TREATING INFLAMMATORY COMMON VARIABLE IMMUNODEFICIENCY WITH A BRUTON'S TYROSINE KINASE INHIBITOR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is an International Application filed pursuant to the Patent Cooperation Treaty and claims benefit of priority from U.S. Provisional Patent Application No. 63/631,500, which was filed April 9, 2024, the entire contents of which are incorporated herein by reference.

BACKGROUND

[0002] Common variable immunodeficiency (CVID) is a primary antibody deficiency disorder. Due to the significant lack of immunoglobulins, CVID patients are susceptible to severe and recurrent infections. Additionally, in ~50% of CVID patients, CVID leads to systemic immune activation and multi-organ inflammatory diseases in the lungs, gastrointestinal tract, liver, and/or lymphoid organs. Additionally, systemic immune activation in CVID leads to an increased risk for autoimmune cytopenia. In aggregate, chronic systemic immune activation and its consequences lead to an 11-fold increase in morbidity and mortality in CVID.

[0003] Immunologically, inflammatory complications in CVID (referred to as "inflammatory-CVID") is notably distinguished by an overarching IFN-γ signature. In addition, however, these patients exhibit broader cytokine dysregulation (e.g., elevated TNF-α, IL-6) and monocyte activation, such as elevated soluble CD14 (sCD14) levels. Pathologic proliferation of B cell subsets and granulomatous inflammation are also prevalent. Addressing inflammatory-CVID has posed a significant conundrum due to the broad scope of implicated immune mediators, underlying immune deficiency, and infection risks. To-date, there is no approved therapy for modulating systemic immune activation in CVID.

[0004] The present disclosure is directed to overcoming these and other deficiencies in the art.

SUMMARY

[0005] In an aspect, provided is method for treating common variable immunodeficiency (CVID) in a human patient in need of such treatment, including administering to the human patient a therapeutically effective amount of (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-

methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof.

[0006] In another aspect provided is a method for treating an inflammatory-CVID condition resulting from bacterial translocation in a human patient in need of such treatment, including administering to the human patient a therapeutically effective amount of (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof, optionally wherein the bacterial translocation is associated with a human immunodeficiency infection in the patient, an inflammatory bowel disease in the patient, and liver fibrosis in the patient.

[0007] In an example, the patient has or has had inflammatory-CVID and the administration includes treating the inflammatory complications. In another example, the inflammatory-CVID condition is thrombocytopenia. In still another example, rilzabrutinib is administered at least once a day.

[0008] In another example, the inflammatory-CVID includes one or more of a CVID-associated autoimmunity, a CVID-associated chronic lung disease, a CVID-associated splenomegaly, a CVID-associated inflammatory gastrointestinal disease, a CVID-associated inflammatory liver disease, a CVID-associated lymphocytic interstitial lung disease, a CVID-associated interstitial lung disease, a CVID-associated granulomatous lung disease, a CVID-associated enteropathy, a CVID-associated gastropathy, a CVID-associated nodular regenerative hyperplasia of the liver, a CVID-associated granulomatous liver disease, a CVID-associated biliary cholangitis, a CVID-associated lymphoproliferation, a CVID-associated splenomegaly, and a CVID-associated systemic granulomatous disease.

[0009] In still another example, the administration includes decreasing a stimulatory effect of bacterial DNA, lipopolysaccharide (LPS), endotoxin, or any combination of two or more of the foregoing, on peripheral blood mononuclear cells (PBMC) secretion of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin 1-alpha (IL-1A), interleukin 6 (IL-6), interleukin-12 subunit beta (IL-12B), interleukin 18 (IL-18), soluble cluster of differentiation 14 (sCD14), signaling lymphocytic activation molecule 1 (SLAMF1), colony stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), chemokine ligand 9 (CXCL9), or any combination of two or more of the foregoing.

[0010] In yet another example, the administration includes decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of IFN-γ. In a further

example, the administration includes decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of TNF-α. In another further example, the administration includes decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of IL-6. In still a further example, the administration includes decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC secretion of sCD14.

[0011] I another aspect, provided is a method of inhibiting secretion of an inflammatory factor by peripheral blood mononuclear cells (PBMC), including contacting the PBMC with (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof, and the inflammatory factor is one or both of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6). In an example, contacting the PBMC includes administering rilzabrutinib to a subject. In another example, rilzabrutinib is administered at least once a day. In still another example, the inhibiting includes decreasing a stimulatory effect of bacterial DNA, lipopolysaccharide (LPS), endotoxin, or any combination of two or more of the foregoing, on the secretion. In yet another example, the inflammatory factor is TNF-α. In a further example, the inflammatory factor is IL-6. In another further example, the inhibition is by up to about 50%.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] These and other features, aspects, and advantages of the present disclosure will become better understood when the following detailed description is read with reference to the accompanying drawings, wherein:

[0013] FIGs. 1A-1I depict examples of serum cytokine profiling revealing distinct inflammatory differentials between common variable immunodeficiency (CVID) and x-linked agammaglobulinemia (XLA), in accordance with aspects of the present disclosure. High dimensional cytokine profiling of serum samples from CVID patients (N = 20) and XLA (N = 12). (A) Heatmap of global protein expression profile in CVID and XLA participants. The proteins were ordered by their statistical significance in group comparisons and the magnitude of fold-changes. Participants were ordered by the total protein expression levels. Top bar annotation corresponds to the patient group (CVID in red, XLA in blue). (B) Volcano plot showing fold changes (FC) of protein expressions in CVID relative to XLA, with the corresponding adjusted P values in group comparisons. The filled dots indicated significant (s) findings, and the open dots indicated non-significant (n.s.) findings. The black

dashed line corresponded to the adjusted P-value cutoff (false discovery rate, FDR = 0.05). (C-G) Serum proteins related to (C) IFN-γ pathway, (D) pro-inflammatory mediators, (E) chemotaxis, (F) monocyte function, and (G) T cell activation, proliferation, and function. Black asterisks indicated serum proteins that were significantly different between groups when adjusted for multiple testing (Wilcoxon rank sum test; Benjamini-Hochberg method); red asterisks indicated serum proteins that failed to pass significance after adjusted for multiple testing. The Y-axis corresponds to Normalized Protein eXpression (NPX, log 2 scale). Lower and upper hinges of the boxplots corresponded to the 25th to 75th percentiles, respectively, and the whiskers extended to the minimum and maximum values. (H) Principal component analysis (PCA) of subjects based on overall protein expressions. CVID participants are in red and XLA participants are in blue. (I) Bar-plot showing the top 20 loading proteins and the scores from the principal component analysis between CVID and XLA participants.

[0014] FIGs. 2A-2I depict examples of differential host response to bacterial translocation between XLA and CVID in accordance with aspects of the present disclosure. (A) In vivo examination of serum protein expressions via Olink inflammation panel in CVID (n = 25) and XLA (n = 12) participants in relation to serum bacterial translocation (BT) levels. Bar plots showing the fold changes, FC, (log 2) of top 20 protein expression differences in $CVID^{high\ BT}$ relative to $CVID^{low\ BT}$ participants (left panel), along with expression differences of each protein in $XLA^{high\ BT}$ relative to $XLA^{low\ BT}$ participants (right panel). (B-E) Secreted inflammatory proteins from bacterial DNA (bDNA)-stimulated CVID (red square) and XLA (blue triangle) participants' peripheral blood mononuclear cells (PBMCs) as measured by ELISA: (B) IFN-γ, (C) TNF-α, (D) IL-6, and (E) sCD14. (F-I) secreted inflammatory proteins from lipopolysaccharide (LPS)-stimulated CVID (red squares) and XLA (blue triangles) PBMCs as measured by ELISA: (F) IFN-γ, (G) TNF-α, (H) IL-6, and (I) sCD14. Red asterisks indicated significant differences between BT-stimulated vs. not stimulated CVID PBMCs. Black asterisks indicated significant differences between BT-stimulated CVID vs BT-stimulated XLA samples. P * <0.05, ** <0.01, *** <0.001 **** <0.0001 by Mann-Whitney test. n.s. not significant.

[0015] FIGs. 3A-3H depict examples of Bruton's tyrosine kinase (BTK) inhibitors reducing key bacterial translocation-induced pro-inflammatory cytokines and monocyte activation markers in CVID in accordance with aspects of the present disclosure. (A-D) Quantification of (A) IFN-γ, (B) TNF-α, (C) IL-6, and (D) sCD14 secretion in bacterial DNA-stimulated CVID (n = 10) PBMCs, with or without a BTK inhibitor (rilzabrutinib, or

PCI 29732). Right panels show the dose effect of rilzabrutinib for each respective inflammatory cytokine/activation marker (minimum n = 5). (E-H) Quantification of (E) IFN-γ, (F) TNF-α, (G) IL-6, and (H) sCD14 secretion in LPS-stimulated CVID (n = 5) peripheral blood mononuclear cells (PBMCs), with or without a BTK inhibitor (rilzabrutinib, or PCI 29732). Right panels show the dose effect of rilzabrutinib for each respective inflammatory cytokine/activation marker (n = 4). P *<0.05, **<0.01, ****<0.0001 by Mann-Whitney test.

[0016] FIGs. 4A-4E depict examples of rilzabrutinib modulating broad bacterial translocation-associated inflammatory signature in CVID in accordance with aspects of the present disclosure. Secreted protein expressions via Olink inflammation panel in inflammatory-CVID PBMCs (n = 7) stimulated with bacterial DNA, with or without rilzabrutinib treatment. (A) Bar plots showing the fold changes (log 2) of top 30 secreted protein expression differences in rilzabrutinib-treated vs. untreated CVID PBMCs. (B-E) Fold changes (log 2) of secreted proteins related to (B) IFN-γ pathway, (C) pro-inflammatory mediators, (D) monocyte function, and (G) T cell activities, in rilzabrutinib-treated vs. untreated CVID PBMCs. Black asterisks indicated serum proteins that were significantly different between groups when adjusted for multiple testing (Wilcoxon rank sum test; Benjamini-Hochberg method); red asterisks indicated serum proteins that failed to pass significance after adjusted for multiple testing. Lower and upper hinges of the boxplots corresponded to 25th to 75th percentiles, respectively, and the whiskers extended to the minimum and maximum value.

[0017] FIGs. 5A and 5B depict Bacterial translocation in CVID and XLA in accordance with aspects of the present disclosure. Serum bacterial DNA (bDNA; A), and LPS-binding protein (LBP; B) levels in CVID (n = 25), XLA (n = 12), and healthy controls (HC). P **<0.01, ****<0.0001 by Kruskal-Wallis test.

DETAILED DESCRIPTION

[0018] This disclosure relates to a method of treating inflammatory-CVID by administering to a patient in need of such treatment a BTK inhibitor. Also provided is a method of inhibiting secretion of inflammatory factors (tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6)) from peripheral blood mononuclear cells by contacting such cells, in vivo or ex vivo, with (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile. (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile is also

known and referred to herein as rilzabrutinib Included in the foregoing is a use of rilzabrutinib in the treatment of inflammatory-CVID.

[0019] Bacterial translocation, i.e., increased translocation of commensal microbial products from the intestinal lumen into systemic circulation, promotes immune activation and inflammatory complications in multiple disease states, including HIV, cirrhosis and inflammatory bowel diseases. In the settings of an inadequate humoral immunity, microbial translocation is also observed in CVID and X-linked agammaglobulinemia (XLA), another primary antibody deficiency disorder in which lack of immunoglobulins leave patients susceptible to severe and recurrent infections. In CVID, there is increased translocation of pro-inflammatory commensal products, with increased bacterial DNA in the circulation, and elevated markers of lipopolysaccharide (LPS) exposure (elevated LPS-binding protein, LBP). Bacterial translocation in CVID is associated with systemic immune activation/dysregulation (monocytes, adaptive immunity), high IFN-γ levels *in vivo*, and the development of inflammatory complications. Microbial translocation is therefore believed to contribute to systemic inflammation in CVID ("inflammatory-CVID").

[0020] XLA, on the other hand, is a primary antibody deficiency due to the loss of function mutations in Bruton's tyrosine kinase (BTK), a signaling molecule crucial for the development, survival, and activation of B cells. Given the essential role of BTK in B cells, BTK inhibitors have been developed and successfully used in the treatment of B cell malignancies. BTK is also expressed in most hematopoietic cells. Aside from its role in B cell receptor signaling, BTK is involved in toll-like receptors (TLR) and chemokine receptor signaling pathways.

[0021] While bacterial translocations are prominent in both CVID and XLA, clinical observations indicate a relative lack of inflammatory manifestations in XLA. Disclosed herein is a comparison of inflammation-related responses in CVID and XLA patients, including for identification of factors that differentiate between patients with inflammatory-CVID and those with XLA. As disclosed herein, lack of BTK in XLA may modify host response to bacterial translocation as compared to such a response in CVID. Whereas inflammatory-CVID and XLA are both immunodeficiencies, the former is characterized by inflammation-related pathologies, including those resulting from bacterial translocation, differentiating inflammatory-CVID from XLA. Thus, differences between inflammatory factors exhibited by the two may identify factors responsible for inflammatory pathologies seen in inflammatory-CVID.

[0022] As disclosed herein, a large national immunodeficiency registry was assessed to compare the development of inflammatory manifestations in XLA vs. CVID. Underlying immune proteome of XLA vs. CVID was assessed, and responses thereof to bacterial translocation stimuli compared. Surprisingly, as disclosed herein, the lack of BTK signaling in XLA appears to be protective against inflammation resulting from bacterial translocation. As further shown herein, inhibition of BTK in inflammatory-CVID counteracts otherwise pro-inflammatory effects of bacterial challenge and, surprisingly, identifies inhibition of BTK as a treatment for inflammatory-CVID. Nevertheless, as disclosed herein, BTK inhibition, a treatment used in other contexts to curb immune cell, e.g. B cell function, for therapeutic purposes, surprisingly is therapeutic for inflammatory-CVID as well.

[0023] CVID is a primary immunodeficiency characterized by low serum antibody levels, including some or all of IgG, IgA, and IgM, and lack of production of specific IgG antibodies. CVID's primary features are hypogammaglobulinemia not due to other causes such as XLA. Complications of inflammatory-CVID include without limitation, inflammatory-CVID autoimmunity, inflammatory-CVID granulomatous infiltrations, inflammatory-CVID interstitial lung disease, inflammatory-CVID lymphoid hyperplasia, inflammatory-CVID lymphoma, inflammatory-CVID liver disease, and inflammatory-CVID enteropathy. Diagnosis includes eliminating diagnoses of other hypogammaglobulinemias such as XLA, drug-induced (e.g., immunosuppressants, glucocorticoids, anti-CD20 therapy, antiepileptic treatments, or other pharmacologically induced immunodeficiencies), malignancies, nephrotic syndrome, protein loss resulting from burn wounds, primary hypogammaglobulinemias such as an IgG subclass deficiency or a hyper IgM syndrome, and a combined immunodeficiency such as adenosis deaminase deficiency.

[0024] Inflammatory-CVID may involve recurrent infections of the gastrointestinal tract, upper and lower respiratory tracts, recurrent sinusitis, otitis, bronchitis, bronchiectasis, and interstitial lung disease. Inflammatory-CVID may also involve development of an autoimmunity, a hematologic disorder such as, autoimmune neutropenia, or Evan's syndrome. autoimmune neutropenia, splenomegaly, lymphadenopathy, and autoimmune disease symptoms such as skin rashes, arthritis, etc.

[0025] Diagnostic criteria for CVID may include decrease in serum IgG levels (<4.5 g/L) and a marked decrease below the lower limit of normal for age in at least one of the isotypes IgM or IgA; a patient's age of at least four years; and an antibody immune response to immunizations or protein antigens being absent. CVID patients may exhibit no immunoglobulin production, production of immunoglobulin (Ig) M only, or normal IgM and

IgG production. B cell levels may be undetectable, reduced, or within the normal range. Naive B cell frequency coupled with a lower frequency of class-switched memory B cells may be seen, and other B cell populations and T cell behavior may also be affected.

[0026] Examples of such lymphomas and of other such neoplastic disease in inflammatory-CVID include B cell lymphoma, ALK negative anaplastic large cell lymphoma, non-Hodgkin's lymphoma, and Hodgkin's lymphoma, secondary lymphoma of unknown B cell type, diffuse large B cell lymphoma, T cell rich B cell lymphoma, plasmacytoid lymphoma, marginal zone lymphoma, extranodal marginal zone lymphoma, diffuse mixed small and large cell lymphoma, diffuse small cleaved cell lymphoma, diffuse poorly differentiated lymphoma, and follicular mixed cell lymphoma, and solid organ malignancy.

[0027] Examples of such granulomatous disease and lymphoid proliferation include for example granulomas of the lung, liver, skin, lymph nodes, brain, bone marrow, parotid gland, and the mesentery, and lymphoid hyperplasia and/or splenomegaly.

[0028] Bacterial translocation contributes to inflammatory conditions and symptoms of inflammatory CVID. As disclosed herein, rilzabrutinib inhibits pro-inflammatory effects of bacterial compositions.. The present disclosure includes administering rilzabrutinib to a subject who has inflammatory CVID, relating to or resulting from bacterial translocation. Administering rilzabrutinib to such a subject may treat such inflammatory symptoms or bacterial translocation-induced inflammatory symptom or condition.

[0029] As disclosed herein, a contributing factor to inflammatory aspects of inflammatory CVID include pathological bacterial translocation, the passage to extraintestinal sites of viable bacteria from the gastrointestinal tract. As disclosed herein, circulating bacterial debris (e.g., 16S rDNA belonging to gut commensals) is increased in serum from CVID patients, particularly for patients with inflammatory CVID. And serum bacterial DNA levels in inflammatory CVID are associated with parameters of systemic immune activation, increased serum IFN-γ, and the lowest isotype-switched memory B cell levels. Also as disclosed herein, bacterial DNA is bioactive ex vivo and induces IFN-γ secretion from peripheral blood mononuclear cells, particularly from inflammatory CVID patients. XLA patients, lacking in BTK, also show increased serum bacterial DNA levels, but not the indicia of inflammatory responsiveness seen in inflammatory CVID, implicating BTK as promoting pathological inflammatory indicia that distinguish inflammatory CVID from XLA. Consistent with this possibility, and as further disclosed herein, inhibiting BTK with a

BTK inhibitor blunts the inflammatory markers induced by bacterial challenges such as bacterial DNA, identifying BTK inhibitors as treatment for inflammatory CVID.

[0030] References disclosing chemical structure, pharmacological characteristics, and other clinical uses of PRN1008 include the following, Murrell et al., Proof of concept for the clinical effects of oral rilzabrutinib, the first Bruton tyrosine kinase inhibitor for pemphigus vulgaris: the phase II BELIEVE study. Br J Dermatol. 2021 Oct;185(4):745-755; Robak, E.; Robak, T. Bruton's Kinase Inhibitors for the Treatment of Immunological Diseases: Current Status and Perspectives. J. Clin. Med. 2022, 11, 2807; Lewis et al., Non-Covalent BTK Inhibitors-The New BTKids on the Block for B-Cell Malignancies. J Pers Med. 2021 Aug 3;11(8):764; Kuter et al., Oral Rilzabrutinib, a Bruton Tyrosine Kinase Inhibitor, Showed Clinically Active and Durable Platelet Responses and Was Well-Tolerated in Patients with Heavily Pretreated Immune Thrombocytopenia, Blood, Volume 136, Supplement 1, 2020, Pages 13-14; Ran et al., Review of the development of BTK inhibitors in overcoming the clinical limitations of ibrutinib, European Journal of Medicinal Chemistry, Volume 229, 2022, 114009; Kuter et al., Rilzabrutinib, an Oral BTK Inhibitor, in Immune Thrombocytopenia. N Engl J Med. 2022 Apr 14;386(15):1421-1431; Langrish et al., Preclinical Efficacy and Anti-Inflammatory Mechanisms of Action of the Bruton Tyrosine Kinase Inhibitor Rilzabrutinib for Immune-Mediated Disease. J Immunol 1 April 2021; 206 (7): 1454–1468; Smith et al., 2017, A phase I trial of PRN1008, a novel reversible covalent inhibitor of Bruton's tyrosine kinase, in healthy volunteers. Br J Clin Pharmacol, 83: 2367–2376.

[0031] The terms "subject" or "patient" or "subject in need thereof" or "patient in need thereof" or the like are used interchangeably herein. These terms refer to a patient with CVID or inflammatory-CVID. The subject may currently be experiencing complications associated with the disorder or may have experienced complications in the past. Additionally, a "subject in need thereof" may be a patient at risk of developing inflammatory-CVID, or to a patient reporting one or more of the complications thereof, even though a diagnosis of may not have been made. As used herein, the terms "treatment" or "treating" are used interchangeably. These terms refer to an approach for obtaining beneficial or desired results including, but not limited to, therapeutic benefit. Therapeutic benefit includes eradication or amelioration of the underlying disorder or complication being treated; it also includes the eradication or amelioration of one or more of the complications associated with the underlying disorder such that an improvement is observed in the patient. In an example, treatment may include administering rilzabrutinib, a pharmaceutically acceptable salt thereof,

or pharmaceutical composition including one or more of the foregoing, to a subject in need thereof.

[0032] Formulations of the rilzabrutinib suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of rilzabrutinib; Other formulations are also available. In certain embodiments, rilzabrutinib may be incorporated with excipients and used in the form of ingestible tablets. Skilled artisans will know how to prepare such tablets. See for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990.

[0033] The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Unless otherwise specified, reference herein to rilzabrutinib, or to any such compound in particular, includes reference to a pharmaceutically acceptable salt thereof. Rilzabrutinib may be formulated into a composition in a free base, neutral or salt form. See for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990.

[0034] As used herein, the term "effective amount" means an amount of a rilzabrutinib that may elicit a biological or medical response. The term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function

[0035] A pharmaceutical composition including rilzabrutinib optionally may include one or more additional agents dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a human subject and/or meet the standards of the FDA. The preparation of a pharmaceutical composition including rilzabrutinib and optionally one or more additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990.

[0036] As disclosed herein, exposure to bacteria or bacterial compositions causes secretion of various cellular factors involved in inflammatory responses from peripheral blood mononuclear cells. Such bacterial compositions may include bacterial DNA such as

bacterial polynucleotides such as bacterial DNA, endotoxin, or lipopolysaccharide. Exposure of blood cells of an inflammatory-CVID patient to such factors, in vivo or ex vivo, causes secretion of such inflammatory factors or factors whose secretion indicates activation of inflammatory pathways, as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin 1-alpha (IL-1A), interleukin 6 (IL-6), interleukin-12 subunit beta (IL-12B), expression of interleukin 18 (IL-18), soluble cluster of differentiation 14 (sCD14), signaling lymphocytic activation molecule 1 (SLAMF1), colony stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), chemokine ligand 9 (CXCL9), or any two or more of the foregoing, in any combination. For example, one or more such inflammatory factor may be secreted from peripheral blood mononuclear cells.

[0037] Thus, a blood sample may be taken from a subject having inflammatory-CVID, and the sample exposed to a bacterial composition, such as but not limited to bacterial DNA, endotoxin, or lipopolysaccharide. Secretion of one or more inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin 1-alpha (IL-1A), interleukin 6 (IL-6), interleukin-12 subunit beta (IL-12B), expression of interleukin 18 (IL-18), soluble cluster of differentiation 14 (sCD14), signaling lymphocytic activation molecule 1 (SLAMF1), colony stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), chemokine ligand 9 (CXCL9), or any two or more of the foregoing, in any combination, in response to exposure to such bacterial composition, and an amount of such secretion, may be measured.

[0038] As further disclosed herein, exposure of blood cells of a subject having inflammatory-CVID to rilzabrutinib decreases an amount of secretion of such inflammatory factors. Thus, as disclosed herein, rilzabrutinib may be administered to such a subject. A blood sample may be taken from a subject having inflammatory-CVID, and the sample exposed to a bacterial composition, such as but not limited to bacterial DNA, endotoxin, or lipopolysaccharide. Secretion of one or more inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin 1-alpha (IL-1A), interleukin 6 (IL-6), interleukin-12 subunit beta (IL-12B), expression of interleukin 18 (IL-18), soluble cluster of differentiation 14 (sCD14), signaling lymphocytic activation molecule 1 (SLAMF1), colony stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), chemokine ligand 9 (CXCL9), or any two or more of the foregoing, in any combination, in response to exposure to such bacterial composition, and an amount of such secretion, may be measured.

[0039] The amount of secretion of one or more of the foregoing inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin 1-alpha (IL-1A), interleukin 6 (IL-6), interleukin-12 subunit beta (IL-12B), expression of interleukin 18 (IL-18), soluble cluster of differentiation 14 (sCD14), signaling lymphocytic activation molecule 1 (SLAMF1), colony stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), chemokine ligand 9 (CXCL9), or any two or more of the foregoing, following exposure of the blood sample to an bacterial factor such as but not limited to bacterial DNA, endotoxin, or lipopolysaccharide, may be lower in the sample from the subject having inflammatory-CVID, to whom rilzabrutinib than the amount of such secretion in the corresponding subject to whom rilzabrutinib was not administered. The blood cells from which bacterial-induced secretion of such inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways is inhibited may include peripheral blood mononuclear cells.

[0040] The subject to whom rilzabrutinib is administered may be the same subject to whom rilzabrutinib was not administered, with blood samples taken before and after rilzabrutinib administration.

[0041] As also disclosed herein, circulating blood levels of one or more inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, may be reduced in a subject having inflammatory-CVID by administering rilzabrutinib to the subject, compared to levels in a subject having inflammatory-CVID to whom rilzabrutinib was not administered. The subject to whom rilzabrutinib is administered may be the same subject as that to whom rilzabrutinib was not administered, with a blood sample taken before and after administration of rilzabrutinib. Or they may be different subjects. Levels of one or more such factor may be measured in blood samples taken from the two subjects. In accordance with the present disclosure, a lower level of such inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, may be lower in the blood sample taken from the subject administered rilzabrutinib. The inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, may be IFN-γ, TNF-α, IL-6, sCD14, or any combination of two or more of the foregoing.

[0042] In any of the following examples, an amount of reduction of the inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, may be by about 5%, or by about 10%, or by about 15%, or by about 20%, or by about 25%, or by about 30%, or by about 35%, or by about 40%, or by about 45%, or by about 50%, or by about

55%, or by about 60%, or by about 65%, or by about 70%, or by about 75%. In any of the following examples, an amount of reduction of the inflammatory factor, or factor whose secretion indicates activation of inflammatory pathways, may be by about 5% or more, or by about 10% or more, or by about 15% or more, or by about 20% or more, or by about 25% or more, or by about 30% or more, or by about 35% or more, or by about 40% or more, or by about 45% or more, or by about 50% or more, or by about 55% or more, or by about 60% or more, or by about 65% or more, or by about 70% or more, or by about 75%.

[0043] As used herein, the term "about" or "approximately" means within 5% of a given value or range. For example, "about 10" includes 10, 9.5, 10.5, and any amount from 9.5 to 10.5, inclusively.

[0044]

EXAMPLES

[0045] The following examples are intended to illustrate particular embodiments of the present disclosure, but are by no means intended to limit the scope thereof.

[0046]

[0047] **EXAMPLE 1: METHODS**

[0048] **USIDNET Registry and Mount Sinai Hospital CVID Cohort**

[0049] We conducted a retrospective cohort study of patients in the USIDNET registry for XLA and CVID. The U.S. Immunodeficiency Network (USIDNET), a program of the Immune Deficiency Foundation (IDF), has been supported by a cooperative agreement, U24AI86837, from the National Institute of Allergy and Infectious Diseases (NIAID). The USIDNET Registry was established in 2003 and is a national patient-consented data base with clinical data from patients with primary immunodeficiency diseases. For this study, the USIDNET Registry was queried for clinical manifestations reported in patients with XLA and CVID, through December 14, 2020. CVID is defined according to the International Consensus Document (ICON).[77] The data analyzed here includes all the information compiled in the USIDNET Registry at the time of our query. Patients were excluded if they did not have a known investigator designated BTK defect. Demographic, clinical, and laboratory information was obtained. Hypogammaglobulinemia subjects with thymoma were also excluded. Hematologic autoimmunity associated with inflammatory-CVID and/or XLA the following diagnosis entries: autoimmune neutropenia, or Evan's syndrome. Interstitial lung diseases included the following diagnosis entries: interstitial lung disease, bronchiolitis obliterans organizing pneumonia, lymphoid interstitial pneumonia, granulomatous lung

diseases, Granulomatous-lymphocytic interstitial lung disease. Inflammatory/autoimmune liver diseases included the following diagnosis entries: autoimmune hepatitis, nodular regenerative hyperplasia, granulomatous hepatitis, lymphoid hyperplasia of the liver, sclerosing cholangitis. Enteropathy/inflammatory bowel disease included the following diagnosis entries: inflammatory bowel disease, ulcerative colitis, Crohn's disease, lymphocytic colitis, autoimmune enteropathy, protein-losing gastroenteropathy.

[0050] For the Mount Sinai Hospital CVID Cohort, clinical and laboratory data from participants seen in the Immune Deficiency Clinic at Mount Sinai Hospital from 1986 through the present were collected and reviewed. Clinical information was obtained during patient visits; for subjects no longer receiving care at Mount Sinai, follow-up information was obtained from patients, their physicians, chart and medical records review.

[0051] **Patient samples**

[0052] All participants recruited for this study were patients at the Mount Sinai Clinical Immunology Faculty Practice. PBMC and serum from CVID patients,[77] XLA patients with confirmed BTK mutations,[78] and healthy controls were collected. Serum was drawn into 6 mL gold top rubber-sealed sterile Vacutainer SST II tubes (BD Diagnostics). The tubes were maintained in the upright position until the serum was aseptically removed in a sterile hood for bacterial and other assays. In the CVID cohort, inflammatory-CVID complications included lymphoid hyperplasia/splenomegaly, hematologic autoimmunity, chronic lung disease (interstitial lung disease and/or granulomatous lung disease), enteropathy, and granulomas.

[0053] **High-dimensional cytokine profiling**

[0054] Olink inflammation panel (92 paired oligonucleotide antibody-labeled probes targeting cytokines, chemokines, and soluble receptors) was used to quantify the study samples based on Proximity Extension Assay technology following the standard Olink-certified protocol. Briefly, 1 ul of patient sera was mixed with 3 ul of an Olink incubation mix in a 96-well plate format and incubated at 4°C overnight. All patient sera were run in a single batch. For culture supernatant, 1 ul of supernatant was mixed with 3 ul of an Olink incubation mix. Next day, Olink extension reagent mix (containing PCR polymerase) was added to each well, vortexed, spun down and placed into thermal cycler for pre-amplification (1.5 h). In the detection phase, 2.8 µl from each well were then mixed with 7.2 µl of a detection mix and placed on a 96-96 Dynamic Array Integrated Fluidic Circuit (IFC) chip (primed in the IFC controller for 30 min before usage) along with the corresponding ninety-two oligonucleotide pairs. Next, the chip was processed through the Fluidigm BioMark qPCR

reader using standard protocol provided by the supplier. Details regarding assay limitations, validations, and protocols may be obtained from the Olink supplier (https://www.olink.com). Sample data quality control and normalization was done using the Olink's Normalized Protein eXpression Manager software. Data is reported as Normalized Protein eXpression (NPX) unit in log2 scale values.

[0055] **DNA extraction and quantitative PCR for bacterial 16S rDNA**

[0056] DNA was extracted from CVID and XLA serum samples following handling guidelines for microbiome studies as previously described.[79] Briefly, collected serum samples were introduced into a biosafety cabinet that was decontaminated prior to introduction of the samples by UV treatment for 1 hour and subsequently decontaminated with 5% bleach. Personnel handling the samples were wearing isolation gowns, clean gloves that were sprayed beforehand with 5% bleach and 70% ethanol, and a face mask. DNA was extracted using the DNeasy UltraClean Microbial Kit (Qiagen).

[0057] Quantitative PCR was performed blinded for clinical characteristics. The amplification reaction mixture was composed of 10 µL of LightCycler 480 SYBR Green (Roche), 1 µL of 16S bacterial rDNA forward primer (5′-AAC AGG ATT AGA TAC CCT GGT AG-3′, 1 µL of reverse primer (5′-GGT TCT KCG CGT TGC WTC-3′),[80] 1 µL of dsDNAase (Thermo Fisher Scientific), 1 µL of 10× dsDNAase buffer (Thermo Fisher Scientific), and 5 µL of SYBR Green reaction mix (Roche). Then, 19 µL of the reaction mixture was added to each well of a 384-qPCR plate, followed by 1 µL of DNA isolated from 200 µL of serum (DNeasy Blood and Tissue Kit, Qiagen) or the E. coli bacterial DNA standard. The DNA was amplified in triplicate, and mean values were calculated. The reaction conditions for amplification of DNA were 95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, 60°C for 20 seconds, and 72°C for 5 seconds. The bacterial DNA standard was prepared from E. coli–competent cells (Thermo Fisher Scientific) using DNeasy Blood and Tissue Kit (Qiagen) and concentrations were measured by NanoDrop (Thermo Fisher Scientific). Using the E. coli genome length (4,700,000 bp), the number of E. coli DNA copies was calculated (https://cels.uri.edu/gsc/cndna.html) and serial dilutions of this standard were used to define DNA copy numbers in a standard curve.

[0058] **Enzyme-linked immunosorbent assays (ELISA) for LBP, IFN-γ, TNF-α, IL-6, sCD14**

[0059] Concentrations of LBP were determined in serum with dilutions of 1:1000 using immunoassay (R&D Systems). Cell culture supernatant IFN-γ, TNF-α, IL-6, sCD14 concentrations were measured at the following concentrations, 1:2, 1:10, 1:100, and 1:20,

respectively using separate immunoassays (R&D Systems). FLUOstar Omega multimode microplate reader (BMG Labtech) was employed for optical density measurements.

[0060] **PBMC cultures and BTK inhibition**

[0061] Bacterial DNA and LPS stimulation in CVID or XLA PBMC cultures were performed. Bacterial DNA was prepared from E. coli–competent cells (Thermo Fisher Scientific) using DNeasy Blood and Tissue Kit (Qiagen). PBMCs freshly isolated from each study participant (5×10^5 cells) were co-cultured with E. coli bacterial DNA (3×10^4 DNA copies [7.5 pg/mL]) as described previously.[12] PBMC (5×10^5 cells) were also co-cultured with LPS (Invitrogen eBioscience) separately. Culture supernatants were collected and analyzed on day 3. For BTK inhibition, rilzabrutinib (final concentration 0.001-1μM, Sanofi S.A.) or PCI 29732 (final concentration 0.5 nM, Tocris Bioscience) was added to PBMC cultures at 2, 8, and 12 hours prior to the addition of E. coli bacterial DNA or LPS.

[0062] **Statistics**

[0063] All statistical analyses related to high-dimensional cytokine profiling were performed in R (version 4.0.4). Wilcoxon Rank Sum Test was used to compare differential protein expression between two groups. P values were adjusted to control the false discovery rate (FDR) using the Benjamini-Hochberg (BH) method across the 92-protein panel.

[0064] Principal component analysis (PCA) was performed in R (version 4.0.4) using the protein-wised z-score. The top loadings were selected by the maximum score of the first 2 PCs.

[0065] Statistical analyses related to ELISA results were performed in GraphPad Prism (version 10.1.0) via Mann-Whittney test or Kruskal-Wallis test. Although some non-limiting examples have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the present disclosure and these are therefore considered to be within the scope of the present disclosure as defined in the claims that follow.

[0066] **EXAMPLE 2: LACK OF INFLAMMATORY MANIFESTATIONS IN XLA COMPARED TO CVID**

[0067] Systemic immune activation in CVID ("inflammatory-CVID") has been linked to bacterial translocation.[12] While XLA patients are also characterized by severe antibody defects and bacterial translocation, it is not known whether they are similarly at risk for developing inflammatory complications in absence of functional BTK. To examine this, we queried the United States Immunodeficiency Network (USIDNET), a national registry

database for rare primary immunodeficiency (PI) disorders, for complications occurring in XLA vs. CVID. As an additional comparator, we examined clinical data from the Mount Sinai Hospital (MSH) CVID cohort, which offered further diagnostic specificities on end-organ diseases.

[0068] We identified 230 XLA and 990 CVID patients in the USIDNET registry, as well as 740 CVID participants in the MSH cohort (Table 1). Overall, the frequency of developing any inflammatory manifestations was significantly lower in XLA (4.3%) compared to CVID (37.5% in USIDNET, 67.8% in MSH cohort; $P < 0.0001$ for both). Mortality in CVID was previously associated with the presence of inflammatory lung, liver, and gastrointestinal diseases.[2] The frequency of interstitial lung disease, inflammatory liver disease, and inflammatory bowel diseases were all significantly lower in XLA compared to CVID ($P<0.0001$ for all, compared to MSH cohort; $P<0.0001$-0.0015, compared to USIDNET cohort). Additionally, nearly none of XLA patients developed lymphoproliferative diseases, granulomatous diseases and hematologic autoimmunity ($P<0.0001$ for all, compared to CVID cohorts). In contrast, there were no significant differences in the sequelae of recurrent lung infections (i.e., bronchiectasis) or irregular bowel habits between XLA and CVID cohorts. Together, these data demonstrated a striking lack of a broad range of inflammatory manifestations in XLA relative to CVID, suggesting the lack of BTK in XLA may be protective for developing such complications.

[0069] **EXAMPLE 3: CYTOKINE PROFILING REVEALS INFLAMMATORY DIFFERENTIALS BETWEEN XLA AND INFLAMMATORY-CVID**

[0070] To determine the immune mediators underlying the clinical differences observed between XLA and CVID, we performed high-dimensional serum cytokine profiling of 92 analytes using the Olink platform in 12 XLA and 20 CVID subjects with inflammatory complications ("inflammatory-CVID"). Overall, there were significant differences across multiple cytokine families between XLA and inflammatory-CVID (FIG. 1A, B). For cytokines, IFN-γ and related IL-12b demonstrated the largest fold-change increases in inflammatory-CVID vs. XLA (FIG. 1B, C). Other mediators involved in the IFN-γ pathways: IL-15Ra, IL-18, SLAMF1, and the chemokine CXCL9 were also prominent in inflammatory-CVID relative to XLA (FIG. 1B, C, E).[28–32] The cytokine differentials extended beyond IFN-γ clusters. There were also significant elevations in other families of pro-inflammatory mediators, including TNF-α and TNF-β, in inflammatory-CVID vs. XLA (FIG. 1B, D). IL-6, a cytokine previously associated with inflammatory complications in inflammatory-CVID,[9,10] was similarly increased in inflammatory-CVID relative to XLA, though it did not reach

statistical significance in this cohort (FIG. 1D). Likewise, there were higher levels of circulating mediators involved in immune cell recruitments (CCL19, CCL23, CCL3, and CXCL9) in CVID relative to XLA (FIG. 1B, E). These included multiple chemokines typically expressed at the junctures of host-commensal interactions, including in the liver (CCL23), lungs (CCL23/CCL3), and lymph nodes (CCL19/CCL3).[33–36]

[0071] In addition, we found that mediators of monocyte chemotaxis (CCL3),[37,38] proliferation, and functions (CSF-1, TNFRSF9)[39–41] were elevated in inflammatory-CVID vs. XLA (FIG. 1B, E, F). Furthermore, there were higher levels of proteins involved in T cell activation, proliferations (CD5, CD8A),[42–44] and recognition of bacterial-associated molecular patterns (CD6; FIG. 1B, G)[45] in inflammatory-CVID. In turn, a profile of immune exhaustion and regulation was evident in inflammatory-CVID relative to XLA, with elevated soluble PD-L1 and IL-10 (FIG. 1A, B),[46,47] likely reflecting compensatory response to inflammation in inflammatory-CVID patients.

[0072] Overall, XLA patients were characterized by a relatively quiescent cytokine profile compared to inflammatory-CVID, and this distinct signature was consistent among patients with a lack of functional BTK. Using a global analytic approach with principal component analysis (PCA), XLA patients reliably clustered together and were separated from inflammatory-CVID patients (FIG. 1H). PCA loading analyses similarly identified differences in the IFN-γ pathways (IFN-γ, IL12-B, IL18, CXCL9), TNF family (TNF-α, TNF-β), and monocyte-related proteins (CSF-1, CCL3, TNFRSF9) as the top components that separated XLA from inflammatory-CVID patients (FIG. 1I).

[0073] **EXAMPLE 4: DIFFERENTIAL HOST RESPONSE TO BACTERIAL TRANSLOCATION BETWEEN XLA AND CVID**

[0074] Despite prominent bacterial translocation in both conditions, we had found significant differences in inflammatory manifestations and cytokine profiles between XLA and CVID. We therefore asked whether XLA and CVID patients exhibited differential host response to bacterial translocation stimuli.

[0075] For *in vivo* examination, we simultaneously measured bacterial translocation markers (bacterial DNA/16S rDNA, LBP) and cytokine profiles (Olink platform) in serum samples. We obtained parallel measurements in 25 CVID and 12 XLA patients. Serum bacterial translocation markers were significantly elevated in CVID and XLA relative to healthy volunteers (FIG. 5). Next, we compared cytokine signatures in those with highest bacterial translocation levels (high BT, bacterial DNA and LBP > 2 standard deviations from the healthy mean) in CVID and XLA relative to the rest of their respective cohorts. In

CVID$^{high\ BT}$, we found that IFN-γ and related proteins (IL-12b, IL-18, IL-18R1, CXCL9, CXCL10, CXCL11) were the most prominent cytokine family elevated compared to other CVID patients (FIG. 2A, left panel). In addition, increased TNF family (TNF-α, TNF-β) and IL-6 were observed in CVID$^{high\ BT}$. Notably, FGF-21, a hepatic peptide whose circulating level was linked to bacterial inflammation,[48] was the top signature observed in the CVID$^{high\ BT}$ group. Lastly, CCL20 upregulation, previously linked to endotoxemia.[49] was also found in the CVID$^{high\ BT}$ group. In XLA (FIG. 2A, right panel), IL-6 and IL-17A were similarly increased in those with the highest bacterial translocation (XLA$^{high\ BT}$) compared to other XLA patients. In contrast, however, most of the upregulated inflammatory cytokines in CVID$^{high\ BT}$ were either minimally (IFN-γ, IL-12b) or *not* increased (CXCL9, CXCL10, CXCL11, TNF-α, TNF-β) in XLA$^{high\ BT}$.

[0076] Next, to directly compare host response to specific bacterial translocation stimuli, we examined the effects of microbial products (bacterial DNA, LPS) exposure in XLA and CVID peripheral blood mononuclear cells (PBMCs) *ex vivo*. Based on literature[50] and our *in vivo* findings (FIG. 1), we measured, via ELISA, four key inflammatory-CVID mediators as the main outcomes: 1) IFN-γ, 2) TNF-α, 3) IL-6, and 4) monocyte activation (soluble CD14, sCD14). Bacterial DNA, found in abundance in the XLA and CVID sera,[12] was strongly immune-stimulating in CVID. Bacterial DNA exposure induced high levels of IFN-γ, TNF-α, and IL-6 secretion, as well as monocyte activation in CVID PBMCs (FIG. 3A-D). In contrast, host response to bacterial DNA was significantly blunted in XLA PBMCs relative to CVID (IFN-γ: $P<0.001$; TNF-α, IL-6, sCD14: $P<0.01$; FIG. 2B-E). LPS is another common bacterial translocation product, and there is evidence of systemic LPS exposure in CVID.[12,20,21] Likewise, we found that LPS exposure was broadly immune-activating in CVID, leading to increased IFN-γ, TNF-α, and IL-6, and monocyte activation (IFN-γ: $P<0.05$; TNF-α, IL-6, sCD14: $P<0.01$; FIG. 2F-I). In comparison, however, XLA PBMCs secreted significantly less IFN-γ, TNF-α, and sCD14 after LPS exposure when compared to CVID (IFN-γ: $P<0.05$; TNF-α, sCD14: $P<0.01$, FIG. 2 F, G, I). With LPS stimulation, mean IL-6 secretion was also lower in XLA vs. CVID, though the differences did not reach statistical significance (FIG. 2H). Together, these *in vivo* and *ex vivo* data demonstrated differential host response to bacterial translocation between XLA and CVID. Specifically, XLA patients, who lack of endogenous BTK, exhibited blunted inflammatory responses to bacterial translocation compared to CVID.

[0077] **EXAMPLE 5: BTK INHIBITORS MODULATE SYSTEMIC IMMUNE ACTIVATION TO BACTERIAL TRANSLOCATION IN INFLAMMATORY-CVID**

[0078] To assess the therapeutic implications of these differences between XLA and inflammatory-CVID, we examined the ability of BTK inhibitors to regulate bacterial translocation-induced immune activation in inflammatory-CVID. The effects of two BTK inhibitors, PCI-29732 and rilzabrutinib (Sanofi), were studied in PBMCs from CVID participants with inflammatory manifestations ("inflammatory-CVID"). Rilzabrutinib was chosen for its high BTK-specificity and its safety profile.[4,5] ELISA measurements of key immune mediators of interest in inflammatory-CVID[50,51] were first performed.

[0079] We found that BTK inhibitors effectively modulated key inflammatory mediators linked to bacterial translocation stimuli in inflammatory-CVID. Both rilzabrutinib and PCI-29732 effectively blunted bacterial DNA-driven IFN-γ by inflammatory-CVID PBMCs – the major cytokine abnormality in inflammatory-CVID[6,7] ($P<0.0001$, FIG. 3A). Additionally, BTK inhibitors significantly reduced bacterial DNA-driven TNF-α, IL-6, and monocyte activation (sCD14) in inflammatory-CVID samples ($P<0.0001$ for all, FIG. 3B-D). In the same manner, rilzabrutinib and PCI-29732 modulated LPS-associated inflammation in inflammatory-CVID. These included the significant reduction of IFN-γ ($P<0.05$), TNF-α ($P<0.01$), IL-6 ($P<0.01$), and monocyte activation (sCD14, $P<0.01$) with rilzabrutinib or PCI-29732 treatment (FIG. 3E-H)

[0080] Lastly, given the wide array of cytokine dysregulation in inflammatory-CVID (FIG. 1), we performed high-dimensional cytokine profiling (Olink platform) on rilzabrutinib treated inflammatory-CVID samples to assess the scope of its therapeutic impacts. We focused on the ability of the BTK inhibitor to regulate global response to bacterial DNA, which is the circulating bioactive material previously linked to inflammatory manifestations in inflammatory-CVID.[12] We found that the effect of rilzabrutinib treatment in inflammatory-CVID samples (FIG. 4A) paralleled the major *in vivo* differences between XLA and inflammatory-CVID. These included the modulation of not just IFN-γ, but multiple mediators in this pathway: IL12-B, IL18, IL-18R1, SLAMF1 (FIG. 4B). In addition, rilzabrutinib was able to target other pro-inflammatory cytokines induced by bacterial translocation, including TNF-α, IL-6, consistent with our ELISA findings, and IL-1α (FIG. 4C). Overall, rilzabrutinib-treated inflammatory-CVID samples exhibited a notably quiescent inflammatory profile, akin to that of XLA, with reduced mediators related to monocyte proliferation/function (CSF-1, TNFRSF9; FIG. 4D) and T cell activation/proliferation (CD8A, CD6; FIG. 4E). Together, these data showed that BTK inhibition effectively modified pathogenic host responses to bacterial translocation, making it a compelling therapeutic target to address the impacts of this phenomenon in inflammatory-CVID.

[0081] It should be appreciated that all combinations of the foregoing concepts and additional concepts discussed in greater detail herein (provided such concepts are not mutually inconsistent) are contemplated as being part of the inventive subject matter disclosed herein. In particular, all combinations of claimed subject matter appearing at the end of this disclosure are contemplated as being part of the inventive subject matter disclosed herein and may be used to achieve the benefits and advantages described herein.

[0082] **IMPLEMENTATIONS**

[0083] Following are non-limiting implementations of subject matter in accordance with the present disclosure.

[0084] Implementation 1: A method for treating common variable immunodeficiency (CVID) in a human patient in need of such treatment, comprising administering to the human patient a therapeutically effective amount of (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof.

[0085] Implementation 2: A method for treating an inflammatory-CVID condition resulting from bacterial translocation in a human patient in need of such treatment, comprising administering to the human patient a therapeutically effective amount of (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof, optionally wherein the bacterial translocation is associated with a human immunodeficiency infection in the patient, an inflammatory bowel disease in the patient, and liver fibrosis in the patient.

[0086] Implementation 3: The method of implementation 1, wherein the patient has or has had inflammatory-CVID and the administration comprises treating the inflammatory complications.

[0087] Implementation 4: The method of implementation 2 or 3, wherein the inflammatory-CVID condition is thrombocytopenia.

[0088] Implementation 5: The method of any one of implementations 1-4, wherein rilzabrutinib is administered at least once a day.

[0089] Implementation 6: The method of any one of implementations 2-5, wherein the inflammatory-CVID comprises one or more of a CVID-associated autoimmunity, a CVID-associated chronic lung disease, CVID-associated splenomegaly, a CVID-associated inflammatory gastrointestinal disease, a CVID-associated inflammatory liver disease, a

CVID-associated lymphocytic interstitial lung disease, a CVID-associated interstitial lung disease, a CVID-associated granulomatous lung disease, a CVID-associated enteropathy, a CVID-associated gastropathy, a CVID-associated nodular regenerative hyperplasia of the liver, a CVID-associated granulomatous liver disease, a CVID-associated biliary cholangitis, a CVID-associated lymphoproliferation, a CVID-associated splenomegaly, and a CVID-associated systemic granulomatous disease.

[0090] Implementation 7: The method of any one of implementations 1-6, wherein the administration comprises decreasing a stimulatory effect of bacterial DNA, lipopolysaccharide (LPS), endotoxin, or any combination of two or more of the foregoing, on peripheral blood mononuclear cells (PBMC) secretion of interferon-? (IFN-?), tumor necrosis factor-? (TNF-?), interleukin 1-alpha (IL-1A), interleukin 6 (IL-6), interleukin-12 subunit beta (IL-12B), interleukin 18 (IL-18), soluble cluster of differentiation 14 (sCD14), signaling lymphocytic activation molecule 1 (SLAMF1), colony stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), chemokine ligand 9 (CXCL9), or any combination of two or more of the foregoing.

[0091] Implementation 8: The method of any one of implementations 1-7, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of IFN-?.

[0092] Implementation 9: The method of any one of implementations 1-8, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of TNF-?.

[0093] Implementation 10: The method of any one of implementations 1-9, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of IL-6.

[0094] Implementation 11: The method of any one of implementations 1-10, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC secretion of sCD14.

[0095] Implementation 12: A method of inhibiting secretion of an inflammatory factor by peripheral blood mononuclear cells (PBMC), comprising contacting the PBMC with (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof, and the inflammatory factor is one or both of tumor necrosis factor ? (TNF-?) and interleukin 6 (IL-6).

[0096] Implementation 13: The method of implementation 12, wherein contacting the PBMC comprises administering rilzabrutinib to a subject.

[0097] Implementation 14: The method of implementation 13, wherein rilzabrutinib is administered at least once a day.

[0098] Implementation 15: The method of any one of implementations 12 or 14 , wherein the inhibiting comprises decreasing a stimulatory effect of bacterial DNA, lipopolysaccharide (LPS), endotoxin, or any combination of two or more of the foregoing, on the secretion.

[0099] Implementation 16: The method of any one of implementations 12-15, wherein the inflammatory factor is TNF-?.

[0100] Implementation 17: The method of any one of implementations 12-15, wherein the inflammatory factor is IL-6.

[0101] Implementation 18: The method of any one of implementations 12-15, wherein the inhibition is by up to about 50%.

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WHAT IS CLAIMED IS:

1. A method for treating common variable immunodeficiency (CVID) in a human patient in need of such treatment, comprising administering to the human patient a therapeutically effective amount of (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof.

2. A method for treating an inflammatory-CVID condition resulting from bacterial translocation in a human patient in need of such treatment, comprising administering to the human patient a therapeutically effective amount of (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof, optionally wherein the bacterial translocation is associated with a human immunodeficiency infection in the patient, an inflammatory bowel disease in the patient, and liver fibrosis in the patient.

3. The method of claim 1, wherein the patient has or has had inflammatory-CVID and the administration comprises treating the inflammatory complications.

4. The method of claim 2 or 3, wherein the inflammatory-CVID condition is thrombocytopenia.

5. The method of any one of claims 1-3, wherein rilzabrutinib is administered at least once a day.

6. The method of any one of claims 1-3, wherein the inflammatory-CVID comprises one or more of a CVID-associated autoimmunity, a CVID-associated chronic lung disease, CVID-associated splenomegaly, a CVID-associated inflammatory gastrointestinal disease, a CVID-associated inflammatory liver disease, a CVID-associated lymphocytic interstitial lung disease, a CVID-associated interstitial lung disease, a CVID-associated granulomatous lung disease, a CVID-associated enteropathy, a CVID-associated gastropathy, a CVID-associated nodular regenerative hyperplasia of the liver, a CVID-associated granulomatous liver disease, a CVID-associated biliary cholangitis, a CVID-associated lymphoproliferation, a CVID-associated splenomegaly, and a CVID-associated systemic granulomatous disease.

7. The method of any one of claims 1-3, wherein the administration comprises decreasing a stimulatory effect of bacterial DNA, lipopolysaccharide (LPS),

endotoxin, or any combination of two or more of the foregoing, on peripheral blood mononuclear cells (PBMC) secretion of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin 1-alpha (IL-1A), interleukin 6 (IL-6), interleukin-12 subunit beta (IL-12B), interleukin 18 (IL-18), soluble cluster of differentiation 14 (sCD14), signaling lymphocytic activation molecule 1 (SLAMF1), colony stimulating factor 1 (CSF-1), tumor necrosis factor receptor superfamily member 9 (TNFRSF9), chemokine ligand 9 (CXCL9), or any combination of two or more of the foregoing.

8. The method of any one of claims 1-3, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of IFN-γ.

9. The method of any one of claims 1-3, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of TNF-α.

10. The method of any one of claims 1-3, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC expression of IL-6.

11. The method of any one of claims 1-3, wherein the administration comprises decreasing a stimulatory effect of one or both of bacterial DNA and LPS on PBMC secretion of sCD14.

12. A method of inhibiting secretion of an inflammatory factor by peripheral blood mononuclear cells (PBMC), comprising contacting the PBMC with (R)-2-[3-[4-amino-3-(2-fluoro-4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidine-1-carbonyl]-4-methyl-4-[4-(oxetan-3-yl)piperazin-1-yl]pent-2-enenitrile (rilzabrutinib) or a pharmaceutically acceptable salt thereof, and the inflammatory factor is one or both of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6).

13. The method of claim 12, wherein contacting the PBMC comprises administering rilzabrutinib to a subject.

14. The method of claim 13, wherein rilzabrutinib is administered at least once a day.

15. The method of any one of claims 12 or 13 , wherein the inhibiting comprises decreasing a stimulatory effect of bacterial DNA, lipopolysaccharide (LPS), endotoxin, or any combination of two or more of the foregoing, on the secretion.

16. The method of any one of claims 12-14, wherein the inflammatory factor is TNF-α.

17. The method of any one of claims 12-14, wherein the inflammatory factor is IL-6.

18. The method of any one of claims 12-14, wherein the inhibition is by up to about 50%.