(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 September 2022 (15.09.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/192531 A2

(51) International Patent Classification:
C12Q 1/6883 (2018.01) *G01N 33/53* (2006.01)

(21) International Application Number:
PCT/US2022/019742

(22) International Filing Date:
10 March 2022 (10.03.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/160,135 12 March 2021 (12.03.2021) US
63/255,148 13 October 2021 (13.10.2021) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2022/192531 A2

(54) **Title:** BIOMARKERS ASSOCIATED WITH ANTI-IL-36R ANTIBODY TREATMENT IN GENERALIZED PUSTULAR PSORIASIS

(57) **Abstract:** This invention generally relates to biomarkers associated with anti-IL-36R antibody treatment in generalized pustular psoriasis (GPP). The invention also relates to methods of using the biomarkers disclosed.

BIOMARKERS ASSOCIATED WITH ANTI-IL-36R ANTIBODY TREATMENT IN GENERALIZED PUSTULAR PSORIASIS

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 1, 2022 is named 09-0718-WO-1-2022-3-10-SL.txt and is 146,122 bytes in size.

Technical Field of the Invention

This invention generally relates to biomarkers associated with anti-IL-36R antibody treatment in generalized pustular psoriasis (GPP). More specifically, the invention relates to biomarkers associated with spesolimab treatment in GPP. The invention also relates to methods of using the biomarkers disclosed herein.

Background of the Invention

Pustular psoriasis comprises a spectrum of severe chronic or relapsing inflammatory skin conditions with recurrent or persistent eruptions of painful neutrophilic sterile pustules. Patients with pustular psoriasis can present with different clinical phenotypes and a predominant recognised subtype is generalized pustular psoriasis (GPP). GPP is a rare disease characterised by episodes of widespread eruption of macroscopically visible pustules and may be accompanied by systemic inflammation. It is associated with significant morbidity and can be life-threatening without appropriate treatment.

Several studies in GPP have reported overexpression of interleukin-36 (IL-36) in skin lesions and loss-of-function mutations in the gene encoding for the IL-36 receptor (IL-36R) antagonist (IL36RN), as well as mutations in other genes with functional connection with the IL-36 pathway, among others, namely CARD14, APS1S3 and SERPINA3. IL36RN mutations alter the normal function of the IL-36 receptor antagonist, leading to reduced inhibition of the IL-36R pathway due to an imbalanced competitive binding against IL-36α,

IL-36β and IL-36γ. This in turn leads to induction of the downstream inflammatory cascade and recruitment of neutrophils, in addition to other innate and adaptive immune cells. IL36RN mutations have been reported in 10–82% of patients with GPP, and an earlier age of onset has been described in patients with GPP who have defective IL36RN mutations. Furthermore, a study investigating the impact of different IL36RN mutations on IL-36Ra protein expression and regulatory function showed that null mutants tend to be preferentially associated with GPP, whereas hypomorphic mutations were found in GPP.

The key role of the IL-36 axis in GPP is further supported by studies demonstrating significant contributions for IL-17A, IL-23, tumour necrosis factor (TNF), IL-1, IL-36 and type 1 interferon in the pathogenesis of GPP lesions. Among the upregulated genes, predominance of IL-1- and IL-36-related transcripts was reported. In addition, strong expression of IL-36α and IL-36γ in keratinocytes proximal to neutrophilic pustules have also been detected in GPP lesions.

Overall, there is sufficient evidence to suggest that the blockade of IL-36R signalling is an appealing targeted therapeutic approach for patients with GPP. Previously, blockade of IL-36R signalling with a single intravenous dose of 10 mg/kg spesolimab, a novel humanised anti-IL-36R monoclonal antibody, in a proof-of-concept Phase I, multicentre, single-arm, open-label study (ClinicalTrials.gov identifier: NCT02978690) showed rapid skin and pustular clearance in patients presenting with an acute GPP flare. However, the inflammatory circuits and cellular interactions driving the pathogenesis of GPP are not yet fully elucidated, and the mechanisms by which these are disrupted by IL-36R blockade are unknown.

There is therefore a need for improved means to follow the efficacy of treatment options against GPP, identify patients that will most benefit from these treatments in GPP, and to determine and adjust the dosages of therapies for patients as may be needed.

Summary of the Invention

The present invention addresses the above needs and provides biomarkers associated with anti-IL-36R antibody treatment in generalized pustular psoriasis (GPP).

In one embodiment, the present invention provides a method for detecting the presence or absence of a beneficial response in a patient after administration of an anti-interleukin 36 receptor antibody (anti-IL-36R antibody) (e.g., spesolimab), comprising: a) obtaining a biological sample from the patient; b) measuring in said sample the level of expression of one or more biomarkers; c) comparing the level to control value of the level of the biomarkers; and d) determining whether or not the difference in levels between the sample and the control reflects a beneficial response in the patient, wherein the one or more biomarkers comprise genes/ proteins associated with the pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*). In a related embodiment, the one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2.

In one embodiment, the presence or absence of a beneficial response in the patient is detected prior to and after administration of an anti-IL36R antibody or spesolimab.

In one embodiment, the control value of the value of a patient treated with a placebo. In one embodiment, the control value of the value of a patient treated with a placebo and the difference is the difference between the sample from a patient treated with an anti-IL36R antibody or spesolimab and the placebo.

In one embodiment, the level of the gene or the protein of said one or more biomarker is measured. In one embodiment, the patient suffers from GPP.

In one embodiment, the control value is calculated using samples from subjects that do not suffer from GPP. In one embodiment, the control value, for example the placebo value, is determined using samples from known GPP patients, for example from placebo-treated GPP patients. In one embodiment, the control value is determined using at least one previous sample taken from the patient.

In one embodiment, the method further comprises continuing the administration of the anti-IL-36R antibody to the patient if the difference in levels between the sample and the control reflects a beneficial response in the patient. In one embodiment, the method further comprises continuing the administration of the anti-IL-36R antibody to the patient if the difference in levels from a patient treated with the anitbody versus the placebo reflects a beneficial response in the patient.

In a further embodiment, the present invention provides a method of determining whether a potential therapeutic agent is efficacious in the treatment of GPP comprising: a) obtaining a first biological sample from a GPP patient prior to being treated with the potential therapeutic agent; b) treating the GPP patient with the potential therapeutic agent; c) obtaining a second biological sample from the GPP patient after being treated with the potential therapeutic agent; d) measuring in said first and second sample the levels of expression of one or more biomarkers; and e) comparing the biomarker levels in the second sample to the levels in the first sample, wherein changes in biomarker levels in the second sample than in the first sample indicate that the potential therapeutic agent is efficacious, and further wherein the one or more biomarkers comprise genes associated with pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*). In a related embodiment, the one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2.

In one embodiment, said step e) comprises comparing the biomarker levels in the second sample to the levels in the first sample, wherein changes in biomarker levels in the second sample than in the first sample and correlation with improvement in a clinical efficacy measure, e.g., GPPASI scores in case of GPP indicates the potential therapeutic agent is efficacious.

In one embodiment, the method further comprises continuing the treatment of the patient if biomarker levels in the second sample change (e.g., are higher or lower) as compared to the first sample.

In a further embodiment, the present invention provides a method of treating GPP in a subject comprising: determining whether to initiate treatment of the subject, modify the treatment dose, modify the dosing interval, or discontinue treatment, based on the method of any of the preceding claims.

In a further embodiment, the present invention provides a method of monitoring patient response to a GPP treatment comprising:

a) obtaining a first biological sample from the patient;

b) measuring the level of one or more biomarkers in said first biological sample, wherein said one or more biomarkers comprise genes/ proteins associated with pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*) or wherein said one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2;

c) administering a treatment compound to the patient;

d) obtaining a second biological sample from the patient;

e) measuring the level of said one or more biomarkers in said second biological sample; and

f) comparing the levels of the one or more biomarkers obtained from first and second biological samples;

wherein a change in the level of the one or more biomarkers in the second biological sample indicates an effective response. In one aspect, a change in the level of the one or more biomarkers in the second biological sample and correlation with improvement in a clinical efficacy measure, e.g., GPPASI scores in case of GPP indicates an effective response.

In a further embodiment, the present invention provides a method for monitoring patient compliance with a drug treatment protocol for GPP comprising:

a) obtaining a biological sample from said patient;

b) measuring the level of one or more biomarkers, wherein the one or more biomarkers comprise genes/ proteins associated with pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*) or wherein said one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2; and

c) determining if the level is changed in the patient sample compared to the level in a control untreated sample;

wherein a change in the level indicates patient compliance with said drug treatment protocol.

In one embodiment, in any one of the methods above, the level of the one or more biomarkers in the second biological sample is changedby at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more as compared to the level in the first biological sample.

In one embodiment, in any one of the methods above, the biological sample is a skin biopsy, blood, plasma or serum sample. In one embodiment, in any one of the methods above, the anti-IL-36R antibody is spesolimab. In one embodiment, in any one of the methods above, the levels of biomarkers are determined by RNA sequencing or ELISA or another protein assay. In one embodiment, the biomarkers are the differentially expressed genes in skin.

In one embodiment, the present invention further provides a method of selecting a patient, for example using a method disclosed herein. In one embodiment, the present invention

further provides a method of enriching a patient population for patients expected to have a beneficial response after treatment with an anti-IL-36R antibody, for example using a method of the present invention. In one embodiment, the present invention further provides a method of enriching a patient population for patients expected to have a beneficial response prior to or early after treatment with an anti-IL-36R antibody, for example using a method of the present invention.

In one embodiment, in any one of the methods above, the anti-IL-36R antibody or an antigen binding fragment thereof as disclosed below.

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 35, 102, 103, 104, 105 106 or 140 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises:

I. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 102 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

II. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 103 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ

ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

III. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 104 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

IV.a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 105 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

V. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 106 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

VI.a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 140 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises:

(i) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 87;or

(ii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 88; or

(iii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 89; or

(iv) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 87;or

(v) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 88; or

(vi) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 89; or

(vii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 85; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 100; or

(viii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 85; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:101; or

(ix) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 86; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 100; or

(x) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 86; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:101.

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises:

i. a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 125; or

ii. a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 126; or

iii. a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 127; or

iv. a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 125; or

v. a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 126; or

vi. a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 127; or

vii. a light chain comprising the amino acid sequence of SEQ ID NO: 123; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 138; or

viii. a light chain comprising the amino acid sequence of SEQ ID NO: 123; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 139; or

ix. a light chain comprising the amino acid sequence of SEQ ID NO: 124; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 138.

In one embodiment, the anti-IL-36R antibody is spesolimab, Antibody B1, Antibody B2, Antibody B3, Antibody B4, Antibody B5, Antibody B6, Antibody C1, Antibody C2, or Antibody C3.

In one embodiment, the anti-IL-36R antibody is an antibody as disclosed in WO2013/074569, WO2016/168542 or WO2020/018503, the content of each of which is incorporated herin by reference.

In another embodiment, the present invention relates to a composition comprising a biological sample from a pateint with GPP and an agent for detecting absence or presence or level of one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2. In a related emboidment, the agent is, for example, an antibody against any of the biomarkers listed in Table 1 and 2.

Brief Description of the Figures

Figure 1 shows the differentially expressed genes (DEGs) in GPP skin lesions after treatment with spesolimab. Panel (A) shows a heatmap of 71 DEGs between lesional skin 1 week after treatment with spesolimab compared with baseline lesional skin (fold change ≥2, adjusted P ≤ .05). Panel (B) shows spesolimab treatment inverts over-activated pathways 1 week after treatment. Ingenuity Pathway Analysis of DEGs in lesional versus non-lesional skin at baseline and in lesional skin 1 week after treatment compared with baseline. Panel (C) shows percent improvement of pathway/process representative marker genes (*P ≤ .05). BL, baseline; DEG, differentially expressed gene; FCH, fold change; GPP, generalized pustular psoriasis; L, lesional; NL, non-lesional.

Figure 2 shows the change in immunohistochemical biomarker scores after treatment with spesolimab in GPP skin lesions. Panel (A) shows a heatmap of changes in clinical scores and immunohistochemical biomarkers from baseline to Week 1 in each patient following spesolimab treatment. Red cells denote per cent change from baseline ≥100; grey cells denote missing values. Panle (B) shows the changes in expression of neutrophil elastase, IL-36γ, CD3, CD11c and lipocalin-2 expressed in GPP lesions before (baseline) and after (Weeks 1 and 4) treatment with spesolimab. Representative histopathology is shown. BL,

baseline; D, dermis; E, epidermis; GPP, generalized pustular psoriasis; GPPASI, Generalized Pustular Psoriasis Area and Severity Index.

Figure 3 shows the changes in peripheral biomarkers in blood and plasma following treatment with spesolimab in patients with GPP. Panel (A) shows the changes (adjusted P ≤ .05, fold change ≥2) in selected genes in blood of patients with GPP (n = 7) after spesolimab treatment throughout Week 1 (Visits 2–9), Week 2 (Visit 10) and Week 4 (Visit 12). Panel (B) shows the Ingenuity Pathway Analysis of DEGs in blood at baseline and at Weeks 1, 2 and 4 after treatment with spesolimab. Panel (C) shows the heatmap of changes in clinical scores and serum biomarkers from baseline over time in each patient after spesolimab treatment. Red cells denote percent change from baseline ≥100. At Weeks 12 and 20, one patient (Patient 5) received methotrexate and was classified as receiving rescue treatment. BL, baseline; CPM, counts per million; DEG, differentially expressed gene; GPP, generalized pustular psoriasis; GPPASI, Generalized Pustular Psoriasis Area and Severity Index.

Detailed Description

GPP symptoms of varying severity occur in most patients and may be idiopathic or triggered by external stimuli, such as infection, corticosteroid use or withdrawal, stress or pregnancy. Moderate or severe GPP cause significant morbidity and mortality due to tender, painful skin lesions, extreme fatigue, high fever, peripheral blood neutrophilia and acute phase response and sepsis. The acute phase is associated with a mean duration of hospitalization of 10 days (range 3-44 days). The observed mortality rate of 7% reported in a retrospective study with 102 GPP cases seen in a tertiary hospital in Johor, Malaysia is likely an underestimate as not all GPP patients were included in the study. Mortality rates are also likely underestimated due to lack of identifying the cause of death as GPP and are largely driven by infectious complications and extra-cutaneous organ manifestations such as renal, hepatic, respiratory and cardiac failure. After responding to treatment or spontaneous flare cessation, it is estimated that up to 50% of patients may suffer from chronic GPP characterized by persistent erythema and scaling that may also include joint symptoms. Based on these limitations, current therapeutic options are not suitable for life-long treatment and do not provide sustained responses in most patients.

The classic presentation of GPP flares as described by von Zumbusch is strongly correlated with polymorphisms in the IL36-R signaling pathway. Individuals with loss-of-function mutations of the IL36RN gene which encodes an endogenous IL36R antagonist (IL-36RN) have dramatically higher incidence of GPP, indicating that uncontrolled upregulation of IL36 signaling due to defective IL36RN antagonism leads to the inflammatory episodes observed in GPP. Genetic human studies have demonstrated the occurrence of GPP clusters in families with a loss of function mutation in IL36RN, which results in uncontrolled IL36R signaling. Mutations in other genes linked to the IL36 pathway such as CARD14 also lead to GPP. Moreover, a recent meta-analysis investigated 233 published GPP cases. They found that 49 (21.0%) of 233 cases carried recessive IL36RN alleles. Those 49 recessive IL36RN alleles defined a GPP phenotype characterized by early onset and high risk of systemic inflammation.

As stated before, there is sufficient evidence to suggest that the blockade of IL-36R signalling is an appealing targeted therapeutic approach for patients with GPP. Previously, blockade of IL-36R signalling with a single intravenous dose of 10 mg/kg spesolimab, a novel humanised anti-IL-36R monoclonal antibody, in a proof-of-concept Phase I, multicentre, single-arm, open-label study (ClinicalTrials.gov identifier: NCT02978690) showed rapid skin and pustular clearance in patients presenting with an acute GPP flare. However, the inflammatory circuits and cellular interactions driving the pathogenesis of GPP are not yet fully elucidated, and the mechanisms by which these are disrupted by IL-36R blockade are unknown. To investigate this, the molecular profiles from skin biopsies were examined in patients with GPP and compared with those from healthy volunteers. In addition, changes in the molecular, histopathological and protein expression profiles in blood and skin post spesolimab treatment in patients with GPP participating in the aforementioned clinical trial were examined and are reported herein.

Therefore, the present invention provides biomarkers associated with anti-IL36R antibody (e.g., spesolimab) treatment in GPP.

In one embodiment, the present invention provides a method for detecting the presence or absence of a beneficial response in a patient after administration of an anti-interleukin 36 receptor antibody (anti-IL-36R antibody) (e.g., spesolimab), comprising: a) obtaining a

biological sample from the patient; b) measuring in said sample the level of expression of one or more biomarkers; c) comparing the level to control value of the level of the biomarkers; and d) determining whether or not the difference in levels between the sample and the control reflects a beneficial response in the patient, wherein the one or more biomarkers comprise genes/ proteins associated with the pro-inflammatory mediators (TNF, IL1B, IL6), neutrophil recruitment mediators (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2), neutrophil-expressed transcripts (NCF1, NCF2, NCF4, ELANE) or in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*), CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP, IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5. In a related embodiment, the one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2.
In one embodiment, the presence or absence of a beneficial response in the patient is detected prior to and after administration of an anti-IL36R antibody or spesolimab.

In one embodiment, the control value of the value of a patient treated with a placebo. In one embodiment, the control value of the value of a patient treated with a placebo and the difference is the difference between the sample from a patient treated with an anti-IL36R antibody or spesolimab and the placebo.

In one embodiment, the level of the gene or the protein of said one or more biomarker is measured. In one embodiment, the patient suffers from GPP.

In one embodiment, the control value is calculated using samples from subjects that do not suffer from GPP. In one embodiment, the control value, for example the placebo value, is determined using samples from known GPP patients, for example from placebo-treated GPP patients. In one embodiment, the control value is determined using at least one previous sample taken from the patient.

In one embodiment, the method further comprises continuing the administration of the anti-IL-36R antibody to the patient if the difference in levels between the sample and the control reflects a beneficial response in the patient. In one embodiment, the method further comprises continuing the administration of the anti-IL-36R antibody to the patient if the difference in levels from a patient treated with the anitbody versus the placebo reflects a beneficial response in the patient.

In a further embodiment, the present invention provides a method of determining whether a potential therapeutic agent is efficacious in the treatment of GPP comprising: a) obtaining a first biological sample from a GPP patient prior to being treated with the potential therapeutic agent; b) treating the GPP patient with the potential therapeutic agent; c) obtaining a second biological sample from the GPP patient after being treated with the potential therapeutic agent; d) measuring in said first and second sample the levels of expression of one or more biomarkers; and e) comparing the biomarker levels in the second sample to the levels in the first sample, wherein changes (e.g., lower or higher) in biomarker levels in the second sample than in the first sample indicate that the potential therapeutic agent is efficacious, and further wherein the one or more biomarkers comprise genes associated with pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*) , CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP, IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5. In a related embodiment, the one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2. In one embodiment, said step e) comprises comparing the biomarker levels in the second sample to the levels in the first sample, wherein changes (e.g., lower or higher) in biomarker levels in the second sample than in the first sample and correlation with improvement in a clinical efficacy measure, e.g., GPPASI scores in case of GPP indicates the potential therapeutic agent is

efficacious. In one embodiment, the method further comprises continuing the treatment of the patient if biomarker levels in the second sample change (e.g., are higher or lower) as compared to the first sample.

In a further embodiment, the present invention provides a method of treating GPP in a subject comprising: a) determining whether to initiate treatment of the subject, modify the treatment dose, modify the dosing interval, or discontinue treatment, based on the method of any of the preceding claims; and b) modifying the treatment regimen based on the determination.

In a further embodiment, the present invention provides a method of monitoring patient response to a GPP treatment comprising:

a) obtaining a first biological sample from the patient;

b) measuring the level of one or more biomarkers in said first biological sample, wherein said one or more biomarkers comprise genes/ proteins associated with pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*), CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP, IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5; or wherein said one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2;

c) administering a treatment compound to the patient;

d) obtaining a second biological sample from the patient;

e) measuring the level of said one or more biomarkers in said second biological sample; and

f) comparing the levels of the one or more biomarkers obtained from first and second biological samples;

wherein a change (e.g., high, low) in the level of the one or more biomarkers in the second biological sample indicates an effective response. In one aspect, a change (e.g., high, low) in the level of the one or more biomarkers in the second biological sample and correlation with improvement in a clinical efficacy measure, e.g., GPPASI scores in case of GPP indicates an effective response.

In a further embodiment, the present invention provides a method for monitoring patient compliance with a drug treatment protocol for GPP comprising:

a) obtaining a biological sample from said patient;

b) measuring the level of one or more biomarkers, wherein the one or more biomarkers comprise genes/ proteins associated with pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*), CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP, IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5; or wherein said one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2; and

c) determining if the level is changed in the patient sample compared to the level in a control untreated sample;

wherein a decreased level indicates patient compliance with said drug treatment protocol.

In one embodiment, in any one of the methods above, the level of the one or more biomarkers in the second biological sample is decreased by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more as compared to the level in the first biological sample.

In one embodiment, in any one of the methods above, the biological sample is a skin biopsy, blood, plasma or serum sample. In one embodiment, in any one of the methods above, the anti-IL-36R antibody is spesolimab. In one embodiment, in any one of the methods above, the levels of biomarkers are determined by RNA sequencing or ELISA or another protein assay. In one embodiment, the biomarkers are the differentially expressed genes in skin.

In one embodiment, the present invention further provides a method of selecting a patient, for example using a method disclosed herein. In one embodiment, the present invention further provides a method of enriching a patient population for patients expected to have a beneficial response after treatment with an anti-IL-36R antibody, for example using a method of the present invention. In one embodiment, the present invention further provides a method of enriching a patient population for patients expected to have a beneficial response prior to or early after treatment with an anti-IL-36R antibody, for example using a method of the present invention.

In one embodiment, in any one of the methods above, the anti-IL-36R antibody or an antigen binding fragment thereof as disclosed below.

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 35, 102, 103, 104, 105 106 or 140 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1);

the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises:

I. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 102 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

II. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 103 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

III. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 104 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

IV. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 105 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

V. a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 106 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

VI.a) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 26 (L-CDR1); the amino acid sequence of SEQ ID NO: 140 (L-CDR2); the amino acid sequence of SEQ ID NO: 44 (L-CDR3); and b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 (H-CDR1); the amino acid sequence of SEQ ID NO: 62, 108, 109, 110 or 111 (H-CDR2); the amino acid sequence of SEQ ID NO: 72 (H-CDR3).

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises:

(i) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 87;or

(ii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 88; or

(iii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 89; or

(iv) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 87;or

(v) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 88; or

(vi) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 80; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 89; or

(vii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 85; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 100; or

(viii) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 85; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:101; or

(ix) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 86; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 100; or

(x) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 86; and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:101.

In one embodiment, the anti-IL-36R antibody or antigen-binding fragment thereof comprises:

i. a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 125; or

ii. a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 126; or

iii. a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 127; or

iv. a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 125; or

v. a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 126; or

vi. a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 127; or

vii.a light chain comprising the amino acid sequence of SEQ ID NO: 123; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 138; or

viii. a light chain comprising the amino acid sequence of SEQ ID NO: 123; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 139; or

ix. a light chain comprising the amino acid sequence of SEQ ID NO: 124; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 138.

In one embodiment, the anti-IL-36R antibody is spesolimab, Antibody B1, Antibody B2, Antibody B3, Antibody B4, Antibody B5, Antibody B6, Antibody C1, Antibody C2, or Antibody C3.

In one embodiment, the anti-IL-36R antibody is an antibody as disclosed in WO2013/074569, WO2016/168542 or WO2020/018503, the content of each of which is incorporated herin by reference.

In one embodiment, the ELISA or other protein assay kit further comprises instructions for use of the kit prior to treatment or for monitoring GPP.

In another embodiment, the present invention relates to a composition comprising a biological sample from a pateint with GPP and an agent for detecting absence or presence or level of one or more biomarkers comprise the genes or proteins listed in Tables 1 and 2. In a related emboidment, the agent is, for example, an antibody against any of the biomarkers listed in Table 1 and 2.

In one embodiment, in any one of the methods above, the anti-IL-36R antibody or an antigen binding fragment thereof as disclosed below.

In one aspect, the anti-IL-36R antibody is a humanized antibody. In one aspect, the anti-IL-36R antibody is a monoclonal antibody. In one aspect, the anti-IL-36R antibody is a full length antibody. In one aspect, the anti-IL-36R antibody is a humanized monoclonal antibody, for example a full length humanized monoclonal antibody. In one aspect, the anti-IL-36R antibody is spesolimab. Representative anti-IL36R antibodies are disclosed in in WO2013/074569, WO2016/168542 or WO2020/018503, the entire content of each of which is incorporated herein by reference.

IL-36R is also known as IL-1RL2 and IL-1Rrp2. It has been reported that agonistic IL-36 ligands (α, β, or γ) initiate the signaling cascade by engaging the IL-36 receptor which then forms a heterodimer with the IL-1 receptor accessory protein (IL-1RAcP). IL-36 antagonist ligands (IL-36RA/IL1F5, IL-38/ILF10) inhibit the signaling cascade.

Variable regions and CDRs of representative anti-IL-36R antibodies are disclosed below:

Anti-IL-36R Mouse Antibody Sequences

Variable regions and CDRs of representative mouse lead antibodies of the present invention (mouse leads) are shown below:

Light Chain Variable Region (VK) Amino Acid Sequences

>33D10B12vK Protein (antibody 33D10)
QIVLTQSPAIMSASLGERVTMTCTASSSVSSSYLHWYQKKPGSSPKLWVYSTSNLASGVPVRF SGSGSGTSYSLTISSMEAEDAATYYCHQHHRSPVTFGSGTKLEMK (SEQ ID NO: 1)
>172C8B12 vK protein (antibody 172C8)
DIQMTQSPASQSASLGESVTFTCLASQTIGTWLAWYQQRPGKSPQLLIYAATSLADGVPSRFSG SGSGTQFSFNIRSLQAEDFASYYCQQVYTTPLTFGGGTKLEIK (SEQ ID NO: 2)
>67E7E8 vK protein (antibody 67E7)
DIQMTQSPASQSASLGESVTFTCLASQTIGTWLGWYQQKPGKSPQLLIYRSTTLADGVPSRFS GSGSGTKFSFKISSLQAADFASYYCQQLYSAPYTFGGGTKLEIR (SEQ ID NO: 3)

>78C8D1 vK Protein (antibody 78C8)
DVLLTQTPLSLPVSLGDQASISCRSSQNIVHSNGNTYLQWYLQKPGQSPKLLIYKVSNRFSGVP DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPFTFGAGTKLELK (SEQ ID NO: 4)
>81A1D1 vK Protein (antibody 81A1)
DIQMTQTTSSLSASLGDRVTISCRASQDIYKYLNWYQQKPDGTLKLLIYYTSGLHSGVPSRFSGS GSGTDFSLTISNLEPEDIATYFCQQDSKFPWTFGGDTKLEIK (SEQ ID NO: 5)
>81B4E11 vK Protein (antibody 81B4)
QIVLTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQQKPGSSPKLWIYRTSNLASGVPGRF SGSGSGTSYSLTISSMEAEDAATYYCHQFHRSPLTFGAGTKLELK (SEQ ID NO: 6)
>73C5C10 vK protein (antibody 73C5)
DIVMTQSQKFLSTSVGVRVSVTCKASQDVGTNVLWYQQKIGQSPKPLIYSASYRHSGVPDRFT GSGSGTDFTLIISNVQSEDLAEYFCQQYSRYPLTFGPGTKLELK (SEQ ID NO: 7)
>73F6F8 vK protein (antibody 73F6)
DIVMTQSQKFLSTSVGVRVSVTCKASQDVGTNVLWYQQKIGQSPKALIYSASYRHSGVPDRFT GSGSGTDFTLIITNVQSEDLAEYFCQQYSRYPLTFGPGTKLELK (SEQ ID NO: 8)
>76E10E8 vK protein (antibody 76E10)
DIVMTQSQKFMSATVGGRVNITCKASQNVGRAVAWYQQKPGQSPKLLTHSASNRYTGVPDRF TGSGSGTDFTLTITNMQSEDLADYFCQQYSSYPLTFGAGTKLDLK (SEQ ID NO: 9)
>89A12B8 vK protein (antibody 89A12)
DIQMTQSPASQSASLGESVTFSCLASQTIGTWLGWYQQKPGKSPQLLIYRATSLADGVPSRFS GSGSGTNFSFKISSLQAEDLASYYCQQLYSGPYTFGGGTKLEIR (SEQ ID NO: 10)

Heavy Chain Variable Region (VH) Amino Acid Sequences

>33D10B12vH Protein (antibody 33D10)
QVQLQQSGTELLKPGASVKLSCKASGNTVTSYWMHWVKQRPGQGLEWIGEILPSTGRTNYNE NFKGKAMLTVDKSSSTAYMQLSSLASEDSAVYYCTIVYFGNPWFAYWGQGTLVTVSA (SEQ ID NO: 11)
>172C8B12 vH protein (antibody 172C8)
EVQLQQSGPELVKPGASVKLSCKASGYTFTDNYMNWVRQSHGKSLEWIGRVNPSNGDTKYN QNFKGKATLTVDKSLSTAYMQLNGLTSEDSAVYYCGRTKNFYSSYSYDDAMDYWGQGTSVTV SS (SEQ ID NO: 12)
>67E7E8 vH protein (antibody 67E7)
EVQLQQSGAEFVRPGASVKFSCTASGFNIKDDYIHWVRQRPEQGLEWVGRIDPANGNTKYAP KFQDKATITADTSSNTAYLQLSSLTSEDTAVYYCAKSFPNNYYSYDDAFAYWGQGTLVTVSA (SEQ ID NO: 13)

>78C8D1 vH Protein (antibody 78C8)
QVQLKESGPVLVAPSQSLSITCTVSGFSLTKFGVHWIRQTPGKGLEWLGVIWAGGPTNYNSAL MSRLTISKDISQSQVFLRIDSLQTDDTAMYYCAKQIYYSTLVDYWGQGTSVTVSS (SEQ ID NO: 14)
>81A1D1 vH Protein (antibody 81A1)
QVQLKESGPGLVAPSQSLFITCTVSGFSLSSYEINWVRQVPGKGLEWLGVIWTGITTNYNSALIS RLSISKDNSKSLVFLKMNSLQTDDTAIYYCARGTGTGFYYAMDYWGQGTSVTVSS (SEQ ID NO: 15)
>81B4E11 vH Protein (antibody 81B4)
QVQLQQPGADFVRPGASMRLSCKASGYSFTSSWIHWVKQRPGQGLEWIGEINPGNVRTNYNE NFRNKATLTVDKSSTTAYMQLRSLTSADSAVYYCTVVFYGEPYFPYWGQGTLVTVSA (SEQ ID NO: 16)
>73C5C10 vH Protein (antibody 73C5)
QVQLKESGPGLVAPSQSLSITCTVSGFSLTNYAVHWVRQFPGKGLEWLGVIWSDGSTDFNAPF KSRLSINKDNSKSQVFFKMNSLQIDDTAIYYCARKGGYSGSWFAYWGQGTLVTVSA (SEQ ID NO: 17)
>73F6F8 vH protein (antibody 73F6)
QVQLKESGPGLVAPSQSLSITCTVSGFSLTNYAVHWVRQFPGKGLEWLGVIWSDGSTDYNAPF KSRLSINKDNSKSQVFFKMNSLQTDDTAIYYCARKGGYSGSWFAYWGQGTLVTVSA (SEQ ID NO: 18)
>76E10E8 vH protein (antibody 76E10)
QVQLKESGPVLVAPSQSLSITCTVSGFSLTNYGVHWVRQPPGKGLEWLGVIWPVGSTNYNSAL MSRLSIHKDNSKSQVFLRMNSLQTDDTAIYYCAKMDWDDFFDYWGQGTTLTVSS(SEQ ID NO: 19)
>89A12B8 vH Protein (antibody 89A12)
EVQLQQSGAELVRPGASVRLSCTASGFNIKDDYIHWVRQRPKQGLEWLGRIDPANGNTKYDPR FQDKATITADTSSNTAYLHLSSLTSEDTAVYYCAKSFPDNYYSYDDAFAYWGQGTLVTVSA (SEQ ID NO: 20)

Light chain CDR-1 (L-CDR1) Amino Acid Sequences

>33D10G1 L-CDR1
TASSSVSSSYLH (SEQ ID NO: 21)

>172C8B12 L-CDR1
LASQTIGTWLA (SEQ ID NO: 22)

>67E7E8 L-CDR1

LASQTIGTWLG (SEQ ID NO: 23)

>78C8D1 L-CDR1
RSSQNIVHSNGNTYLQ (SEQ ID NO: 24)

>81A1D1 L-CDR1
RASQDIYKYLN (SEQ ID NO: 25)

>81B4E11 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>73C5C10 L-CDR1
KASQDVGTNVL (SEQ ID NO: 27)

>73F6F8 L-CDR1
KASQDVGTNVL (SEQ ID NO: 27)

>76E10E8 L-CDR1
KASQNVGRAVA (SEQ ID NO: 28)

>89A12B8 L-CDR1
LASQTIGTWLG (SEQ ID NO: 29)

Light chain CDR-2 (L-CDR2) Amino Acid Sequences

>33D10B12 L-CDR2
STSNLAS (SEQ ID NO: 30)

>172C8B12 L-CDR2
AATSLAD (SEQ ID NO: 31)

>67E7E8 L-CDR2
RSTTLAD (SEQ ID NO: 32)

>78C8D1 L-CDR2
KVSNRFS (SEQ ID NO: 33)

>81A1D1 L-CDR2
YTSGLHS (SEQ ID NO: 34)

>81B4E11 L-CDR2
RTSNLAS (SEQ ID NO: 35)

>73C5C10 L-CDR2
SASYRHS (SEQ ID NO: 36)

>73F6F8 L-CDR2
SASYRHS (SEQ ID NO: 36)

>76E10E8 L-CDR2
SASNRYT (SEQ ID NO: 37)

>89A12B8 L-CDR2
RATSLAD (SEQ ID NO: 38)

Light chain CDR-3 (L-CDR3) Amino Acid Sequences

>33D10B12 L-CDR3
HQHHRSPVT (SEQ ID NO: 39)

>172C8B12 L-CDR3
QQVYTTPLT (SEQ ID NO: 40)

>67E7E8 L-CDR3
QQLYSAPYT (SEQ ID NO: 41)

>78C8D1 L-CDR3
FQGSHVPFT (SEQ ID NO: 42)

>81A1D1 L-CDR3
QQDSKFPWT (SEQ ID NO: 43)

>81B4E11 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>73C5C10 L-CDR3
QQYSRYPLT (SEQ ID NO: 45)

>73F6F8 L-CDR3
QQYSRYPLT (SEQ ID NO: 45)

>76E10E8 L-CDR3
QQYSSYPLT (SEQ ID NO: 46)

>89A12B8 L-CDR3
QQLYSGPYT (SEQ ID NO: 47)

Heavy chain CDR-1 (H-CDR1) Amino Acid Sequences

>33D10B12 H-CDR1
GNTVTSYWMH (SEQ ID NO: 48)

>172C8B12 H-CDR1
GYTFTDNYMN (SEQ ID NO: 49)

>67E7E8 H-CDR1
GFNIKDDYIH (SEQ ID NO: 50)

>78C8D1 H-CDR1
GFSLTKFGVH (SEQ ID NO: 51)

>81A1D1 H-CDR1
GFSLSSYEIN (SEQ ID NO: 52)

>81B4E11 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>73C5C10 H-CDR1
GFSLTNYAVH (SEQ ID NO: 54)

>73F6F8 H-CDR1
GFSLTNYAVH (SEQ ID NO: 54)

>76E10E8 H-CDR1
GFSLTNYGVH (SEQ ID NO: 55)

>89A12B8 H-CDR1
GFNIKDDYIH (SEQ ID NO: 56)

Heavy chain CDR-2 (H-CDR2) Amino Acid Sequences

>33D10B12 H-CDR2
EILPSTGRTNYNENFKG (SEQ ID NO: 57)

>172C8B12 H-CDR2
RVNPSNGDTKYNQNFKG (SEQ ID NO: 58)

>67E7E8 H-CDR2
RIDPANGNTKYAPKFQD (SEQ ID NO: 59)

>78C8D1 H-CDR2
VIWAGGPTNYNSALMS (SEQ ID NO: 60)

>81A1D1 H-CDR2
VIWTGITTNYNSALIS (SEQ ID NO: 61)

>81B4E11 H-CDR2

EINPGNVRTNYNENF (SEQ ID NO: 62)

>73C5C10 H-CDR2
VIWSDGSTDFNAPFKS (SEQ ID NO: 63)

>73F6F8 H-CDR2
VIWSDGSTDYNAPFKS (SEQ ID NO: 64)

>76E10E8 H-CDR2
VIWPVGSTNYNSALMS (SEQ ID NO: 65)

>89A12B8 H-CDR2
RIDPANGNTKYDPRFQD (SEQ ID NO: 66)

Heavy chain CDR-3 (H-CDR3) Amino Acid Sequences

>33D10B12 H-CDR3
VYFGNPWFAY (SEQ ID NO: 67)

>172C8B12 H-CDR3
TKNFYSSYSYDDAMDY (SEQ ID NO: 68)

>67E7E8 H-CDR3
SFPNNYYSYDDAFAY (SEQ ID NO: 69)

>78C8D1 H-CDR3
QIYYSTLVDY (SEQ ID NO: 70)

>81A1D1 H-CDR3
GTGTGFYYAMDY (SEQ ID NO: 71)

>81B4E11 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>73C5C10 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

>73F6F8 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

>76E10E8 H-CDR3
MDWDDFFDY (SEQ ID NO: 74)

>89A12B8 H-CDR3
SFPDNYYSYDDAFAY (SEQ ID NO: 75)

Anti-IL-36R Mouse CDR Sequences

A summary of the CDR sequences of the lead mouse antibodies is shown below:

Antibody	H-CDR Sequences	L-CDR Sequences
33D10	GNTVTSYWMH (H-CDR1) SEQ ID No: 48 EILPSTGRTNYNENFKG (H-CDR2) SEQ ID No: 57 VYFGNPWFAY (H-CDR3) SEQ ID No: 67	TASSSVSSSYLH (L-CDR1) SEQ ID No: 21 STSNLAS (L-CDR2) SEQ ID No: 30 HQHHRSPVT (L-CDR3) SEQ ID No: 39
172C8	GYTFTDNYMN (H-CDR1) SEQ ID No: 49 RVNPSNGDTKYNQNFKG (H-CDR2) SEQ ID No: 58 TKNFYSSYSYDDAMDY (H-CDR3) SEQ ID No: 68	LASQTIGTWLA (L-CDR1) SEQ ID No: 22 AATSLAD (L-CDR2) SEQ ID No: 31 QQVYTTPLT (L-CDR3) SEQ ID No: 40
67E7	GFNIKDDYIH (H-CDR1) SEQ ID No: 50 RIDPANGNTKYAPKFQD (H-CDR2) SEQ ID No: 59 SFPNNYYSYDDAFAY (H-CDR3) SEQ ID No: 69	LASQTIGTWLG (L-CDR1) SEQ ID No: 23 RSTTLAD (L-CDR2) SEQ ID No: 32 QQLYSAPYT (L-CDR3) SEQ ID No: 41
78C8	GFSLTKFGVH (H-CDR1) SEQ ID No: 51 VIWAGGPTNYNSALMS (H-CDR2) SEQ ID No: 60 QIYYSTLVDY (H-CDR3) SEQ ID No: 70	RSSQNIVHSNGNTYLQ (L-CDR1) SEQ ID No: 24 KVSNRFS (L-CDR2) SEQ ID No: 33 FQGSHVPFT (L-CDR3) SEQ ID No: 42
81A1	GFSLSSYEIN (H-CDR1) SEQ ID No: 52	RASQDIYKYLN (L-CDR1) SEQ ID No: 25 YTSGLHS (L-CDR2) SEQ ID No: 34

	VIWTGITTNYNSALIS (H-CDR2) SEQ ID No: 61 GTGTGFYYAMDY (H-CDR3) SEQ ID No: 71	QQDSKFPWT (L-CDR3) SEQ ID No: 43
81B4	GYSFTSSWIH (H-CDR1) SEQ ID No: 53 EINPGNVRTNYNENF (H-CDR2) SEQ ID No: 62 VFYGEPYFPY (H-CDR3) SEQ ID No: 72	TASSSVSSSYFH (L-CDR1) SEQ ID No: 26 RTSNLAS (L-CDR2) SEQ ID No: 35 HQFHRSPLT (L-CDR3) SEQ ID No: 44
73C5	GFSLTNYAVH (H-CDR1) SEQ ID No: 54 VIWSDGSTDFNAPFKS (H-CDR2) SEQ ID No: 63 KGGYSGSWFAY (H-CDR3) SEQ ID No: 73	KASQDVGTNVL (L-CDR1) SEQ ID No: 27 SASYRHS (L-CDR2) SEQ ID No: 36 QQYSRYPLT (L-CDR3) SEQ ID No: 45
73F6	GFSLTNYAVH (H-CDR1) SEQ ID No: 54 VIWSDGSTDYNAPFKS (H-CDR2) SEQ ID No: 64 KGGYSGSWFAY (H-CDR3) SEQ ID No: 73	KASQDVGTNVL (L-CDR1) SEQ ID No:27 SASYRHS (L-CDR2) SEQ ID No: 36 QQYSRYPLT (L-CDR3) SEQ ID No: 45
76E10	GFSLTNYGVH (H-CDR1) SEQ ID No: 55 VIWPVGSTNYNSALMS (H-CDR2) SEQ ID No: 65 MDWDDFFDY (H-CDR3) SEQ ID No: 74	KASQNVGRAVA (L-CDR1) SEQ ID No: 28 SASNRYT (L-CDR2) SEQ ID No: 37 QQYSSYPLT (L-CDR3) SEQ ID No: 46
89A12	GFNIKDDYIH (H-CDR1) SEQ ID No: 56 RIDPANGNTKYDPRFQD (H-CDR2) SEQ ID No: 66	LASQTIGTWLG (L-CDR1) SEQ ID No: 29 RATSLAD (L-CDR2) SEQ ID No: 38 QQLYSGPYT (L-CDR3) SEQ ID No: 47

	SFPDNYYSYDDAFAY (H-CDR3) SEQ ID No: 75	

Anti-IL-36R Humanized Antibody Sequences

Human framework sequences were selected for the mouse leads based on the framework homology, CDR structure, conserved canonical residues, conserved interface packing residues and other parameters to produce humanized variable regions (see Example 5).

Representative humanized variable regions derived from antibodies 81B4 and 73C5 are shown below.

Light Chain Variable Region (VK) Amino Acid Sequences

>81B4vK32_3 vK protein
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSTLASGI PDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGQGTKLEIK (SEQ ID NO: 76)
>81B4vK32_105 vK protein
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSILASGV PDRFSGSGSGTDFTLTISRLEPEDFATYYCHQFHRSPLTFGQGTKLEIK (SEQ ID NO: 77)
>81B4vK32_116 vK protein
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLWIYRTSRLASG VPDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGQGTKLEIK (SEQ ID NO: 78)
>81B4vK32_127 vK protein
EIVLTQSPGTLSLSPGERATMTCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSRLASGV PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQFHRSPLTFGQGTKLEIK (SEQ ID NO: 79)
>81B4vK32_138 vK protein
QIVLTQSPGTLSLSPGERATMTCTASSSVSSSYFHWYQQKPGQAPRLWIYRTSRLASG VPDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGAGTKLEIK (SEQ ID NO: 80)
>81B4vK32_140 vK protein
QIVLTQSPGTLSLSPGERVTMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSQLASGI PDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGQGTKLEIK (SEQ ID NO: 81)

>81B4vK32_141 vK protein
QIVLTQSPGTLSLSPGERATMTCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSKLASG VPDRFSGSGSGTDFTLTISRLEPEDFATYYCHQFHRSPLTFGQGTKLEIK (SEQ ID NO: 82)
>81B4vK32_147 vK protein
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSHLASGI PGRFSGSGSGTDFTLTISRLEPEDAAVYYCHQFHRSPLTFGQGTKLEIK (SEQ ID NO: 83)

>73C5vK39_2 vK protein
EIVMTQSPATLSVSPGVRATLSCKASQDVGTNVLWYQQKPGQAPRPLIYSASYRHSGI PDRFSGSGSGTEFTLTISSLQSEDFAEYFCQQYSRYPLTFGQGTKLEIK (SEQ ID NO: 84)
>73C5vK39_7 vK protein
EIVMTQSPATLSVSPGVRATLSCKASQDVGTNVLWYQQKPGQAPRPLIYSASYRHSGI PDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSRYPLTFGQGTKLEIK (SEQ ID NO: 85)
>73C5vK39_15 vK protein
EIVMTQSPATLSVSPGVRATLSCKASQDVGTNVLWYQQKPGQAPRPLIYSASYRHSGI PARFSGSGSGTEFTLTISSLQSEDFAEYYCQQYSRYPLTFGQGTKLEIK (SEQ ID NO: 86)

Heavy Chain Variable Region (VH) Amino Acid Sequences

>81B4vH33_49 vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGNVR TNYNENFRNKATMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV TVSS (SEQ ID NO: 87)
>81B4vH33_85T vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWIGEINPGNVR TNYNENFRNRVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTLV TVSS (SEQ ID NO: 88)
>81B4vH33_90 vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVKQAPGQGLEWMGEINPGNV RTNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTL VTVSS (SEQ ID NO: 89)
>81B4vH33_93 vH Protein

QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWMGEINPGNV RTNYNENFRNRATLTRDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTL VTVSS (SEQ ID NO: 90)
>81B4vH50_22 vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWMGEILPGVV RTNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTL VTVSS (SEQ ID NO: 91)
>81B4vH50_30 vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWIGEINPGAVR TNYNENFRNRVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTLV TVSS (SEQ ID NO: 92)
>81B4vH51_13 vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGLVR TNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV TVSS (SEQ ID NO: 93)
>81B4vH51_15 vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGAVR TNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV TVSS (SEQ ID NO: 94)
>81B4vH52_83 vH Protein
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGSVR TNYNENFRNKATMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV TVSS (SEQ ID NO: 95)

>73C5vH46_4 vH Protein
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTINKDTSKSQVSFKMSSVQAADTAVYYCARKGGYSGSWFAYWGQGTLV TVSS (SEQ ID NO: 96)
>73C5vH46_19 vH Protein
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDTSKNQVSLKMNSLTTDDTAVYYCARKGGYSGSWFAYWGQGTLV TVSS (SEQ ID NO: 97)
>73C5vH46_40 vH Protein

QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDNSKSQVSLKMNSVTVADTAVYYCARKGGYSGSWFAYWGQGTLV TVSS (SEQ ID NO: 98)
>73C5vH47_65 vH Protein
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWVRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDTSKNQVSFKLSSVTVDDTAVYYCARKGGYSGSWFAYWGQGTLV TVSS (SEQ ID NO: 99)
>73C5vH47_77 vH Protein
QVQLQESGPGLVAPSETLSLTCTVSGFSLTDYAVHWIRQFPGKGLEWIGVIWSDGSTD FNAPFKSRVTISKDTSKNQVSFKLSSVTTDDTAVYYCARKGGYSGSWFAYWGQGTLV TVSS (SEQ ID NO: 100)
>73C5vH58_91 vH Protein
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDNSKSQVSFKMSSVTADDTAVYYCARKGGYSGSWFAYWGQGTLV TVSS (SEQ ID NO: 101)

The CDR sequences from the humanized variable regions derived from antibodies 81B4 and 73C5 shown above are depicted below.

<u>L-CDR1 Amino Acid Sequences</u>

>81B4vK32_3 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>81B4vK32_105 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>81B4vK32_116 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>81B4vK32_127 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>81B4vK32_138 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>81B4vK32_140 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>81B4vK32_141 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>81B4vK32_147 L-CDR1
TASSSVSSSYFH (SEQ ID NO: 26)

>73C5vK39_2 L-CDR1
KASQDVGTNVL (SEQ ID NO: 27)

>73C5vK39_7 L-CDR1
KASQDVGTNVL (SEQ ID NO: 27)

>73C5vK39_15 L-CDR1
KASQDVGTNVL (SEQ ID NO: 27)

L-CDR2 Amino Acid Sequences
>81B4vK32_3 L-CDR2 (SEQ ID 102)
RTSTLAS

>81B4vK32_105 L-CDR2 (SEQ ID 103)
RTSILAS

>81B4vK32_116 L-CDR2 (SEQ ID 104)
RTSRLAS

>81B4vK32_127 L-CDR2 (SEQ ID 104)
RTSRLAS

>81B4vK32_138 L-CDR2 (SEQ ID 104)
RTSRLAS

>81B4vK32_140 L-CDR2 (SEQ ID 105)
RTSQLAS

>81B4vK32_141 L-CDR2 (SEQ ID 106)
RTSKLAS

>81B4vK32_147 L-CDR2 (SEQ ID 140)
RTSHLAS

>73C5vK39_2 L-CDR2
SASYRHS (SEQ ID NO: 36)

>73C5vK39_7 L-CDR2
SASYRHS (SEQ ID NO: 36)

>73C5vK39_15 L-CDR2
SASYRHS (SEQ ID NO: 36)

L-CDR3 Amino Acid Sequences
>81B4vK32_3 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>81B4vK32_105 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>81B4vK32_116 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>81B4vK32_127 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>81B4vK32_138 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>81B4vK32_140 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>81B4vK32_141 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>81B4vK32_147 L-CDR3
HQFHRSPLT (SEQ ID NO: 44)

>73C5vK39_2 L-CDR3
QQYSRYPLT (SEQ ID NO: 45)

>73C5vK39_7 L-CDR3
QQYSRYPLT (SEQ ID NO: 45)

>73C5vK39_15 L-CDR3
QQYSRYPLT (SEQ ID NO: 45)

<u>H-CDR1 Amino Acid Sequences</u>
>81B4vH33_49 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH33_85T H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH33_90 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH33_93 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH50_22 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH50_30 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH51_13 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH51_15 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>81B4vH52_83 H-CDR1
GYSFTSSWIH (SEQ ID NO: 53)

>73C5vH46_4 H-CDR1
GFSLTDYAVH (SEQ ID NO: 107)

>73C5vH46_19 H-CDR1
GFSLTDYAVH (SEQ ID NO: 107)

>73C5vH46_40 H-CDR1
GFSLTDYAVH (SEQ ID NO: 107)

>73C5vH47_65 H-CDR1
GFSLTDYAVH (SEQ ID NO: 107)

>73C5vH47_77 H-CDR1
GFSLTDYAVH (SEQ ID NO: 107)

>73C5vH58_91 H-CDR1
GFSLTDYAVH (SEQ ID NO: 107)

H-CDR2 Amino Acid Sequences
>81B4vH33_49 H-CDR2
EINPGNVRTNYNENF (SEQ ID NO: 62)

>81B4vH33_85T H-CDR2
EINPGNVRTNYNENF (SEQ ID NO: 62)

>81B4vH33_90 H-CDR2
EINPGNVRTNYNENF (SEQ ID NO: 62)

>81B4vH33_93 H-CDR2
EINPGNVRTNYNENF (SEQ ID NO: 62)

>81B4vH50_22 H-CDR2
EILPGVVRTNYNENF (SEQ ID NO: 108)

>81B4vH50_30 H-CDR2

EINPGAVRTNYNENF (SEQ ID NO: 109)

>81B4vH51_13 H-CDR2
EINPGLVRTNYNENF (SEQ ID NO: 110)

>81B4vH51_15 H-CDR2
EINPGAVRTNYNENF (SEQ ID NO: 109)

>81B4vH52_83 H-CDR2
EINPGSVRTNYNENF (SEQ ID NO: 111)

>73C5vH46_4 H-CDR2
VIWSDGSTDYNAPFKS (SEQ ID NO: 64)

>73C5vH46_19 H-CDR2
VIWSDGSTDYNAPFKS (SEQ ID NO: 64)

>73C5vH46_40 H-CDR2
VIWSDGSTDYNAPFKS (SEQ ID NO: 64)

>73C5vH47_65 H-CDR2
VIWSDGSTDYNAPFKS (SEQ ID NO: 64)

>73C5vH47_77 H-CDR2
VIWSDGSTDFNAPFKS (SEQ ID NO: 63)

>73C5vH58_91 H-CDR2
VIWSDGSTDYNAPFKS (SEQ ID NO: 64)

H-CDR3 Amino Acid Sequences
>81B4vH33_49 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH33_85T H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH33_90 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH33_93 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH50_22 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH50_30 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH51_13 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH51_15 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>81B4vH52_83 H-CDR3
VFYGEPYFPY (SEQ ID NO: 72)

>73C5vH46_4 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

>73C5vH46_19 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

>73C5vH46_40 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

>73C5vH47_65 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

>73C5vH47_77 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

>73C5vH58_91 H-CDR3
KGGYSGSWFAY (SEQ ID NO: 73)

Heavy Chain Constant region linked downstream of a humanized variable heavy region:
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 112)
Light Chain Constant region linked downstream of a humanized variable light region:
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:113)

Representative light chain and heavy chain sequences of the present invention are shown below (humanized variable regions derived from antibodies 81B4 and 73C5 linked to constant regions).

Light Chain Amino Acid Sequences

>81B4vK32_3 Light Chain
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSTLASGI PDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 114)
>81B4vK32_105 Light Chain
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSILASGV PDRFSGSGSGTDFTLTISRLEPEDFATYYCHQFHRSPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 115)
>81B4vK32_116 Light Chain
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLWIYRTSRLASG VPDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGQGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 116)
>81B4vK32_127 Light Chain
EIVLTQSPGTLSLSPGERATMTCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSRLASGV PDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQFHRSPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 117)
>81B4vK32_138 Light Chain
QIVLTQSPGTLSLSPGERATMTCTASSSVSSSYFHWYQQKPGQAPRLWIYRTSRLASG VPDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGAGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 118)
>81B4vK32_140 Light Chain
QIVLTQSPGTLSLSPGERVTMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSQLASGI PDRFSGSGSGTDFTLTISRLEPEDAATYYCHQFHRSPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 119)
>81B4vK32_141 Light Chain
QIVLTQSPGTLSLSPGERATMTCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSKLASG VPDRFSGSGSGTDFTLTISRLEPEDFATYYCHQFHRSPLTFGQGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 120)

>81B4vK32_147 Light Chain
EIVLTQSPGTLSLSPGERATMSCTASSSVSSSYFHWYQQKPGQAPRLLIYRTSHLASGI PGRFSGSGSGTDFTLTISRLEPEDAAVYYCHQFHRSPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 121)

>73C5vK39_2 Light Chain
EIVMTQSPATLSVSPGVRATLSCKASQDVGTNVLWYQQKPGQAPRPLIYSASYRHSGI PDRFSGSGSGTEFTLTISSLQSEDFAEYFCQQYSRYPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 122)
>73C5vK39_7 Light Chain
EIVMTQSPATLSVSPGVRATLSCKASQDVGTNVLWYQQKPGQAPRPLIYSASYRHSGI PDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSRYPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 123)
>73C5vK39_15 Light Chain
EIVMTQSPATLSVSPGVRATLSCKASQDVGTNVLWYQQKPGQAPRPLIYSASYRHSGI PARFSGSGSGTEFTLTISSLQSEDFAEYYCQQYSRYPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 124)

Heavy Chain Amino Acid Sequences

>81B4vH33_49 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGNVR TNYNENFRNKATMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 125)
>81B4vH33_85T Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWIGEINPGNVR TNYNENFRNRVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 126)

>81B4vH33_90 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVKQAPGQGLEWMGEINPGNV RTNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 127)
>81B4vH33_93 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWMGEINPGNV RTNYNENFRNRATLTRDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 128)
>81B4vH50_22 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWMGEILPGVV RTNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 129)
>81B4vH50_30 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQRPGQGLEWIGEINPGAVR TNYNENFRNRVTMTVDTSISTAYMELSRLRSDDTAVYYCTVVFYGEPYFPYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 130)
>81B4vH51_13 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGLVR TNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV

TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 131)
>81B4vH51_15 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGAVR TNYNENFRNKVTMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 132)
>81B4vH52_83 Heavy Chain
QVQLVQSGAEVKKPGASVKVSCKASGYSFTSSWIHWVRQAPGQGLEWIGEINPGSVR TNYNENFRNKATMTVDTSISTAYMELSRLRSDDTAVYYCAVVFYGEPYFPYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 133)

>73C5vH46_4 Heavy Chain
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTINKDTSKSQVSFKMSSVQAADTAVYYCARKGGYSGSWFAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 134)
>73C5vH46_19 Heavy Chain
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDTSKNQVSLKMNSLTTDDTAVYYCARKGGYSGSWFAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 135)
>73C5vH46_40 Heavy Chain
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDNSKSQVSLKMNSVTVADTAVYYCARKGGYSGSWFAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 136)
>73C5vH47_65 Heavy Chain
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWVRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDTSKNQVSFKLSSVTVDDTAVYYCARKGGYSGSWFAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 137)
>73C5vH47_77 Heavy Chain
QVQLQESGPGLVAPSETLSLTCTVSGFSLTDYAVHWIRQFPGKGLEWIGVIWSDGSTD FNAPFKSRVTISKDTSKNQVSFKLSSVTTDDTAVYYCARKGGYSGSWFAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 138)
>73C5vH58_91 Heavy Chain
QVQLQESGPGLVKPSETLSITCTVSGFSLTDYAVHWIRQPPGKGLEWIGVIWSDGSTD YNAPFKSRVTISKDNSKSQVSFKMSSVTADDTAVYYCARKGGYSGSWFAYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 139)

The CDRs listed above are defined using the Chothia numbering system (Al-Lazikani et al., (1997) JMB 273, 927-948).

In one aspect, an antibody of the present invention comprises 3 light chain CDRs and 3 heavy chain CDRs, for example as set forth above.

In one aspect, an antibody of the present invention comprises a light chain and a heavy chain variable region as set forth above. In one aspect, a light chain variable region of the invention is fused to a light chain constant region, for example a kappa or lambda constant region. In one aspect, a heavy chain variable region of the invention is fused to a heavy chain constant region, for example IgA, IgD, IgE, IgG or IgM, in particular, IgG_1, IgG_2, IgG_3 or IgG_4.

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 125 (Antibody B1).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 126 (Antibody B2).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 115; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 127 (Antibody B3).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 125 (Antibody B4).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 126 (Antibody B5).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 118; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 127 Antibody B6).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 123; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 138 (Antibody C3).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 123; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 139 (Antibody C2).

The present invention provides an anti-IL-36R antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 124; and a heavy chain comprising the amino acid sequence of SEQ ID NO: 138 (Antibody C1)

Representative antibodies of the present invention are shown below.

Table B.

Antibody	**Light Chain Sequences**	**Heavy Chain Sequences**
B1	EIVLTQSPGTLSLSPGERATMSCT ASSSVSSSYFHWYQQKPGQAPR LLIYRTSILASGVPDRFSGSGSGT DFTLTISRLEPEDFATYYCHQFHR SPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 115)	QVQLVQSGAEVKKPGASVKVSCKASGY SFTSSWIHWVRQAPGQGLEWIGEINPGN VRTNYNENFRNKATMTVDTSISTAYMEL SRLRSDDTAVYYCAVVFYGEPYFPYWG QGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 125)

B2	EIVLTQSPGTLSLSPGERATMSCT ASSSVSSSYFHWYQQKPGQAPR LLIYRTSILASGVPDRFSGSGSGT DFTLTISRLEPEDFATYYCHQFHR SPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 115)	QVQLVQSGAEVKKPGASVKVSCKASGY SFTSSWIHWVRQRPGQGLEWIGEINPG NVRTNYNENFRNRVTMTVDTSISTAYME LSRLRSDDTAVYYCTVVFYGEPYFPYWG QGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 126)
B3	EIVLTQSPGTLSLSPGERATMSCT ASSSVSSSYFHWYQQKPGQAPR LLIYRTSILASGVPDRFSGSGSGT DFTLTISRLEPEDFATYYCHQFHR SPLTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 115)	QVQLVQSGAEVKKPGASVKVSCKASGY SFTSSWIHWVKQAPGQGLEWMGEINPG NVRTNYNENFRNKVTMTVDTSISTAYME LSRLRSDDTAVYYCTVVFYGEPYFPYWG QGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 127)
B4	QIVLTQSPGTLSLSPGERATMTCT ASSSVSSSYFHWYQQKPGQAPR LWIYRTSRLASGVPDRFSGSGSG TDFTLTISRLEPEDAATYYCHQFH RSPLTFGAGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 118)	QVQLVQSGAEVKKPGASVKVSCKASGY SFTSSWIHWVRQAPGQGLEWIGEINPGN VRTNYNENFRNKATMTVDTSISTAYMEL SRLRSDDTAVYYCAVVFYGEPYFPYWG QGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVV

		SVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 125)
B5	QIVLTQSPGTLSLSPGERATMTCT ASSSVSSSYFHWYQQKPGQAPR LWIYRTSRLASGVPDRFSGSGSG TDFTLTISRLEPEDAATYYCHQFH RSPLTFGAGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 118)	QVQLVQSGAEVKKPGASVKVSCKASGY SFTSSWIHWVRQRPGQGLEWIGEINPG NVRTNYNENFRNRVTMTVDTSISTAYME LSRLRSDDTAVYYCTVVFYGEPYFPYWG QGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 126)
B6	QIVLTQSPGTLSLSPGERATMTCT ASSSVSSSYFHWYQQKPGQAPR LWIYRTSRLASGVPDRFSGSGSG TDFTLTISRLEPEDAATYYCHQFH RSPLTFGAGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO: 118)	QVQLVQSGAEVKKPGASVKVSCKASGY SFTSSWIHWVKQAPGQGLEWMGEINPG NVRTNYNENFRNKVTMTVDTSISTAYME LSRLRSDDTAVYYCTVVFYGEPYFPYWG QGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 127)

Table C

Antibody	Light Chain Sequences	Heavy Chain Sequences
C1	EIVMTQSPATLSVSPGVRATLSCK ASQDVGTNVLWYQQKPGQAPRP LIYSASYRHSGIPARFSGSGSGTE FTLTISSLQSEDFAEYYCQQYSRY PLTFGQGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 124)	QVQLQESGPGLVAPSETLSLTCTVSGFS LTDYAVHWIRQFPGKGLEWIGVIWSDGS TDFNAPFKSRVTISKDTSKNQVSFKLSSV TTDDTAVYYCARKGGYSGSWFAYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 138)
C2	EIVMTQSPATLSVSPGVRATLSCK ASQDVGTNVLWYQQKPGQAPRP LIYSASYRHSGIPDRFSGSGSGTE FTLTISSLQSEDFAVYYCQQYSRY PLTFGQGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 123)	QVQLQESGPGLVKPSETLSITCTVSGFSL TDYAVHWIRQPPGKGLEWIGVIWSDGST DYNAPFKSRVTISKDNSKSQVSFKMSSV TADDTAVYYCARKGGYSGSWFAYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 139)
C3	EIVMTQSPATLSVSPGVRATLSCK ASQDVGTNVLWYQQKPGQAPRP LIYSASYRHSGIPDRFSGSGSGTE FTLTISSLQSEDFAVYYCQQYSRY PLTFGQGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEK	QVQLQESGPGLVAPSETLSLTCTVSGFS LTDYAVHWIRQFPGKGLEWIGVIWSDGS TDFNAPFKSRVTISKDTSKNQVSFKLSSV TTDDTAVYYCARKGGYSGSWFAYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKRVEPKSCD

	HKVYACEVTHQGLSSPVTKSFNR GEC (SEQ ID NO: 123)	KTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSL SPGK (SEQ ID NO: 138)

The invention is further described in the following examples, which are not intended to limit the scope of the invention.

Examples

Example 1: Pustular psoriasis: molecular pathways and effects of spesolimab in generalized pustular psoriasis

ABSTRACT

Background: The IL-36 pathway plays a key role in the pathogenesis of generalized pustular psoriasis (GPP). In a proof-of-concept clinical trial, treatment with spesolimab, an anti-IL-36 receptor antibody, resulted in rapid skin and pustular clearance in patients presenting with acute GPP flares.

Objective: To compare the molecular profiles of lesional and non-lesional skin from patients with GPP with skin from healthy volunteers, and to investigate the molecular changes after spesolimab treatment in the skin and blood of patients with acute GPP flares.

Methods: Pre- and post-treatment skin and blood samples were collected from patients with GPP who participated in a single-arm, Phase I study (n = 7). Biomarkers were assessed by RNA sequencing, histopathology and immunohistochemistry.

Results: In GPP lesions, 1287 transcripts were commonly up- or downregulated. Selected transcripts from the IL-36 signalling pathway were upregulated in untreated GPP lesions.

In patients with GPP, IL-36 pathway-related signatures, T helper (Th)1/Th17 and innate inflammation signalling, neutrophilic mediators and keratinocyte-driven inflammation pathways, were downregulated by spesolimab as early as Week 1. Spesolimab also decreased related serum biomarkers and cell populations in the skin lesions from patients with GPP, including CD3+ T, CD11c+, IL-36γ+ cells and lipocalin-2-expressing cells.

Conclusion: In patients with GPP, spesolimab showed rapid modulation of commonly dysregulated molecular pathways in GPP, which may be associated with improved clinical outcomes. A single intravenous dose of spesolimab resulted in strong and rapid downregulation of biomarkers linked to key inflammatory processes, highlighting the relevance of anti-IL-36 receptor-targeted therapy for patients with GPP.

CAPSULE SUMMARY

This is the first study to show that spesolimab, an anti-IL-36 receptor monoclonal antibody, can modulate differentially expressed genes in GPP lesions, including IL-36 pathway signatures, in patients with a GPP flare.

ABBREVIATIONS

DEG: differentially expressed gene

IHC: immunohistochemistry

GPP: generalized pustular psoriasis

PPP: palmoplantar pustulosis

RNA-seq: RNA sequencing

METHODS AND ANALYSIS

Study samples and studies

Skin biopsies of 3—4 mm punch were taken adjacent to pustules, and blood samples were collected from adult patients with GPP (limbs/torso, n = 7) and healthy volunteers (limbs [arms/legs], n = 10; palms/soles, n = 6)(23, 25). In a Phase I, multicentre, single-arm, open-label, proof-of-concept study, patients with GPP received a single intravenous dose of 10 mg/kg spesolimab and were followed for 20 weeks (NCT02978690, Fig E1). Patients

had a GPP flare involving ≥10% of their body surface area with erythema, including the presence of pustules, and a Generalized Pustular Psoriasis Physician Global Assessment (GPPGA) score of ≥3 (moderate-to-severe disease). Informed consent was obtained from all participants.

Tissue and blood sampling and biomarker analyses in patients with GPP

Global transcriptome-wide RNA sequencing (RNA-seq; Illumina Hi-Seq 4000, Illumina, San Diego, CA) of lesional and non-lesional skin biopsy specimens and whole blood was used to characterise cellular and molecular response to spesolimab. Skin biopsies were performed at baseline (lesional and non-lesional skin) and at Week 1 (lesional skin), with an optional biopsy performed at Week 2 (lesional skin). Lesional skin biopsies were collected at the site of the most inflamed lesion, that is the deepest red erythema. RNA extraction from skin samples were performed with RNeasy Fibrous Tissue Mini Kit (Qiagen, Valencia, CA). Whole blood and serum were collected at multiple timepoints. RNA-seq was performed at baseline for both non-lesional and lesional skin types, at Weeks 1 and 2 for lesional skin, and at baseline and Weeks 1, 2 and 4 in whole blood samples. RNA extraction from blood samples was performed with the PAXgene® Blood mRNA Kit (Qiagen, Valencia, CA). Skin biopsies were evaluated for histopathology and immunohistochemistry (IHC) using specific antibodies, and evaluated with a semi-quantitative scoring method that has been previously published; with this method, each biopsy sample was given a global histopathological score. Biomarkers associated with GPP disease, including neutrophil elastase, lipocalin-2, IL-36γ, CD11c and CD3, were assessed in skin biopsies by IHC at baseline, Week 1 and Week 2 (optional). Serum samples were assayed for multiple biomarkers using the Randox Biochip Array platform at baseline and at Weeks 2, 4 and 12. The biomarkers assessed were β-defensin 4A, CCL20, CXCL1, IL-17A, IL-19, IL-1RN, IL-6, IL-8 and C-reactive protein. The Ingenuity Pathway Analysis (IPA) library of canonical pathways was used to identify pathways that were most significant to the data set.

Statistical analysis

Due to the small sample size, mainly descriptive analyses for continuous IHC biomarkers and serum biomarkers were conducted. For patients with GPP, the main focus was on changes over time, particularly for patients with elevated biomarker values at baseline.

Link to clinical endpoints were mainly assessed via graphical visualisations on an individual patient level (heatmaps). Since the various clinical endpoints and biomarkers are on different scales, these figures are presented on a relative scale via percentage change from baseline allowing for a meaningful comparison.

Exploratory analyses of over time changes in gene expression levels over time were performed to identify differentially expressed genes (DEGs). This analysis was conducted using the limma package. Briefly, only genes with counts per million ≥1 in at least half the samples in at least one subgroup were included in the analysis. Data were normalised using the TMM method described by Robinson and Oshlack and voom-transformed. To account for correlation between subjects, the duplicate Correlation function was used with subject as a blocking factor, a linear model was fit using the lmFit-function and, finally, moderated t-statistics were computed to derive log2 fold change and corresponding Benjamini-Hochberg FDR-adjusted P-values.

RESULTS

Baseline comparison of the molecular profile in skin from patients with GPP and healthy volunteers

Gene expression in biopsies of lesional skin compared with non-lesional skin from patients with GPP (limbs/torso) and these were also compared with healthy volunteer's samples (limbs or palms/soles). Clear distinct patterns of DEGs were observed between thin (limbs/torso) and thick (palms/soles) skin associated with GPP.

Using an absolute fold change of ≥2 and an adjusted P ≤ .01 biopsies from patients with GPP had 7614 DEGs. Disease-relevant genes that were increased >5-fold in GPP lesional skin compared with healthy donors included S-100 (A7A, A7, A8, A9, A12), DEFB4A, VNN3, CCL18, IL19, IL20, IL22, IL17A, IL36G, IL36A, CXCL8, CXCL10, MMP12 and LCN2. Most DEGs in GPP lesional skin (>log 5-fold) were more highly expressed in lesional than non-lesional skin. Up-regulation of S100A8/9 and IL36A was measured in GPP. Increased expression of molecular pathways across GPP lesional skin

included acute phase response signalling, IL-6, IL-8 and IL-23 signalling in addition to Th1, Th2 and dendritic cell signalling and keratinocyte-driven inflammation. Multiple consensus disease-relevant pathways are modulated across GPP, including IL-17/TNF-induced transcripts in keratinocytes and psoriatic pathways.

Modulation of genes and proteins in patients with GPP after treatment with spesolimab

At baseline, global transcriptome analysis identified 3276 DEGs between lesional and non-lesional skin, 1885 upregulated and 1391 downregulated transcripts (adjusted $P \leq .05$, fold change ≥2; Fig 1, A). Among those, all IL-36 ligands were strongly upregulated (IL36A: 90-fold; IL36B: 2.6-fold; IL36G: 13-fold).

The expression of 987 genes in lesional skin reached near non-lesional levels by Week 1 after a single infusion with spesolimab (adjusted $P \leq .05$, fold change ≥2) (Fig 1, A). DEGs were associated with innate (e.g. IL6, TNFα) and Th1/Th17-mediated inflammation (e.g. IL1B, IL12B, IL23A) and proinflammatory processes of keratinocyte activation (e.g. IL17C, IL24). Activation z-scores obtained for canonical pathways by Ingenuity Pathway Analysis (IPA) showed that the higher activity in lesional skin at baseline was downregulated by spesolimab by Week 1 (Fig 1, B). Significant decreases in pro-inflammatory mediators (TNF, IL1B, IL6), neutrophil recruitment mediators (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2), neutrophil-expressed transcripts (NCF1, NCF2, NCF4, ELANE) or in keratinocyte activation, differentiation and mediated inflammation transcripts (IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24) were found in lesional skin after 1 week of spesolimab treatment (Fig 1, C). Strong inhibition of T-cell activity was observed after spesolimab treatment, including T helper (Th)1/Th2 pathways and CD28 signalling in Th cells (Fig 1, B), and this was associated with the downregulation of elevated IL17C (Fig 1, C).

In addition, as early as Week 1, spesolimab treatment selectively decreased the expression of IHC biomarkers, including the number of CD11c+ dendritic cells, CD3+ T cells, neutrophil elastase-, lipocalin-2- and IL-36γ-expressing cells (Fig 2, A and B). These reductions were accompanied by decreases in clinical severity (Fig 2, A).

RNA-seq performed in whole blood detected DEGs at Weeks 1, 2, and 4 compared with baseline (364, 476 and 568 genes, respectively; adjusted P ≤ .05, fold change ≥2), with 319 commonly DEGs between Weeks 2 and 4. Proinflammatory mediators involved in neutrophil activation, including IL10, CD177, S100A8/9, S100A12 and MMP9, were among the genes identified to be most strongly downregulated at different timepoints after treatment (Fig 3, A). Comparisons of activity at baseline to Weeks 1, 2 and 4 post spesolimab treatment showed sustained downregulation of immune response pathways, as identified using IPA (Fig 3, B).

Baseline levels of serum biomarkers β-defensin 4A, CCL20, CXCL1, IL-17A, IL-1RN, IL-6 and IL-8 were variable across the seven patients (median [quartile 1, quartile 3], ng/L: β-defensin 4A, 28,804.8 [22,424.8, 28,804.8]; CCL20, 38.2 [12.1, 111.8]; CXCL1, 341.5 [187.4, 525.2]; IL-17A, 2.5 [0.7, 2.9]; IL-1RN, 560.6 [163.2, 993.0]; IL-6, 41.2 [14.2, 188.7]; IL-8, 50.5 [18.5, 134.6]). Treatment with spesolimab led to marked downregulation of select serum biomarkers linked to inflammatory (e.g. C-reactive protein, β-defensin 4A), neutrophilic (e.g. CXCL1, IL-8), innate (e.g. IL-1RN, IL-6) and Th17 (e.g. IL-17A, CCL20) pathways as early as Week 2 in select patients; these reductions were accompanied by decreases in clinical disease severity (Fig 3, C). See also Table 1.

Table 1. Top 50 differentially expressed genes (incldung fold change, pvalue and adjusted pvalue) in lesional skin pre and post treatment with spesolimab.

Ensembl gene ID	HGNC	Log2 Fold change VISIT9 vs VISIT3	pValues VISIT9 vs VISIT3	Adjusted pValues VISIT9 vs VISIT3
ENSG00000108342	CSF3	-4.74088102	0.0079781	0.03637123
ENSG00000162892	IL24	-4.35708057	0.00487161	0.02786815
ENSG00000142224	IL19	-3.9660122	0.00496594	0.02810991
ENSG00000162891	IL20	-3.7524392	0.00164141	0.01735926
ENSG00000136244	IL6	-3.67317327	0.0044921	0.02666965
ENSG00000124391	IL17C	-3.60900729	0.00426816	0.02596593
ENSG00000113302	IL12B	-3.42466655	0.00068099	0.01255625
ENSG00000263426	RN7SL471P	-3.40390066	0.00248866	0.02048129
ENSG00000163661	PTX3	-3.33345876	0.00528475	0.02895902
ENSG00000179826	MRGPRX3	-3.07363966	0.00190363	0.01838095
ENSG00000129988	LBP	-2.97584579	0.00704837	0.03399448
ENSG00000164047	CAMP	-2.92409414	0.00389863	0.02491137
ENSG00000110944	IL23A	-2.8952076	0.00346823	0.02354341
ENSG00000172602	RND1	-2.84842563	0.00076866	0.01293981
ENSG00000158859	ADAMTS4	-2.8454902	0.00016702	0.01094397
ENSG00000134668	SPOCD1	-2.81598394	0.00189836	0.01836339
ENSG00000262814	MRPL12	-2.67437157	0.01314382	0.04935877

ENSG00000163739	CXCL1	-2.67052924	0.00326973	0.02305719
ENSG00000123689	G0S2	-2.57158919	0.00491452	0.02796448
ENSG00000231123	SPATA20P1	-2.51714251	0.00739987	0.03495342
ENSG00000189410	SH2D5	-2.43001543	0.00135778	0.01599897
ENSG00000184557	SOCS3	-2.37954148	0.00198251	0.01871687
ENSG00000181649	PHLDA2	-2.37824334	0.00075578	0.01290546
ENSG00000257335	MGAM	-2.36888182	0.00901432	0.03907333
ENSG00000091137	SLC26A4	-2.33328282	0.0069774	0.0338197
ENSG00000100985	MMP9	-2.31713739	0.00132499	0.01594817
ENSG00000159339	PADI4	-2.30791137	0.00747457	0.03514
ENSG00000175592	FOSL1	-2.2870487	0.00261917	0.02095618
ENSG00000188389	PDCD1	-2.28592236	0.00073004	0.01282036
ENSG00000125148	MT2A	-2.28138957	3.4863E-05	0.01094397
ENSG00000229035	SPRR2C	-2.28015765	0.00520157	0.02872806
ENSG00000171631	P2RY6	-2.26336359	1.5754E-05	0.01094397
ENSG00000198535	C2CD4A	-2.26163584	0.00198179	0.01871687
ENSG00000099985	OSM	-2.26061026	0.00575847	0.03042149
ENSG00000125538	IL1B	-2.2585177	0.01328167	0.04963035
ENSG00000259230		-2.21902494	0.0003323	0.01110959
ENSG00000111012	CYP27B1	-2.21701925	0.00047675	0.0116585
ENSG00000005001	PRSS22	-2.21093725	0.00215497	0.01915111
ENSG00000275395	FCGBP	-2.20402093	1.3103E-07	0.00242084
ENSG00000187116	LILRA5	-2.19638079	0.00909475	0.03923293
ENSG00000196136	SERPINA3	-2.16337735	0.00288674	0.02174291
ENSG00000124216	SNAI1	-2.14783417	9.652E-05	0.01094397
ENSG00000277048		-2.1414334	0.00859644	0.03790642
ENSG00000198959	TGM2	-2.13859752	0.00135537	0.01599897
ENSG00000070729	CNGB1	-2.12908082	0.01266926	0.04814421
ENSG00000177943	MAMDC4	-2.12658565	1.9058E-05	0.01094397
ENSG00000125144	MT1G	-2.10288036	0.00082735	0.01330472
ENSG00000171223	JUNB	-2.09814442	0.00020919	0.01094397
ENSG00000185338	SOCS1	-2.09194354	1.0638E-05	0.01094397
ENSG00000137757	CASP5	-2.09138024	0.00050731	0.01177523

For example, differentially expressed genes/ proteins associated with the pro-inflammatory mediators (*TNF, IL1B, IL6*), neutrophil recruitment mediators (*CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2*), neutrophil-expressed transcripts (*NCF1, NCF2, NCF4, ELANE) or* in keratinocyte activation, differentiation and mediated inflammation transcripts (*IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24*) were found in lesional skin after 1 week of spesolimab treatment (Fig 1, C). Strong inhibition of T-cell activity was observed after spesolimab treatment, including T helper (Th)1/Th2 pathways and CD28 signalling in Th cells (Fig 1, A and B), and this was associated with the downregulation of elevated *IL17C* (Fig 1, C).

DISCUSSION

To our knowledge, this is the first study conducted to assess the global transcriptome profiles and gene set analysis in GPP. Here, we show that compared with healthy samples, GPP skin lesions modulated genes linked to Th1 and Th17 and innate inflammation signalling, neutrophilic activation and recruitment mediators, IL-36 and keratinocyte-driven inflammation pathways. Interestingly, most of the DEGs in the GPP transcriptome were unique to this disease (83.1%).

These results are consistent with genetic mutations and dysregulated gene expression previously reported in GPP. Genetic studies in patients with GPP have identified loss-of-function mutations in IL36RN and other genes functionally-associated with the IL-36 pathway, (e.g. AP1S3) and gain-of-function mutations in CARD14. In addition, transcriptome analysis of GPP lesions has shown an increase in innate immune inflammation response over Th1/Th17-related transcripts, including increased expression of genes encoding for IL-1β, IL-36α and IL-36γ, and enriched neutrophil and monocyte transcripts such as CXCL1, CXCL2, CXCL8.

Previously, the central role of IL-36 in the immunopathology of GPP was further supported by results from a proof-of-concept study to evaluate the efficacy and safety of spesolimab in patients with GPP. In this study, a single dose of 10 mg/kg intravenous spesolimab resulted in rapid (within 7 days) and sustained improvements (up to Week 20) in the clinical signs and symptoms of GPP. Further comparisons of the cellular and molecular expression patterns in non-lesional and lesional skin or blood from those patients revealed that spesolimab treatment resulted in a rapid normalisation or strong downregulation in levels of DEGs. This was characterised by reductions in the expression of pro-inflammatory mediators, neutrophil recruitment mediators, neutrophil-expressed transcripts and in keratinocyte activation; differentiation transcripts were found in lesional skin after 1 week of spesolimab treatment.

In addition, spesolimab induced rapid reductions of different highly infiltrated cell populations, including neutrophils, CD3+ T cells, CD11c+ cells, lipocalin-2-expressing cells, and decreased the expression of IL-36γ in lesional skin. The effects of spesolimab on T-cell subsets were consistent with the downregulation of elevated IL17C causing inhibition of the feed-forward inflammatory response and strongly affecting T-cell activation (Fig 1, C). Of relevance, these decreased gene and protein expression profiles

correlated with parallel improvement in clinical disease severity and pustulation clearance (Generalized Pustular Psoriasis Area and Severity Index and pustulation severity scores); this behaviour was in line with the strong and rapid reduction of C-reactive protein values compared with baseline levels in patients who achieved pustulation clearance (GPPGA pustule subscore of 0) after treatment with spesolimab in the proof-of-concept study. This highlights the importance of IL-36 pathway inhibition in the skin and blood of patients with GPP.

Based on these biomarker results in samples collected from patients presenting with acute GPP flares, we have identified that GPP involved key dysregulated pathways associated with IL-36, Th17, neutrophilic and keratinocyte-driven inflammation. In addition, the intervention by blocking IL-36R with spesolimab resulted in a rapid normalisation/downregulation of those genes in patients with GPP, with matched clinical improvement.

Example 2: Changes in the molecular profile of lesional skin and blood of patients with generalized pustular psoriasis treated with spesolimab are associated with clinical response

Generalized pustular psoriasis (GPP) is a rare, chronic inflammatory skin and systemic disease characterized by acute onset of disseminated pustular eruptions. GPP is associated with significant morbidity, and GPP flares can be life-threatening if untreated. The pathogenesis of GPP involves dysregulated interleukin (IL)-36 signaling. Spesolimab, a monoclonal antibody that targets the IL-36 receptor, was efficacious in patients experiencing a GPP flare. In the Effisayil™ 1 study, spesolimab resulted in rapid pustular and skin clearance within 1 week versus placebo in patients experiencing a GPP flare. We identified 5208 gene transcripts that were differentially expressed (2861 decreased, 2347 elevated) in lesional versus non-lesional skin biopsies (adjusted p-value ≤0.05, log2 fold change ≥1) at baseline. These included genes associated with the IL-36 family (IL36A, IL36B, IL36G), neutrophilic recruitment (CXCL1, CXCL6, CXCL8), proinflammatory cytokines (IL6, IL19, IL20), and skin inflammation (DEFB4a, S100A7, S100A8, S100A9). A significant number of genes in lesional skin were modulated 1 week after (324

decreased, 622 increased; adjusted p-value ≤0.05, log2 fold change ≥1) and 7–8 weeks after (1115 decreased, 1425 increased; adjusted p-value ≤0.05, log2 fold change ≥1) a single dose of spesolimab (900 mg intravenously). Patients who achieved the primary endpoint (GPP Physician Global Assessment pustulation subscore of 0 by Week 1) demonstrated significant changes from baseline in differentially expressed genes in lesional skin. Histopathological changes in select biomarkers (NE, K16, beta defensin 2, IL-17C) were observed in lesional versus non-lesional skin pre- versus post-treatment at Week 8. There were also reductions in serum biomarker levels, including IL-17C, IL-20, TGF-α, IL-24, CCL4, CCL19, IL1-RN, and CCL20, which were sustained until Week 12 and correlated with primary endpoint achievement. In summary, the clinical efficacy of spesolimab in patients with a GPP flare in the Effisayil™ 1 study was associated with modulation of key pathogenic pathways in skin and blood. See Table 2.

Table 2. Correlation of change from baseline of protein biomarker with GPPGA score of 0 or 1 at week 1 per treatment group – SAF (sorted by p-value)

Parameter	Treatment	N	Correlation coefficient	95% Confidence interval		p-value
				Lower	Upper	
Interleukin-17A [NPX]	Speso 900 mg IV SD	26	-0.699	-0.855	-0.427	<.001
Interleukin-17C [NPX]	Speso 900 mg IV SD	26	-0.662	-0.835	-0.369	<.001
Interleukin-20 [NPX]	Speso 900 mg IV SD	26	-0.635	-0.821	-0.329	<.001
Trail receptor 1 [NPX]	Speso 900 mg IV SD	27	-0.578	-0.786	-0.254	0.001
Interleukin-24 [NPX]	Speso 900 mg IV SD	24	-0.603	-0.810	-0.264	0.001
Trail receptor 2 [NPX]	Speso 900 mg IV SD	27	-0.565	-0.778	-0.235	0.001
MMP [NPX]	Speso 900 mg IV SD	27	-0.559	-0.775	-0.226	0.002
IL-12B [NPX]	Speso 900 mg IV SD	26	-0.566	-0.782	-0.229	0.002
IL-27 [NPX]	Speso 900 mg IV SD	27	-0.555	-0.772	-0.222	0.002
FGF-21 [NPX]	Speso 900 mg IV SD	26	-0.559	-0.777	-0.217	0.002
C-C motif chemokine 19 [NPX]	Speso 900 mg IV SD	26	-0.543	-0.768	-0.196	0.003
OSP-1 [NPX]	Speso 900 mg IV SD	26	-0.521	-0.756	-0.167	0.005
C-C motif chemokine 20 [NPX]	Speso 900 mg IV SD	26	-0.490	-0.741	-0.138	0.008
IL-1RN [NPX]	Speso 900 mg IV SD	27	-0.482	-0.739	-0.125	0.009
C-C motif chemokine 4 [NPX]	Speso 900 mg IV SD	26	-0.473	-0.727	-0.124	0.012
CDPR [NPX]	Speso 900 mg IV SD	27	-0.469	-0.715	-0.098	0.013
Placenta growth factor [NPX]	Speso 900 mg IV SD	27	-0.457	-0.712	-0.093	0.014
TGF-alpha [NPX]	Speso 900 mg IV SD	26	-0.458	-0.718	-0.085	0.016
Oncostatin-M [NPX]	Speso 900 mg IV SD	26	-0.453	-0.715	-0.079	0.017
Interleukin-18 receptor 1 [NPX]	Speso 900 mg IV SD	26	-0.443	-0.708	-0.066	0.023
Hepatocyte growth factor [NPX]	Speso 900 mg IV SD	26	-0.441	-0.707	-0.064	0.021

Pearson correlation coefficient with Fisher's Z test p-values.
OC approach is used for the protein biomarkers. NRI imputation is applied for GPPGA.
Correlation coefficient for placebo group was omitted due to very small number of responders in the placebo group.

While certain aspects and embodiments of the invention have been described, these have been presented by way of example only, and are not intended to limit the scope of the invention. Indeed, the novel methods and systems described herein may be embodied in a variety of other forms without departing from the spirit thereof. The accompanying claims and their equivalents are intended to cover such forms or modifications as would fall within the scope and spirit of the invention.

All patents and/or publications including journal articles cited in this disclosure are expressly incorporated herein by reference.

Claims

1. A method for detecting the presence or absence of a beneficial response in a patient after administration of an anti-interleukin-36 receptor antibody (anti-IL-36R antibody), comprising:

   a) obtaining a biological sample from the patient;

   b) measuring in said sample the level of one or more biomarkers, or the level of expression of one or more biomarkers;

   c) comparing the level to control value of the level of the biomarkers; and

   d) determining whether or not the difference in levels between the sample and the control reflects a beneficial response in the patient,

   wherein the one or more biomarkers comprise genes/ proteins associated with pro-inflammatory processes (TNF, IL1B, IL6), neutrophil recruitment (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2), neutrophil occurrence (NCF1, NCF2, NCF4, ELANE) or in keratinocyte activation, differentiation and mediated inflammation transcripts (IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24), CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP, IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5.

2. The method of claim 1, wherein the level of the gene or the protein of said one or more biomarker is measured.

3. The method of any one of claims 1 to 2, wherein the patient suffers from generalized pustular psoriasis (GPP).

4. The method of any one of claims 1 to 3, wherein the control value is calculated using samples from subjects that do not suffer from GPP.

5. The method of any one of claims 1 to 3, wherein the control value is determined using samples from known GPP patients.

6. The method of any one of claims 1 to 3, wherein the control value is determined using at least one previous sample taken from the patient.

7. The method of any one of claims 1 to 7, wherein the biological sample is a skin biopsy, blood, plasma or serum sample.

8. The method of any one of claims 1 to 8, wherein the anti-IL-36R antibdoy is spesolimab.

9. The method any one of claims 1 to 9, wherein the levels of biomarkers are determined by RNA sequencing or ELISA and IHC.

10. The method according to any one of claims 1 to 10, further comprising continuing the administration of the IL-36R antagonist to the patient if the difference in levels between the sample and the control reflects a beneficial response in the patient.

11. A method of determining whether a potential therapeutic agent is efficacious in the treatment of GPP comprising:

a) obtaining a first biological sample from a GPP patient prior to being treated with the potential therapeutic agent;

b) treating the GPP patient with the potential therapeutic agent;

c) obtaining a second biological sample from the GPP patient after being treated with the potential therapeutic agent;

d) measuring in said first and second sample the levels of expression of one or more biomarkers; and

e) comparing the biomarker levels in the second sample to the levels in the first sample,

wherein changes (e.g., lower or higher) in biomarker levels in the second sample than in the first sample indicate that the potential therapeutic agent is efficacious, and further wherein the one or more biomarkers comprise genes associated with pro-inflammatory processes (TNF, IL1B, IL6), neutrophil recruitment (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2), neutrophil occurrence (NCF1, NCF2, NCF4, ELANE) or in keratinocyte activation, differentiation and mediated inflammation transcripts (IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24), CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP, IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5.

12. The method according to claim 12, wherein changes (e.g., lower or higher) in biomarker levels in the second sample than in the first sample correlate with improvement in clinical efficacy measures.

13. The method according to claims 12 or 13, further comprising continuing the treatment of the patient if biomarker levels in the second sample change (e.g., are higher or lower) as compared to the first sample.

14. A method of treating GPP in a subject comprising:

a) determining whether to initiate treatment of the subject, modify the treatment dose, modify the dosing interval, or discontinue treatment, based on the method of any of the preceding claims; and

b) modifying the treatment regimen based on the determination.

15. A method of monitoring patient response to a GPP treatment comprising:

a) obtaining a first biological sample from the patient;

b) measuring the level of one or more biomarkers in said first biological sample, wherein said one or more biomarkers comprise genes/ proteins associated with pro-inflammatory processes (TNF, IL1B, IL6), neutrophil recruitment (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2), neutrophil occurrence (NCF1, NCF2, NCF4, ELANE) or in keratinocyte activation, differentiation and mediated inflammation transcripts (IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24), CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP, IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5;

c) administering a treatment compound to the patient;

d) obtaining a second biological sample from the patient;

e) measuring the level of said one or more biomarkers in said second biological sample; and

f) comparing the levels of the one or more biomarkers obtained from first and second biological samples;

wherein a change (e.g., high, low) in the level of the one or more biomarkers in the second biological sample indicates an effective response.

16. A method for monitoring patient compliance with a drug treatment protocol for GPP comprising:

a) obtaining a biological sample from said patient;

b) measuring the level of one or more biomarkers, wherein the one or more biomarkers comprise genes/ proteins associated with pro-inflammatory processes (TNF, IL1B, IL6), neutrophil recruitment (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCR1, CXCR1, CXCR2), neutrophil occurrence (NCF1, NCF2, NCF4, ELANE) or in keratinocyte activation, differentiation and mediated inflammation transcripts (IL36A, IL36G, IL17C, IL19, IL20, IL22, IL24), CSF3, IL24, IL19, IL20, IL6, IL17C, IL12B, RN7SL471P, PTX3, MRGPRX3, LBP, CAMP,

IL23A, RND1, ADAMTS4, SPOCD1, MRPL12, CXCL1, G0S2, SPATA20P1, SH2D5, SOCS3, PHLDA2, MGAM, SLC26A4, MMP9, PADI4, FOSL1, PDCD1, MT2A, SPRR2C, P2RY6, C2CD4A, OSM, IL1B, CYP27B1, PRSS22, FCGBP, LILRA5, SERPINA3, SNAI1, TGM2, CNGB1, MAMDC4, MT1G, JUNB, SOCS1or CASP5; and

c) determining if the level is changed in the patient sample compared to the level in a control untreated sample;

wherein a decreased level indicates patient compliance with said drug treatment protocol.

17. The method of any one of claims 15 to 17, wherein the level of the one or more biomarkers in the second biological sample is decreased by at least about 50%, 55%, 60%, 65%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more as compared to the level in the first biological sample.

18. The method of any one of claims 15 to 17, wherein the biological sample is a skin biopsy, blood, plasma or serum sample.

19. The method of any one of claims 15 to 17, wherein the anti-IL-36R antibody is spesolimab.

20. The method of any one of claims 15 to 17, wherein the levels of biomarkers are determined by RNA sequencing or ELISA and IHC.

FIG. 1A

FIG. 1AA

FIG. 1AB

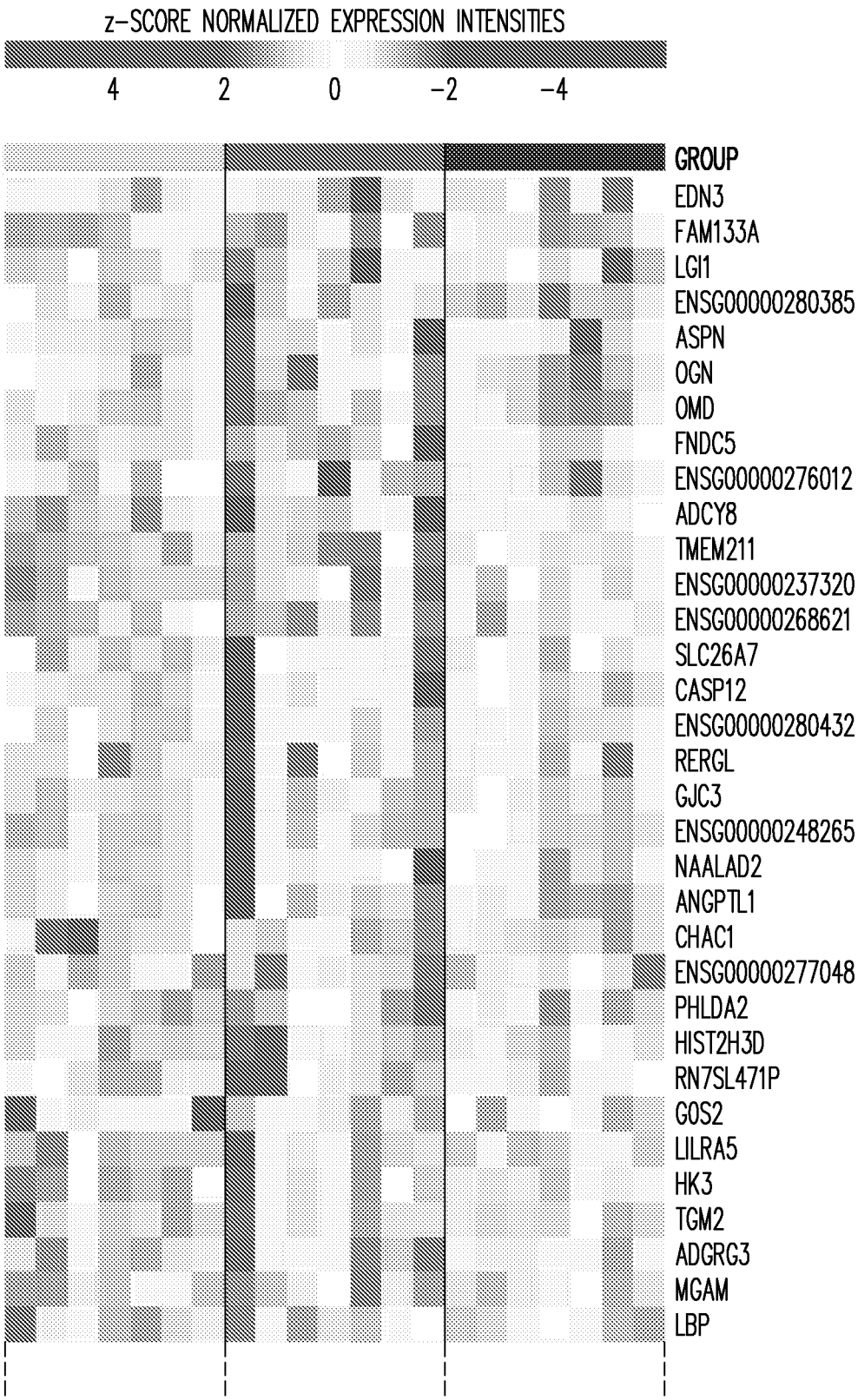

FIG. 1AA

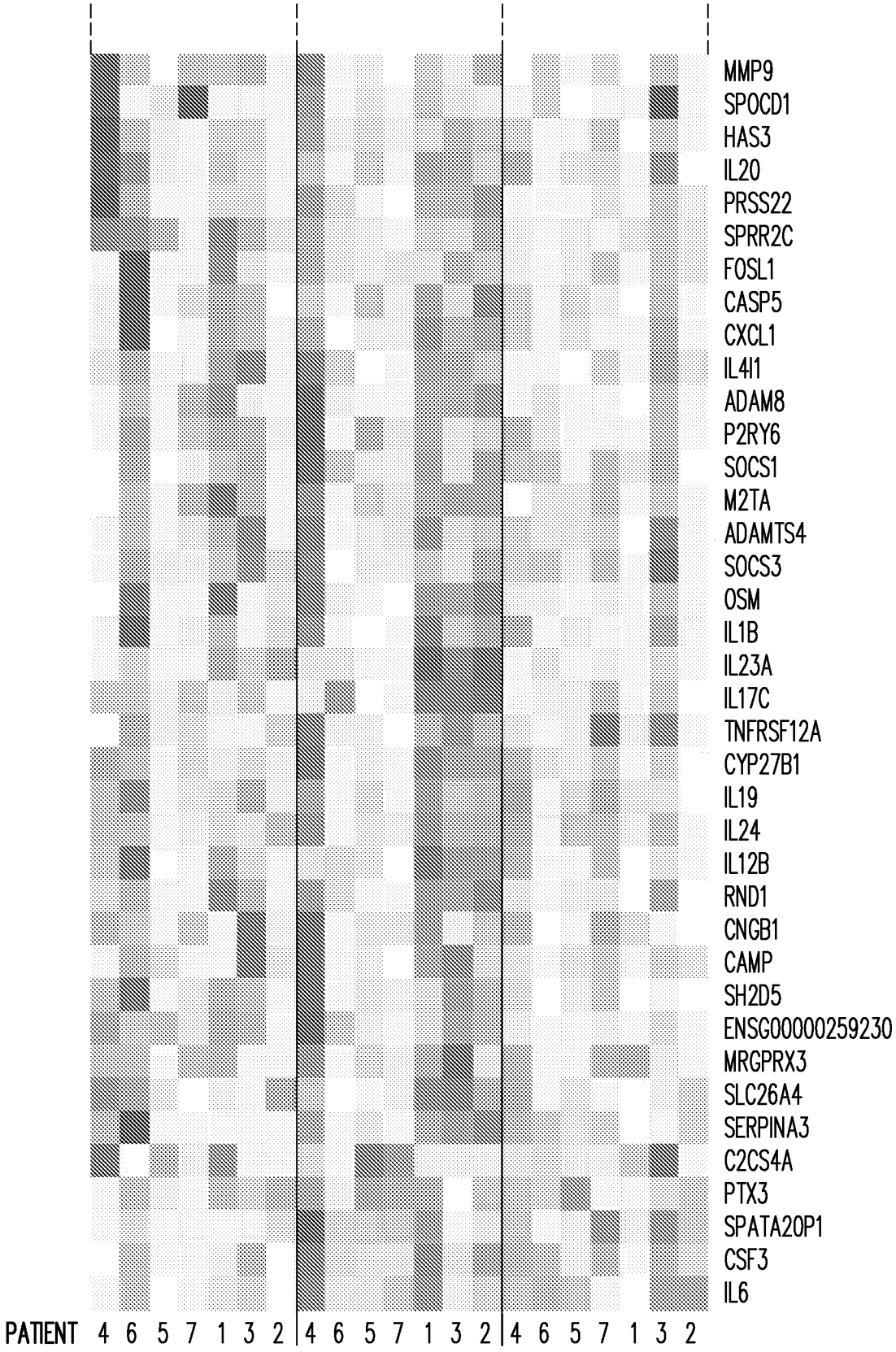
MMP9
SPOCD1
HAS3
IL20
PRSS22
SPRR2C
FOSL1
CASP5
CXCL1
IL4I1
ADAM8
P2RY6
SOCS1
M2TA
ADAMTS4
SOCS3
OSM
IL1B
IL23A
IL17C
TNFRSF12A
CYP27B1
IL19
IL24
IL12B
RND1
CNGB1
CAMP
SH2D5
ENSG00000259230
MRGPRX3
SLC26A4
SERPINA3
C2CS4A
PTX3
SPATA20P1
CSF3
IL6
PATIENT 4 6 5 7 1 3 2 4 6 5 7 1 3 2 4 6 5 7 1 3 2

FIG. 1AB

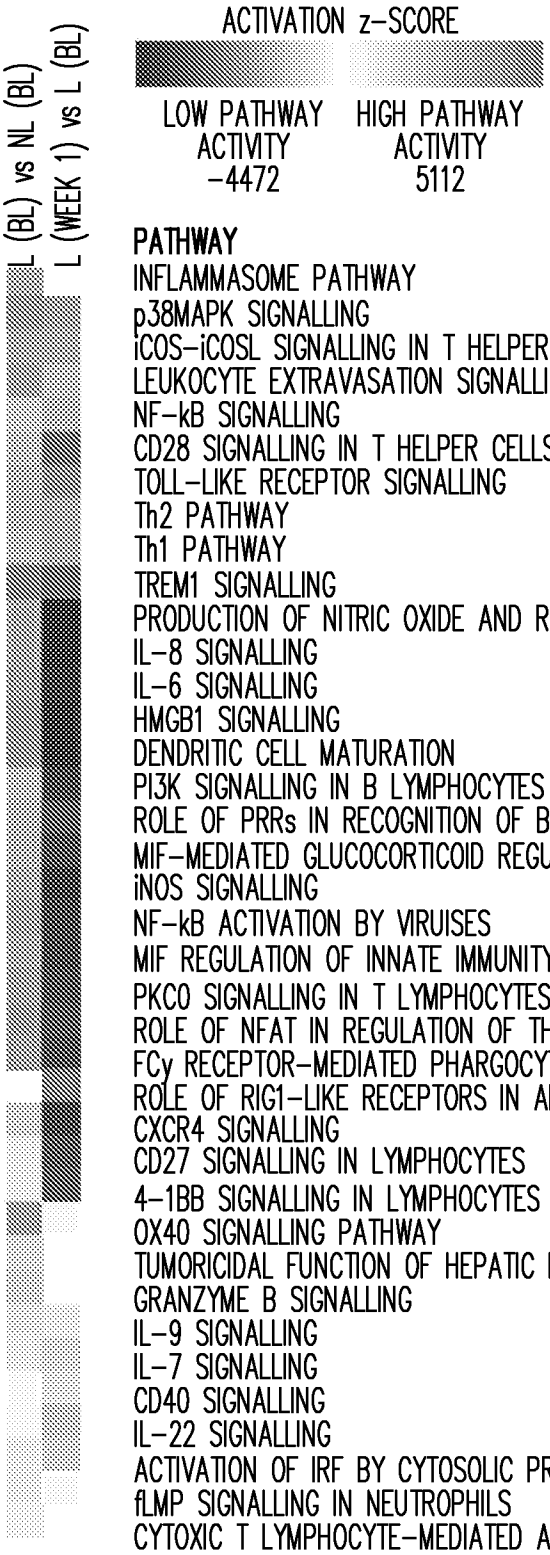
L (BL) vs NL (BL)
L (WEEK 1) vs L (BL)
ACTIVATION z-SCORE
LOW PATHWAY ACTIVITY -4472
HIGH PATHWAY ACTIVITY 5112
PATHWAY
INFLAMMASOME PATHWAY
p38MAPK SIGNALLING
iCOS-iCOSL SIGNALLING IN T HELPER CELLS
LEUKOCYTE EXTRAVASATION SIGNALLING
NF-kB SIGNALLING
CD28 SIGNALLING IN T HELPER CELLS
TOLL-LIKE RECEPTOR SIGNALLING
Th2 PATHWAY
Th1 PATHWAY
TREM1 SIGNALLING
PRODUCTION OF NITRIC OXIDE AND REACTIVE OXYGEN SPECIES IN MACROPHAGES
IL-8 SIGNALLING
IL-6 SIGNALLING
HMGB1 SIGNALLING
DENDRITIC CELL MATURATION
PI3K SIGNALLING IN B LYMPHOCYTES
ROLE OF PRRs IN RECOGNITION OF BACTERIA AND VIRUSES
MIF-MEDIATED GLUCOCORTICOID REGULATION
iNOS SIGNALLING
NF-kB ACTIVATION BY VIRUISES
MIF REGULATION OF INNATE IMMUNITY
PKCO SIGNALLING IN T LYMPHOCYTES
ROLE OF NFAT IN REGULATION OF THE IMMUNE RESPONSE
FCγ RECEPTOR-MEDIATED PHARGOCYTOSIS IN MACROPHAGES AND MONOCYTES
ROLE OF RIG1-LIKE RECEPTORS IN ANTIVIRAL INNATE IMMUNITY
CXCR4 SIGNALLING
CD27 SIGNALLING IN LYMPHOCYTES
4-1BB SIGNALLING IN LYMPHOCYTES
OX40 SIGNALLING PATHWAY
TUMORICIDAL FUNCTION OF HEPATIC NATURAL KILLER CELLS
GRANZYME B SIGNALLING
IL-9 SIGNALLING
IL-7 SIGNALLING
CD40 SIGNALLING
IL-22 SIGNALLING
ACTIVATION OF IRF BY CYTOSOLIC PRRs
fLMP SIGNALLING IN NEUTROPHILS
CYTOXIC T LYMPHOCYTE-MEDIATED APOPTOSIS OF TARGET CELLS

FIG. 1B

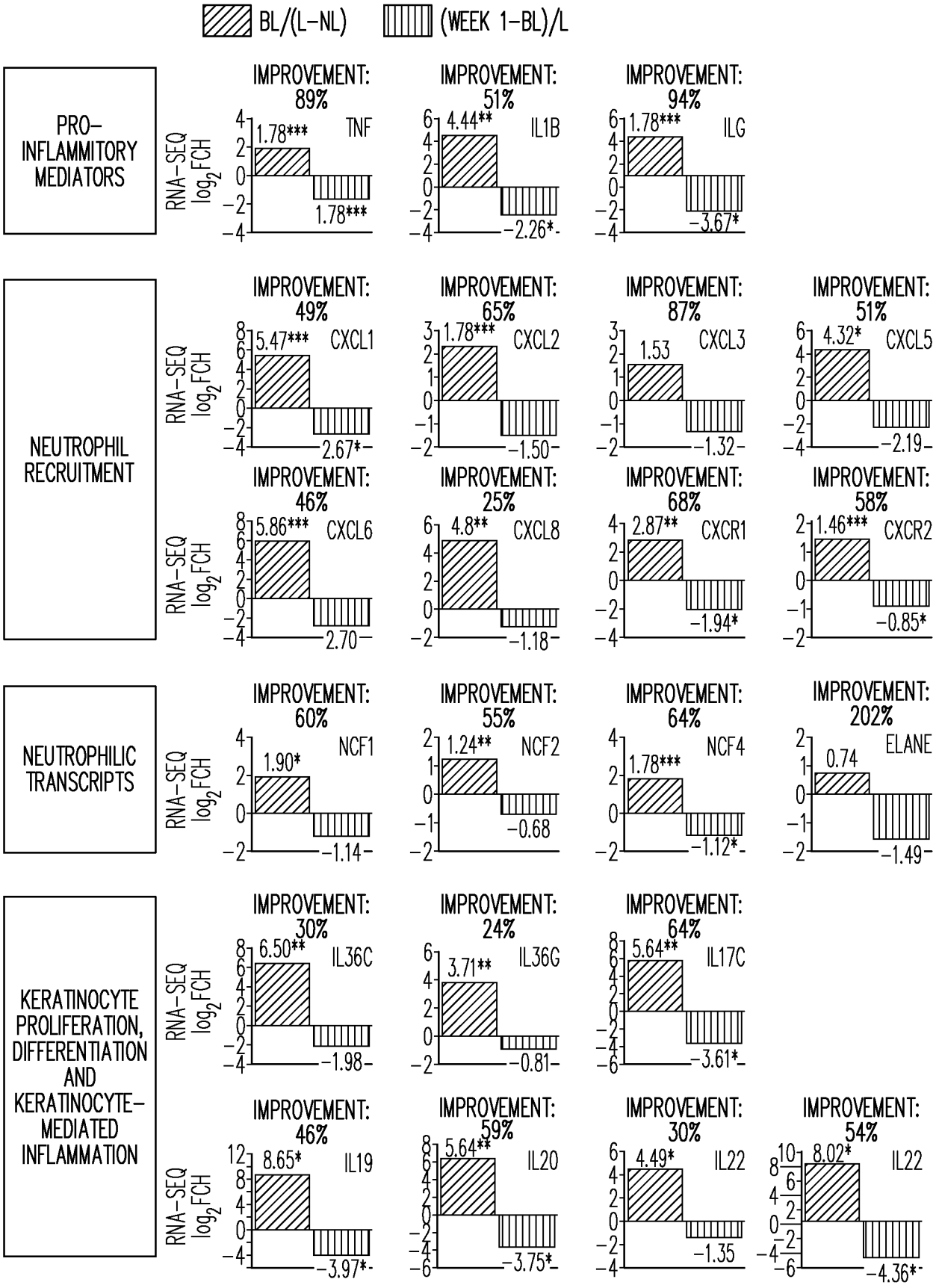
BL/(L-NL)
(WEEK 1-BL)/L
PRO-INFLAMMITORY MEDIATORS
NEUTROPHIL RECRUITMENT
NEUTROPHILIC TRANSCRIPTS
KERATINOCYTE PROLIFERATION, DIFFERENTIATION AND KERATINOCYTE-MEDIATED INFLAMMATION
RNA-SEQ log2FCH
IMPROVEMENT: 89%
TNF
1.78***
1.78***
IMPROVEMENT: 51%
IL1B
4.44**
-2.26*
IMPROVEMENT: 94%
ILG
1.78***
-3.67*
IMPROVEMENT: 49%
CXCL1
5.47***
2.67*
IMPROVEMENT: 65%
CXCL2
1.78***
-1.50
IMPROVEMENT: 87%
CXCL3
1.53
-1.32
IMPROVEMENT: 51%
CXCL5
4.32*
-2.19
IMPROVEMENT: 46%
CXCL6
5.86***
2.70
IMPROVEMENT: 25%
CXCL8
4.8**
-1.18
IMPROVEMENT: 68%
CXCR1
2.87**
-1.94*
IMPROVEMENT: 58%
CXCR2
1.46***
-0.85*
IMPROVEMENT: 60%
NCF1
1.90*
-1.14
IMPROVEMENT: 55%
NCF2
1.24**
-0.68
IMPROVEMENT: 64%
NCF4
1.78***
-1.12*
IMPROVEMENT: 202%
ELANE
0.74
-1.49
IMPROVEMENT: 30%
IL36C
6.50**
-1.98
IMPROVEMENT: 24%
IL36G
3.71**
-0.81
IMPROVEMENT: 64%
IL17C
5.64**
-3.61*
IMPROVEMENT: 46%
IL19
8.65*
-3.97*
IMPROVEMENT: 59%
IL20
5.64**
-3.75*
IMPROVEMENT: 30%
IL22
4.49*
-1.35
IMPROVEMENT: 54%
IL22
8.02*
-4.36*

FIG. 1C

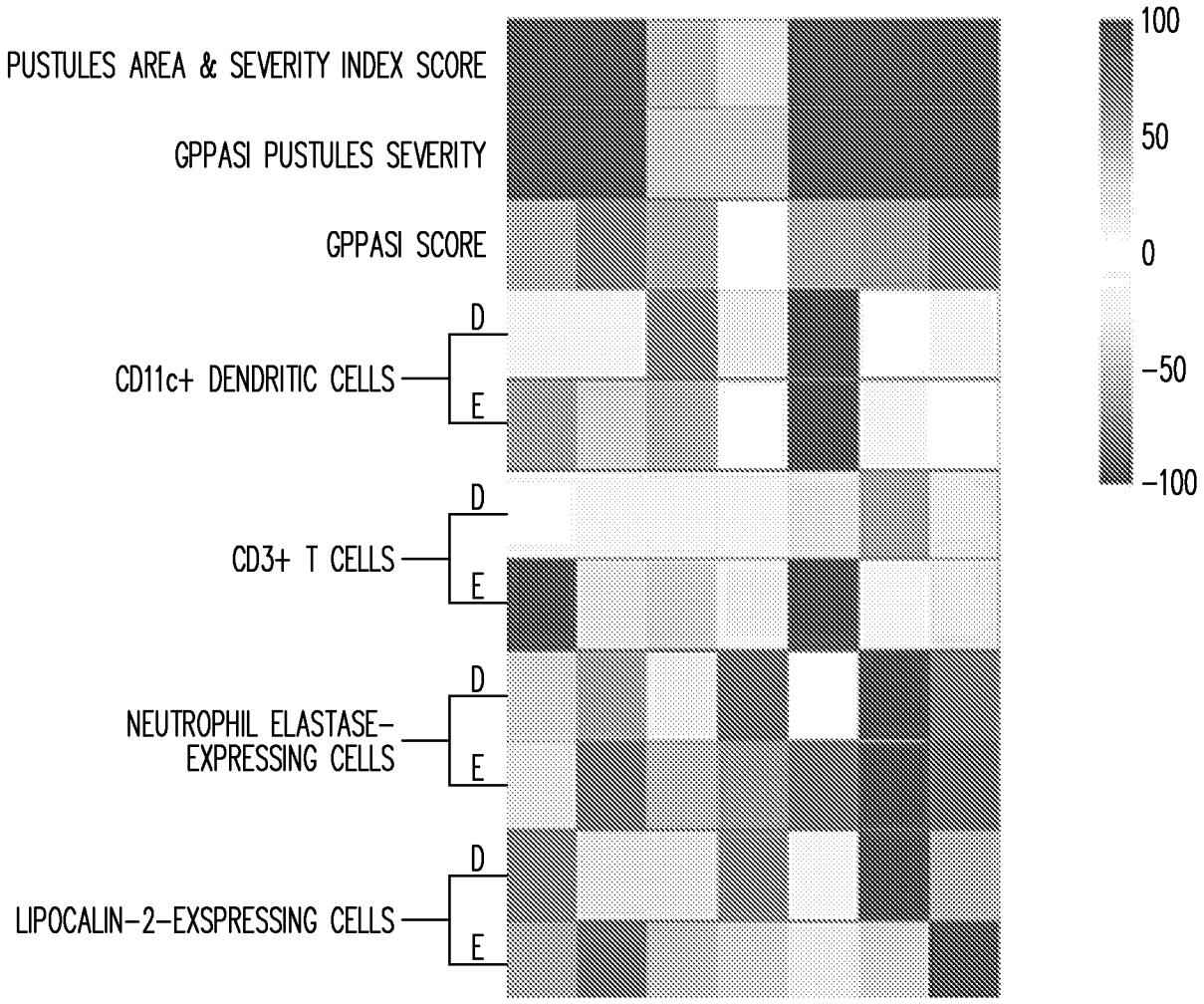
PUSTULES AREA & SEVERITY INDEX SCORE
GPPASI PUSTULES SEVERITY
GPPASI SCORE
CD11c+ DENDRITIC CELLS
D
E
CD3+ T CELLS
D
E
NEUTROPHIL ELASTASE-EXPRESSING CELLS
D
E
LIPOCALIN-2-EXSPRESSING CELLS
D
E
100
50
0
-50
-100

FIG. 2A

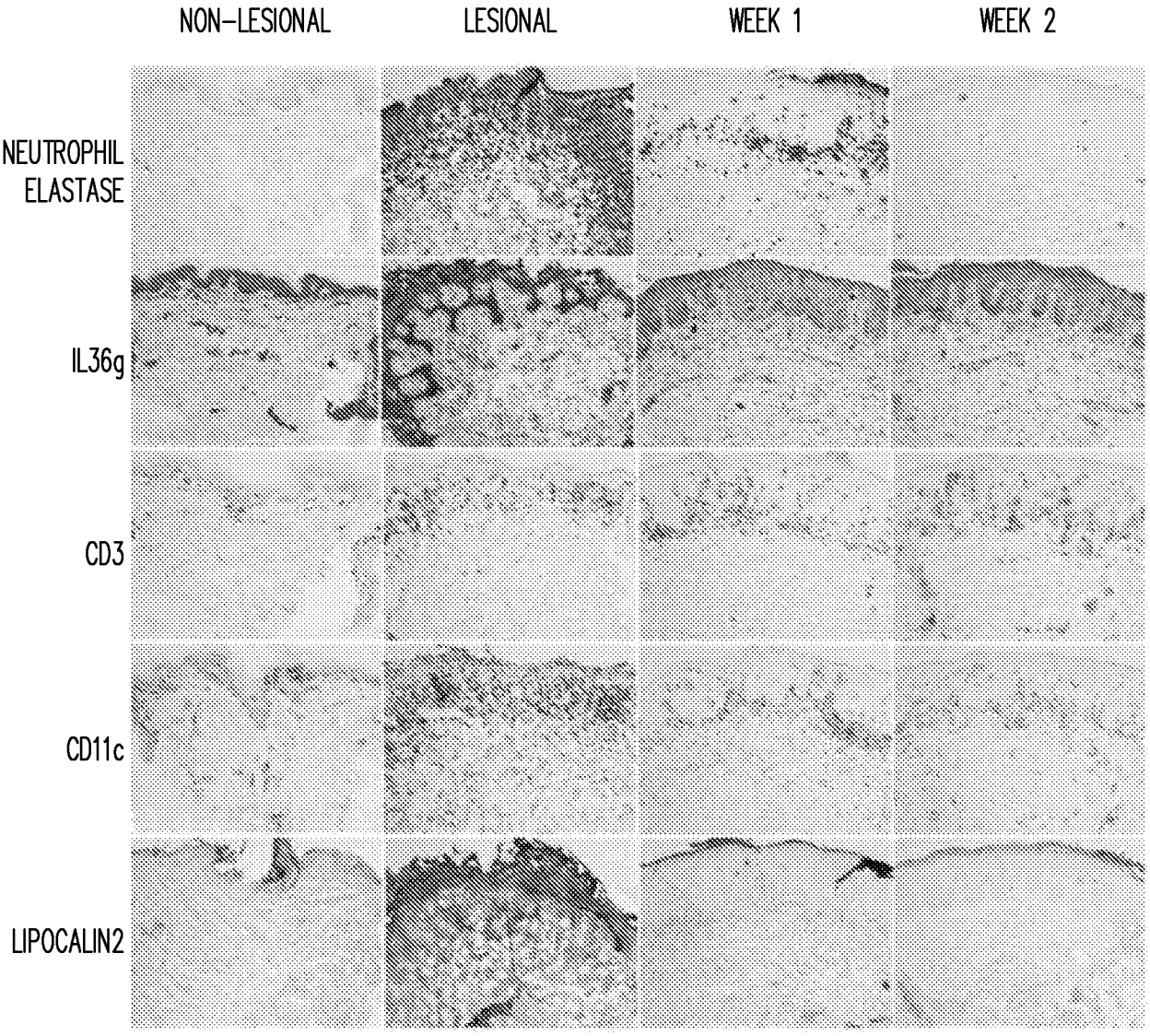
NON-LESIONAL
LESIONAL
WEEK 1
WEEK 2
NEUTROPHIL ELASTASE
IL36g
CD3
CD11c
LIPOCALIN2

FIG. 2B

FIG. 3A

FIG. 3AA

FIG. 3AB

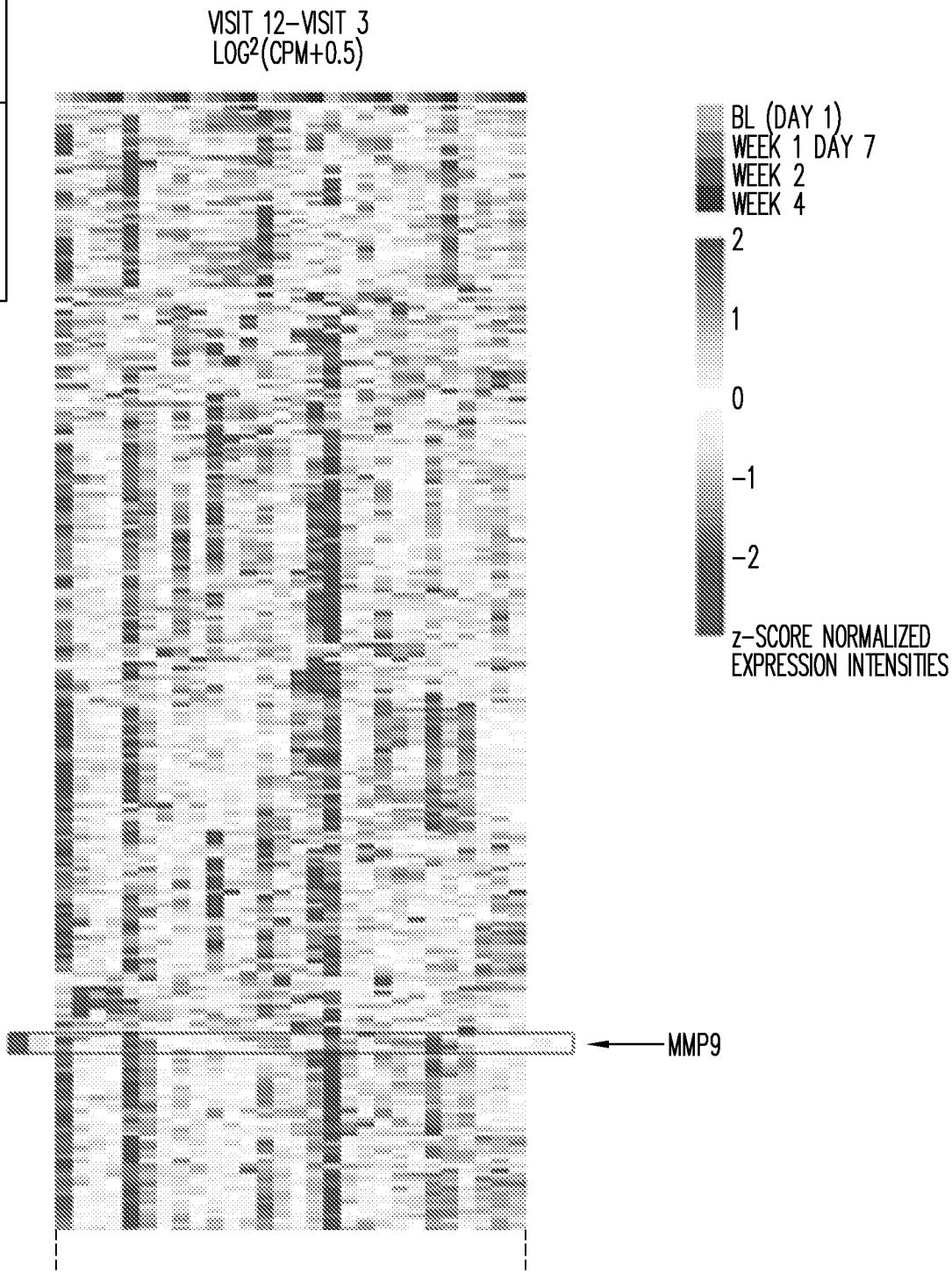

FIG. 3AA

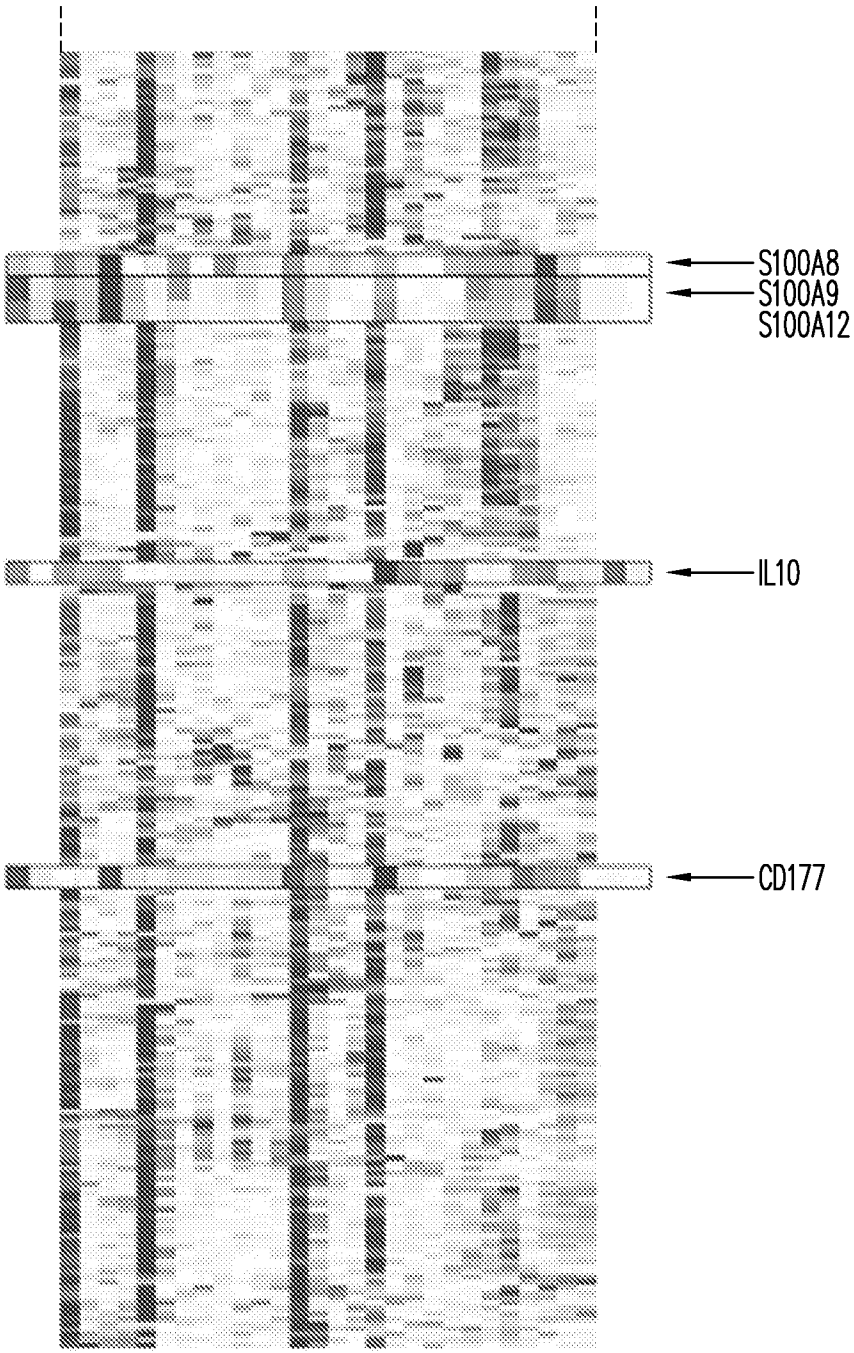

FIG. 3AB

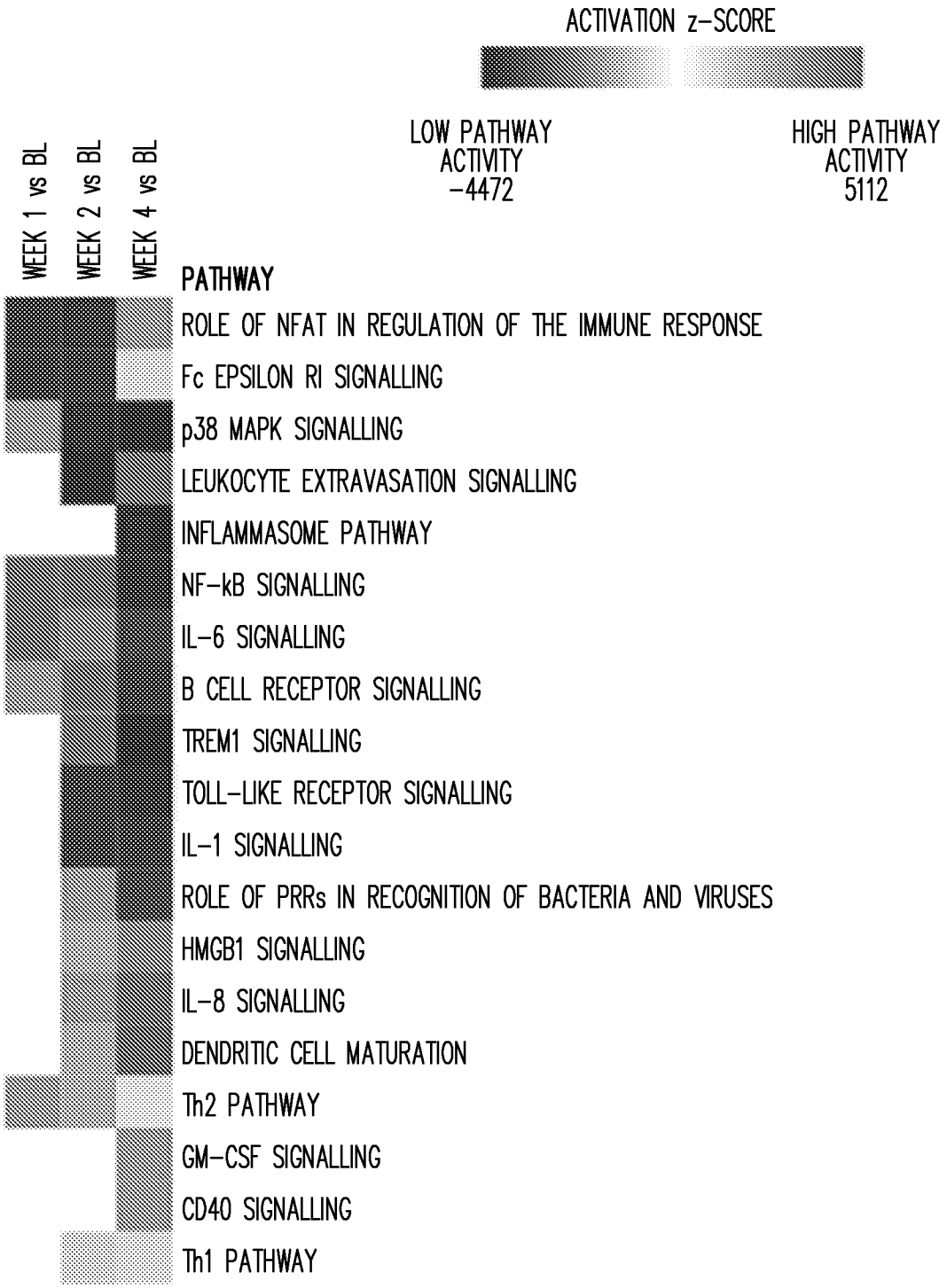
ACTIVATION z-SCORE
LOW PATHWAY ACTIVITY -4472
HIGH PATHWAY ACTIVITY 5112
WEEK 1 vs BL
WEEK 2 vs BL
WEEK 4 vs BL
PATHWAY
ROLE OF NFAT IN REGULATION OF THE IMMUNE RESPONSE
Fc EPSILON RI SIGNALLING
p38 MAPK SIGNALLING
LEUKOCYTE EXTRAVASATION SIGNALLING
INFLAMMASOME PATHWAY
NF-kB SIGNALLING
IL-6 SIGNALLING
B CELL RECEPTOR SIGNALLING
TREM1 SIGNALLING
TOLL-LIKE RECEPTOR SIGNALLING
IL-1 SIGNALLING
ROLE OF PRRs IN RECOGNITION OF BACTERIA AND VIRUSES
HMGB1 SIGNALLING
IL-8 SIGNALLING
DENDRITIC CELL MATURATION
Th2 PATHWAY
GM-CSF SIGNALLING
CD40 SIGNALLING
Th1 PATHWAY

FIG. 3B

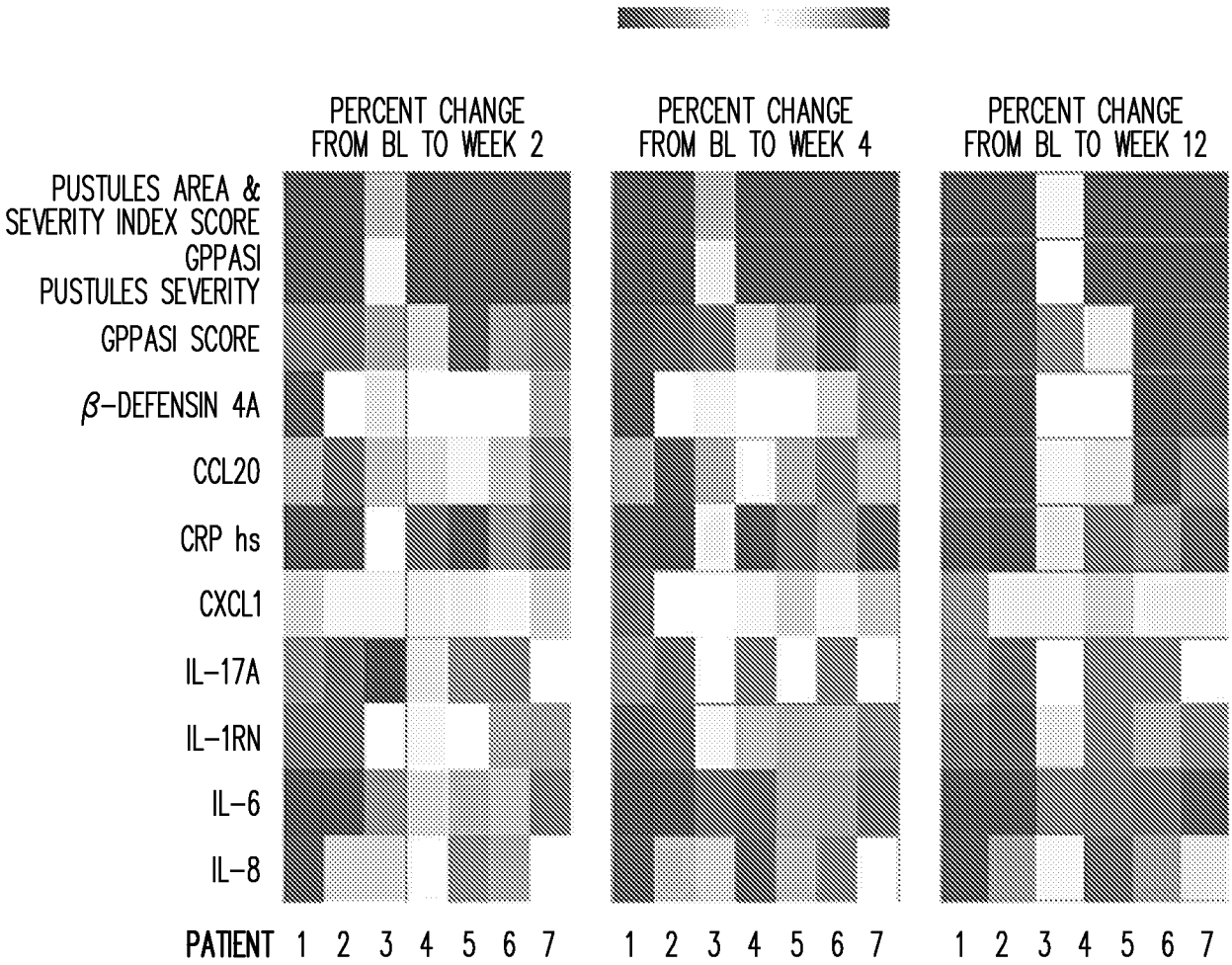
PERCENT CHANGE FROM BL TO WEEK 2
PERCENT CHANGE FROM BL TO WEEK 4
PERCENT CHANGE FROM BL TO WEEK 12
PUSTULES AREA & SEVERITY INDEX SCORE
GPPASI PUSTULES SEVERITY
GPPASI SCORE
β-DEFENSIN 4A
CCL20
CRP hs
CXCL1
IL-17A
IL-1RN
IL-6
IL-8
PATIENT 1 2 3 4 5 6 7 1 2 3 4 5 6 7 1 2 3 4 5 6 7

FIG. 3C