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(54) Title: COMPOSITIONS AND METHODS FOR TREATING VITILIGO

(57) Abstract: In its various aspects and embodiments, the invention comprises a method for treating vitiligo in a patient comprising administering a composition comprising an effective amount of at least one Janus kinase inhibitor and phototherapy.

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TITLE OF THE INVENTION

Compositions and Methods for Treating Vitiligo

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 62/490,304 filed April 26, 2017, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under TR001453 and AR069114 awarded by National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Vitiligo is a chronic autoimmune disease that results from the destruction of melanocytes, causing white spots on the skin. Vitiligo affects approximately 1% of people worldwide and can affect both adults and children, causing diminished quality of life and marked psychological distress. The pathogenesis of vitiligo involves the destruction of melanocytes via cell mediated immunity, and studies show that IFN-γ and $CD8^+$ T cells play a key role in this process.

Treatments for vitiligo include topical corticosteroids and calcineurin inhibitors, but the most effective treatment and mainstay of therapy is narrowband ultraviolet B (nbUVB) phototherapy. However, incomplete response to treatment is common.

Accordingly, there is a need in the art for methods and compositions that reduce the symptoms of vitiligo and reverse depigmentation in affected areas. Such methods and compositions should allow for maintenance of repigmentation in patients under treatment. The present disclosure addresses that need.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a method for treating vitiligo in a subject. In another aspect, the invention provides a kit comprising an effective amount of at least one Janus kinase inhibitor.

In various embodiments, the method comprises administering a therapeutically effective amount of at least one Janus kinase inhibitor to the subject, wherein the subject is further administered phototherapy.

In various embodiments, the at least one Janus kinase inhibitor is selected from the group consisting of tofacitinib, ruxolitinib, oclacitinib, baricitinib, filgotinib, gandotinib, lestaurtinib, momelotinib, pacritinib, upadacitinib (ABT-494), peficitinib, cucurbitacin I, CHZ868, fedratinib, cerdulatinib, ATI-50001, Leo-124429, or a salt or solvate thereof.

In various embodiments, the at least one Janus kinase inhibitor is tofacitinib or a salt or solvate thereof. In various embodiments, the subject is administered about 5-20 mg/day of tofacitinib.

In various embodiments, the at least one Janus kinase inhibitor is ruxolitinib or a salt or solvate thereof. In various embodiments, the subject is administered about 5-50 mg/day of ruxolitinib.

In various embodiments, the phototherapy comprises low-dose narrow band UVB phototherapy. In various embodiments, the phototherapy comprises a dose equal to or less than about 150 mJ/cm^2 of nbUVB. In various embodiments, the phototherapy comprises a dose equal to or greater than about 150 mJ/cm^2 of nbUVB. In various embodiments, the phototherapy is applied at least to the subject's skin section that is afflicted by vitiligo. In various embodiments, the phototherapy is substantially restricted to the subject's skin section that is afflicted by vitiligo. In various embodiments, the at least one Janus kinase inhibitor is administered topically.

In various embodiments, the intensity, duration and/or frequency of the phototherapy used is ineffective in treating vitiligo in a subject who is not being co-administered the at least one Janus kinase inhibitor. In various embodiments, the subject continues to be administered the at least one Janus kinase inhibitor after the phototherapy is discontinued.

In various embodiments, the at least one Janus kinase inhibitor is part of a pharmaceutical composition further comprising at least one pharmaceutically acceptable excipient. In various embodiments, the at least one Janus kinase inhibitor is administered orally or topically to the subject.

In various embodiments, the at least one Janus kinase inhibitor is the only agent that treats vitiligo that is administered to the subject. In various embodiments, the at least one Janus kinase inhibitor is the only agent that treats vitiligo that is administered to the subject in a therapeutically effective amount.

In various embodiments, the invention further provides instructional material comprising instructions for the concomitant administration of the at least one JAKi with phototherapy to treat vitiligo in a subject. In various embodiments, the kit further comprises a handheld phototherapy device.

BRIEF DESCRIPTION OF THE DRAWINGS

For the purpose of illustrating the invention, there are depicted in the drawings certain embodiments of the invention. However, the invention is not limited to the precise arrangements and instrumentalities of the embodiments depicted in the drawings.

FIGS. 1A-1B depict clinical images of patient 1 in Example 1 with vitiligo. FIG. 1A shows the sun-exposed forearm and dorsal hand, before and during treatment, and demonstrates repigmentation. FIG. 1B shows the chest, before and during treatment, under room light (top panels) and Wood's lamp (bottom panels), and demonstrates repigmentation of sun-exposed lesions involving the upper chest but not sun-protected lesions involving the intermammary chest.

FIGS. 2A-2D depict $CD8^+$ T cell quantification and chemokine protein levels in blister fluid in patient 1 in Example 1. FIG. 2A depicts representative $CD8^+$ T cells in the $CD8^+$ gate for each site at baseline and after treatment. $CD8^+$ T cells were first gated on singlets and then by physical parameters SSC and FSC, all live cells, all $CD45^+$ cells, all $CD3^+$ cells and all $CD8^+$ T cells. FIGS. 2B-2D show a comparison of $CD8^+$ T cell number (FIG. 2B), CXCL9 (FIG. 2C) and CXCL10 (FIG. 2D) in each site before and after treatment. R = responding site, NR = Non-responding site, NL = non-lesional site.

FIG. 3 depicts a model of vitiligo treatment with JAK inhibitor plus light exposure. Interferon (IFN)-γ signals through the IFN-γ Receptor (IFN-γR) via Janus kinase (JAK) 1 and JAK2, initiating phosphorylation of Signal Transducer and Activator of Transcription 1 (STAT1) and translocation to the nucleus where IFN-γ dependent genes, including C-X-C motif chemokine ligand (CXCL) 9 and CXCL10 are transcribed. CXCL9 and CXCL10 recruit autoreactive $CD8^+$ T cells to the skin where they attack melanocytes, leading to depigmentation (white keratinocytes). Tofacitinib inhibits this process by blocking JAK signaling. Even under JAK inhibitor suppression of vitiligo, regimentation depends on photoactivation to stimulate melanocytes to leave their stem cell niche in the hair follicle bulge and seed the epidermis to make pigment.

FIGS. 4A-4B depict data from the study described herein. FIG. 4A shows mart-1 $Pentamter^+$ $CD8^+$ T cells in the peripheral blood mononuclear cells (PBMCs) before and after

treatment. Fluorescent minus-one samples are shown on the left, the full staining is shown on the right. FIG. 4B depicts CXCL9 and CXCL10 chemokine levels in the serum at baseline and post-treatment.

FIGS. 5A-5B depict images of the patient discussed in Example 2, case 1, before (FIG. 5A) and after (FIG. 5B) treatment with tofacitinib and low-dose, narrowband UV-B Phototherapy.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The instant invention is most clearly understood with reference to the following definitions.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. "About" can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

As used in the specification and claims, the terms "comprises," "comprising," "containing," "having," and the like can have the meaning ascribed to them in U.S. patent law and can mean "includes," "including," and the like.

The terms "biomarker" or "marker," as used herein, refers to a molecule that can be detected. Therefore, a biomarker according to the present invention includes, but is not limited to, a nucleic acid, polypeptide, carbohydrate, lipid, inorganic molecule, and/or

organic molecule, each of which may vary widely in size and properties. A "biomarker" can be a bodily substance relating to a bodily condition or disease. A "biomarker" can be detected using any means known in the art or by a previously unknown means that only becomes apparent upon consideration of the marker by the skilled artisan.

A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

The term "depigmentation" as used herein refers to any significant depletion of melanocytes and/or skin pigmentation in a specific area of skin due to vitiligo. In certain embodiments, depigmentation occurs due to complete or partial loss of melanocytes or their function.

The phrase "inhibit," as used herein, means to reduce a molecule, reaction, interaction, gene, mRNA, and/or protein's expression, stability, function or activity by a measurable amount or to prevent entirely. Inhibitors are compounds that, *e.g.*, bind to, partially or totally block stimulation, decrease, prevent, delay activation, inactivate, desensitize, or downregulate a protein, a gene, and an mRNA stability, expression, function and activity, *e.g.*, antagonists.

By "inhibitory nucleic acid" is meant a double-stranded RNA, siRNA, shRNA, or antisense RNA, or a portion thereof, or a mimetic thereof, that when administered to a mammalian cell results in a decrease (e.g., by 10%, 25%, 50%, 75%, or even 90-100%) in the expression of a target gene. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. For example, an inhibitory nucleic acid molecule comprises at least a portion of any or all of the nucleic acids delineated herein.

"Instructional material," as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the composition and/or compound of the invention in a kit. The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition of the invention or be shipped together with a container which contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the

instructional material and the compound cooperatively. Delivery of the instructional material may be, for example, by physical delivery of the publication or other medium of expression communicating the usefulness of the kit, or may alternatively be achieved by electronic transmission, for example by means of a computer, such as by electronic mail, or download from a website.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components that normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (*e.g.*, a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. The preparation can be at least 75%,

at least 90%, and at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

As used herein "Janus kinase" or "JAK" means a member of the tyrosine kinase family of genes or proteins that are important for cytokine signaling.

By "Janus kinase 1" or "Janus kinase type 1" or "JAK1" is meant the member of the JAK family having the following sequence for the human polypeptide, SEQ ID NO: 1

```
                10         20         30         40         50
        MQYLNIKEDC NAMAFCAKMR SSKKTEVNLE APEPGVEVIF YLSDREPLRL
                60         70         80         90        100
        GSGEYTAEEL CIRAAQACRI SPLCHNLFAL YDENTKLWYA PNRTITVDDK
               110        120        130        140        150
        MSLRLHYRMR FYFTNWHGTN DNEQSVWRHS PKKQKNGYEK KKIPDATPLL
               160        170        180        190        200
        DASSLEYLFA QGQYDLVKCL APIRDPKTEQ DGHDIENECL GMAVLAISHY
               210        220        230        240        250
        AMMKKMQLPE LPKDISYKRY IPETLNKSIR QRNLLTRMRI NNVFKDFLKE
               260        270        280        290        300
        FNNKTICDSS VSTHDLKVKY LATLETLTKH YGAEIFETSM LLISSENEMN
               310        320        330        340        350
        WFHSNDGGNV LYYEVMVTGN LGIQWRHKPN VVSVEKEKNK LKRKKLENKH
               360        370        380        390        400
        KKDEEKNKIR EEWNNFSYFP EITHIVIKES VVSINKQDNK KMELKLSSHE
               410        420        430        440        450
        EALSFVSLVD GYFRLTADAH HYLCTDVAPP LIVHNIQNGC HGPICTEYAI
               460        470        480        490        500
        NKLRQEGSEE GMYVLRWSCT DFDNILMTVT CFEKSEQVQG AQKQFKNFQI
               510        520        530        540        550
        EVQKGRYSLH GSDRSFPSLG DLMSHLKKQI LRTDNISFML KRCCQPKPRE
               560        570        580        590        600
        ISNLLVATKK AQEWQPVYPM SQLSFDRILK KDLVQGEHLG RGTRTHIYSG
               610        620        630        640        650
        TLMDYKDDEG TSEEKKIKVI LKVLDPSHRD ISLAFFEAAS MMRQVSHKHI
               660        670        680        690        700
        VYLYGVCVRD VENIMVEEFV EGGPLDLFMH RKSDVLTTPW KFKVAKQLAS
               710        720        730        740        750
        ALSYLEDKDL VHGNVCTKNL LLAREGIDSE CGPFIKLSDP GIPITVLSRQ
               760        770        780        790        800
        ECIERIPWIA PECVEDSKNL SVAADKWSFG TTLWEICYNG EIPLKDKTLI
               810        820        830        840        850
        EKERFYESRC RPVTPSCKEL ADLMTRCMNY DPNQRPFFRA IMRDINKLEE
               860        870        880        890        900
        QNPDIVSEKK PATEVDPTHF EKRFLKRIRD LGEGHFGKVE LCRYDPEGDN
               910        920        930        940        950
        TGEQVAVKSL KPESGGNHIA DLKKEIEILR NLYHENIVKY KGICTEDGGN
               960        970        980        990       1000
        GIKLIMEFLP SGSLKEYLPK NKNKINLKQQ LKYAVQICKG MDYLGSRQYV
              1010       1020       1030       1040       1050
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HRDLAARNVL VESEHQVKIG DFGLTKAIET DKEYYTVKDD RDSPVFWYAP
      1060       1070       1080       1090       1100
ECLMQSKFYI ASDVWSFGVT LHELLTYCDS DSSPMALFLK MIGPTHGQMT
      1110       1120       1130       1140       1150
VTRLVNTLKE GKRLPCPPNC PDEVYQLMRK CWEFQPSNRT SFQNLIEGFE

ALLK
```

By "Janus kinase 2" or "Janus kinase type 2" or "JAK2" is meant the member of the JAK family having the following sequence for the human polypeptide, SEQ ID NO: 2

```
        10         20         30         40         50
MGMACLTMTE MEGTSTSSIY QNGDISGNAN SMKQIDPVLQ VYLYHSLGKS
        60         70         80         90        100
EADYLTFPSG EYVAEEICIA ASKACGITPV YHNMFALMSE TERIWYPPNH
       110        120        130        140        150
VFHIDESTRH NVLYRIRFYF PRWYCSGSNR AYRHGISRGA EAPLLDDFVM
       160        170        180        190        200
SYLFAQWRHD FVHGWIKVPV THETQEECLG MAVLDMMRIA KENDQTPLAI
       210        220        230        240        250
YNSISYKTFL PKCIRAKIQD YHILTRKRIR YRFRRFIQQF SQCKATARNL
       260        270        280        290        300
KLKYLINLET LQSAFYTEKF EVKEPGSGPS GEEIFATIII TGNGGIQWSR
       310        320        330        340        350
GKHKESETLT EQDLQLYCDF PNIIDVSIKQ ANQEGSNESR VVTIHKQDGK
       360        370        380        390        400
NLEIELSSLR EALSFVSLID GYYRLTADAH HYLCKEVAPP AVLENIQSNC
       410        420        430        440        450
HGPISMDFAI SKLKKAGNQT GLYVLRCSPK DFNKYFLTFA VERENVIEYK
       460        470        480        490        500
HCLITKNENE EYNLSGTKKN FSSLKDLLNC YQMETVRSDN IIFQFTKCCP
       510        520        530        540        550
PKPKDKSNLL VFRTNGVSDV PTSPTLQRPT HMNQMVFHKI RNEDLIFNES
       560        570        580        590        600
LGQGTFTKIF KGVRREVGDY GQLHETEVLL KVLDKAHRNY SESFFEAASM
       610        620        630        640        650
MSKLSHKHLV LNYGVCVCGD ENILVQEFVK FGSLDTYLKK NKNCINILWK
       660        670        680        690        700
LEVAKQLAWA MHFLEENTLI HGNVCAKNIL LIREEDRKTG NPPFIKLSDP
       710        720        730        740        750
GISITVLPKD ILQERIPWVP PECIENPKNL NLATDKWSFG TTLWEICSGG
       760        770        780        790        800
DKPLSALDSQ RKLQFYEDRH QLPAPKWAEL ANLINNCMDY EPDFRPSFRA
       810        820        830        840        850
IIRDLNSLFT PDYELLTEND MLPNMRIGAL GFSGAFEDRD PTQFEERHLK
       860        870        880        890        900
FLQQLGKGNF GSVEMCRYDP LQDNTGEVVA VKKLQHSTEE HLRDFEREIE
       910        920        930        940        950
ILKSLQHDNI VKYKGVCYSA GRRNLKLIME YLPYGSLRDY LQKHKERIDH
       960        970        980        990       1000
IKLLQYTSQI CKGMEYLGTK RYIHRDLATR NILVENENRV KIGDFGLTKV
      1010       1020       1030       1040       1050
LPQDKEYYKV KEPGESPIFW YAPESLTESK FSVASDVWSF GVVLYELFTY
      1060       1070       1080       1090       1100
IEKSKSPPAE FMRMIGNDKQ GQMIVFHLIE LLKNNGRLPR PDGCPDEIYM
      1110       1120       1130
IMTECWNNNV NQRPSFRDLA LRVDQIRDNM AG
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By "Janus kinase 3" or "Janus kinase type 3" or "JAK3" is meant the member of the JAK family having the following sequence for the human polypeptide, SEQ ID NO: 3

```
                10         20         30         40         50
MAPPSEETPL IPQRSCSLLS TEAGALHVLL PARGPGPPQR LSFSFGDHLA
        60         70         80         90        100
EDLCVQAAKA SGILPVYHSL FALATEDLSC WFPPSHIFSV EDASTQVLLY
       110        120        130        140        150
RIRFYFPNWF GLEKCHRFGL RKDLASAILD LPVLEHLFAQ HRSDLVSGRL
       160        170        180        190        200
PVGLSLKEQG ECLSLAVLDL ARMAREQAQR PGELLKTVSY KACLPPSLRD
       210        220        230        240        250
LIQGLSFVTR RRIRRTVRRA LRRVAACQAD RHSLMAKYIM DLERLDPAGA
       260        270        280        290        300
AETFHVGLPG ALGGHDGLGL LRVAGDGGIA WTQGEQEVLQ PFCDFPEIVD
       310        320        330        340        350
ISIKQAPRVG PAGEHRLVTV TRTDNQILEA EFPGLPEALS FVALVDGYFR
       360        370        380        390        400
LTTDSQHFFC KEVAPPRLLE EVAEQCHGPI TLDFAINKLK TGGSRPGSYV
       410        420        430        440        450
LRRSPQDFDS FLLTVCVQNP LGPDYKGCLI RRSPTGTFLL VGLSRPHSSL
       460        470        480        490        500
RELLATCWDG GLHVDGVAVT LTSCCIPRPK EKSNLIVVQR GHSPPTSSLV
       510        520        530        540        550
QPQSQYQLSQ MTFHKIPADS LEWHENLGHG SFTKIYRGCR HEVVDGEARK
       560        570        580        590        600
TEVLLKVMDA KHKNCMESFL EAASLMSQVS YRHLVLLHGV CMAGDSTMVQ
       610        620        630        640        650
EFVHLGAIDM YLRKRGHLVP ASWKLQVVKQ LAYALNYLED KGLPHGNVSA
       660        670        680        690        700
RKVLLAREGA DGSPPFIKLS DPGVSPAVLS LEMLTDRIPW VAPECLREAQ
       710        720        730        740        750
TLSLEADKWG FGATVWEVFS GVTMPISALD PAKKLQFYED RQQLPAPKWT
       760        770        780        790        800
ELALLIQQCM AYEPVQRPSF RAVIRDLNSL ISSDYELLSD PTPGALAPRD
       810        820        830        840        850
GLWNGAQLYA CQDPTIFEER HLKYISQLGK GNFGSVELCR YDPLGDNTGA
       860        870        880        890        900
LVAVKQLQHS GPDQQRDFQR EIQILKALHS DFIVKYRGVS YGPGRQSLRL
       910        920        930        940        950
VMEYLPSGCL RDFLQRHRAR LDASRLLLYS SQICKGMEYL GSRRCVHRDL
       960        970        980        990       1000
AARNILVESE AHVKIADFGL AKLLPLDKDY YVVREPGQSP IFWYAPESLS
      1010       1020       1030       1040       1050
DNIFSRQSDV WSFGVVLYEL FTYCDKSCSP SAEFLRMMGC ERDVPALCRL
      1060       1070       1080       1090       1100
LELLEEGQRL PAPPACPAEV HELMKLCWAP SPQDRPSFSA LGPQLDMLWS
      1110       1120
GSRGCETHAF TAHPEGKHHS LSFS
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As used herein, "tyrosine kinase 2" or "TYK2" is meant the member of the JAK family having the following sequence for the human polypeptide, SEQ ID NO: 4

```
10         20         30         40         50
MPLRHWGMAR GSKPVGDGAQ PMAAMGGLKV LLHWAGPGGG EPWVTFSESS
        60         70         80         90        100
LTAEEVCIHI AHKVGITPPC FNLFALFDAQ AQVWLPPNHI LEIPRDASLM
       110        120        130        140        150
LYFRIRFYFR NWHGMNPREP AVYRCGPPGT EASSDQTAQG MQLLDPASFE
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       160        170        180        190        200
YLFEQGKHEF VNDVASLWEL STEEEIHHFK NESLGMAFLH LCHLALRHGI
       210        220        230        240        250
PLEEVAKKTS FKDCIPRSFR RHIRQHSALT RLRLRNVFRR FLRDFQPGRL
       260        270        280        290        300
SQQMVMVKYL ATLERLAPRF GTERVPVCHL RLLAQAEGEP CYIRDSGVAP
       310        320        330        340        350
TDPGPESAAG PPTHEVLVTG TGGIQWWPVE EEVNKEEGSS GSSGRNPQAS
       360        370        380        390        400
LFGKKAKAHK AVGQPADRPR EPLWAYFCDF RDITHVVLKE HCVSIHRQDN
       410        420        430        440        450
KCLELSLPSR AAALSFVSLV DGYFRLTADS SHYLCHEVAP PRLVMSIRDG
       460        470        480        490        500
IHGPLLEPFV QAKLRPEDGL YLIHWSTSHP YRLILTVAQR SQAPDGMQSL
       510        520        530        540        550
RLRKFPIEQQ DGAFVLEGWG RSFPSVRELG AALQGCLLRA GDDCFSLRRC
       560        570        580        590        600
CLPQPGETSN LIIMRGARAS PRTLNLSQLS FHRVDQKEIT QLSHLGQGTR
       610        620        630        640        650
TNVYEGRLRV EGSGDPEEGK MDDEDPLVPG RDRGQELRVV LKVLDPSHHD
       660        670        680        690        700
IALAFYETAS LMSQVSHTHL AFVHGVCVRG PENIMVTEYV EHGPLDVWLR
       710        720        730        740        750
RERGHVPMAW KMVVAQQLAS ALSYLENKNL VHGNVCGRNI LLARLGLAEG
       760        770        780        790        800
TSPFIKLSDP GVGLGALSRE ERVERIPWLA PECLPGGANS LSTAMDKWGF
       810        820        830        840        850
GATLLEICFD GEAPLQSRSP SEKEHFYQRQ HRLPEPSCPQ LATLTSQCLT
       860        870        880        890        900
YEPTQRPSFR TILRDLTRLQ PHNLADVLTV NPDSPASDPT VFHKRYLKKI
       910        920        930        940        950
RDLGEGHFGK VSLYCYDPTN DGTGEMVAVK ALKADCGPQH RSGWKQEIDI
       960        970        980        990       1000
LRTLYHEHII KYKGCCEDQG EKSLQLVMEY VPLGSLRDYL PRHSIGLAQL
      1010       1020       1030       1040       1050
LLFAQQICEG MAYLHAQHYI HRDLAARNVL LDNDRLVKIG DFGLAKAVPE
      1060       1070       1080       1090       1100
GHEYYRVRED GDSPVFWYAP ECLKEYKFYY ASDVWSFGVT LYELLTHCDS
      1110       1120       1130       1140       1150
SQSPPTKFLE LIGIAQGQMT VLRLTELLER GERLPRPDKC PCEVYHLMKN
      1160       1170       1180
CWETEASFRP TFENLIPILK TVHEKYQGQA PSVFSVC
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As used herein, "microRNA" or "miRNA" describes small non-coding RNA molecules, generally about 15 to about 50 nucleotides in length, preferably 17-23 nucleotides, which can play a role in regulating gene expression through, for example, a process termed RNA interference (RNAi). RNAi describes a phenomenon whereby the presence of an RNA sequence that is complementary or antisense to a sequence in a target gene messenger RNA (mRNA) results in inhibition of expression of the target gene. miRNAs are processed from hairpin precursors of about 70 or more nucleotides (pre-miRNA), which are derived from primary transcripts (pri-miRNA) through sequential cleavage by RNAse III enzymes. miRBase is a comprehensive microRNA database located at www *dot* mirbase *dot* org, incorporated by reference herein in its entirety for all purposes.

The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 60 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (*i.e.*, A, U, G, C) in which "U" replaces "T."

Unless specifically stated or obvious from context, the term "or" as used herein is understood to be inclusive.

As used herein, the term "pharmaceutical composition" or "composition" refers to a mixture of at least one compound and/or composition useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound and/or composition to a subject.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound and/or composition useful within the invention within or to the patient such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including the compound and/or composition useful within the invention, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound and/or composition useful within the invention, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions. The "pharmaceutically

acceptable carrier" may further include a pharmaceutically acceptable salt of the compound and/or composition useful within the invention. Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

As used herein, the language "pharmaceutically acceptable salt" refers to a salt of the administered compound and/or composition prepared from pharmaceutically acceptable non-toxic acids and bases, including inorganic acids, inorganic bases, organic acids, inorganic bases, solvates, hydrates, and clathrates thereof. Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include sulfate, hydrogen sulfate, hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β-hydroxybutyric, salicylic, galactaric and galacturonic acid. Suitable pharmaceutically acceptable base addition salts of compounds and/or compositions of the invention include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylene-diamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared from the corresponding compound and/or composition by reacting, for example, the appropriate acid or base with the compound and/or composition.

The terms "pharmaceutically effective amount" and "effective amount" refer to a nontoxic but sufficient amount of an agent to provide the desired biological result. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease or disorder, or any other desired alteration of a biological system. An appropriate effective amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. By "pharmaceutical formulation" it is further meant that the carrier,

solvent, excipient(s) and/or salt must be compatible with the active ingredient of the formulation (*e.g.*, a compound of the invention). It is understood by those of ordinary skill in this art that the terms "pharmaceutical formulation" and "pharmaceutical composition" are generally interchangeable, and they are so used for the purposes of this application.

The term "phototherapy" as used herein refers to controlled and/or prescribed application of light to an area of or the entire body of a patient in order to derive a therapeutic benefit. Deliberate exposure of the body to sunlight is within the scope of this term, however exposure to light occurring during the course of ordinary life is not.

As used herein, "polynucleotide" includes cDNA, RNA, DNA/RNA hybrid, antisense RNA, siRNA, miRNA, snoRNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified to contain non-natural or derivatized, synthetic, or semi-synthetic nucleotide bases. Also, included within the scope of the invention are alterations of a wild type or synthetic gene, including but not limited to deletion, insertion, substitution of one or more nucleotides, or fusion to other polynucleotide sequences.

As used herein, the term "prevent," "prevention," or "preventing" refers to any method to partially or completely prevent or delay the onset, worsening, and/or recurrence of one or more symptoms or features of a disease, disorder, and/or condition. Prevention is causing the clinical symptoms of the disease state not to develop, worsen, and/or recur, *i.e.*, inhibiting the onset, spread, and/or recurrence of disease, in a subject that may be exposed to or predisposed to the disease state or that has been successfully treated already, but does not yet experience or display symptoms of the disease state. Prevention may be administered to a subject who does not exhibit signs or symptoms of a disease, disorder, and/or condition.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

The term "repigmentation" as used herein refers to any significant replenishment of melanocytes and/or skin pigmentation in a specific area of skin. In certain embodiments, repigmentation occurs due to generation of functional melanocytes.

By "siRNA" is meant a double stranded RNA. Optimally, an siRNA is 18, 19, 20, 21, 22, 23 or 24 nucleotides in length and has a 2 base overhang at its 3' end. These dsRNAs can be introduced to an individual cell or to a whole animal; for example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity.

By "specifically binds" is meant an agent that recognizes and binds a polypeptide or polynucleotide of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polynucleotide of the invention. In some embodiments, the agent is a nucleic acid molecule.

As used herein, the term "subject," "patient" or "individual" to which administration is contemplated includes, but is not limited to, humans (*i.e.*, a male or female of any age group, *e.g.*, a pediatric subject (e.g., infant, child, adolescent) or adult subject (*e.g.*, young adult, middle-aged adult or senior adult)) and/or other primates (*e.g.*, cynomolgus monkeys, rhesus monkeys); mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, goats, cats, and/or dogs; and/or birds, including commercially relevant birds such as chickens, ducks, geese, quail, and/or turkeys.

As used herein, the term "therapeutically effective amount" is an amount of a compound and/or composition of the invention, that when administered to a patient, treats, minimizes and/or ameliorates a symptom of the disease or disorder. The amount of a compound and/or composition of the invention that constitutes a "therapeutically effective amount" will vary depending on the compound, the disease state and its severity, the age of the patient to be treated, and the like. The therapeutically effective amount can be determined routinely by one of ordinary skill in the art having regard to his own knowledge and to this disclosure.

The terms "treat," "treating," and "treatment," refer to therapeutic or preventative measures described herein. The methods of "treatment" employ administration to a subject, in need of such treatment, a compound and/or composition of the present invention, for example, a subject afflicted with a disease or disorder, or a subject who ultimately may acquire such a disease or disorder, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

As used herein, the terms "ultraviolet light" or "UV" refers to light with a wavelength between 10 and 400 nm, including but not limited to, ultraviolet B (UVB, 280-320 nm) and ultraviolet A (UVA, 320-400 nm) and narrow regions thereof, e.g., narrowband ultraviolet B (nbUVB, 311-312 nm) and UVA1 (340-400 nm), with any intensity or from any source, including a manufactured device or the sun.

As used herein, the term "vitiligo" refers to the autoimmune disease associated with loss of functional melanocytes resulting in loss of pigment in circumscribed and/or diffuse areas of the skin.

Selected abbreviations used herein include: nbUVB, narrowband ultraviolet B; IFN-γ, Interferon-γ; AA, alopecia areata; JAK, Janus kinase; TYK, tyrosine kinase; BSA, body surface area; SD, standard deviation; C-X-C, motif chemokine ligand, CXCL; PBMCs, peripheral blood mononuclear cells.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

In one aspect, the invention comprises a method for treating vitiligo in a subject. In certain embodiments, the method comprises administering at least one Janus kinase inhibitor (JAKi) to the subject, wherein the subject is further administered phototherapy. In other embodiments, the administration of the at least one JAKi and phototherapy is initially approximately contemporaneous. In other embodiments, after at least partial repigmentation is achieved, phototherapy is discontinued. In yet other embodiments, after at least partial repigmentation is achieved, administration of the at least one JAKi continues in order to maintain pigmentation. In yet other embodiments, the method reduces the area of depigmentation.

The pathogenesis of vitiligo involves $CD8^+$ T cell production of IFN-γ, which leads to CXCL9/10 expression by keratinocytes and further recruitment of $CD8^+$ T cells, resulting in melanocyte destruction. As reported herein, treatment with tofacitinib only led to repigmentation when there was concomitant light exposure, either sunlight or nbUVB phototherapy. The low doses of nbUVB that were beneficial would not typically be effective as monotherapy in vitiligo. Adverse events were mild and infrequent.

Without wishing to be bound by any particular theory, treatment of vitiligo with JAKi's appears to require light exposure, which can be relatively low in intensity, such as regular exposure to sunlight.

The model shown in FIG. 3 summarizes a non-limiting mechanism by which treatment with JAKi's combined with phototherapy promotes repigmentation in areas affected by vitiligo. Repigmentation requires both suppression of inflammation in the skin, which is achieved with JAK inhibitor treatment, and melanocyte stimulation via light exposure, which is most effectively accomplished by phototherapy. In certain non-limiting embodiments, low doses of nbUVB can be sufficient to promote melanocyte regeneration. Although higher doses are within the scope of certain embodiments of the invention and may under some circumstances provide benefits, in other embodiments treatment with heretofore sub-clinical dosing can provide benefits and avoid adverse effects associated with more intensive exposure to UV.

In certain embodiments, the JAKi is any known or heretofore unknown JAKi. In other embodiments, the JAKi is at least one selected from the group consisting of:

tofacitinib (3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino) piperidin-1-yl)-3-oxopropanenitrile),

ruxolitinib ((R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentyl propanenitrile),

oclacitinib (N-Methyl-1-(trans-4-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino) cyclohexyl)methanesulfonamide),

baricitinib (2-(3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-1-(ethylsulfonyl) azetidin-3-yl)acetonitrile),

filgotinib (N-(5-(4-((1,1-dioxidothiomorpholino)methyl)phenyl)-[1,2,4]triazolo[1,5-a] pyridin-2-yl)cyclopropane carboxamide),

gandotinib (3-(4-chloro-2-fluorobenzyl)-2-methyl-N-(5-methyl-1H-pyrazol-3-yl)-8-(morpholinomethyl)imidazo(1,2-b)pyridazin-6-amine),

lestaurtinib (9,12-Epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6] benzodiazocin-1-one, 2,3,9,10,11,12-hexahydro-10-hydroxy-10-(hydroxymethyl)-9-methyl-, (9S,10S,12R)-),

momelotinib (*N*-(cyanomethyl)-4-{2-[4-(morpholin-4-yl)anilino]pyrimidin-4-yl}benzamide),

pacritinib (11-(2-pyrrolidin-1-ylethoxy)-14,19-dioxa-5,7,26-triazatetracyclo (19.3.1.1(2,6).1(8,12))heptacosa-1(25),2(26),3,5,8,10,12(27),16,21,23-decaene),

upadacitinib (ABT-494, or (3S,4R)-3-ethyl-4-(3H-imidazo[1,2-a]pyrrolo[2,3-e]pyrazin-8-yl)-N-(2,2,2-trifluoroethyl)pyrrolidine-1-carboxamide),

peficitinib (4-[[(1R,3S)-5-hydroxy-2-adamantyl]amino]-1H-pyrrolo[2,3-b]pyridine-5-carboxamide),

cucurbitacin I (2,16α,20,25-Tetrahydroxy-9-methyl-19-Nor-9β,10α-lanosta-1,5,23-triene-3,11,22-trione),

CHZ868 (N-(4-((2-((2,4-difluorophenyl)amino)-1,4-dimethyl-1H-benzo[d]imidazol-5-yl)oxy)pyridin-2-yl)acetamide),

fedratinib (Benzenesulfonamide, N-(1,1-dimethylethyl)-3-[[5-methyl-2-[[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]amino]-4-pyrimidinyl]amino]-),

cerdulatinib (4-(cyclopropylamino)-2-((4-(4-(ethylsulfonyl)piperazin-1-yl)phenyl)amino) pyrimidine-5-carboxamide)

ATI-50001 (Aclaris Therapeutics's JAKi),

Leo-124429 (LeoPharma's JAKi),

or a salt or solvate thereof.

In certain embodiments, the JAKi is tofacitinib, or a salt or solvate thereof. In other embodiments, the JAKi is ruxolitinib, or a salt or solvate thereof.

In certain embodiments, the at least one JAKi is a specific inhibitor to one or more of JAK1, JAK2, JAK3, or tyrosine kinase (Tyk) 2. In other embodiments, the at least one JAKi is a non-specific inhibitor. In embodiments including more than one JAKi, multiple inhibitor types can be utilized. In certain embodiments, the JAKi is administered in a pharmaceutical composition comprising one or more pharmaceutically acceptable excipients. In other embodiments, the JAKi is administered orally. In yet other embodiments, the JAKi is administered topically. In yet other embodiments, topical ruxolitinib is administered to the subject as a 1.5% cream. In yet other embodiments, the JAKi is administered intralesionally. In yet other embodiments, the JAKi is administered subcutaneously.

In certain embodiments, the phototherapy is ultraviolet B (UVB, 280-320 nm) phototherapy. In other embodiments, the phototherapy is narrowband ultraviolet B (nbUVB, 311-312 nm) phototherapy. In yet other embodiments, the phototherapy is ultraviolet A (UVA, 320-340 nm) phototherapy. In yet other embodiments, the phototherapy is ultraviolet A1 (UVA1, 340-400 nm) phototherapy. In yet other embodiments, the phototherapy is visible light (400-700 nm) phototherapy. In yet other embodiments, the phototherapy is a combination of phototherapies, including but not limited to those listed above.

In certain embodiments, the phototherapy comprises a dose that is insufficient for use as a monotherapy for vitiligo, including but not limited to 150 mJ/cm^2 of nbUVB or less. In other embodiments, the phototherapy comprises a dose of, in non-limiting embodiments, 50–4000 mJ/cm^2 of nbUVB and/or 10–6,000 mJ/cm^2 of UVB and/or 0.5–40 J/cm^2 of UVA and/or 10–200 J/cm^2 of UVA1. In yet other embodiments, the phototherapy has a duration

that is insufficient (non-therapeutically effective) for use as a monotherapy for vitiligo, including but not limited to 4 weeks. In yet other embodiments, the phototherapy comprises a number of phototherapy treatments that is insufficient (non-therapeutically effective) for use as a monotherapy for vitiligo, including but not limited to 12 treatments.

In certain embodiments, phototherapy is applied to the subject's entire body. In other embodiments, phototherapy is limited to specific areas of the subject's body, including but not limited to areas of depigmentation. Accordingly, in certain embodiments, phototherapy is delivered by a full body-sized unit or a smaller unit or a handheld device. In yet other embodiments, phototherapy is delivered by controlled or prescribed exposure to sunlight.

In certain embodiments, the at least one JAKi includes other methods for inhibiting the activity of JAK, including but not limited to siRNA, antibodies, isolated peptides or isolated polynucleotides, which bind JAK at the level of protein or nucleic acid and reduce the level of JAK activity or any other known or heretofore unknown method for modulating the activity of JAK.

Kits

In another aspect, the invention comprises a kit comprising an effective amount of at least one JAKi and instructional material comprising instructions for the concomitant administration of the at least one JAKi with phototherapy. In certain embodiments, the JAKi is any of the above described inhibitors. The instructional material can contain any information necessary to allow the practice of the above described method. In particular, the instructional material can include directions and information directing the concomitant administration of the at least one JAKi and phototherapy. Possible means and methods for delivering phototherapy are described elsewhere herein and includes sunlight. In certain embodiments, the kit also includes a UV emitting device for phototherapy. In general, the UV emitting device may be any that a person of skill in the art would recognize as suitable for the purpose.

Administration/Dosage/Formulations

The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the subject either prior to or after the onset of a disease or disorder contemplated in the invention. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic

formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a patient, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder contemplated in the invention. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the state of the disease or disorder in the patient; the age, sex, and weight of the patient; and the ability of the therapeutic compound to treat a disease or disorder contemplated in the invention. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 0.1 and 5,000 mg/kg of body weight/per day. The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of from 1 ng/kg/day and 100 mg/kg/day. In certain embodiments, the invention envisions administration of a dose which results in a concentration of the compound of the present invention from 1 μM and 10 μM in a mammal. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

In particular, the selected dosage level depends upon a variety of factors including the activity of the particular compound employed, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds or materials used in combination with the compound, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well, known in the medical arts.

A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required

in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/ formulating such a therapeutic compound for the treatment of a disease or disorder contemplated in the invention.

In certain embodiments, the compositions of the invention are formulated using one or more pharmaceutically acceptable excipients or carriers. In other embodiments, the pharmaceutical compositions of the invention comprise a therapeutically effective amount of a compound of the invention and a pharmaceutically acceptable carrier.

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

In certain embodiments, the compositions of the invention are administered to the patient in dosages that range from one to five times per day or more. In other embodiments, the compositions of the invention are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It is readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of the invention varies from individual to individual depending on many factors including, but not limited to, age,

disease or disorder to be treated, gender, overall health, and other factors. Thus, the invention should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient is determined by the attending physical taking all other factors about the patient into account.

Compounds of the invention for administration may be in the range of from about 1 μg to about 10,000 mg, about 20 μg to about 9,500 mg, about 40 μg to about 9,000 mg, about 75 μg to about 8,500 mg, about 150 μg to about 7,500 mg, about 200 μg to about 7,000 mg, about 3050 μg to about 6,000 mg, about 500 μg to about 5,000 mg, about 750 μg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 30 mg to about 1,000 mg, about 40 mg to about 900 mg, about 50 mg to about 800 mg, about 60 mg to about 750 mg, about 70 mg to about 600 mg, about 80 mg to about 500 mg, and any and all whole or partial increments therebetween.

In some embodiments, the dose of a compound of the invention is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound of the invention used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

In certain embodiments, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the invention, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of a disease or disorder contemplated in the invention.

Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of

administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like.

Routes of administration of any of the compositions of the invention include oral, nasal, rectal, intravaginal, parenteral, buccal, sublingual or topical. The compounds for use in the invention may be formulated for administration by any suitable route, such as for oral or parenteral, for example, transdermal, transmucosal (*e.g.*, sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration.

Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein.

Oral Administration

For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gelcaps. The compositions intended for oral use may be prepared according to any method known in the art and such compositions may contain one or more agents selected from the group consisting of inert, non-toxic pharmaceutically excipients that are suitable for the manufacture of tablets. Such excipients include, for example an inert diluent such as lactose; granulating and disintegrating agents such as cornstarch; binding agents such as starch; and lubricating agents such as magnesium stearate. The tablets may be uncoated or they may be coated by known techniques for elegance or to delay the release of the active ingredients. Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert diluent.

For oral administration, the compounds of the invention may be in the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, polyvinylpyrrolidone, hydroxypropylcellulose or

hydroxypropylmethylcellulose); fillers (*e.g.*, cornstarch, lactose, microcrystalline cellulose or calcium phosphate); lubricants (*e.g.*, magnesium stearate, talc, or silica); disintegrates (*e.g.*, sodium starch glycollate); or wetting agents (*e.g.*, sodium lauryl sulfate). If desired, the tablets may be coated using suitable methods and coating materials such as OPADRY™ film coating systems available from Colorcon, West Point, Pa. (*e.g.*, OPADRY™ OY Type, OYC Type, Organic Enteric OY-P Type, Aqueous Enteric OY-A Type, OY-PM Type and OPADRY™ White, 32K18400). Liquid preparation for oral administration may be in the form of solutions, syrups or suspensions. The liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agent (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); and preservatives (*e.g.*, methyl or propyl p-hydroxy benzoates or sorbic acid).

Granulating techniques are well known in the pharmaceutical art for modifying starting powders or other particulate materials of an active ingredient. The powders are typically mixed with a binder material into larger permanent free-flowing agglomerates or granules referred to as a "granulation". For example, solvent-using "wet" granulation processes are generally characterized in that the powders are combined with a binder material and moistened with water or an organic solvent under conditions resulting in the formation of a wet granulated mass from which the solvent must then be evaporated.

Melt granulation generally consists in the use of materials that are solid or semi-solid at room temperature (*i.e.* having a relatively low softening or melting point range) to promote granulation of powdered or other materials, essentially in the absence of added water or other liquid solvents. The low melting solids, when heated to a temperature in the melting point range, liquefy to act as a binder or granulating medium. The liquefied solid spreads itself over the surface of powdered materials with which it is contacted, and on cooling, forms a solid granulated mass in which the initial materials are bound together. The resulting melt granulation may then be provided to a tablet press or be encapsulated for preparing the oral dosage form. Melt granulation improves the dissolution rate and bioavailability of an active (*i.e.* drug) by forming a solid dispersion or solid solution.

U.S. Patent No. 5,169,645 discloses directly compressible wax-containing granules having improved flow properties. The granules are obtained when waxes are admixed in the melt with certain flow improving additives, followed by cooling and granulation of the admixture. In certain embodiments, only the wax itself melts in the melt combination of the wax(es) and additives(s), and in other cases both the wax(es) and the additives(s) melt.

The present invention also includes a multi-layer tablet comprising a layer providing for the delayed release of one or more compounds of the invention, and a further layer providing for the immediate release of a medication for treatment of a disease or disorder contemplated in the invention. Using a wax/pH-sensitive polymer mix, a gastric insoluble composition may be obtained in which the active ingredient is entrapped, ensuring its delayed release.

Parenteral Administration

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intravenous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multidose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In certain embodiments of a formulation for parenteral administration, the active ingredient is provided in dry (*i.e.*, powder or granular) form for reconstitution with a suitable vehicle (*e.g.*, sterile pyrogen free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-

toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butanediol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Topical Administration

An obstacle for topical administration of pharmaceuticals is the stratum corneum layer of the epidermis. The stratum corneum is a highly resistant layer comprised of protein, cholesterol, sphingolipids, free fatty acids and various other lipids, and includes cornified and living cells. One of the factors that limit the penetration rate (flux) of a compound through the stratum corneum is the amount of the active substance that can be loaded or applied onto the skin surface. The greater the amount of active substance which is applied per unit of area of the skin, the greater the concentration gradient between the skin surface and the lower layers of the skin, and in turn the greater the diffusion force of the active substance through the skin. Therefore, a formulation containing a greater concentration of the active substance is more likely to result in penetration of the active substance through the skin, and more of it, and at a more consistent rate, than a formulation having a lesser concentration, all other things being equal.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically administrable formulations may, for example, comprise from about 0.05% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Enhancers of permeation may be used. These materials increase the rate of penetration of drugs across the skin. Typical enhancers in the art include ethanol, glycerol monolaurate, PGML (polyethylene glycol monolaurate), dimethylsulfoxide, and the like. Other enhancers include oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone.

One acceptable vehicle for topical delivery of some of the compositions of the

invention may contain liposomes. The composition of the liposomes and their use are known in the art (for example, see Constanza, U.S. Patent No. 6,323,219).

In alternative embodiments, the topically active pharmaceutical composition may be optionally combined with other ingredients such as adjuvants, anti-oxidants, chelating agents, surfactants, foaming agents, wetting agents, emulsifying agents, viscosifiers, buffering agents, preservatives, and the like. In other embodiments, a permeation or penetration enhancer is included in the composition and is effective in improving the percutaneous penetration of the active ingredient into and through the stratum corneum with respect to a composition lacking the permeation enhancer. Various permeation enhancers, including oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone, are known to those of skill in the art. In another aspect, the composition may further comprise a hydrotropic agent, which functions to increase disorder in the structure of the stratum corneum, and thus allows increased transport across the stratum corneum. Various hydrotropic agents such as isopropyl alcohol, propylene glycol, or sodium xylene sulfonate, are known to those of skill in the art.

The topically active pharmaceutical composition should be applied in an amount effective to affect desired changes. As used herein "amount effective" shall mean an amount sufficient to cover the region of skin surface where a change is desired. An active compound should be present in the amount of from about 0.0001% to about 15% by weight volume of the composition. More preferable, it should be present in an amount from about 0.0005% to about 5% of the composition; most preferably, it should be present in an amount of from about 0.001% to about 1% of the composition. Such compounds may be synthetically-or naturally derived.

Additional Administration Forms

Additional dosage forms of this invention include dosage forms as described in U.S. Patents Nos. 6,340,475; 6,488,962; 6,451,808; 5,972,389; 5,582,837; and 5,007,790. Additional dosage forms of this invention also include dosage forms as described in U.S. Patent Applications Nos. 20030147952; 20030104062; 20030104053; 20030044466; 20030039688; and 20020051820. Additional dosage forms of this invention also include dosage forms as described in PCT Applications Nos. WO 03/35041; WO 03/35040; WO 03/35029; WO 03/35177; WO 03/35039; WO 02/96404; WO 02/32416; WO 01/97783; WO 01/56544; WO 01/32217; WO 98/55107; WO 98/11879; WO 97/47285; WO 93/18755; and WO 90/11757.

Controlled Release Formulations and Drug Delivery Systems

In certain embodiments, the formulations of the present invention may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, delayed release and pulsatile release formulations.

The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that may, although not necessarily, result in substantially constant blood levels of a drug over an extended time period. The period of time may be as long as a month or more and should be a release which is longer that the same amount of agent administered in bolus form.

For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material that provides sustained release properties to the compounds. As such, the compounds for use the method of the invention may be administered in the form of microparticles, for example, by injection or in the form of wafers or discs by implantation.

In certain embodiments, the compounds of the invention are administered to a patient, alone or in combination with another pharmaceutical agent, using a sustained release formulation.

The term delayed release is used herein in its conventional sense to refer to a drug formulation that provides for an initial release of the drug after some delay following drug administration and that may, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours.

The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration.

The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

As used herein, short-term refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes and any or all whole or partial increments thereof after drug administration after drug administration.

As used herein, rapid-offset refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes, and any and all whole or partial increments thereof after drug administration.

Dosing

The therapeutically effective amount or dose of a compound of the present invention depends on the age, sex and weight of the patient, the current medical condition of the patient and the progression of a disease or disorder contemplated in the invention. The skilled artisan is able to determine appropriate dosages depending on these and other factors.

A suitable dose of a compound of the present invention may be in the range of from about 0.01 mg to about 5,000 mg per day, such as from about 0.1 mg to about 1,000 mg, for example, from about 1 mg to about 500 mg, such as about 5 mg to about 250 mg per day. The dose may be administered in a single dosage or in multiple dosages, for example from 1 to 4 or more times per day. When multiple dosages are used, the amount of each dosage may be the same or different. For example, a dose of 1 mg per day may be administered as two 0.5 mg doses, with about a 12-hour interval between doses.

It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on.

In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the compound of the invention is optionally given continuously; alternatively, the dose of drug being administered is temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday optionally varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday includes from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, is reduced, as a function of the disease or disorder, to a level at which the improved disease is retained. In certain embodiments, patients require intermittent treatment on a long-term basis upon any recurrence of symptoms and/or infection.

The compounds for use in the method of the invention may be formulated in unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosage for patients undergoing treatment, with each unit containing a predetermined

quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical carrier. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*, about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

Toxicity and therapeutic efficacy of such therapeutic regimens are optionally determined in cell cultures or experimental animals, including, but not limited to, the determination of the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD_{50} and ED_{50}. The data obtained from cell culture assays and animal studies are optionally used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with minimal toxicity. The dosage optionally varies within this range depending upon the dosage form employed and the route of administration utilized.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

Methods & Materials

This study is a retrospective case series of 10 patients seen between July 2014 and January 2017. Medical records of patients with vitiligo, aged 18 years and older, treated with tofacitinib for at least 3 months were reviewed. Clinical and demographic information, including biological sex, age, disease duration and course, medical history, family history, and prior treatments were collected. Before initiating tofacitinib, all patients underwent baseline laboratory evaluation including comprehensive metabolic panel, complete blood cell count with differential, fasting lipid panel, QuantiFERON-TB Gold® (Cellestis Limited, Melbourne, Australia), and screening for HIV and hepatitis B and C. Body surface area (BSA) of depigmentation was assessed prior to and at the end of treatment. Serial laboratory monitoring, physical exams, and review of systems were used to monitor for adverse events. Suction blister sampling was performed in one patient before beginning tofacitinib and then again 10 months later to assess changes in T cell recruitment and chemokine expression. Three sites were selected for sampling – one was a site of vitiligo that did not previously respond to treatment with tofacitinib, one was a recently active site (featuring confetti depigmentation) that previously responded to tofacitinib, and one was unaffected skin.

Suction Blister Sampling and Processing

The Negative Pressure Instrument Model NP-4 (Electronic Diversities®, Finksburg, MD) was used to induce suction blisters (1 cm in diameter). Blisters formed at a pressure between 10-15 mm Hg negative pressure and at a constant temperature of 40°C. Once blisters formed, the blister fluid was aspirated 135 through the roof using a 1 mL insulin syringe. Cells in the blister fluid were pelleted at 330 x g for 10 minutes and prepared for flow cytometry. The supernatant of the blister fluid was stored at -80°C until analysis by enzyme-linked immunosorbent-assays (ELISA) for chemokines. None of the blisters were hemorrhagic.

ELISA

Chemokines were assayed using the Human CXLC9/MIG DuoSet ELISA (DY392) and Human CXCL10/IP-10 DuoSet ELISA (DY266, R&D Systems) per the manufacturer's

instructions. Optical densities were measured using a Perkin Elmer EnVision 2102® multilabel reader and analyzed using a 4 parameter logarithmic standard curve.

Flow Cytometry

Cells were blocked with Human TruStain and incubated with an antibody cocktail for 30 minutes at 4°C. Anti-human CD45 (2D1) and CD8 (SK1) were used at 1:20 dilution. Anti-human CD3 (OKT3) was used at 1:200 dilution (BioLegend) and Fixable Viability Dye eFluor 455UV at 1:1000 (Ebioscience). Prior to cell staining in the PBMCs, 10^6 PBMCs were treated with desatinib for 30 minutes at 36°C, pelleted and incubated with Mart-1 Pentamer (ProImmune, 082) for 10 minutes at room temperature.

Example 1

Patient characteristics

All 10 patients in this case series were adults. Duration of disease was 4-33 years (mean 16.6, SD, standard deviation, 8.8). Eight patients had generalized vitiligo and 2 patients had primarily acral involvement, with 1-100% BSA. Additional patient characteristics are described in Table 1. Previous treatments included nbUVB (9 patients), topical calcineurin inhibitors (7), topical steroids (4), psoralen with ultraviolet A (4), excimer laser (2), blister grafting (1), cryotherapy (1), Fraxel laser (1), pseudocatalase cream (1), prednisone (1), and secukinumab (1).

Table 1. Clinical characteristics of patients

Patient No.	Age	Sex	Race	BSA prior to tofacitinib	BSA after to tofacitinib	Tofacitinib treatment duration (months)	Vitiligo disease duration (years)	Responder Status
1	54	F	Caucasian	10%	4%	10	4	R
2	45	M	Caucasian	28%	24%	8	23	R
3	46	F	Caucasian	39%	24%	11	16	R
4	55	F	Caucasian	10%	8%	14	24	R
5	45	M	East Indian	2%	2%*	10	5	R
6	28	M	Caucasian	7%	7%	3	14	NR
7	47	F	Hispanic	1%	1%	9	18	NR

8	49	M	Caucasian	4%	4%	15	17	NR
9	32	M	Hispanic	6%	6%	4	12	NR
10	73	F	Caucasian	100%	100%	11	33	NR

BSA, body surface area; R, responder; NR, non-responder

*Islands of repigmentation, apparent after 12 treatments with nbUVB phototherapy did not change the BSA appreciably at this early time point.

Clinical course and results

Ten patients underwent treatment with tofacitinib 5-10 mg QD-BID for an average of 9.5 months (SD 3.8, range 3-15). A mean decrease of 5.4% BSA involvement with vitiligo was observed in 5/10 patients, while the other 5 patients did not achieve any repigmentation. In the 5 patients who achieved some reversal of disease, repigmentation occurred only in sun-exposed areas of skin in 3 of them, diffusely in another patient undergoing concomitant full-body nbUVB phototherapy, and to the dorsal hands in another patient after starting concomitant hand nbUVB phototherapy.

Patient 1 is a 45-year-old Caucasian female who had generalized vitiligo affecting approximately 10% BSA. She had previously been treated with tofacitinib 5 mg daily and, over 5 months, experienced near complete repigmentation of her face, forearms, and dorsal hands, with only minimal repigmentation at other body sites, including the trunk. Subsequently, she stopped tofacitinib for 8 months and, during this period, depigmentation recurred in the areas that had previously responded. Prior to re-treatment with tofacitinib and again after 10 months of treatment, she underwent suction blister sampling in order to evaluate the effect of this treatment on vitiligo pathogenesis.

Treatment with tofacitinib 5 mg daily over 6 months, started in late spring, again led to repigmentation of the face, forearms and dorsal hands, and the upper back and shoulders, but not the lower back or other sun-protected body sites.

Patient 2 is a 45-year-old Caucasian male who had generalized vitiligo affecting approximately 28% BSA. In his occupation as an athletics coach, his days were spent in the sun, usually wearing shorts and short-sleeved shirts. Treatment with tofacitinib 5 mg twice daily, started in early spring, led to partial repigmentation of the face, lateral and posterior neck, and forearms over 3 months. After 8 months of treatment, near-complete repigmentation was noted on the face, neck (but not in the submental area), and forearms, as well as islands of repigmentation involving the dorsal hands and lower legs; sun-protected

body sites, however, remained unchanged. Repigmentation was maintained while taking tofacitinib during the winter, when sunlight exposure was minimal.

Patient 3 is a 46-year-old Caucasian female who had generalized vitiligo affecting approximately 39% BSA. She reported some repigmentation with nbUVB in the past, but out of concern for skin cancer, she decreased the dose and had been treating herself at a dose of 150 mJ every other day with no improvement, and this was continued without modification after initiation of tofacitinib 5 mg twice daily. After 11 months of treatment, complete repigmentation of the face and >75% repigmentation of the back was observed, and innumerable islands of repigmentation involving all other body sites were apparent.

Patient 4 is a 55-year-old Caucasian female who had generalized vitiligo affecting approximately 10% BSA. After 14 months of therapy, near-complete repigmentation of the face was noted; neither the hands nor sun-protected body sites, however, showed evidence of repigmentation.

Patient 5 is a 45-year-old East Indian male who had vitiligo affecting his dorsal hands, elbows, lower back, and buttocks. He wore dark brown makeup on his hands to disguise the white patches. His vitiligo had been unresponsive to tofacitinib 5-10 mg twice daily for 1 year when he initiated concomitant nbUVB hand phototherapy, and, after approximately 12 treatments (most recent dose 350 mJ), islands of repigmentation were apparent.

Of the remaining 5 patients, who did not experience repigmentation, only one reported significant sunlight exposure, and the others either avoided sunlight or practiced photoprotection.

Adverse events

The most common side effect was upper respiratory infection in 2 patients. One patient reported weight gain of 5 pounds and one patient reported arthralgias. Mild elevations of lipids were noted in 4 patients. There were no serious adverse events.

Suction Blister Sampling

Previous analysis of suction blisters of active vitiligo lesions demonstrated that T cell number and CXCL9 protein level were both sensitive and specific to predict disease activity. This method was used to measure the treatment response of patient 1. Prior to re-treatment with tofacitinib, she underwent suction blister sampling of a treatment unresponsive site (intermammary chest), a treatment-responsive site (forearm), and skin unaffected by vitiligo (arm). After 10 months of tofacitinib, she underwent blister sampling again at the same sites.

A small number of T cells was detected in all sites at baseline as well as during treatment. At baseline, there were 29 $CD8^+$ T cells in the previously non-responding lesion and a greater number in the recently developed lesion (69 cells). Both lesional sites had more cells than in unaffected skin. Following treatment with tofacitinib, absolute numbers in each site were reduced (FIGS. 2A and 2B). Similarly, melanocyte-specific T cells (HLA pentamer positive) were detected in the PBMCs at baseline, and this percentage did not change with tofacitinib treatment, and even increased slightly (FIG. 1A).

CXLC9 was elevated in all 4 lesional sites compared to unaffected skin at baseline (FIG. 2, panel C), and CXCL10 was elevated in 3 of 4 lesional sites (FIG. 2D). Following treatment, chemokine levels became undetectable in both the blister fluid (FIGS. 2C-2D) and serum (FIG. 4B).

In summary, tofacitinib effectively decreased T cell numbers and chemokine proteins (a measure of IFN-γ signaling) in the skin. In the blood, tofacitinib did not affect the number of autoreactive T cells but did decrease chemokines.

Example 2

Case 1. A Hispanic woman in her 30s presented with a 12-year history of vitiligo. Facial lesions appeared during her first pregnancy and spread over the following year to her neck, torso, and extremities. Her family history was unremarkable. Treatment with liquid nitrogen and topical corticosteroids had been unsuccessful, and a 3-month course of topical MBEH failed to induce depigmentation. On physical examination, there were white patches involving about 75% of her face (FIG. 5A) as well as white patches on the neck, chest, forearms, hands, and shins. Treatment was begun with tofacitinib, 5mg, twice daily and full-body narrowband UV-B (400-500 mJ) twice weekly. After 3 months of treatment, there was nearly complete repigmentation of her face (FIG. 5B), 75% or greater repigmentation of her neck, chest, forearms, and shins, and only minimal freckling of the dorsal hands.

Case 2. A white man in his 50s presented with long-standing vitiligo. Treatment with topical corticosteroids, topical calcineurin inhibitors, excimer laser, and narrowband UV-B had been unsuccessful. Four years prior to presentation, he had achieved depigmentation of the face using topical MBEH. On physical examination, there were white patches involving about 90% of his face as well as white patches on the torso and arms. Treatment was begun with tofacitinib, 5mg, twice daily and narrowband UV-B (360-500 mJ) 2 to 3 times weekly to only the face. After 3 months of treatment, there was about 50%

repigmentation of the face, and, after 6 months, about 75% facial repigmentation. No repigmentation occurred at the other body sites.

Both patients have continued this treatment regimen, and at last follow-up there were no adverse effects, and complete blood cell count, liver function tests, serum creatinine, and fasting lipids revealed no abnormalities.

In the present cases, treatment of 2 patients with vitiligo with significant facial involvement using a combination of tofacitinib and low-dose, narrowband UV-B led to rapid repigmentation in both. The results support the hypothesis that photoactivation is required to stimulate melanocytes to leave their stem cell niche and seed the epidermis, while tofacitinib suppresses the autoimmune response. In contrast to narrowband UV-B monotherapy, repigmentation using narrowband UV-B together with tofacitinib required relatively low-dose light exposure. Repigmentation in patient 2 is particularly interesting and unexpected given his prior successful depigmentation with MBEH. Without wishing to be limited by any theory, this result suggests that melanocytes of the interfollicular epidermis, but not melanocyte stem cells, are destroyed by MBEH. Combination therapy with JAK inhibitors and low-dose, narrowbandUV-Bmay be effective targeted treatment for vitiligo.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A method for treating vitiligo in a subject, the method comprising administering a therapeutically effective amount of at least one Janus kinase inhibitor to the subject, wherein the subject is further administered phototherapy.

2. The method of claim 1, wherein the at least one Janus kinase inhibitor is selected from the group consisting of tofacitinib, ruxolitinib, oclacitinib, baricitinib, filgotinib, gandotinib, lestaurtinib, momelotinib, pacritinib, upadacitinib (ABT-494), peficitinib, cucurbitacin I, CHZ868, fedratinib, cerdulatinib, ATI-50001, Leo-124429, or a salt or solvate thereof.

3. The method of any one of claims 1-2, wherein the at least one Janus kinase inhibitor is tofacitinib, or a salt or solvate thereof.

4. The method of any one of claims 1-3, wherein the subject is administered about 5-20 mg/day of tofacitinib.

5. The method of any one of claims 1-2, wherein the at least one Janus kinase inhibitor is ruxolitinib, or a salt or solvate thereof.

6. The method of any one of claims 1-2 and 5, wherein the subject is administered about 5-50 mg/day of ruxolitinib.

7. The method of any one of claims 1-6, wherein the phototherapy comprises low-dose narrow band UVB phototherapy.

8. The method of any one of claims 1-7, wherein the phototherapy comprises a dose equal to or less than about 150 mJ/cm^2 of nbUVB.

9. The method of any one of claims 1-7, wherein the phototherapy comprises a dose equal to or greater than about 150 mJ/cm^2 of nbUVB.

10. The method of any one of claims 1-9, wherein the phototherapy is applied at least to the subject's skin section that is afflicted by vitiligo.

11. The method of any one of claims 1-10, wherein the phototherapy is substantially restricted to the subject's skin section that is afflicted by vitiligo.

12. The method of any one of claims 1-11, wherein the at least one Janus kinase inhibitor is administered topically to the subject.

13. The method of any one of claims 1-11, wherein the at least one Janus kinase inhibitor is administered orally to the subject.

14. The method of any one of claims 1-13, wherein the intensity, duration, and/or frequency of the phototherapy used is substantially ineffective in treating vitiligo in a subject who is not being co-administered the at least one Janus kinase inhibitor.

15. The method of any one of claims 1-14, wherein the subject continues to be administered the at least one Janus kinase inhibitor after the phototherapy is discontinued.

16. The method of any one of claims 1-15, wherein the at least one Janus kinase inhibitor is part of a pharmaceutical composition further comprising at least one pharmaceutically acceptable excipient.

17. A kit comprising an effective amount of at least one Janus kinase inhibitor and instructional material comprising instructions for the concomitant administration of the at least one JAKi with phototherapy to treat vitiligo in a subject.

18. The kit of claim 17, wherein the at least one Janus kinase inhibitor comprises tofacitinib.

19. The kit of any one of claims 17-18, wherein the Janus kinase inhibitor comprises ruxolitinib.

20. The kit of any one of claims 17-19, further comprising a handheld phototherapy device.

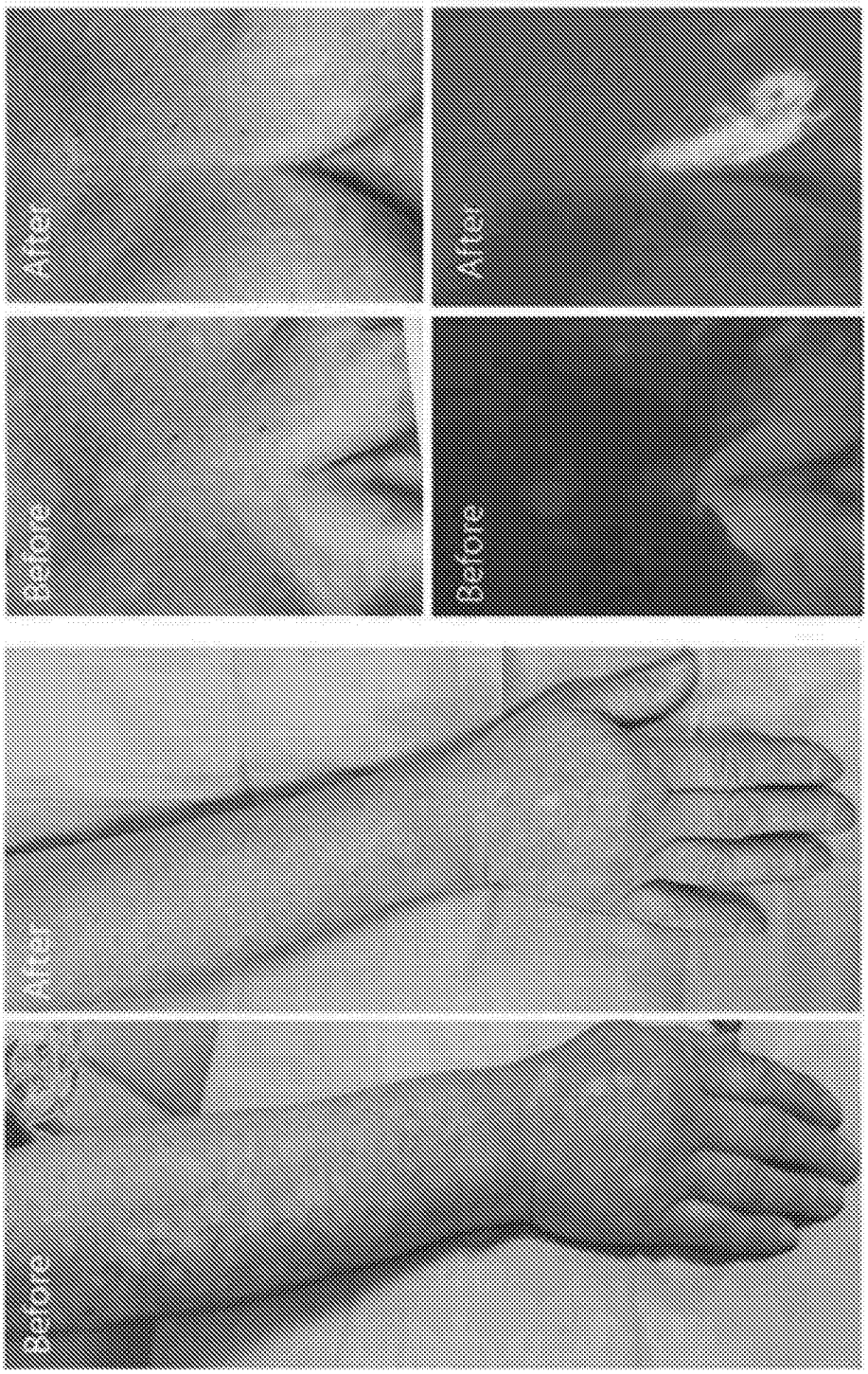


FIG. 1B

FIG. 1A

FIG. 2A

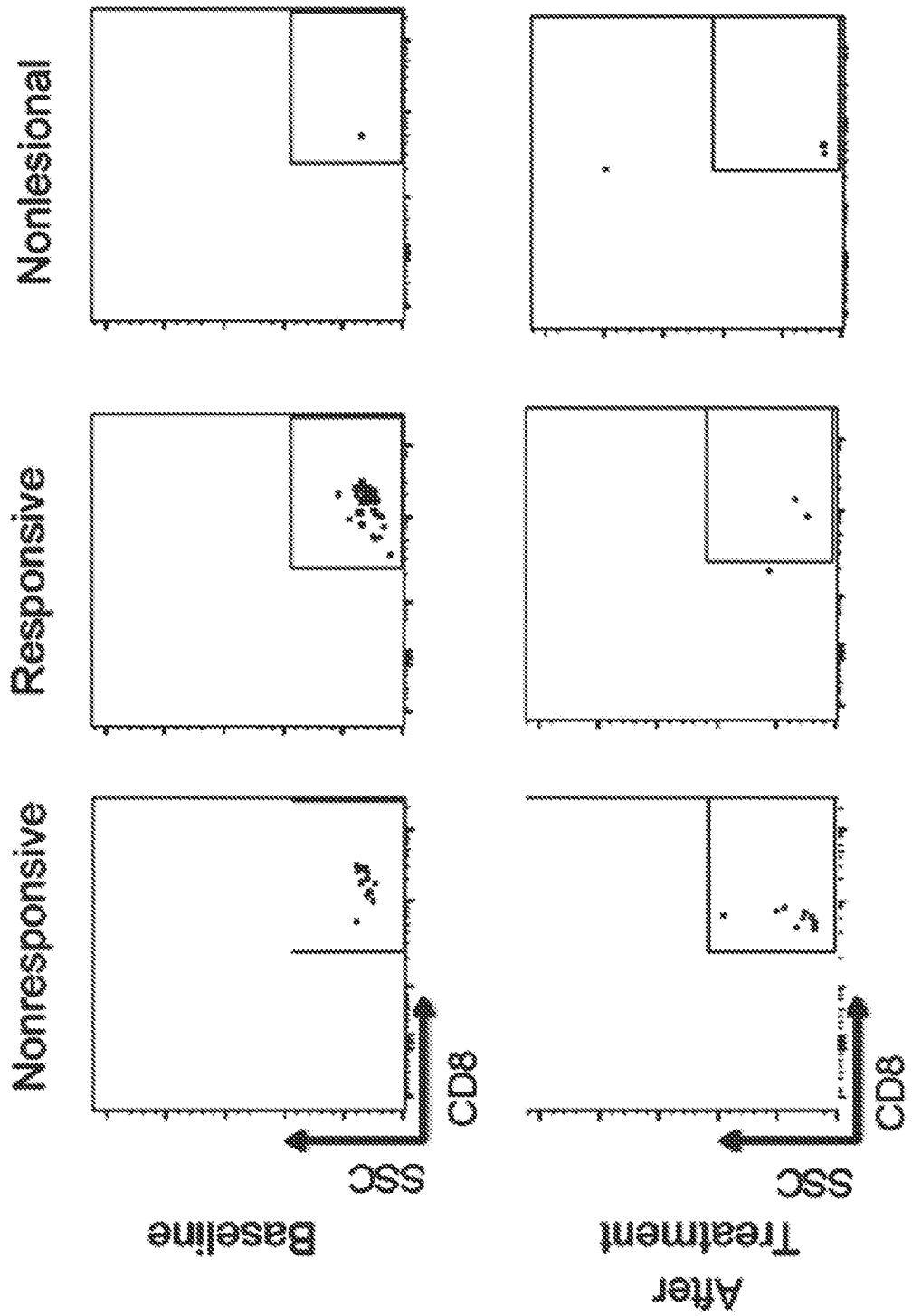

Nonresponsive
Responsive
Nonlesional
Baseline
After Treatment
SSC
CD8

FIG. 2B

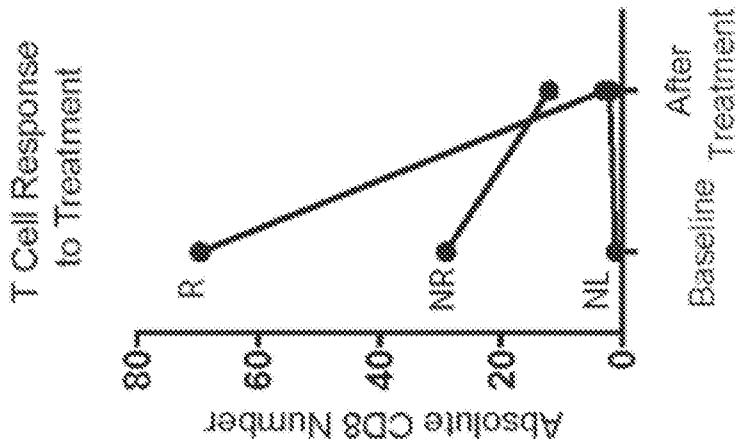


FIG. 2C

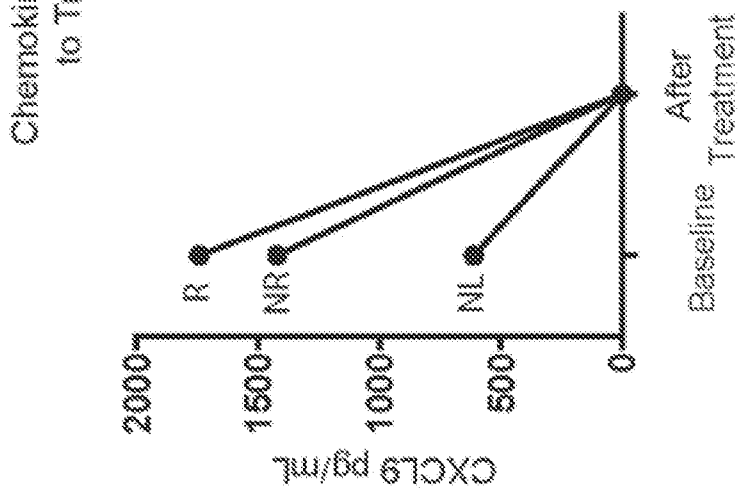


FIG. 2D

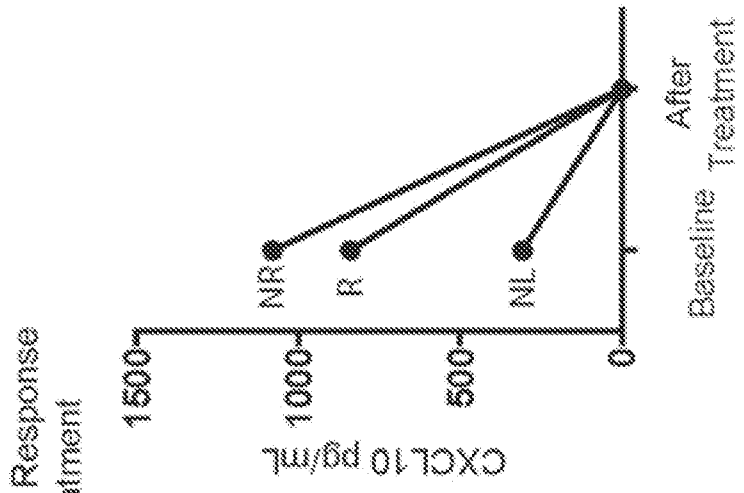


FIG. 3

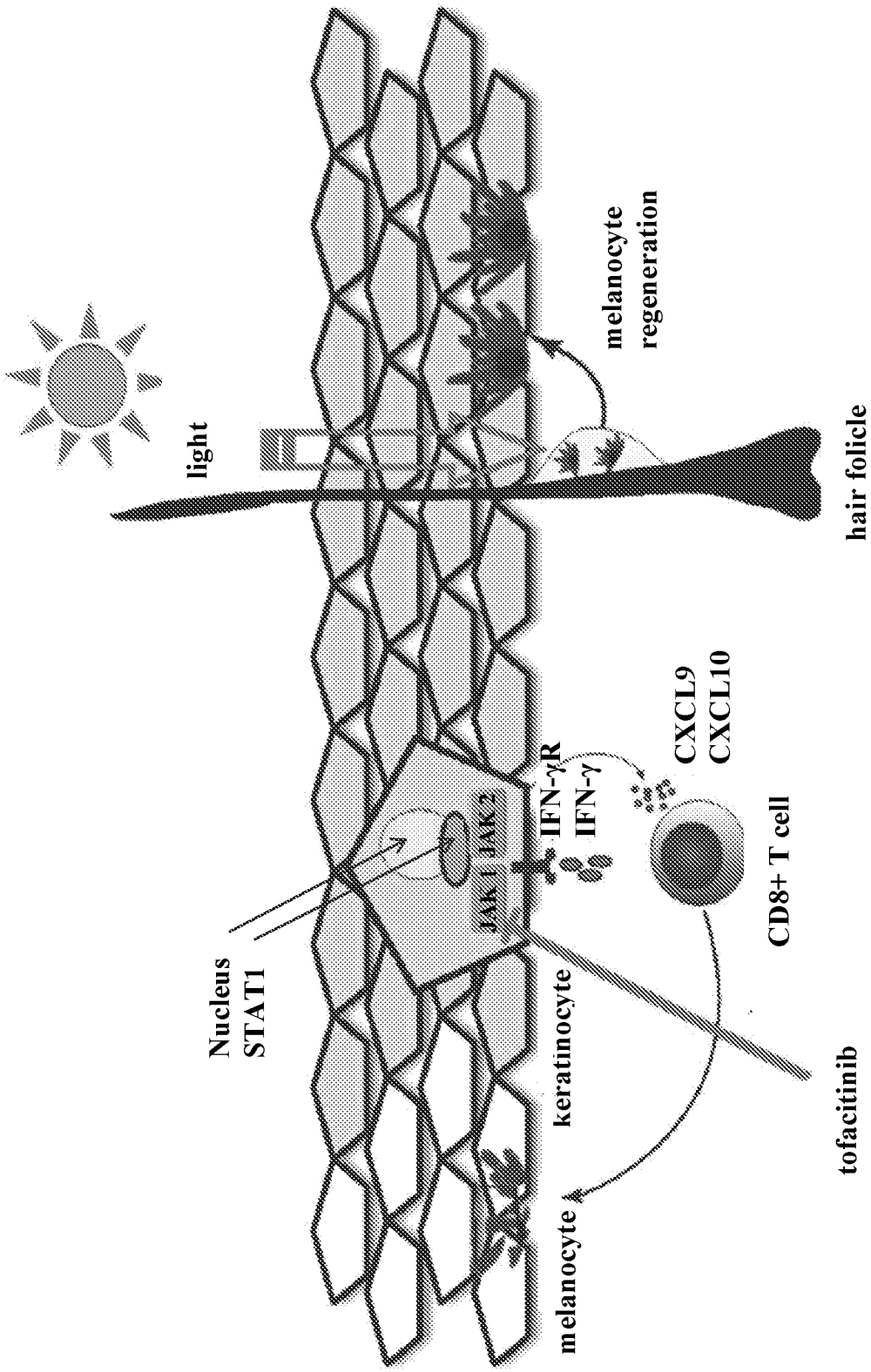

light
melanocyte regeneration
hair folicle
Nucleus STAT1
JAK 1 JAK 2
IFN-γR
IFN-γ
CXCL9 CXCL10
CD8+ T cell
keratinocyte
melanocyte
tofacitinib

FIG. 4B

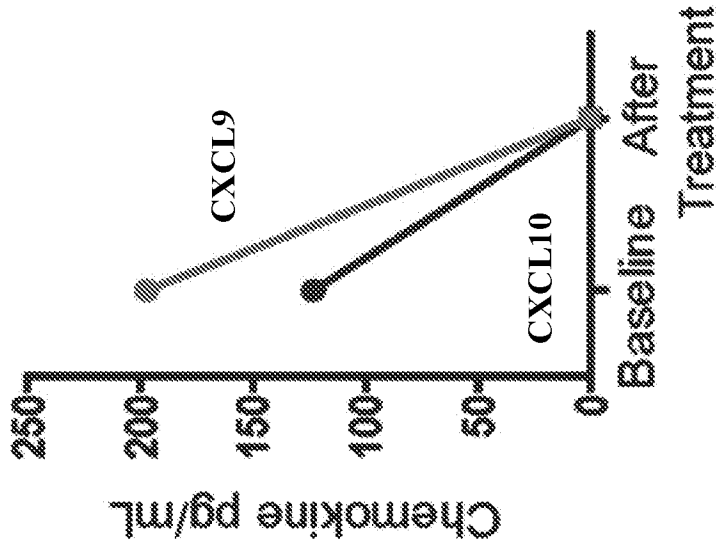

Serum
CXCL9
CXCL10
250
200
150
100
50
0
Chemokine pg/mL
Baseline
After Treatment

FIG. 4A

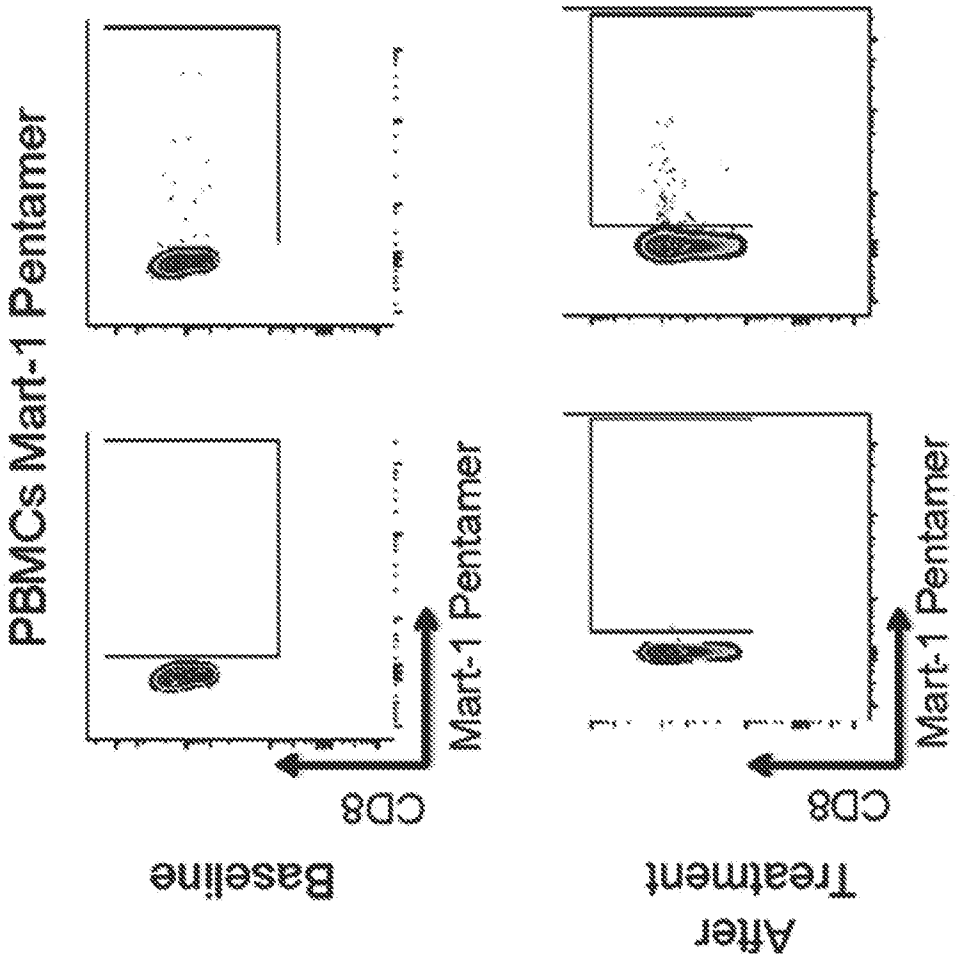

PBMCs Mart-1 Pentamer
CD8
Mart-1 Pentamer
Baseline
After Treatment



FIG. 5A FIG. 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/29531

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/403, A61K 31/513, A61K 9/00 (2018.01)
CPC - A61K 31/519, A61K 31/403, A61K 2300/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROTHSTEIN et al. "Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib", April 5, 2017, J AM ACAD DERMATOL, Vol. 76(6): pgs. 1054-1060.e1 Pg. 1055, Full Para [1]; Pg. 1055, Full Para [3]; Pg. 1060, Full Para [2]	1-3, 5, 17-19
A	WO 2016/083433 A1 (BAYER PHARMA) 02 June 2016 (02.06.2016) Entire Document	1-3, 5, 17-19
A	LEVY et al. "Treatment of recalcitrant atopic dermatitis with the oral Janus kinase inhibitor tofacitinib citrate", July 17, 2015, J AM ACAD DERMATOL, pgs. 395-399 Entire Document	1-3, 5, 17-19
A	MANGA et al. "Recent advances in understanding vitiligo", September 06, 2016, F1000 Research, pgs. 1-9 Entire Document	1-3, 5, 17-19
A	CRAIGLOW et al. "Tofacitinib Citrate for the Treatment of Vitiligo A Pathogenesis-Directed Therapy", October 2015, JAMA Dermatology, Vol. 151(10) Entire Document	1-3, 5, 17-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2018	13 JUL 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/29531

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/29531

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4, 6-16, 20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.