Title: **Targeting the IL-36 Receptor with Spesolimab Mitigates Residual Inflammation and Prevents Generalized Pustular Psoriasis Flares**

Authors: James G. Krueger[1], Mrinal K. Sarkar[2], Mark G. Lebwohl[3], Akimichi Morita[4], Kenneth Gordon[5], Rachael Bogle[2], Christopher Cole[2], Anthony Coon[2], Richard G. Langley[6], Richard B. Warren[7], Arash Mostaghimi[8], Bruce Strober[9], A. David Burden[10], Min Zheng[11], Aaron R. Mangold[12], Milan J. Anadkat[13], Jonathan N. Barker[14], Joseph F. Merola[15], Lam C. Tsoi[2], Ming Tang[16], Kolja Becker[17], Denis Delic[17], Christian Thoma[17], Johann E. Gudjonsson[2,18]

1. Laboratory for Investigative Dermatology, Rockefeller University, New York City, NY, USA
2. University of Michigan, Ann Arbor, MI, USA
3. Icahn School of Medicine at Mount Sinai, New York City, NY, USA
4. Nagoya City University, Nagoya, Japan
5. Medical College of Wisconsin, Milwaukee, WI, USA
6. Dalhousie University, Halifax, NS, Canada
7. Dermatology Centre, Northern Care Alliance NHS Foundation Trust & Division of Musculoskeletal and Dermatological Sciences, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK
8. Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
9. Yale University and Central Connecticut Dermatology Research, Cromwell, CT, USA
10. School of Infection and Immunity, University of Glasgow, Glasgow, UK
11. School of Medicine, Second Affiliated Hospital, Zhejiang University, Hangzhou, Zhejiang, China
12. Mayo Clinic, Scottsdale, AZ, USA
13. Washington University School of Medicine, St. Louis, MO, USA
14. St. John's Institute of Dermatology, King's College London, London, UK
15. UT Southwestern Medical Center, Dallas, TX, USA

16. Boehringer Ingelheim (China) Investment Co. Ltd, Shanghai, China

17. Boehringer Ingelheim International GmbH, Biberach, Germany

18. Taubman Medical Research Institute, Ann Arbor, MI, USA

Corresponding Author:

Name: Johann E. Gudjonsson, Address: 1500 E Medical Center Dr, Ann Arbor, MI 48109, USA. Email address: johanng@med.umich.edu. Telephone: +1 734-936-4054

Generalized pustular psoriasis (GPP) is a rare, chronic, neutrophilic inflammatory disease (1,2). GPP is associated with mutations in genes related to interleukin (IL)-36 signaling including *IL36RN* (3*)*, *CARD14* (4)*, and AP1S3* (5), which contribute to unregulated activation of the IL-36 inflammatory axis in the epidermis, resulting in a neutrophil-rich inflammatory infiltrate and pustule formation (1-5).

Spesolimab, an anti-IL-36 receptor (IL-36R) monoclonal antibody, is the first targeted and approved therapy for the comprehensive treatment of GPP (1,2). Spesolimab has been shown to reduce the pathogenic molecular profile associated with GPP flares, showing robust suppression of IL-36 pathway-related signatures and neutrophil mediators as early as week 1 (6). Here, we analyzed the effect of spesolimab on the molecular profile of skin in the absence of flares.

Of 123 patients enrolled in the EFFISAYIL® 2 clinical trial (2), 18 patients consented to having (optional) biopsies and participated in a biomarker sub-study; seven provided data for all time points throughout the study period. Figure 1 shows biomarker and gene expression data (n=7). Of these, 5 patients did not experience a flare during the 48-week study period; 4 patients received spesolimab 300 mg subcutaneous (s.c.) every 4 weeks after a 600 mg loading dose, and 1 patient received 150 mg spesolimab s.c. every 12 weeks after a 300 mg loading dose. Two patients, both of whom received placebo, experienced a flare. At randomization, all patients had a Generalized Pustular Psoriasis Physician Global Assessment (GPPGA) score of 1 (almost clear skin), with some patients also having low-grade inflammation visible on clinical assessment (Supplementary Figure 1).

Using non-supervised clustering of the entire patient cohort from EFFISAYIL® 2, along with associated disease parameters, all patients in the biomarker sub-study were evenly distributed amongst the larger cohort, suggesting that they are representative of the full study cohort (Supplementary Figure 2). Supplementary Figure 3 shows the overall effect of spesolimab on gene expression and distinct inflammatory pathways during the 48-week treatment period.

To highlight the variability of gene expression, clinical features, and immunohistochemistry, we visualized this to a single-patient view of the 7 patients (Supplementary Figure 1). Six patients had increased expression of a broad range of pro-inflammatory genes at baseline, some of which have been previously associated with pustular psoriasis and defined as IL-36 response genes (2,6); this indicates that ongoing subclinical inflammation can be present between flares in the majority of patients. Of note, these 6 patients received systemic non-biologic treatment for GPP before randomization.

GPP flares were accompanied by increased expression of various pro-inflammatory genes, which normalized after treatment with intravenous (i.v.) spesolimab within 4 weeks and was maintained at week 48. Of note, most (3 of 4) patients receiving the higher dose of spesolimab (spesolimab 300 mg s.c. every 4 weeks after a 600 mg loading dose) had near-complete normalization of their pro-inflammatory gene expression at week 48 (Figure 1) and did not experience flares. No significant impact of *IL36RN*, *CARD14*, or *AP1S3* mutation status on spesolimab efficacy and transcriptomic changes was observed.

CRISPR/Cas9 knock-outs for *IL36RN* and *AP1S3* in keratinocytes (Supplementary Figure 4) revealed that keratinocytes show heightened sensitivity to low-intensity pro-inflammatory stimuli with either IL-17A or IL-36G. Thus, these mutations may predispose these patients to a more rapid and amplified inflammatory response in the skin.

Importantly, our data demonstrate that patients with a history of GPP flares have subclinical residual inflammatory activity in their skin during periods without flare. We further demonstrate that this inflammatory activity is effectively suppressed with spesolimab s.c. maintenance therapy, decreasing the risk of spontaneous flares, which is consistent with the findings from the EFFISAYIL® 2 trial (2).

This study has several limitations. One is the small number of patients, which represent only a small fraction of the overall EFFISAYIL® 2 trial. However, the 7 patients were shown to be representative of the overall trial. Another limitation is that only few patients had mutations in

genes such as *IL36RN*, *CARD14* and *AP1S3*. However, the data demonstrate the effectiveness of anti-IL-36R inhibition with spesolimab regardless of *IL36RN* mutation status.

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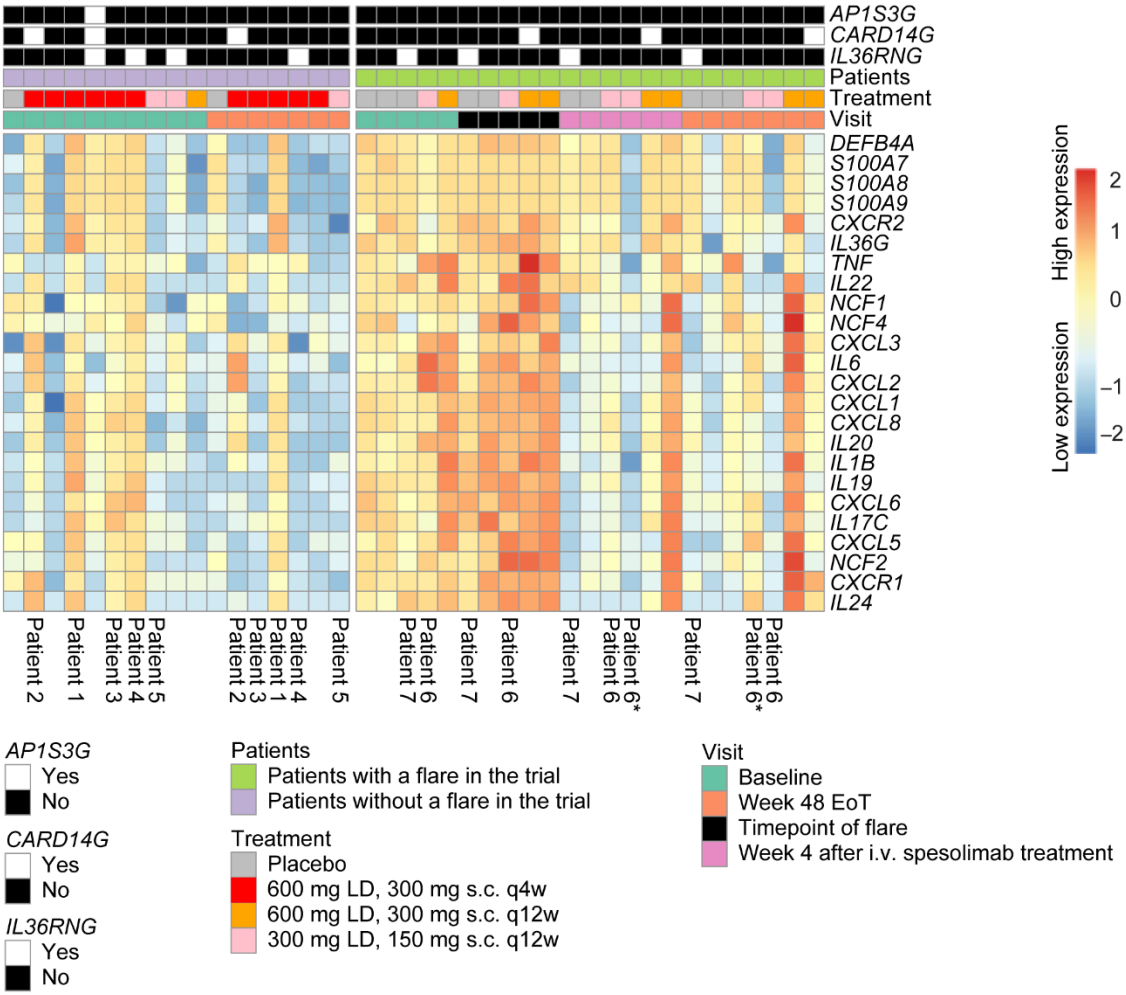

Figure 1. Changes in pro-inflammatory gene expression during the study period.

*Alternative biopsies.

This graph shows the gene expression of pro-inflammatory mediators in all 18 patients who participated in the biomarker study at all time points, including flare and post-flare treatment and at the end of the study (week 48). Patients who had a complete series of biopsies throughout the studies are shown (Patient 1–7). One patient had more than one biopsy (Patient 6).

DEFB4A, defensin beta 4A; EoT, end of trial; IL, interleukin; i.v., intravenous; LD, loading dose; NCF, neutrophil cytosolic factor; q12w, every 12 weeks; q4w, every 4 weeks; s.c., subcutaneous; TNF, tumor necrosis factor.