3 study. *Dermatol Ther.* (2023) 13:1517–34. doi: 10.1007/s13555-023-00942-y

102. Lytvyn Y, Gooderham M. Targeting interleukin 13 for the treatment of atopic dermatitis. *Pharmaceutics.* (2023) 15:568.

103. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell S, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol.* (2004) 5:752–60.

104. Ishii T, Wang J, Zhang W, Mascarenhas J, Hoffman R, Dai Y, et al. Pivotal role of mast cells in pruritogenesis in patients with myeloproliferative disorders. *Blood.* (2009) 113:5942–50. doi: 10.1182/blood-2008-09-179416

105. Niyonsaba F, Ushio H, Hara M, Yokoi H, Tominaga M, Takamori K, et al. Antimicrobial peptides human beta-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. *J Immunol.* (2010) 184:3526–34. doi: 10.4049/jimmunol.0900712

106. Cornelissen C, Brans R, Czaja K, Skazik C, Marquardt Y, Zwadlo-Klarwasser G, et al. Ultraviolet B radiation and reactive oxygen species modulate interleukin-31 expression in T lymphocytes, monocytes and dendritic cells. *Br J Dermatol.* (2011) 165:966–75. doi: 10.1111/j.1365-2133.2011.10487.x

107. Rabenhorst A, Hartmann K. Interleukin-31: A novel diagnostic marker of allergic diseases. *Curr Allergy Asthma Rep.* (2014) 14:423. doi: 10.1007/s11882-014-0423-y

108. Hermanns HM. Oncostatin M and interleukin-31: Cytokines, receptors, signal transduction and physiology. *Cytokine Growth Factor Rev.* (2015) 26:545–58.

109. Horejs-Hoeck J, Schwarz H, Lamprecht S, Maier E, Hainzl S, Schmittner M, et al. Dendritic cells activated by IFN-γ/STAT1 express IL-31 receptor and release proinflammatory mediators upon IL-31 treatment. *J Immunol.* (2012) 188:5319–26.

110. Takamori A, Nambu A, Sato K, Yamaguchi S, Matsuda K, Numata T, et al. IL-31 is crucial for induction of pruritus, but not inflammation, in contact hypersensitivity. *Sci Rep.* (2018) 8:6639. doi: 10.1038/s41598-018-25094-4

111. Arai I, Tsuji M, Miyagawa K, Takeda H, Akiyama N, Saito S. Repeated administration of IL-31 upregulates IL-31 receptor A (IL-31RA) in dorsal root ganglia and causes severe itch-associated scratching behaviour in mice. *Exp Dermatol.* (2015) 24:75–8. doi: 10.1111/exd.12587

112. Arai I, Tsuji M, Takeda H, Akiyama N, Saito S. A single dose of interleukin-31 (IL-31) causes continuous itch-associated scratching behaviour in mice. *Exp Dermatol.* (2013) 22:669–71. doi: 10.1111/exd.12222

113. Takaoka A, Arai I, Sugimoto M, Honma Y, Futaki N, Nakamura A, et al. Involvement of IL-31 on scratching behavior in NC/Nga mice with atopic-like dermatitis. *Exp Dermatol.* (2006) 15:161–7. doi: 10.1111/j.1600-0625.2006.00405.x

114. Bilsborough J, Mudri S, Chadwick E, Harder B, Dillon SR. IL-31 receptor (IL-31RA) knockout mice exhibit elevated responsiveness to oncostatin M. *J Immunol.* (2010) 185:6023–30. doi: 10.4049/jimmunol.0902769

115. Grimstad O, Sawanobori Y, Vestergaard C, Bilsborough J, Olsen U, Grønhøj-Larsen C, et al. Anti-interleukin-31-antibodies ameliorate scratching behaviour in NC/Nga mice: A model of atopic dermatitis. *Exp Dermatol.* (2009) 18:35–43. doi: 10.1111/j.1600-0625.2008.00766.x

116. Kasraie S, Niebuhr M, Baumert K, Werfel T. Functional effects of interleukin 31 in human primary keratinocytes. *Allergy.* (2011) 66:845–52.

117. Kasraie S, Niebuhr M, Werfel T. Interleukin (IL)-31 activates signal transducer and activator of transcription (STAT)-1, STAT-5 and extracellular signal-regulated kinase 1/2 and down-regulates IL-12p40 production in activated human macrophages. *Allergy.* (2013) 68:739–47. doi: 10.1111/all.12152

118. Dai X, Okazaki H, Hanakawa Y, Murakami M, Tohyama M, Shirakata Y, et al. Eccrine sweat contains IL-1α, IL-1β and IL-31 and activates epidermal keratinocytes as a danger signal. *PLoS One.* (2013) 8:e67666. doi: 10.1371/journal.pone.0067666

119. Kim HJ, Zeidi M, Bonciani D, Pena S, Tiao J, Sahu S, et al. Itch in dermatomyositis: The role of increased skin interleukin-31. *Br J Dermatol.* (2018) 179:669–78. doi: 10.1111/bjd.16498

120. Sonkoly E, Muller A, Lauerma A, Pivarcsi A, Soto H, Kemeny L, et al. IL-31: A new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol.* (2006) 117:411–7.

121. Kabashima K, Matsumura T, Komazaki H, Kawashima M, Nemolizumab-JP01 Study Group. Trial of nemolizumab and topical agents for atopic dermatitis with pruritus. *N Engl J Med.* (2020) 383:141–50.

122. Kabashima K, Matsumura T, Komazaki H, Kawashima M, Nemolizumab JP01 andJP02 Study Group. Nemolizumab plus topical agents in patients with atopic dermatitis (AD) and moderate-to-severe pruritus provide improvement in pruritus and signs of AD for up to 68 weeks: Results from two phase III, long-term studies. *Br J Dermatol.* (2022) 186:642–51. doi: 10.1111/bjd.20873

123. Silverberg JI, Pinter A, Alavi A, Lynde C, Bouaziz J, Wollenberg A, et al. Nemolizumab is associated with a rapid improvement in atopic dermatitis signs and symptoms: Subpopulation (EASI = 16) analysis of randomized phase 2B study. *J Eur Acad Dermatol Venereol.* (2021) 35:1562–8. doi: 10.1111/jdv.17218

124. Silverberg JI, Pinter A, Pulka G, Poulin Y, Bouaziz J, Wollenberg A, et al. Phase 2B randomized study of nemolizumab in adults with moderate-to-severe atopic dermatitis and severe pruritus. *J Allergy Clin Immunol.* (2020) 145:173–82. doi: 10.1016/j.jaci.2019.08.013

125. Bonnekoh H, Butze M, Metz M. Characterization of the effects on pruritus by novel treatments for atopic dermatitis. *J Dtsch Dermatol Ges.* (2022) 20:150–6.

126. Xie MH, Aggarwal S, Ho W, Foster J, Zhang Z, Stinson J, et al. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem.* (2000) 275:31335–9. doi: 10.1074/jbc.M005304200

127. Hayashida S, Uchi H, Takeuchi S, Esaki H, Moroi Y, Furue M. Significant correlation of serum IL-22 levels with CCL17 levels in atopic dermatitis. *J Dermatol Sci.* (2011) 61:78–9. doi: 10.1016/j.jdermsci.2010.08.013

128. Meephansan J, Ruchusatsawat K, Sindhupak W, Thorner PS, Wongpiyabovorn J. Effect of methotrexate on serum levels of IL-22 in patients with psoriasis. *Eur J Dermatol.* (2011) 21:501–4. doi: 10.1684/ejd.2011.1335

129. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. *Immunity.* (2004) 21:241–54.

130. Jin M, Yoon J. From bench to clinic: The potential of therapeutic targeting of the IL-22 signaling pathway in atopic dermatitis. *Immune Netw.* (2018) 18:e42. doi: 10.4110/in.2018.18.e42

131. Zhang W, Dang E, Shi X, Jin L, Feng Z, Hu L, et al. The pro-inflammatory cytokine IL-22 up-regulates keratin 17 expression in keratinocytes via STAT3 and ERK1/2. *PLoS One.* (2012) 7:e40797. doi: 10.1371/journal.pone.0040797

132. Yoon J, Leyva-Castillo J, Wang G, Galand C, Oyoshi M, Kumar L, et al. IL-23 induced in keratinocytes by endogenous TLR4 ligands polarizes dendritic cells to drive IL-22 responses to skin immunization. *J Exp Med.* (2016) 213:2147–66. doi: 10.1084/jem.20150376

133. Guttman-Yassky E, Lowes M, Fuentes-Duculan J, Zaba L, Cardinale I, Nograles K, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. *J Immunol.* (2008) 181:7420–7.

134. Nograles KE, Zaba L, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, et al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J Allergy Clin Immunol.* (2009) 123:1244–52.e2. doi: 10.1016/j.jaci.2009.03.041

135. Gittler JK, Shemer A, Suárez-Fariñas M, Fuentes-Duculan J, Gulewicz K, Wang C, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol.* (2012) 130:1344–54. doi: 10.1016/j.jaci.2012.07.012

136. Boniface K, Bernard F, Garcia M, Gurney A, Lecron J, Morel F. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J Immunol.* (2005) 174:3695–702. doi: 10.4049/jimmunol.174.6.3695

137. Sa SM, Valdez P, Wu J, Jung K, Zhong F, Hall L, et al. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J Immunol.* (2007) 178:2229–40. doi: 10.4049/jimmunol.178.4.2229

138. Brunner PM, Pavel A, Khattri S, Leonard A, Malik K, Rose S, et al. Baseline IL-22 expression in patients with atopic dermatitis stratifies tissue responses to fezakinumab. *J Allergy Clin Immunol.* (2019) 143:142–54. doi: 10.1016/j.jaci.2018.07.028

139. Guttman-Yassky E, Brunner P, Neumann A, Khattri S, Pavel A, Malik K, et al. Efficacy and safety of fezakinumab (an IL-22 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis inadequately controlled by conventional treatments: A randomized, double-blind, phase 2a trial. *J Am Acad Dermatol.* (2018) 78: 872–81.e6. doi: 10.1016/j.jaad.2018.01.016

140. Wilson NJ, Boniface K, Chan J, McKenzie B, Blumenschein W, Mattson J, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol.* (2007) 8:950–7.

141. Borowczyk J, Shutova M, Brembilla NC, Boehncke W-H. IL-25 (IL-17E) in epithelial immunology and pathophysiology. *J Allergy Clin Immunol.* (2021) 148:40–52.

142. Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Nakamizo S, Moniaga C, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. *J Invest Dermatol.* (2014) 134:2122–30.

143. Salimi M, Barlow J, Saunders S, Xue L, Gutowska-Owsiak D, Wang X, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med.* (2013) 210:2939–50. doi: 10.1084/jem.20130351

144. Fort MM, Cheung J, Yen D, Li J, Zurawski S, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity.* (2001) 15:985–95.

145. Spidale NA, Malhotra N, Frascoli M, Sylvia K, Miu B, Freeman C, et al. Neonatal-derived IL-17 producing dermal γδ T cells are required to prevent spontaneous atopic dermatitis. *Elife.* (2020) 9:e51188. doi: 10.7554/eLife.51188

146. Nakai K, He Y, Nishiyama F, Naruse F, Haba R, Kushida Y, et al. IL-17A induces heterogeneous macrophages, and it does not alter the effects of lipopolysaccharides on macrophage activation in the skin of mice. *Sci Rep.* (2017) 7:12473.

147. Krzysiek J, Lesiak A, Szybka M, Michalak A, Pastuszak-Lewandoska D, Grzegorczyk J, et al. The role of heterodimer IL-17-A/F in atopic dermatitis. *Postepy Dermatol Alergol.* (2022) 39:1093–100. doi: 10.5114/ada.2022.122604

148. Klonowska J, Gleń J, Nowicki RJ, Trzeciak M. Combination of FLG mutations and SNPs of IL-17A and IL-19 influence on atopic dermatitis occurrence. *Postepy Dermatol Alergol.* (2022) 39:200–8. doi: 10.5114/ada.2021.105412

149. Hvid M, Vestergaard C, Kemp K, Christensen G, Deleuran B, Deleuran M. IL-25 in atopic dermatitis: A possible link between inflammation and skin barrier dysfunction? *J Invest Dermatol.* (2011) 131:150–7. doi: 10.1038/jid.2010.277

150. Kim BE, Bin L, Ye Y-M, Ramamoorthy P, Leung DYM. IL-25 enhances HSV-1 replication by inhibiting filaggrin expression, and acts synergistically with Th2 cytokines to enhance HSV-1 replication. *J Invest Dermatol.* (2013) 133:2678–85. doi: 10.1038/jid.2013.223

151. Borowczyk J, Buerger C, Tadjrischi N, Drukala J, Wolnicki M, Wnuk D, et al. IL-17E (IL-25) and IL-17A differentially affect the functions of human keratinocytes. *J Invest Dermatol.* (2020) 140:1379–89.e2. doi: 10.1016/j.jid.2019.12.013

152. Wang Y-H, Angkasekwinai P, Lu N, Voo K, Arima K, Hanabuchi S, et al. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J Exp Med.* (2007) 204:1837–47. doi: 10.1084/jem.20070406

153. Aktar MK, Kido-Nakahara M, Furue M, Nakahara T. Mutual upregulation of endothelin-1 and IL-25 in atopic dermatitis. *Allergy.* (2015) 70:846–54. doi: 10.1111/all.12633

154. Prakoeswa CRS, Bonita L, Karim A, Herwanto N, Umborowati M, Setyaningrum T, et al. Beneficial effect of Lactobacillus plantarum IS-10506 supplementation in adults with atopic dermatitis: A randomized controlled trial. *J Dermatol Treat.* (2022) 33:1491–8. doi: 10.1080/09546634.2020.1836310

155. Thaçi D, Singh D, Lee M, Timmis H, Jacobs D, Passier P, et al. Phase 1 and 2 randomized clinical studies determine lack of efficacy for anti-IL-17C antibody MOR106 in moderate-severe atopic dermatitis. *J Clin Med Res.* (2022) 11:7244. doi: 10.3390/jcm11237244

156. Ungar B, Pavel A, Li R, Kimmel G, Nia J, Hashim P, et al. Phase 2 randomized, double-blind study of IL-17 targeting with secukinumab in atopic dermatitis. *J Allergy Clin Immunol.* (2021) 147:394–7. doi: 10.1016/j.jaci.2020.04.055

157. Oppmann B, Lesley R, Blom B, Timans J, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity.* (2000) 13:715–25. doi: 10.1016/s1074-7613(00)00070-4

158. Andersson A, Kokkola R, Wefer J, Erlandsson-Harris H, Harris RA. Differential macrophage expression of IL-12 and IL-23 upon innate immune activation defines rat autoimmune susceptibility. *J Leukoc Biol.* (2004) 76:1118–24. doi: 10.1189/jlb.0704385

159. Piskin G, Sylva-Steenland RMR, Bos JD, Teunissen MBM. In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: Enhanced expression in psoriatic skin. *J Immunol.* (2006) 176:1908–15. doi: 10.4049/jimmunol.176.3.1908

160. Chamilos G, Ganguly D, Lande R, Gregorio J, Meller S, Goldman W, et al. Generation of IL-23 producing dendritic cells (DCs) by airborne fungi regulates fungal pathogenicity via the induction of T(H)-17 responses. *PLoS One.* (2010) 5:e12955. doi: 10.1371/journal.pone.0012955

161. Rachitskaya AV, Hansen A, Horai R, Li Z, Villasmil R, Luger D, et al. Cutting edge: NKT cells constitutively express IL-23 receptor and RORgammat and rapidly produce IL-17 upon receptor ligation in an IL-6-independent fashion. *J Immunol.* (2008) 180:5167–71. doi: 10.4049/jimmunol.180.8.5167

162. Awasthi A, Riol-Blanco L, Jäger A, Korn T, Pot C, Galileos G, et al. Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol.* (2009) 182:5904–8. doi: 10.4049/jimmunol.0900732

163. Tonel G, Conrad C, Laggner U, Di Meglio P, Grys K, McClanahan T, et al. Cutting edge: A critical functional role for IL-23 in psoriasis. *J Immunol.* (2010) 185:5688–91. doi: 10.4049/jimmunol.1001538

164. McGeachy MJ, Chen Y, Tato C, Laurence A, Joyce-Shaikh B, Blumenschein W, et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol.* (2009) 10:314–24. doi: 10.1038/ni.1698

165. Wu C, Yosef N, Thalhamer T, Zhu C, Xiao S, Kishi Y, et al. Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature.* (2013) 496:513–7. doi: 10.1038/nature11984

166. Graham AC, Carr K, Sieve A, Indramohan M, Break T, Berg R. IL-22 production is regulated by IL-23 during Listeria monocytogenes infection but is not required for bacterial clearance or tissue protection. *PLoS One.* (2011) 6:e17171.

167. Esaki H, Brunner P, Renert-Yuval Y, Czarnowicki T, Huynh T, Tran G, et al. Early-onset pediatric atopic dermatitis is TH2 but also TH17 polarized in skin. *J Allergy Clin Immunol.* (2016) 138:1639–51. doi: 10.1016/j.jaci.2016.07.013

168. Singh S, Kroe-Barrett R, Canada K, Zhu X, Sepulveda E, Wu H, et al. Selective targeting of the IL23 pathway: Generation and characterization of a novel high-affinity humanized anti-IL23A antibody. *MAbs.* (2015) 7:778–91. doi: 10.1080/19420862.2015.1032491

169. Tyring SK, Rich P, Tada Y, Beeck S, Messina I, Liu J, et al. Risankizumab in patients with moderate-to-severe atopic dermatitis: A phase 2, randomized, double-blind, placebo-controlled study. *Dermatol Ther.* (2023) 13:595–608. doi: 10.1007/s13555-022-00876-x

170. Furue M, Ulzii D, Vu Y, Tsuji G, Kido-Nakahara M, Nakahara T. Pathogenesis of atopic dermatitis: Current paradigm. *Iran J Immunol.* (2019) 16:97–107.

171. Fujita H. The role of IL-22 and Th22 cells in human skin diseases. *J Dermatol Sci.* (2013) 72:3–8.

172. Siedlikowski S, Sandhu V, Lynde C. Treatment of atopic dermatitis using JAK inhibitors: A systematic review. *EMJ Dermatol.* (2019) 7:89–100.

173. Tsai H-R, Lu J-W, Chen L-Y, Chen T-L. Application of Janus kinase inhibitors in atopic dermatitis: An updated systematic review and meta-analysis of clinical trials. *J Pers Med.* (2021) 11:279.

174. Ferreira S, Guttman-Yassky E, Torres T. Selective JAK1 inhibitors for the treatment of atopic dermatitis: Focus on upadacitinib and abrocitinib. *Am J Clin Dermatol.* (2020) 21:783–98.

175. Singh R, Heron CE, Ghamrawi RI, Strowd LC, Feldman SR. Emerging role of Janus kinase inhibitors for the treatment of atopic dermatitis. *Immunotargets Ther.* (2020) 9:255–72.

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