Proliferation-associated protein 2G4 promotes keratinocyte proliferation and survival in psoriasis

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Data availability: *Bulk* RNA sequencing data of the skin related to this article can be obtained at GEO (www.ncbi.nlm.nih.gov/) under the accession number GSE154200. All other data will be shared in reasonable request to the corresponding author.

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What is already known about this topic?

- Psoriasis is a chronic inflammatory skin disease characterized by keratinocyte hyperproliferation, aberrant differentiation and reduced cell death, leading to scaly plaques.
- Current treatments mainly target immune cells rather than keratinocytes, and some patients still show suboptimal responses to these therapies.
- In several malignancies, PA2G4 has been identified as a promoter of cell growth as well as tumourigenesis by inhibiting cell death - cellular hallmarks that are also shared with psoriasis.

What does this study add?

- PA2G4 is highly expressed in psoriatic skin and correlates with disease severity and histopathological features of psoriasis.
- PA2G4 was identified as a key regulator of keratinocyte proliferation, differentiation, and survival.

- PA2G4 knockout reduced keratinocyte proliferation, decreased IL-22-induced acanthosis in reconstructed human epidermis models and increased cell death in primary human keratinocytes.
- Pharmacological inhibition of PA2G4 using WS6 reproduced these effects, further supporting its pathological role in psoriasis.

What is the translational message?

- Our complementary in vivo and in vitro findings highlight the pathological role of PA2G4, which disrupts skin homeostasis in psoriasis by promoting keratinocyte proliferation and survival.
- Thus, inhibiting PA2G4 in keratinocytes, for example through topical or systemic treatment, represents a potential therapeutic strategy for psoriasis.

Abstract

Background: Psoriasis is a non-communicable inflammatory skin disease that affects approximately 2%–3% of the world's population. Given its high impact on quality of life and the fact that a subset of patients exhibits suboptimal or secondary loss of response to current treatments, identifying new therapeutic strategies is crucial. Proliferation-associated protein 2G4 (PA2G4) is a transcription factor that has been exclusively studied in cancer research, where it promotes cell growth and enhances tumourigenesis by inhibiting apoptosis. However, its role in inflammatory skin diseases remains largely unknown.

Objectives: This study focused on the pathophysiological and immunological functions of PA2G4 in psoriasis and evaluated its potential as a therapeutic target.

Methods: Bulk, single-cell, and spatial RNA sequencing combined with immunohistochemistry were used to assess *PA2G4* expression in psoriatic skin compared with that in non-lesional controls. Functional studies were performed in primary human keratinocytes and reconstructed human epidermis (RHE) models using the CRISPR/Cas9-mediated knockout (KO) of PA2G4 and pharmacological inhibition of PA2G4 with the small-molecule WS6. The regulatory effects of PA2G4 on cellular processes, such as proliferation, differentiation, and survival, were investigated using RNA-seq, western blot analysis, scratch assays, and annexin V staining.

Results: *PA2G4* was highly abundant in psoriasis, and its expression was predominantly restricted to basal proliferating keratinocytes. Its gene expression is positively correlated with psoriasis severity, the degree of acanthosis, neutrophil infiltration, and genes which are upregulated in psoriasis. PA2G4 KO in primary human keratinocytes activated differentiation pathways while

suppressing proliferation pathways, resulting in the downregulation of proliferation- and inflammation-related genes (e.g. *MKI67, IL20, VEGFA,* and *HIF1A*) and the upregulation of differentiation and cell adhesion markers (e.g. *KRT6C, LCE2C,* and *DSG4*). Functionally, the PA2G4 KO reduced keratinocyte proliferation in scratch assays, attenuated interleukin-22-induced acanthosis in RHE models, and promoted keratinocyte death. Pharmacological inhibition of PA2G4 using the small-molecule inhibitor WS6 similarly downregulated genes associated with proliferation and cell survival.

Conclusions: PA2G4 could promote keratinocyte hyperproliferation and survival in psoriasis, thereby critically influencing epidermal homeostasis. Therefore, inhibition of PA2G4 may represent a new treatment option for psoriasis.

Introduction

Psoriasis is a common immune-mediated inflammatory skin disease (ISD) affecting approximately 2 to 3% of the world's population (*1*). It is a complex genetic disease with a high risk of multiple comorbidities, including psoriatic arthritis, cardiovascular diseases, and metabolic syndromes, all of which substantially impair patients' quality of life (*2, 3*). Psoriasis is primarily driven by a T cell immune response characterised by an exaggerated T helper (Th)17/Th1 axis (*4-6*). Cytokines produced by Th17/Th1 cells promote keratinocyte hyperproliferation, aberrant differentiation, and dysregulated cell death, leading to the formation of characteristic scaly plaques (*4*). However, psoriatic keratinocytes actively contribute to the initiation and maintenance of psoriasis pathogenesis by amplifying type 3 immune response (*7, 8*). These type 3 responses drive the histopathological features of psoriasis, including epidermal thickening (acanthosis), elongated rete ridges, a pronounced neutrophil infiltration, and high metabolic activity (*4, 9*).

Given its high prevalence and profound impact on the quality of life, psoriasis imposes a substantial burden on global health. While therapies targeting interleukin (IL)-17 and IL-23 have improved outcomes, a subset of patients exhibits suboptimal response, highlighting the need to better understand disease mechanisms (*10-16*). Keratinocyte hyperproliferation is a hallmark of psoriasis, driven by transcription factors (TFs), such as MYC, JUN, STAT family members, and PCNA (*17-21*). TF activity can be modulated through expression levels, subcellular trafficking, post-translational modification, or targeted degradation, making TFs tractable hubs for elucidating

pathogenic molecular networks. Understanding these regulatory circuits may decipher the mechanisms underlying keratinocyte hyperproliferation and pathogenic epidermal remodeling, while also identifying TFs as potential intervention points. Such strategies offer the potential to overcome cytokine pathway redundancies while maintaining a manageable safety profile. This suggests that targeting aberrantly expressed TFs may represent an alternative therapeutic approach for psoriasis, with the potential to restore pathogenic transcriptional programs and improve outcomes in patients who fail current therapies (*17, 22*).

Proliferation-associated 2G4 protein (PA2G4, also known as EBP1), is a multifunctional TF that binds to RNA, DNA, and proteins, regulating transcription, RNA biological activity, proteostasis, and epigenetics (*23, 24*). It is ubiquitously expressed in epithelial and mesenchymal cells, and influences cell proliferation, differentiation, and survival. Dysregulated expression of PA2G4 has been linked to abnormal cell development and oncogenesis, highlighting its importance as a regulator of pathogenic processes in various tissues and diseases, particularly cancer (*23, 24*). Alternative splicing generates two isoforms with opposing roles in cell growth and differentiation. PA2G4-p48, is predominantly expressed and localises to both the cytoplasm and nucleus, where it promotes cell growth and enhances tumourigenesis by inhibiting apoptosis. Accordingly, elevated PA2G4-p48 expression levels have been observed in several tumours compared to healthy tissues, and they are associated with tumour progression. By contrast, PA2G4-p42, was less abundant and primarily localised in the cytoplasm. It functions as a tumour suppressor by inhibiting cell proliferation and promoting differentiation, similar to thyroid or salivary gland cancer (*25-27*). Overall, PA2G4 represents a promising therapeutic target in oncology, given its dual function in regulating proliferation, differentiation, cell survival, and processes that are also dysregulated in psoriasis.

In this study, we aimed to investigate the pathophysiological and immunological role of PA2G4 in psoriasis and primary human keratinocytes. Further, we identified PA2G4 inhibition as a novel therapeutic approach in for psoriasis treatment.

Materials and methods

Patient sample collection and ethics compliance

Human skin samples (punch biopsies and keratinocytes isolated from suction blisters) were collected after written informed consent was obtained from Biobank Biederstein, in accordance with the Declaration of Helsinki protocol and local ethics committees: Klinikum Rechts der Isar (44/16S and 5590/12). Punch biopsies (6 mm) of lesional and non-lesional skin were obtained under local anaesthesia. For diagnostic accuracy, independent clinical and histological assessments were performed by a dermatologist and a dermatopathologist.

Cell culture

Primary human epidermal keratinocytes were obtained by suction blister (*28*) and cultured in DermaLife K Keratinocyte Medium Complete Kit (Lifeline Cell Technology, LL-0007) at 37°C, 5% CO_2. Details are provided in the Supplementary Materials and Methods.

RHE skin equivalents

RHE skin equivalents were generated as previously described (*29*). Details are provided in the Supplementary Materials and Methods.

CRISPR-Cas9 KO of PA2G4

CRISPR/Cas9 KO of PA2G4 was performed by applying ribonucleoprotein (RNP) complexes with the 4D-Nucleofector™ device (Lonza, Basel, Switzerland) using the P3 Primary Cell 4D-Nucleofector™ X Kit S (Lonza, Basel, Switzerland) as previously described (*30*). Details are provided in the Supplementary Materials and Methods.

Inhibition of PA2G4 with WS6 treatment

PA2G4 inhibition was achieved after 24 h of treatment with WS6 (Hölzel, HY-12461). A concentration of 1 µM was used according to manufactures instructions and published data (*22*). IC50 values of WS6 are 0.24 nM, 0.21 nM, and 40.48 nM in MV4-11, MOLM13, and K562 cells,

respectively. Functional and molecular effects were assessed using real-time polymerase chain reaction and scratch assays, respectively.

Statistical analysis

Data were analysed using GraphPad Prism 10 software and visualised as mean ± standard deviation (SD) or as boxplots from *n* independent human donors. Details of statistical tests are provided in the figure legends. The significance levels were set at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****).

Supplementary material and methods

Detailed information on *bulk* and single-cell RNA sequencing and spatial transcriptomics of psoriatic skin biopsies, immunohistochemistry, *bulk* RNA sequencing of keratinocytes, the scratch-proliferation assay, IL-20 enzyme-linked immunosorbent assay (ELISA), Annexin V staining, Western blot analysis, and RNA and real-time PCR is provided in the Supplementary Methods section.

Results

PA2G4 is abundant in psoriatic skin and predominantly expressed in basal keratinocytes

Using *bulk* RNA sequencing, *PA2G4* was identified as significantly upregulated in psoriasis (n=90, $p<0.0001$) compared to autologous non-lesional (NL) skin (Figure 1A). Single-cell RNA sequencing revealed strongest expression of *PA2G4* in keratinocytes, predominantly in proliferating (*CDK1*$^+$, *PCNA*$^+$, *KRT1*$^-$, *KRT10*$^-$) and differentiating cells (*KRT1*$^+$, *KRT10*$^+$) (Figure 1B) *(31)*. Consistently, spatial transcriptomics confirmed abundant *PA2G4* expression in the psoriatic epidermis (n=12) compared to non-lesional skin (n=5), with marked enrichment in the basal and suprabasal layers, corresponding to the site of keratinocyte proliferation (Figure 1C and 1D). Immunohistochemistry (IHC) further confirmed high PA2G4 protein expression in psoriatic skin (n=5, 44.3 ± 13.7 cells/10× field), $p=0.0159$). Conversely, non-lesional skin

exhibited weak to no PA2G4 protein expression (n=5, 1.6 ± 0.8 cells/10× field) (Figure 1E and 1F).

PA2G4 gene expression positively correlated with disease severity, histological features, and proliferation/inflammation markers of psoriasis

To assess whether PA2G4 contributes to psoriasis pathogenesis, gene expression from *bulk* RNA sequencing data was correlated with disease severity and histological features. *PA2G4* expression positively correlated (p=0.0001, r=0.3999) with Psoriasis Area and Severity (PASI) scores in patients with psoriasis (n=85) (Figure 1G). Moreover, *PA2G4* levels significantly increased with the intensity of acanthosis, neutrophils, parakeratosis, and hyperkeratosis (intensity 0 to 3: p<0.0001) in skin lesions of patients with ISDs (n=274) (Figure 1H). Consistently, *PA2G4* expression correlated positively with pro-mitogenic cytokines, including *IL20* (p<0.0001, r=0.5399) and *IL22* (p=0.0725, r=0.2032), in patients with psoriasis (n=90). Significant associations were also observed with proliferation markers indicative of keratinocyte hyperproliferation, such as *MKI67* (p<0.0001, r=0.5198) and *PCNA* (p<0.0001, r=0.7520), and keratinocyte TF *EHF* (p<0.0001, r=0.4975) (Figure S1A). Furthermore, *PA2G4* expression strongly correlated with type 3 inflammation markers, including *CXCL1* (p<0.0001, r=0.4467), *CXCL8* (p=0.0015, r=0.3340), *IL36G* (p<0.0001, r=0.6214), *HIF1A* (p<0.0001, r=0.5830), and *S100A9* (p<0.0001, r=0.5206) in patients with psoriasis (n=90) (Figure S1B). *IL17C* (p=0.0538, r=0.2367), *CXCL5* (p=0.3225, r=0.1142), and *VEGFA* (p=0.0725, r=0.2032) levels were not correlated with *PA2G4* expression (Figure 1SC). Thus, PA2G4 likely contributes to psoriasis by regulating cell proliferation, differentiation, and inflammation.

PA2G4 regulated keratinocyte proliferation, differentiation, and inflammation

Following the identification of abundant PA2G4 expression in psoriasis, we characterised its transcriptional landscape and elucidated its function. Highly efficient PA2G4 KO at the gene (log2FC = −2.4, p<0.0001) and protein levels (expression relative to the control: 0.049 vs. 0.42; 88.3% reduction) were generated in keratinocytes using CRISPR–Cas9 ribonucleoprotein complexes (Figure 2A). PA2G4 KO and pulsed wild-type (WT) control keratinocytes (n=6) were cultured under unstimulated or chronic type 3 inflammatory (IL-17A+TNFα) conditions for 24 h

and analysed by *bulk* RNA-seq. Transcriptomic profiling by DESeq2 analysis identified 88 downregulated and 129 upregulated genes in PA2G4 KO keratinocytes under basal conditions, relative to WT keratinocytes (log2FC≥|0.5|, p≤0.05) (Figure 2B). Under type 3 condition, the number of downregulated genes increased to 145, whereas 137 genes were upregulated (Figure S2A). Gene set enrichment analysis (GSEA) provided a global overview of PA2G4-regulated processes. Under basal conditions, GSEA revealed suppression of proliferation- and inflammation-related pathways (e.g., "positive regulation of cell population proliferation", "regulation of cell growth", "cytokine production involved in inflammatory response", "response to type I IFN and IFN-γ"). By contrast, pathways linked to epidermal differentiation (e.g., "keratinocyte and epidermal cell differentiation") and skin development were activated (Figure 2C). Moreover, suppression of the "negative regulation of programmed cell death" pathway indicated activation of cell death processes. A comparable pathway enrichment was observed under type 3 conditions (Figure S2B). Hyperproliferation-associated genes were significantly downregulated in PA2G4 KO cells, including inflammatory cytokines *IL20, VEGFA,* and *TGFB1* (log2FC=−0.97|−0.56|−0.20; p=0.0394|<0.0001|0.02520), as well as transcription factors *TP63, MKI67* and *E2F8* (log2FC=−0.34|−0.90|−0.54; p=<0.0001|0.0111|0.0065) (Figure 2D and S2C). By contrast, genes associated with skin homeostasis, epidermal differentiation, cell adhesion, and tissue remodelling were significantly upregulated. These included keratins *KRT6B* and *KRT6C* (log2FC=0.59|0.79; p=0.0165|0.0023), cell adhesion genes *FLG* and *DSG4* (log2FC=0.75|1.36; p<0.0001 for both), extracellular matrix gene *DCN* (log2FC=1.67; p=0.0405), and late cornified envelope gene *LCE2C* (log2FC=1.32; p=0.0243) (Figure 2E and S2C). Inflammation-associated genes such as the TNF superfamily member lymphotoxin β (*LTB*) (log2FC=−1.48; p=0.0242) as well as *IL33* and *CXCL10* (log2FC=−0.47|−1.20; p=0.0262|0.0041) were suppressed in PA2G4 KO cells. Likewise, interferon-related genes, including *IFNGR2, IFITM1,* and *IRF1* (log2FC=−0.28|−0.79|−0.66; p<0.0001|<0.0001|0.0004) and matrix metalloproteinase *MMP2* (log2FC=−0.89; p<0.0001) were significantly downregulated (Figure 2F and S2C). By contrast, typical psoriasis-associated cytokines and chemokines (*CXCL1, CXCL5, CXCL8, TNF, IL6,* and *IL23A*) remained unchanged (Figure S3). Overall, PA2G4 promoted proliferation and inflammation, while suppressing differentiation, adhesion, and epidermal remodelling in the skin.

PA2G4 promotes keratinocyte proliferation capacity and acanthosis induction in reconstructed human epidermis (RHE)

Functional proliferation-related assays were performed using PA2G4 KO keratinocytes. In scratch-proliferation assays (n=7), PA2G4 KO keratinocytes exhibited significantly impaired proliferation capacity (p=0.0032) with a closing gap of 0.88 ± 0.66 mm^2 compared with 1.44 ± 0.89 mm^2 in WT cells, which nearly closed the scratch (Figure 3A and 3B). In line, PA2G4 KO cells secreted significantly reduced levels of the proliferative cytokine IL-20 (68.5 ± 67.5 pg/ml vs 643.3 ± 917.2 pg/ml in WT, p=0.0073) (Figure 3C). Western blot analysis further revealed decreased HIF-1α protein expression in PA2G4 KO (0.31 ± 0.36 a.u. vs 0.69 ± 0.11 a.u. in WT), suggesting PA2G4 supports hyperproliferation through HIF-1 α regulation (Figure 3D). Consistently, cell cycle phase analysis of scRNA-seq data from patients with psoriasis (n=7) showed highest *PA2G4* expression in keratinocytes in the DNA synthesis (S) phase, indicating its involvement in DNA replication during mitosis and further underscoring its contribution to keratinocyte proliferation (Figure 3E). Finally, IL-22-induced acanthosis in reconstructed human epidermis (RHE) was markedly reduced in PA2G4 KO keratinocytes (33.9 ± 3.1 µm vs 17.0 ± 2.7 µm in WT, p=0.0320), with a tendency toward reduced epidermal thickness under basal conditions (Figure 3F and 3G). Therefore, PA2G4 was identified as a key regulator of keratinocyte proliferation and demonstrated its contribution to the development of acanthosis in psoriasis.

PA2G4 suppressed cell death/apoptosis in keratinocytes

Bulk RNA sequencing initially indicated that PA2G4 may suppress cell death in keratinocytes, as reflected by the suppression of the "negative regulation of programmed cell death" (Figure 2C). Therefore, we examined the effect of PA2G4 on keratinocyte survival. PA2G4 KO keratinocytes showed significant upregulation of pro-apoptotic genes such as *CASP9, DAPK1* and *HRK* (log2FC=0.41|0.67|2.49; p=0.0175|<0.0001|0.0718), the pyroptotic effector *GSMDA* (log2FC=1.00; p<0.0001), and inflammasome-related genes, such as *IL18* and *NLRP10* (log2FC=0.33|0.67; p=0.0171|0.0142). Conversely, the anti-apoptotic protein *BCL11A* (log2FC=−0.38; p=0.0182), ferroptosis inhibitor *GPX4* (log2FC=−0.32; p=0.0008), and complement inhibitor *CFH* (log2FC=−1.55; p=0.0188) were downregulated (Figure 4A), further supporting the role of PA2G4 in restraining cell death. To functionally validate PA2G4-effects on

cell death, annexin V and propidium iodide (PI) staining were performed under basal and type 3 inflammatory conditions. Flow cytometric analysis revealed a marked increase in the frequency of total dead cells (Annexin V^+ PI^+) (p=0.0044 for IL-17A+TNF, p=0.0332 for IL-22) and apoptotic cells (Annexin V^+ PI^-) (p=0.0557 for IL-17A+TNF, p=0.0278 for IL-22) in PA2G4 KO keratinocytes. (Figure 4B and 4C). Thus, PA2G4 could suppress keratinocyte death.

Targeting PA2G4 by WS6 inhibitor treatment reduced psoriasis features

We next investigated the potential of pharmacological PA2G4 inhibition using the selective PA2G4 inhibitor WS6 as a possible treatment approach for psoriasis (*22*). WS6, a tyrosine kinase inhibitor that primarily targets PA2G4, was initially shown to regulate cell proliferation in diabetes (*32, 33*) and more recently, has been reported to exert tumour-suppressive effects in MYCN-driven cancers and acute myeloid leukaemia (AML) (*34, 35*). To investigate the effects of WS6 treatment in psoriasis context, keratinocytes were treated with low-dose WS6 (1 μM), according to the manufacturer's instructions and previous reports (*22*). The expression of psoriasis-associated genes (upregulated in psoriasis, Figure 5A) was measured using real-time PCR. WS6 treatment significantly reduced *PA2G4* expression (61%, p=0.0375) and the proliferation marker *MYC* (44%, p=0.0500); similar trends were observed for *MKI67* (61%, p=0.1608) and *PCNA* (80%, p=0.2917). In addition, WS6 markedly downregulated the expression of *HIF1A*, *S100A7*, and *CXCL8* (51%, 23%, and 67%, respectively; p=0.0827, p=0.0145, and p=0.1930, respectively) (Figure 5B). Consistent with these transcriptomic findings, functional scratch proliferation assays confirmed the severely impaired proliferative capacity of WS6-treated keratinocytes (Figure 5C). WS6-treatment reduced proliferation capacity (p=0.0594), with minimal gap closure of 0.07 ± 0.04 mm^2 to 0.95 ± 0.58 mm^2 in untreated cells, which completely closed the gap (Figure 5D). Overall, WS6 treatment suppressed keratinocyte proliferation and downregulated psoriasis-related inflammatory genes involved in metabolic activity, thereby supporting PA2G4 as a promising therapeutic target for psoriasis.

Discussion

PA2G4 is a multifunctional transcription factor involved in transcriptional regulation, RNA biology, proteostasis, and epigenetic modulation, highlighting its critical function in maintaining cellular homeostasis (*23*). To our knowledge, this study is the first to functionally characterise PA2G4 in psoriasis, identifying it as a regulatory hub of keratinocyte proliferation, differentiation, inflammation, and survival, positioning it as a therapeutic target in psoriasis.

PA2G4 was first identified in 2000 in a yeast two-hybrid screen, and has since attracted considerable interest for its role in tumour progression and poor clinical outcomes (*26, 27, 36-39*). Herein, we demonstrated that PA2G4 was markedly upregulated in psoriasis at both RNA and protein levels, with spatial enrichment in basal keratinocytes, aligning with Skin Atlas proteomics maps, showing predominant epidermal expression (*40*). Moreover, PA2G4 expression positively correlated with disease severity, acanthosis, and neutrophil infiltration, supporting its involvement in psoriasis.

Given that acanthosis reflects dysregulated cell cycle control, a hallmark of tumour biology, we investigated PA2G4´s role in keratinocyte proliferation. PA2G4 has been shown to promote proliferation by interacting with HDAC2 and Sin3A to regulate E2F-mediated transcription (*41-45*). Consistent with this, our bulk RNA-seq of PA2G4 KO keratinocytes demonstrated the downregulation of cell cycle genes (E2F members, AURKB, and BUB1), underscoring the importance of PA2G4 in cell cycle progression. Single-cell RNA-seq data further showed the highest expression in proliferating keratinocytes enriched in the S phase, supporting its involvement in DNA replication and proliferation. Similar effects have been reported in tumour models, where PA2G4 overexpression increased S-phase entry, and knockdown impaired cell cycle progression (*24, 46*).

Beyond cell cycle control, PA2G4 promotes proliferation by stabilising PCNA through MDM2 binding, mediating p53 degradation, and inhibiting MDM2 self-ubiquitination by enhancing Akt activity (*26, 47, 48*). Moreover, the interaction with TIF-1A, it promotes the transcription of PCNA, thereby enhancing rRNA synthesis in T cells (*49*). Accordingly, genetic or pharmacological inhibition of *PA2G4* reduced *MKI67, MYC, HIF1A, TP63* and *PCNA* expression at both the RNA and protein levels. We further determined that the cytokines driving hyperproliferation, including *IL20, VEGFA* and *TGFB1*, were likewise reduced, and *PA2G4* expression correlated positively with proliferation-related genes in psoriasis. The loss of PA2G4 impaired keratinocyte

proliferation in scratch assays and reduced IL-22-induced acanthosis in RHE models. Supporting our data, Li et al. have reported that loss of the post-translation modification lysine 2-hydroxyisobutyrylation (Khib) on PA2G4 enhances its hyperproliferative function in psoriasis (*50*).

PA2G4 modulates adaptive immune responses (*49, 51, 52*), suggesting its involvement in shaping inflammatory responses in psoriasis. We showed that its loss reduced *TGFB1*, a central driver of Th17 differentiation and tissue remodeling, and *LTB*, which contributes to lymphoid tissue organisation (*53, 54*), whereas canonical Th17 cytokines remained unaffected. Instead, interferon-related genes, *IL33,* and *MMP2* were significantly downregulated, indicating modulation beyond the IL-23/IL-17 axis. Although the IL-23/IL-17 axis and tissue-resident memory T cells drive psoriasis chronicity, our findings highlight PA2G4 as a potential link between interferon signaling and chronic inflammation. The role of PA2G4 in the psoriatic microenvironment warrants further investigation, which our group will address in a follow-up study.

Psoriasis pathogenesis is driven by accelerated epidermal turnover and reduced keratinocyte death, processes to which PA2G4 contributes (4). In our PA2G4 KO keratinocytes, GSEA revealed activation of epidermal differentiation and skin development pathways, with upregulation of keratins, adhesion molecules and ECM genes critical for terminal differentiation, and skin homeostasis (*54-58*). Consistent with this, PA2G4 has been linked to cellular differentiation, including pancreatic cancer where its expression correlates with tumour size (*46, 59-62*). Moreover, PA2G4 KO keratinocytes showed increased frequencies of apoptotic and dead cells, alongside upregulation of pro-apoptotic and inflammasome-related genes, including the pyroptosis gene *GSMDA*. Conversely, anti-apoptotic *BCL11A*, ferroptosis inhibitor *GPX4* and complement inhibitor *CFH* were downregulated, indicating survival-promoting function in keratinocytes, consistent with its PKC-δ-Akt-mediated anti-apoptotic signaling (*46, 63-65*).

Given its predominant epidermal expression and multifaceted contribution to psoriatic features, PA2G4 is a promising target for topical interventions. Pharmacological blockade with the tyrosine kinase inhibitor WS6 reduced keratinocyte proliferation and suppressed inflammatory genes linked to metabolic activity and neutrophil recruitment. WS6 targets PA2G4 primarily by interfering with PA2G4-MYC binding (*33*), reducing MYCN stability and tumourigenicity *in vitro* and *in vivo* (*34, 66*). Tumour-suppressive functions have been demonstrated in AML and MYCN-

driven cancers (*39, 40*), where systemic administration decreased proliferation without major toxicity (*22*). While potential off-target effects of WS6 cannot be completely excluded (67), several studies report outcomes consistent with on-target inhibition (22, 34, 68), and PA2G4 KO in our study also confirmed WS6-induced phenotypes. The absence of major off-target readouts (e.g., HDAC activity assays) further strengthens the argument that WS6 is acting through its intended mechanism (22). Recent structure-activity optimisations of WS6 have broadened the therapeutic window and reduced non-specific activities (34), indicating that current limitations are compound specific and do not compromise the translational potential of targeting PA2G4.

In summary, this study implicates the transcription factor PA2G4 as an important factor in psoriasis pathogenesis that could promote proliferation, aberrant differentiation, cell survival, and inflammation. PA2G4 may be an interesting target for psoriasis treatment.

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Figure legends

Figure 1: PA2G4 is abundant in psoriatic skin and positively correlates with disease severity and histological features of psoriasis. A) Normalised *PA2G4* gene counts in non-lesional (NL) and lesional (Pso) skin of psoriasis patients (n=90) from *bulk* RNA sequencing. Padj value was

extracted from DESeq2 analysis. **B)** Cell type-specific expression of *PA2G4* from single cell RNA sequencing analysis of lesional psoriasis skin (n=7). *PA2G4* counts per cell are visualised as UMAP blot. **C, D)** Spatial expression of *PA2G4* in lesional (Pso, n=12) and non-lesional (NL, n=5) psoriatic skin analyzed by spatial transcriptomics. (C) *PA2G4* total counts per spot in HE stainings of a representative patient. (D) Quantification of *PA2G4* counts per spot in the entire cohort, classified by upper, middle and basal epidermis as well as seven different dermis depths. **E, F)** PA2G4 protein expression in non-lesional (NL, n=5) and lesional (Pso, n=5) psoriatic skin analyzed by immunohistochemistry (IHC). (E) IHC staining of PA2G4 of a representative patient with Pso. Scale bar indicates 200 µm. (F) Quantification of PA2G4 positive cells per 10× field using QuPath (version 0.6.0) with the positive cell detection workflow. **G)** Correlation of normalised *PA2G4* gene counts to PASI scores of patients with Pso (n=85). Significance for linear regression was calculated by Pearson correlation. **H)** Association of normalised *PA2G4* gene counts in lesional skin of chronic inflammatory skin diseases (n=274) with psoriasis-associated histological attribute scores for acanthosis, neutrophils, parakeratosis and hyperkeratosis. Attribute scores were determined by histological analysis and collected as categorical ordinary data represented as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Histological score groups were compared using an ordinary one-way analysis of variance.
*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001. *n* indicates the number of independent human donors; NL, non-lesional; Pso, psoriasis; KCs, keratinocytes; VEs, vascular endothelial cells; LEs, lymphatic endothelial cells; Mac, macrophages; MOs, monocytes; DCs, dendritic cells; PASI, Psoriasis Area and Severity Index; r, Pearson correlation score.

Figure 2: PA2G4 regulates keratinocyte proliferation, differentiation and inflammation. PA2G4 was knocked (KO) out via CRISPR-Cas9 in primary human keratinocytes under basal conditions. Subsequently changes on transcriptome level under basal (unstimulated, n=6) or IL-17A+TNF-stimulated (n=6, Fig. S1) conditions were analyzed by *bulk* RNA sequencing. **A)** KO efficiency of PA2G4 on RNA level (top) and protein level analyzed by western blot analysis (bottom) under basal conditions. **B)** Volcano plot displaying differentially regulated genes in PA2G4-KO compared to WT keratinocytes under basal conditions analyzed with DESeq2 (log2FC≥|0.5|, p≤0.05). blue = downregulated, orange = upregulated, grey = non-significant (ns). **C)** Suppressed and activated pathways in PA2G4-KO compared to WT keratinocytes under basal conditions with p≤0.05 analyzed by gene set enrichment analysis. **D–F)** Normalised gene

expression in PA2G4-KO and WT keratinocytes under basal conditions of proliferation-(D), differentiation/cell-adhesion/ECM- (E), and inflammation-related (F) genes regulated by PA2G4. Significant values for differences in normalised gene expression were extracted from DESeq2 analysis.

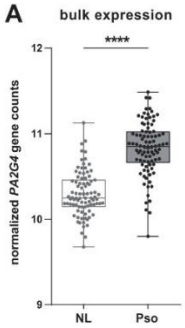
*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001. *n* indicates the number of independent human donors; WT, wild type (pulsed cells); KO, knockout; NES, normalised enrichment score; FC, fold change; ns, not significant.

Figure 3: PA2G4 promotes keratinocyte proliferation capacity and acanthosis induction in reconstructed human epidermis (RHE). PA2G4 was knocked (KO) out via CRISPR-Cas9 in primary human keratinocytes, followed by functional assays to analyze the effects on proliferation capacity. **A, B)** Scratch-proliferation assay (n=7). Confluent keratinocytes under basal conditions were scratched using a pipet tip and proliferation capacity was observed within 24 h. (A) Representative pictures after 0, 16, and 24 h of scratching. Scale bar indicates 1000 µm. The scratched area is outlined with a yellow line and quantified in (B). The Δarea 0–24 h was calculated. **C)** Released IL-20 concentrations from keratinocytes after IL-17A+TNF stimulation for 24 h (n=5), analyzed by enzyme-linked immunosorbent assay. ELISA measurements were performed in technical replicates. **D)** Western blot analysis of HIF-1α in basal keratinocytes (left). Relative protein intensity was quantified to ß-actin (right, n=4). The intensity of the bands was calculated using ImageJ. **E)** Cell cycle phase analysis on scRNAseq data of patients with psoriasis (n=7). Cell cycle phases G1, S, and G2/M are highlighted for each cell in a UMAP plot (left) and correlated to the respective *PA2G4* gene counts/cell in keratinocytes (right). **F, G)** Acanthosis model of reconstructed human epidermis (RHE) (n=3). Keratinocytes were grown in an air-liquid interface to form an RHE. Acanthosis was induced by stimulation with recombinant IL-22 for 72 h or left unstimulated (US) as a control. Representative HE staining's are shown in (E) and epidermal thickness was quantified in (F). Scale bar indicates 50 µm.

Mean KO and WT values were compared using a paired two-sided t-test. *p≤0.05, **p≤0.01. *n* indicates the number of independent human donors; WT, wild-type (pulsed cells); KO, knockout; ELISA, enzyme-linked immunosorbent assay; KCs, keratinocytes; G1, Gap1; S, DNA synthesis; G2/M, Gap2/mitosis; RHE, reconstructed human epidermis; HE, haematoxylin and eosin; US, unstimulated (basal).

Figure 4: PA2G4 suppresses cell death/ apoptosis of keratinocytes. PA2G4 was knocked (KO) out via CRISPR-Cas9 in primary human keratinocytes, followed by functional assays to analyze the effects on cell death/apoptosis. **A)** *Bulk* RNA sequencing (n=5). Normalised gene expression in PA2G4-KO and WT keratinocytes under basal or IL-17A+TNF-stimulated (S) conditions of cell death-related genes regulated by PA2G4. Significant values for differences in normalised gene expression were extracted from DESeq2 analysis. **B, C)** Annexin V staining (n=3). Keratinocytes were stimulated for 72 h with IL-17A+TNF and then stained with Annexin V and propidium iodide (PI). Frequency of apoptotic (Annexin V^+PI^-) and dead (Annexin V^+PI^+) cells was determined by flow cytometry. Representative scatter plots (B) and quantification of all donors (C) are shown. Mean values of KO and WT were compared using a paired two-sided t test.
*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001. *n* indicates the number of independent human donors; WT, wild type (pulsed cells); KO, knockout; S, IL-17A+TNF-stimulated; US, unstimulated (basal); PI, propidium iodide.

Figure 5: Targeting PA2G4 by WS6 inhibitor treatment reduces features of psoriasis. Primary human keratinocytes were treated with 1 µM WS6 inhibitor or left untreated as control (0.1% dimethyl sulfoxide), followed by functional assays to analyze the effects on proliferation and inflammation. **A)** Heatmap of selected upregulated genes in lesional compared to non-lesional skin (log2FC) of patients with psoriasis (n=90) from *bulk* RNA sequencing**. B)** Relative gene expression of psoriasis-related markers 24 h after WS6 treatment of keratinocytes compared to untreated (n=3). **C, D)** Scratch-proliferation assay (n=3). Confluent keratinocytes were scratched using a pipet tip directly after WS6 treatment and proliferation capacity was observed within 24 h. (B) Representative pictures after 0, 16, and 24 h of scratching. Scale bar indicates 1000 µm. The scratched area is outlined with a yellow line and quantified in (C). The Δarea 0–24 h was calculated.
WS6 and untreated mean values were compared using a paired two-sided t-test. *p≤0.05. *n* indicates the number of independent human donors; WS6, PA2G4 inhibitor.



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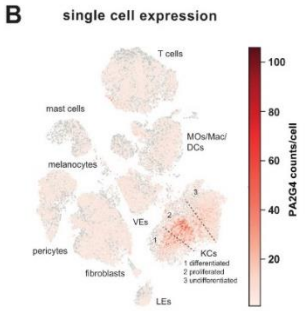

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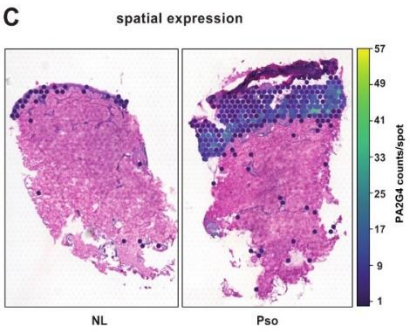

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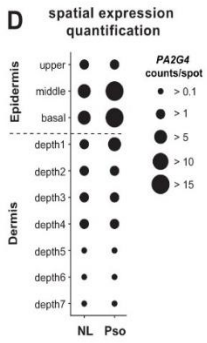

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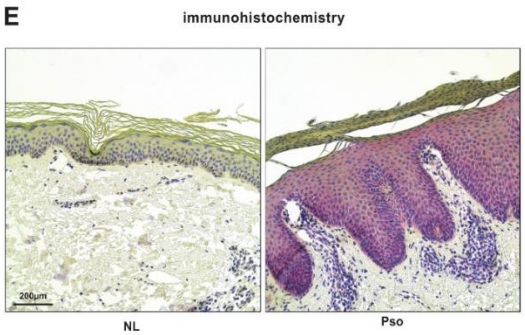

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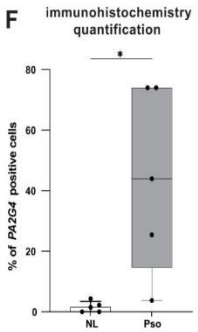

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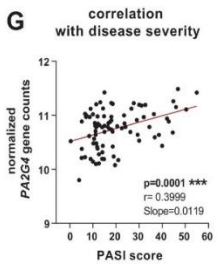

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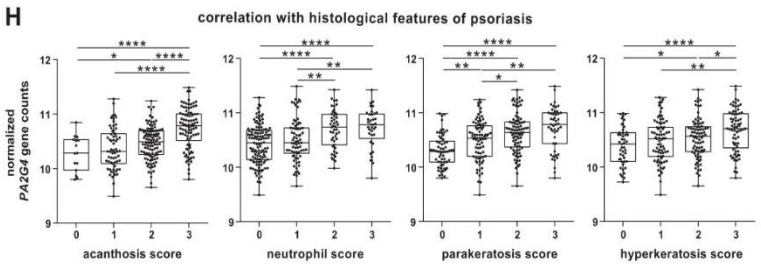

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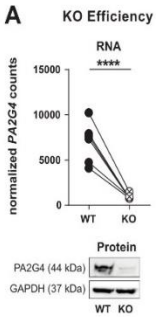

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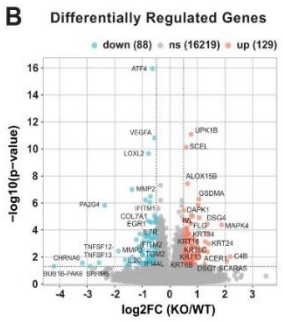

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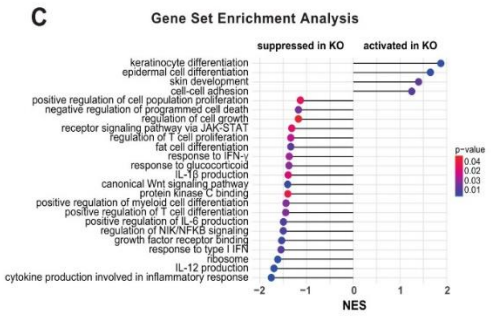

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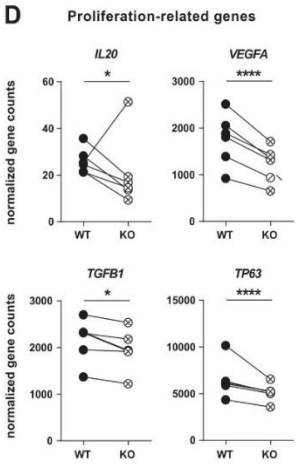

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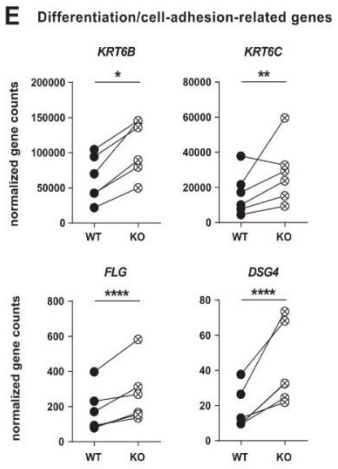

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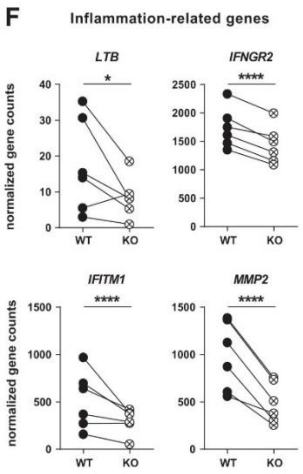

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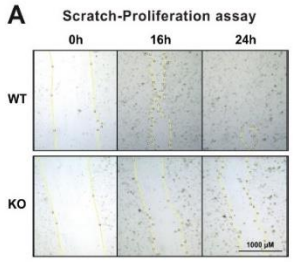

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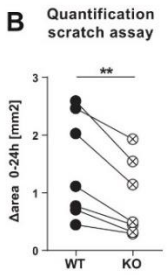

Figure 3B
162x229 mm (x DPI)

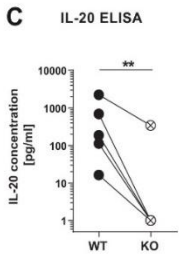

Figure 3C
162x229 mm (x DPI)

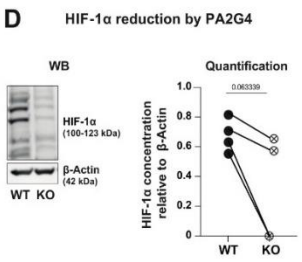

Figure 3D
162x229 mm (x DPI)

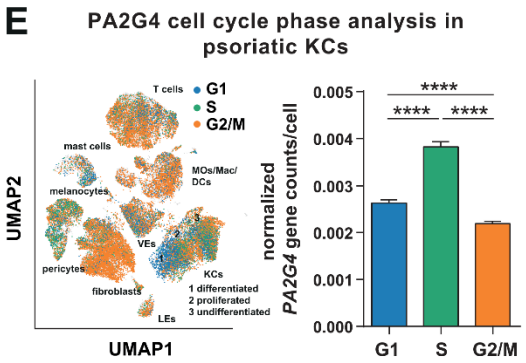

Figure 3E
68x48 mm (x DPI)

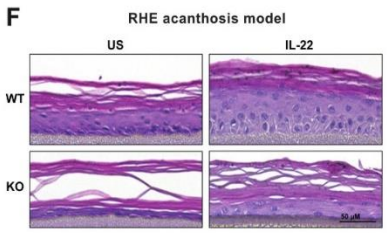

Figure 3F
162x229 mm (x DPI)

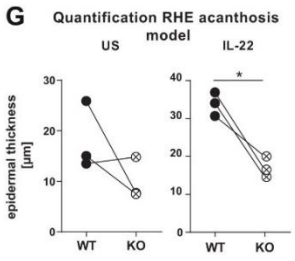

Figure 3G
162x229 mm (x DPI)

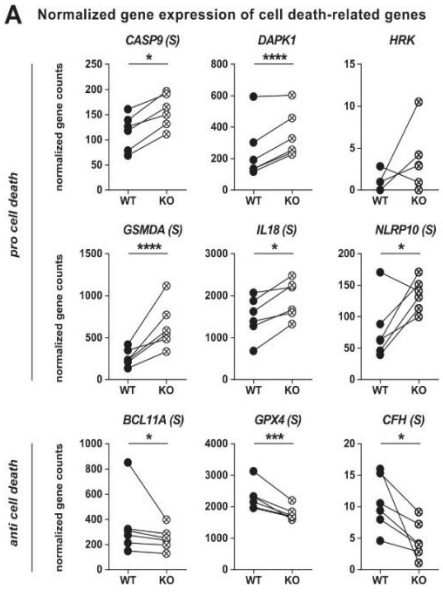

Figure 4A
162x229 mm (x DPI)

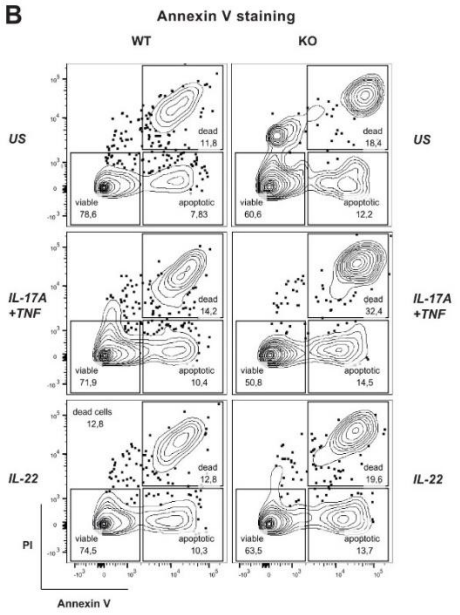

Figure 4B
162x229 mm (x DPI)

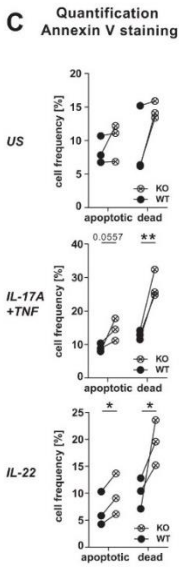

Figure 4C
162x229 mm (x DPI)

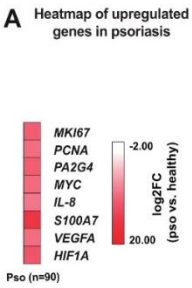

Figure 5A
162x229 mm (x DPI)

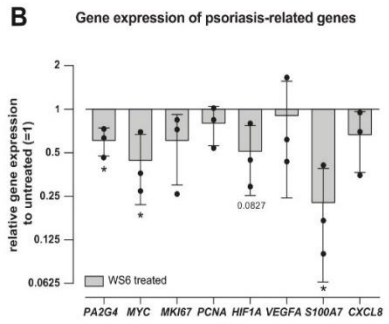

Figure 5B
162x229 mm (x DPI)

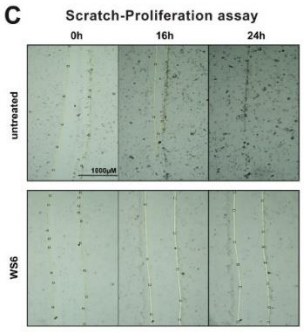

Figure 5C
162x229 mm (x DPI)

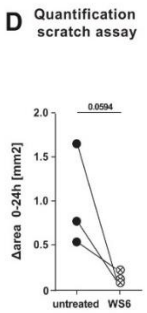

Figure 5Ds
162x229 mm (x DPI)

NO COMPROMISE, JUST CLEARANCE

Bimzelx®▼ (bimekizumab) offers the opportunity for complete, fast, and lasting skin clearance and proven PsA efficacy [1–7]

68.2%
(n=238/349)

of patients with PsO achieved **PASI 100 at Week 16**

(vs 1.2% placebo [n=1/86], p<0.0001)*,**[2]

75.9%
(n=265/349)

of patients with PsO achieved **PASI 75 at Week 4**

(vs 1.2% placebo [n=1/86], p<0.0001)*,**[2]

76.9%
(N=52)†

of patients with PsO achieved **PASI 100 at 5 years**[3]

51.5%
(n=222/431)

and

50.6%
(n=135/267)

of biologic-naïve and TNFi-IR PsA patients achieved **ACR 50 at Week 104/100,** respectively‡[1,4–6]

BIMZELX was well tolerated, the most frequently reported adverse reactions were: upper respiratory tract infections and oral candidiasis. Other common reported adverse reactions include tinea infections, ear infections, herpes simplex infections, oropharyngeal candidiasis, gastroenteritis, folliculitis, headache, rash, dermatitis, eczema, acne, injection site reactions, fatigue, and vulvovaginal mycotic infection (including vulvovaginal candidiasis).[4]

This promotional material has been created and funded by UCB Pharma Ltd and is intended for healthcare professionals in the UK.

BIMZELX is indicated for the treatment of: moderate to severe plaque PsO in adults who are candidates for systemic therapy; active PsA, alone or in combination with methotrexate, in adults who have had an inadequate response, or who have been intolerant, to one or more DMARDs; active nr-axSpA with objective signs of inflammation as indicated by elevated CRP and/or MRI, in adults who have responded inadequately, or are intolerant, to NSAIDs; active AS in adults who have responded inadequately or are intolerant to conventional therapy; and active moderate to severe HS (acne inversa) in adults with an inadequate response to conventional systemic HS therapy.[4]

Prescribing information for United Kingdom click here.
Please refer to the SmPC for further information.

▼This medicine is subject to additional monitoring. This will allow quick identification of new safety information. Adverse events should be reported. Reporting forms and information can be found at www.yellowcard.mhra.gov.uk for the UK. Adverse events should also be reported to UCB Pharma Ltd at UCBCares.UK@UCB.com or 0800 2793177 for UK.

These data are from different clinical trials and cannot be directly compared.

Co-primary endpoints PASI 90 and IGA 0/1 at Week 16 were met.**Secondary endpoints. †N= mNRI, missing data were imputed with mNRI (patients with missing data following treatment discontinuation due to lack of efficacy or a TRAE were counted as non-responders; multiple imputation methodology was used for other missing data). ‡43.9% (n=189/431), and 43.4% (n=116/267) of biologic-naïve and TNFi-IR PsA patients achieved the primary endpoint of ACR 50 at Week 16 in BE OPTIMAL and BE COMPLETE, respectively (vs 10.0% [n=28/281] and 6.8% [n=9/133] placebo, p<0.0001); 54.5% (n=235/431) and 51.7% (n=138/267) maintained it at Week 52 (NRI).[4–6]

ACR 50, ≥50% response in the American College of Rheumatology criteria; **AS**, ankylosing spondylitis; **CRP**, C-reactive protein; **DMARD**, disease-modifying antirheumatic drug; **HS**, hidradenitis suppurativa; **IGA**, Investigator's Global Assessment; **(m)NRI**, (modified) non-responder imputation; **MRI**, magnetic resonance imaging; **nr-axSpA**, non-radiographic axial spondyloarthritis; **NSAID**, non-steroidal anti-inflammatory drug; **PASI 75/90/100**, ≥75/90/100% improvement from baseline in Psoriasis Area and Severity Index; **PsA**, psoriatic arthritis; **PsD**, psoriatic disease; **PsO**, psoriasis; **TNFi-IR**, tumour necrosis factor-α inhibitor – inadequate responder; **TRAE**, treatment-related adverse event.

References: 1. Gordon KB, et al. Lancet. 2021;397(10273):475–486. 2. Blauvelt. 2025. AAD Presentation 62275. 3. Mease PJ, et al. Rheumatol Ther. 2024;11(5):1363–1382. 4. BIMZELX SmPC. 5. Ritchlin CT, et al. Ann Rheum Dis. 2023;82(11):1404–1414. 6. Coates LC, et al. RMD Open. 2024;10(1):e003855. 7. Strober B, et al. AAD 2024;oral presentation.

Inspired by **patients.**
Driven by **science.**

GB-BK-2500315 | July 2025

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(bimekizumab)