**Regular Article**

**Topical Application of BMS-509744, a Selective Inhibitor of Interleukin-2-Inducible T Cell Kinase, Ameliorates Imiquimod-Induced Skin Inflammation in Mice**

Sho Otake,*a,b Tomoko Otsubaki,* Naofumi Uesato, Yoshifumi Ueda, Toshihiko Murayama, and Mikio Hayashi

*a Central Pharmaceutical Research Institute, Japan Tobacco Inc.; 1–1 Murasaki-cho, Takatsuki, Osaka 569–1125, Japan; and b Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University; 1–8–1 Inohana, Chuo-ku, Chiba 260–8675, Japan.

Received October 22, 2020; accepted January 6, 2021; advance publication released online January 20, 2021

Psoriasis is an immune disorder-related inflammatory skin disease. Recent studies have suggested a contribution of T cell activation in the pathogenesis of psoriasis. Interleukin-2 (IL-2)-inducible T cell kinase (ITK) regulates T cell activation, including proliferation, and cytokine production. In this study, we investigated the effect of the topically administered selective ITK inhibitor BMS-509744 on imiquimod (IMQ)-induced psoriasis-like skin inflammation in mice. Topically administered BMS-509744 ameliorated IMQ-induced psoriasis-like skin inflammation as shown by decreased skin lesions, epidermal thickening, and cell infiltration into the dermis. These suppressive effects occurred with lower numbers of cluster of differentiation antigen-3 (CD3)+ and CD8+ T cells and T helper subset 17 (Th17)-related cytokine expression in IMQ-treated skin. IMQ-induced upregulation of proinflammatory cytokine expression was also inhibited by topical application of BMS-509744 in IMQ-treated skin. Our report showed for the first time that topical application of BMS-509744 ameliorated psoriasis-like skin inflammation in mice, which is likely mediated by the inhibition of T cell activation in the skin lesions.

**Key words** interleukin-2-inducible T cell kinase; psoriasis; topical application

**INTRODUCTION**

Psoriasis, which is characterized by red scaly papules or plaques, is an inflammatory skin disease that is commonly found and has a prevalence of up to 2–3% worldwide. Although the pathogenesis of psoriasis is not completely understood, currently available evidence suggests the involvement of the immune system, including interactions between various cells, namely keratinocytes, dendritic cells, and T cells. The identification of activated cluster of differentiation antigen-4 (CD4)+ and CD8+ T cells in lesional skin and blood of psoriasis patients support activated T cells as major players in the development of psoriasis. Additionally, the pathogenesis of psoriasis is known to involve proinflammatory cytokines, namely interleukin (IL)-17, IL-23, and tumor necrosis factor α (TNFα). This cytokine profile indicates that T helper subset 17 (Th17) cells appear to play a critical role in disease pathogenesis. Inhibiting T cell activation and subsequent production of Th17-related cytokines is expected to be an effective treatment for psoriasis.

When imiquimod (IMQ) is applied to the skin of mice, it can induce inflamed scaly lesions, the influx of various immune cells, proinflammatory cytokine secretion, and epidermal hyperplasia. These phenotypes resemble the symptoms of dermatitis found in psoriasis patients, and have been reported to be caused via the IL-23/Th17 axis. Thus, IMQ-induced inflammation in the skin of mice is commonly used as a rodent psoriasis model for analyzing the mechanism of psoriasis and identifying the target pathways for treatment.

IL-2-inducible T cell kinase (ITK), a member of the tyrosine kinase expressed in hepatocellular carcinoma (Tec) family, regulates T cell activation, including proliferation and cytokine production. ITK inhibition is expected to inhibit T cell activation followed by cytokine production. In psoriasis patients, ITK expression is up-regulated in lesions. Treatment with PRN694, an inhibitor of both ITK and resting lymphocyte kinase (RLK), attenuated IMQ-induced psoriasis-like phenotype severity in mice. However, the effects of selective ITK inhibition on psoriasis-like skin inflammation have not been elucidated.

The small molecule, N\{[5-\{[3-(4-acetyl-1-piperazinyl)-carbonyl]-4-methoxy-2-methylphenyl\}thio]-2-thiazolyl\}-4-[[1,2,2-trimethylpropylamino]methyl]benzamide (BMS-509744), binds to ITK and selectively inhibits ITK kinase activity, and BMS-509744 shows high selectivity for other Tec family kinases. BMS-509744 markedly inhibits T cell activation via T cell receptors (TCR), including proliferation and cytokine production. We hypothesized that selective ITK inhibition is a promising treatment for psoriasis. In the present study, we investigated whether BMS-509744, a selective ITK inhibitor, ameliorates psoriasis-like inflammation in the skin of mice.

**MATERIALS AND METHODS**

**Compound** BMS-509744 was purchased from ChemScene (Newark, NJ, U.S.A.). For cell-based experiments, a solution of BMS-509744 was prepared in dimethyl sulfoxide (DMSO) that was then diluted in culture medium immediately before use. For the animal studies, a solution of BMS-509744 was prepared in DMSO and diluted in acetone (10% DMSO/90% methanol) to a concentration of 100 μM.
acetteone). Prednisolone (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) solution was prepared in 0.5% (w/v) aqueous methylcellulose solution.

**Animals** Female BALB/c mice (Japan SLC, Inc., Shizuoka, Japan) were kept in specific-pathogen-free, 23 ± 3 °C, and 55 ± 15% air humidity conditions under a 12-h light/dark cycle with free access to a standard laboratory chow (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and water. All animal experiments in this study complied with the Institutional Animal Care and Use Committee guidelines at Japan Tobacco Inc., and were conducted according to standards published by the National Research Council (Guide for the Care and Use of Laboratory Animals, NIH OACU), and the National Institutes of Health Policy on Human Care and Use of Laboratory Animals.

**IMQ-Induced Psoriasis-Like Skin Inflammation** Five milligrams of cream containing IMQ (5%; Beselna; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was topically administered to the front and back of the left ear of 7–8 week-old female BALB/c mice or for 4 consecutive days, as described previously with modifications. One hour before administering the IMQ, mice received topical 3% BMS-509744 daily on both the front and back of the left or right ear, and oral 5 mg/kg prednisolone daily. Because of the poor bioavailability, BMS-509744 was applied at a concentration close to maximum solubility (i.e., 3% BMS-509744) in this study. On indicated days, left ear thickness was measured with a micrometer (Mitutoyo Corp., Kanagawa, Japan).

**Histology and Immunohistochemistry** After being fixed in 4% paraformaldehyde and soaked in 30% sucrose, ear tissues were embedded in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek Japan Co., Ltd., Tokyo, Japan) and frozen in liquid nitrogen. Next, 7-µm-thick cryosections were obtained using a cryostat (CM1860; Leica Biosystems, Nussloch, Germany) followed by hematoxylin and eosin (H&E) staining. To quantify epidermal thickness and infiltrating cell counts in the dermis, the images were captured using a digital microscope (BIORÉVO BZ-9000; Keyence Corp., Osaka, Japan). Epidermal thickness was determined as the average of the distance from the stratum corneum to the stratum basale using WinROOF software (Mitani Corp., Tokyo, Japan), and the number of infiltrating cells in the dermis was quantified using inForm software (PerkinElmer, Inc./Akoya Biosciences, Inc., Marlborough, MA, U.S.A.). For the immunofluorescence staining, sections of the ear tissue were pre-incubated for 1 h with blocking one histo (Nacalai Tesque, Inc., Kyoto, Japan) at room temperature, followed by 1 h incubation with rabbit anti-mouse CD3 (SP7) monoclonal antibody (Nichirei Biosciences Inc., Tokyo, Japan), diluted 1:100 in 1:20 blocking one histo in phosphate-buffered saline (PBS) followed by 1 h incubation with Alexa Fluor 594 goat anti-rabbit immunoglobulin G (H + L) (Abcam, Cambridge, U.K.), diluted 1:500 in 1:20 blocking one histo in PBS. The counterstained sections were then mounted with 4',6-diamidino-2-phenylindole (DAPI) (Fluoromount-G; SouthernBiotech, Birmingham, AL, U.S.A.); images of immunofluorescence were captured using a confocal laser scanning microscope (FV3000; Olympus Corp., Tokyo Japan) under ×20 magnification; and the number of immunofluorescence-positive cells in the dermis was quantified using the inForm software.

**Quantitative RT-PCR Analysis** Total RNA from the ear tissue was isolated with an RNeasy Lipid Tissue Mini Kit (QIAGEN, Venlo, the Netherlands). Briefly, the collected ear tissue was homogenized with TissueLyser II (QIAGEN) using the buffer included in the kit. After applying the homogenate to the column, the RNA was purified and eluted using sodium acetate (RNAse)-free water. The cDNA was reverse transcribed from the RNA using the High-Capacity cDNA Reverse Transcription Kit in the presence of RNAse Inhibitor (Applied Biosystems, Foster City, CA, U.S.A.). Using the TaqMan Gene Expression Assay with TaqMan Gene Expression Master Mix kit (Applied Biosystems), the mRNA levels of IL-17A (Mm00439618_m1), IL-17F (Mm0521423_m1), IL-22 (Mm01226722_g1), interferon-γ (IFNγ) (Mm01168134_m1), IL-6 (Mm00656925_m1), TNFα (Mm00443258_m1), IL-1β (Mm00434228_m1), IL-6 (Mm00446190_m1), IL-23α (Mm00518984_m1), and IL-12β (Mm01288989_m1) were measured. 18S ribosomal RNA (18S) (Mm03928990_g1) was used to normalize the expression level of each mRNA species.

**Preparation and Culture of Primary Cells from Ear Tissue and Spleen** To prepare the primary cells from ear tissue, mouse ears were amputated at the base. Cartilage was removed from the ears with forceps. The ears were cut into small fragments using scissors and incubated in RPMI-1640 medium (Sigma-Aldrich Co.) with 10% heat-inactivated fetal bovine serum, 0.05 mmol/L of 2-mercaptoethanol, 100 U/mL of penicillin, and 100 µg/mL of streptomycin (complete RPMI) supplemented with 0.1 mg/mL deoxyribonuclease and 1000 U/mL collagenase type 2 (Worthington Biochemical Corp., Freehold, NJ, U.S.A.) for 1 h at 37 °C. Primary cells were obtained by removing the ear tissues from the supernatant using a 40-µm cell strainer. Isolated primary cells were then seeded on a round bottom 96-well plate at 3 × 10^5/well and stimulated using 0.5 µg/mL anti-CD3 antibody (eBioscience, San Diego, CA, U.S.A.) for 18 h.

To prepare the primary cells from spleen, mouse spleens were cut into small fragments using scissors and gently mashed with a syringe plunger through a 40-µm cell strainer. After incubating the sample on ice for 10 min with 1X red blood cell lysis buffer (eBioscience) to lyse red blood cells, the primary cells were re-suspended in complete RPMI. Isolated splenic primary cells were seeded on a round bottom 96-well plate at 1 × 10^6/well and stimulated with 0.03 µg/mL anti-CD3 antibody (eBioscience) for 18 h. The supernatants of the cultured medium were collected, and the level of IL-2 was measured with the Mouse IL-2 Quantikine enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, U.S.A.).

**Statistical Analysis** All results in this study were shown as the mean ± standard deviation (S.D.). Statistical significance was determined with Aspin–Welch’s t-test after the F-test, when two conditions were compared. For comparisons of multiple conditions, statistical significance was analyzed using Dunnett’s test or the Steel test after using Bartlett’s test. A significant difference was set at p values <0.05.

**RESULTS**

**Topical Application of BMS-509744 Reduced Psoriasis-Like Skin Lesions in IMQ-Induced Skin Inflammation in Mice** To examine whether topical application of BMS-509744 ameliorated psoriasis-like skin inflammation, we
administered topical BMS-509744 to the IMQ-treated mice. The schedule of the experiments is summarized in Fig. 1A. After 4 consecutive days of topical application, IMQ treatment induced psoriasis-like lesions with redness, erythema, scaling, and thickening on the left ear of mice (Fig. 1B) that were consistent with previous reports.\textsuperscript{11,13,14} Topical application of BMS-509744 to the IMQ-treated skin markedly reduced psoriasis-like skin lesions, such as redness, erythema, scaling, and thickening on day 5. Daily administration of IMQ on the left ear increased the ear thickness in a time-dependent manner (Fig. 1C), and topical application of BMS-509744 significantly reduced the increase in ear thickness on day 5 in IMQ-treated mice. Oral administration of prednisolone, an anti-inflammatory steroid, also reduced psoriasis-like lesions and the increased ear thickness in the IMQ-treated mice.

Topical Application of BMS-509744 Attenuated Histological Changes in IMQ-Induced Skin Inflammation in Mice The epidermal thickness increased in the IMQ-treated skin of mouse ear on day 5 (Figs. 2A, B), which was attenuated by the topical application of BMS-509744. Excessive cellular infiltration was observed in the dermis in the H&E-stained sections (Figs. 2A, C). The topical application of BMS-509744 reduced the number of infiltrating cells in the dermis, and oral administration of prednisolone reduced the increased epidermal thickness and the number of cells showing infiltration.

Topical Application of BMS-509744 Inhibited Accumulation of CD3\textsuperscript{+} T Cells in IMQ-Treated Skin To assess inhibition of T cell accumulation, we examined whether topical application of BMS-509744 inhibited accumulation of CD3\textsuperscript{+} T cells in the IMQ-treated skin of mice. Immunohistochemical analysis of IMQ-treated mouse skin showed accumulation of CD3\textsuperscript{+} T cells in the dermis (Figs. 3A, B), and topical application of BMS-509744 and oral administration of prednisolone both reduced the number of CD3\textsuperscript{+} T cells.

To determine the effect of BMS-509744 on T cell activation \textit{in vitro}, we examined whether BMS-509744 inhibited anti-CD3 antibody-induced T cell cytokine release in primary cells obtained from mice ear and spleens. The antibody-induced increases in the IL-2 production in primary cells from both ear
Fig. 2. Topical Application of BMS-509744 Attenuated Histological Changes Induced by IMQ Treatment

(A) Typical image of the hematoxylin and eosin (H&E)-stained left ear sections. Ear samples were obtained on day 5 after treatment. Scale bar: 50 µm. (B) Quantified epidermal thickness. Data are shown as means ± S.D. for 5 mice per group. ‡: \( p < 0.01 \) vs. Normal group (Aspin–Welch's t-test), ##: \( p < 0.01 \) vs. Vehicle group (Dunnett’s test). (C) The number of infiltrating cells into the dermis was counted using inForm software. Data are shown as means ± S.D.s of 5 mice per group. ‡: \( p < 0.01 \) vs. Normal group (Aspin–Welch’s t-test); #, ##: \( p < 0.05, 0.01 \), respectively, vs. Vehicle group (Dunnett’s test). IMQ: imiquimod; S.D.: standard deviation.

Fig. 3. Topical Application of BMS-509744 Inhibited Cluster of Differentiation Antigen 3+ (CD3+) T Cell Accumulation in IMQ-Treated Skin

Sections of the left ear were prepared and stained with anti-CD3+ antibody and DAPI. Typical images and quantitative analyses of CD3+ -positive cells in the dermis are shown in A and B, respectively. Scale bar: 50 µm. Data are shown as means ± S.D.s of 5 mice per group. ‡: \( p < 0.01 \) vs. Normal group (Aspin–Welch’s t-test), ##: \( p < 0.01 \) vs. Vehicle group (Dunnett’s test). DAPI: 4'6-diamidino-2-phenylindole; S.D.: standard deviation.
tissue and spleens were concentration-dependently inhibited by BMS-509744 (Suppl. Table 1).

Effect of Topical Application of BMS-509744 on Th17-Related Cytokine and Proinflammatory Cytokine mRNA Expression in IMQ-Treated Skin

We determined the effect of the topical application of BMS-509744 on the Th17-related cytokine mRNA levels in the skin in which inflammation was induced by IMQ. IL-17A, IL-17F, and IL-22 mRNA levels increased following IMQ treatment, while the level of IFNγ mRNA was not affected (Fig. 4A). Furthermore, IMQ-induced upregulated levels of IL-17A, IL-17F, and IL-22 were significantly inhibited by the topical application of BMS-509744. The mRNA levels of S100a8/9, which are antimicrobial peptides induced in keratinocytes after IL-17 stimulation,20 were increased in IMQ-treated skin, and topical application of BMS-509744 inhibited the increase of S100a8/9 (Fig. 4B). The proinflammatory cytokine (TNFα, IL-1β, IL-6, IL-23α (p19 subunit) and IL-12β (p40 subunit)) mRNA levels were also increased by IMQ treatment. IMQ-induced upregulation of these proinflammatory cytokines was inhibited by BMS-509744 (Fig. 4C). Oral administration of prednisolone inhibited the levels of IL-1β and IL-6 mRNA, but did not affect the levels of IL-17A, IL-17F, IL-22, TNFα, IL-23α, and IL-12β in IMQ-treated skin.

Topical Application of BMS-509744 to the Skin of the IMQ-Untreated Right Ear Did Not Reduce the Increase in IMQ-Treated Left Ear Thickness in IMQ-Induced Skin Inflammation in Mice

Daily application of IMQ to the left ear significantly increased the thickness of the left ear by day 5 (Fig. 5), and topical application of BMS-509744 to the left ear skin significantly reduced the increase in the ear thickness by day 5 in the IMQ-treated mice. In contrast, topical application of BMS-509744 to the right ear skin, which was not treated with IMQ, showed no such suppressive effect in mice treated with IMQ in the left ear.

DISCUSSION

The present study investigated the effect of topical application of the selective ITK inhibitor BMS-509744 on IMQ-induced psoriasis-like skin inflammation. IMQ-induced inflammation in the skin of mice has been reported to closely resemble human psoriasis in terms of histological features and phenotypes.11,13 We demonstrated that topical application of BMS-509744 ameliorated IMQ-induced psoriasis-like inflammation of skin, namely the skin lesions, and increased the epidermal thickness and accumulation of infiltrating cells in the dermis (Figs. 1, 2). These suppressive effects were confirmed by the reduction in the number of CD3+ T cells and the mRNA levels of Th17-related cytokines (IL-17A, IL-17F, and IL-22) (Fig. 3). Oral administration of prednisolone inhibited the levels of IL-1β and IL-6 mRNA, but did not affect the levels of IL-17A, IL-17F, IL-22, TNFα, IL-23α, and IL-12β in IMQ-treated skin.

We also demonstrated that topical application of BMS-509744 on the right ear skin, which was not treated with IMQ, showed no such suppressive effect in mice treated with IMQ in the left ear (Fig. 5). These results suggest that topically administered BMS-509744 ameliorated the IMQ-induced psoriasis-like inflammation of the skin and exerted a therapeutic effect in the skin lesions.

Previous studies have shown that CD3+ T cells accumulated...
in the skin lesions of IMQ-treated mice and that depletion of CD3+ T cells attenuated the IMQ-induced skin inflammation.11,13) Similar to these studies, we demonstrated that the IMQ-treated mouse skin also showed CD3+ T cell accumulation (Fig. 3). Topical application of BMS-509744 reduced the number of CD3+ T cells in the skin lesions of IMQ-treated mice.

Several reports supported activated T cells as major players in the pathogenesis of psoriasis.4–7) Additionally, the pathogenesis of psoriasis is known to involve the proinflammatory cytokines IL-17, IL-23, and TNFα, indicating the potential involvement of Th17 cells in disease pathogenesis.8,9) There are some differences in responses to IMQ treatment depending on the mice strain; thus, we used female BALB/c mice exhibit lower survival rate when challenged with intracellular pathogens compared to wild type mice.22) Although there are concerns about the tolerability of complete ITK inhibition by ITK inhibitor, avoiding systemic exposure with topical application is expected to reduce the risk.

In summary, this study showed that topical application of BMS-509744 (Figs. 1, 2), treatment with prednisolone did not affect the mRNA levels of Th17-related cytokines and some proinflammatory cytokines in the IMQ-treated skin lesions (Fig. 4). These observations may be explained by the possibility that suppression of systemic immune activation would contribute to the suppressive effects of prednisolone.

The Tec family tyrosine kinases, TEC, Bruton’s tyrosine kinase (BTK), ITK, RLK, and bone marrow-expressed kinase (BMX) serve as key mediators of antigen receptor signaling in lymphocytes.15,21) ITK, RLK, and TEC, which are three main Tec family members, mediate TCR-induced T cell activation.15,16) A recent report showed that intraperitoneal injection of PRN694, an inhibitor of both ITK and RLK, improved IMQ-induced psoriasis-like inflammation of skin.13) However, the effect of selective inhibition of ITK on psoriasis-like skin inflammation has not been elucidated. BMS-509744 showed at least a 30-fold selectivity for several protein kinases and >200-fold selectivity for other Tec family kinases.18) BMS-509744 appears to be a selective inhibitor of ITK without affecting other kinases, including the Tec family. Our results suggest that ITK is a major player in the IMQ-induced psoriasis-like inflammation of skin.

ITK expression is upregulated in lesional skin from patients with allergic contact dermatitis, atopic dermatitis and psoriasis.13,16) The effect of topical application of ITK inhibitor on other types of skin inflammation, including allergic contact dermatitis and atopic dermatitis, should be determined in the future. ITK deficient mice have reduced T cell activation and show lower survival rate when challenged with intracellular pathogens compared to wild type mice.22) Although there are concerns about the tolerability of complete ITK inhibition by ITK inhibitor, avoiding systemic exposure with topical application is expected to reduce the risk.

**Acknowledgments** We thank Koji Inagaki for his practical advice.

**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**REFERENCES**

1. Armstrong AW, Read C. Pathophysiology, clinical presentation, and treatment of psoriasis: a review. *JAMA*, 323, 1945–1960 (2020).
2. Albanesi C, Madonna S, Gisondi P, Girolomoni G. The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. *Front. Immunol.*, 9, 1549 (2018).
3. Polese B, Zhang H, Thuraiirajah B, King IK. Innate lymphocytes in psoriasis. *Front. Immunol.*, 11, 242 (2020).
4. Bos JD, Hagaenaars C, Das PK, Krieg SR, Voorn WJ, Kapsenberg ML. Predominance of “memory” T cells (CD4+, CDw29+) over “naïve” T cells (CD4+, CD45R+) in both normal and diseased human skin. *Arch. Dermatol. Res.*, 281, 24–30 (1989).
5) De Panfilis G, Manara GC, Ferrari C, Torresani C, Zucchi A, Devoto RM. Further characterization of the “incipient lesion of chronic stationary type psoriasis vulgaris in exacerbation”. The CD4-positive lymphocytes are the prominent cell population infiltrating the dermis. Acta Derm. Venereol. Suppl. (Stockh.), 146, 26–30 (1989).

6) Ferenci K, Burack L, Pope M, Krueger JG, Austin LM. CD69, HLA-DR and the IL-2R identify persistently activated T cells in psoriasis vulgaris lesional skin: blood and skin comparisons by flow cytometry. J. Autoimmun., 14, 63–78 (2000).

7) Vollmer S, Menssen A, Prinz JC. Dominant lesional T cell receptor rearrangements persist in relapsing psoriasis but are absent from nonlesional skin: evidence for a stable antigen-specific pathogenic T cell response in psoriasis vulgaris. J. Invest. Dermatol., 117, 1296–1301 (2001).

8) Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. J. Invest. Dermatol., 129, 1339–1350 (2009).

9) Hawkes JE, Yan BY, Chan TC, Krueger JG. Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. J. Immunol., 201, 1605–1613 (2018).

10) AbuHilal M, Walsh S, Shear N. The role of IL-17 in the pathogenesis of psoriasis and update on IL-17 inhibitors for the treatment of plaque psoriasis. J. Cutan. Med. Surg., 20, 509–516 (2016).

11) van der Fits L, Mourits S, Voerman JSA, Kant M, Boon L, Laman JD, Cornelissen F, Mus AM, Florencia E, Prens EP, Lubberts E. Imiquimod-induced psoriasis-like inflammation in mice is mediated via the IL-23/IL-17 axis. J. Immunol., 182, 5836–5845 (2009).

12) Rahmani F, Rezaei N. Therapeutic targeting of toll-like receptors: a review of Toll-like receptors and their signaling pathways in psoriasis. Expert Rev. Clin. Immunol., 12, 1289–1298 (2016).

13) Fuhriman JM, Winge MCG, Haberstock-Debic H, Funk JO, Bradshaw JM, Marinkovich MP. ITK and RLK inhibitor PRN694 improves skin disease in two mouse models of psoriasis. J. Invest. Dermatol., 138, 864–871 (2018).

14) Kasuha N, Kito A, Dainichi T, Honda T, Otsuka A, Egawa G, Nakajima S, Miyachi Y, Kabashima K. Inhibition of IL-17-committed T cells in a murine psoriasis model by a vitamin D analogue. J. Allergy Clin. Immunol., 141, 972–981e10 (2018).

15) Andreadi AH, Schwartzberg PL, Joseph RE, Berg LJ. T cell signaling regulated by the Tec family kinase, Itk. Cold Spring Harb. Perspect. Biol., 2, a00287 (2010).

16) Kapnick SM, Stinchcombe JC, Griffiths GM, Schwartzberg PL. Inducible T cell kinase regulates the acquisition of cytolytic capacity and degranulation in CD8+ CTLs. J. Immunol., 198, 2699–2711 (2017).

17) von Bonin A, Rausch A, Mengel A, Hitchcock M, Krüger M, von Ahsen O, Merz C, Röse L, Stock C, Martin SF, Leder G, Dücke WD, Asadullah K, Zügel U. Inhibition of the IL-2-inducible tyrosine kinase (Itk) activity: a new concept for the therapy of inflammatory skin diseases. Exp. Dermatol., 20, 41–47 (2011).

18) Lin TA, McIntyre KW, Das J, et al. Selective Itk inhibitors block T-cell activation and murine lung inflammation. Biochemistry, 43, J1056–J1062 (2004).

19) Mori H, Arta K, Yamaguchi T, Hirai M, Kurebayashi Y. Effects of topical application of betamethasone on imiquimod-induced psoriasis-like skin inflammation in mice. Kobe J. Med. Sci., 62, E79–E88 (2016).

20) Liang SC, Tan X-Y, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J. Exp. Med., 203, 2271–2279 (2006).

21) Vargas L, Hamasy A, Nore BF, Smith CJ. Inhibitors of BTK and ITK: state of the new drugs for cancer, autoimmunity and inflammatory diseases. Scand. J. Immunol., 78, 130–139 (2013).

22) Schaeffer EM, Debnath J, Yap G, McVicar D, Liao XC, Littman DR, Schaeffer EM, Debnath J, Yap G, McVicar D, Liao XC, Littman DR. Requirement for Tec kinases Rlk and Itk in T cell receptor signaling and immunity. Science, 284, 638–641 (1999).