

TREATMENT OF HAIR-LOSS DISORDER

REFERENCE TO RELATED APPLICATION

[0001] This Application claims priority to and the benefit of the filing date of U.S. Provisional Patent Application No. 63/516,534, filed on July 31, 2023, the entire content of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Interleukin-7 (IL-7) is a member of the common γ chain (γc) family of cytokines that also includes IL-2, IL-4, IL-9, IL-15, and IL-21. Like other members, IL-7 signals via a ternary complex formed with its unique α -receptor, IL-7R α (CD127), and the common γ receptor (γc , CD132). IL-7 regulates T-cell homeostasis by enhancing survival and proliferation of naïve and memory T cells; however, it appears to be dispensable for B cell development in humans. IL-7R α is expressed on T cells including most mature T cells, on dendritic cells (DCs) and activated monocytes.

[0003] IL-7 / IL-7R interaction stimulates the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) proteins with subsequent activation of the phosphoinositol 3-kinase (PI3K)/Akt, or Src pathways to facilitate target gene transcription. IL-7 R α is also used by thymic stromal-derived lymphopoietin (TSLP) as part of a complex that contains a second receptor chain, TSLPR.

[0004] The TSLPR complex contains CD127 and TSLPR (CRLF2), and is expressed on several types of immune cells, including B cells, T cells, monocytes and DCs. TSLP has been shown to be a potent activator of antigen presenting cells such as DCs to induce TH2-mediated immune responses. For example, TSLP-stimulated DCs increase OX40L expression and production of TH2 chemokines, such as CCL17 and CCL21, leading to the priming of TH2 cell development.

[0005] IL-7 plays a critical role in the development of a normal immune system, as it is essential for the thymic development, peripheral maintenance, and survival of lymphocytes. Thymic T cell precursors require IL-7 for proliferation, differentiation, and survival. In the periphery, IL-7 regulates T cell hemostasis by enhancing the survival and proliferation of naïve and memory T cells.

[0006] IL-7 is a tissue-derived cytokine and is primarily produced from stromal and epithelial cells in various tissues. For instance, in the small and large intestines, IL-7 is

produced by the intestinal goblet epithelial cells and has been described as being essential for the persistence of chronic colitis in animal models. IL-7 has also been shown to interfere with the immunosuppressive capabilities of regulatory T cells (Tregs). Thus, agents that can modulate the activity of IL-7 *in vivo* and thereby decrease the survival/function of pathogenic T cells and/or increase the induction of regulatory T cells are highly desirable for the treatment of inflammatory diseases, such as inflammatory bowel disease.

[0007] Alopecia areata, also known as spot baldness, is a condition in which hair is lost from some or all areas of the body. Often, it results in a few bald spots on the scalp, each about the size of a coin. In general, alopecia areata is characterized by bald patches on the scalp, beard, and, possibly, eyebrows; eyelashes may also fall out. On the other hand, alopecia areata is distinct from pattern hair loss, which is common among males.

[0008] Psychological stress and illness are possible factors in bringing on alopecia areata in individuals at risk, but in most cases, there is no obvious trigger. People with alopecia areata are generally otherwise healthy. In a few cases, all the hair on the scalp is lost (alopecia totalis), or all body hair is lost (alopecia universalis). Hair loss can be permanent, or temporary.

[0009] Alopecia areata is believed to be an autoimmune disease resulting from a breach in the immune privilege of the hair follicles. Risk factors include a family history of the condition. Among identical twins, if one is affected, the other has about a 50% chance of also being affected. The underlying mechanism may involve failure by the body to recognize its own cells, with subsequent immune-mediated destruction of the hair follicle.

[0010] Dai *et al.* (*Sci. Adv.* 2021;7: eabd1866) described that the interleukin-7 (IL-7) signaling pathway plays an important role in regulation of T cell function and survival, and that exogenous IL-7 accelerated the onset of AA by augmenting the expansion of alopecic T cells, while blockade of IL-7 stopped the progression of AA and reversed early AA in C3H/HeJ mice.

[0011] Currently, there is no known cure for this condition. Some treatments, particularly triamcinolone injections and 5% minoxidil topical creams, are effective in speeding hair regrowth. Sunscreen, head coverings to protect from cold and sun, and glasses, if the eyelashes are missing, are also recommended. In more than 50% of cases of sudden-onset localized “patchy” disease, hair regrows within a year. In patients with only one or two patches, this one-year recovery will occur in up to 80%. However, most patients will have more than one episode over the course of a lifetime. In many patients, hair loss and regrowth

occurs simultaneously over the course of several years. Among those in whom all body hair is lost, fewer than 10% recover.

[0012] Baricitinib, a Janus kinase (JAK) inhibitor, is the only FDA-approved treatment for alopecia. Although JAK inhibitors have demonstrated hair regrowth in patients with severe alopecia ($\geq 50\%$ hair loss), increased risk of serious side effects may preclude this option for some patient populations. Other standard of care approaches for alopecia include topical corticosteroids, immunotherapy, and light therapy. Lack of efficacy and side effects of these latter treatments limit their usefulness across the patient population. The development of a safe and efficacious selective treatment for severe alopecia may provide another therapeutic option for patients who cannot tolerate or who are contraindicated for baricitinib.

[0013] About 0.15% of people are affected by alopecia areata at any one time, and 2% of people are affected at some point in time. Alopecia areata incidence appears to increase almost linearly with age, but the mean age of onset appears to be between 25 and 36 years. Females are affected at higher rates than males.

[0014] Thus, there is a need to develop effective treatment to alleviate hair-loss in hair-loss disorders, such as alopecia areata.

SUMMARY OF THE INVENTION

[0015] Provided herein is a method of treating a hair-loss disorder (such as alopecia areata (AA), including autoimmune-driven AA) or reducing hair loss in the hair-loss disorder (such as reducing hair loss associated with AA), in a mammalian (*e.g.*, human) subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 sequence comprising the sequence of SEQ ID NO: 1 (DHAMH), a VH CDR2 sequence of SEQ ID NO: 2 (GISWNSRGIGYADSVKG), and a VH CDR3 sequence of SEQ ID NO: 3 (DEYSRGYYVLDV); and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4 (RASQGISSALA), a VL CDR2 sequence of SEQ ID NO: 5 (DASSLES), and a VL CDR3 sequence of SEQ ID NO: 6 (QQFNSYPLWIT), such that the hair-loss disorder (*e.g.*, AA) is treated and/or the hair loss is reduced in the mammalian subject.

[0016] Also provided herein is a composition for use in treating a hair-loss disorder (such as alopecia areata (AA), including autoimmune-driven AA), or for use in reducing hair loss in

the hair-loss disorder (such as reducing hair loss associated with AA), in a mammalian subject in need thereof, wherein the composition comprises an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 sequence comprising the sequence of SEQ ID NO: 1 (DHAMH), a VH CDR2 sequence of SEQ ID NO: 2 (GISWNSRGIGYADSVKG), and a VH CDR3 sequence of SEQ ID NO: 3 (DEYSRGYYVLDV); and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4 (RASQGISSALA), a VL CDR2 sequence of SEQ ID NO: 5 (DASSLES), and a VL CDR3 sequence of SEQ ID NO: 6 (QQFNSYPLWIT).

[0017] Also provided herein is a method of inducing hair growth in a mammalian subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2, and a VH CDR3 sequence of SEQ ID NO: 3; and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4, a VL CDR2 sequence of SEQ ID NO: 5, and a VL CDR3 sequence of SEQ ID NO: 6, thereby inducing hair growth in the mammalian subject.

[0018] Also provided herein is a composition for use in inducing hair growth in a mammalian subject in need thereof, wherein the composition comprises an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2, and a VH CDR3 sequence of SEQ ID NO: 3; and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4, a VL CDR2 sequence of SEQ ID NO: 5, and a VL CDR3 sequence of SEQ ID NO: 6.

[0019] Also provided herein is a composition for use in a therapeutic method of inducing hair growth in a mammalian subject in need thereof, wherein the composition comprises an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2, and a VH CDR3 sequence of SEQ ID NO: 3; and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4, a VL CDR2 sequence of SEQ ID NO: 5, and a VL CDR3 sequence of SEQ ID NO: 6.

[0020] Further provided herein is a pharmaceutical composition comprising an antibody having two heavy chains and two light chains, wherein each said heavy chain has the amino acid sequence of SEQ ID NO: 23, and each said light chain has the amino acid sequence of SEQ ID NO: 24; and wherein the antibody is formulated in a pharmaceutically acceptable solution comprising about 50-200 mg/mL (*e.g.*, about 100 mg/mL) of the antibody in about 15-25 mM (*e.g.*, about 20 mM) histidine, 200-300 mM (*e.g.*, about 260 mM) sucrose, and about 0.03-0.07% (w/v) (*e.g.*, about 0.05% (w/v)) polysorbate 80 at pH 6.0.

[0021] Additionally provided herein is a container (*e.g.* a polycarbonate bottle) containing about 2 mL of extractable volume of the pharmaceutical composition described herein.

[0022] It should be understood that any embodiment described herein, including those described only in the examples, can be combined with any one or more other embodiments, unless such combination is expressly disclaimed or is improper. Thus, the term “embodiment”, as used herein, is not to be considered as excluding features recited in other embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. 1 is a schema for the Phase 2 clinical trial described in Example 1. The abbreviations used include: EOS - end of study; Q2W - once every 2 weeks; SALT - Severity of Alopecia Tool; SC - subcutaneous. The primary endpoint is the percent change from baseline in SALT score. The last dose of COMPOUND A or placebo is administered at Week 24.

[0024] FIG. 2 provides the SALT assessment tool according to Olson *et al.* (*J Am Acad Dermatol.* 2004;51(3):440-447. doi: 10.1016/j.jaad.2003.09.032). The total SALT Score is calculated based on the formula $[(LRS \times 0.18) + (RRS \times 0.18) + (TRS \times 0.40) + (BRS \times 0.24)]$, in which LRS is Left Quadrant Raw Score, TRS is Top Quadrant Raw Score, RRS is Right Quadrant Raw Score, and BRS is Back Quadrant Raw Score.

[0025] FIG. 3 shows SKINDEX-16 for AA.

[0026] FIGs. 4A-4C show effects of prophylactic treatment with COMPOUND A (Comp. A) and a comparator IL-7R α antibody (A3312F) in the Hu-NSG mouse model of GvHD on body weight loss (FIG. 4A), plasma cytokine levels (ELISA) (FIG. 4B) and percent human T cells (CD45+ CD3+) in blood as measured by flow cytometry (FIG. 4C). Measurements were determined 21 days post PBMC transfer. Abbreviations: CCL1: antimicrobial protein with bactericidal activity; CD: cluster differentiation; GvHD: graft-versus host disease; Hu-NSG:

humanized nod/SCID/IL2r γ null; IFN: interferon; IL-7R α : interleukin-7 receptor alpha; IP: intraperitoneal; PBMC: peripheral blood mononuclear cells; TNF: tumor necrosis factor.

[0027] FIGs. 5A-5B show target engagement and inhibition of pSTAT5 levels on human T cells in blood (FIG. 5A) and spleens (FIG. 5B) of Hu-NSG mice after treatment with (Comp. A), a comparator IL-7R α antibody (A3312F) or vehicle (PBS). Antibodies were administered in vivo at concentrations of 0.2, 1, and 5 mg/kg. IL-7-induced pSTAT5 was evaluated in an ex vivo assay and included a non-targeting IL-7 negative control sample (CTL Unstim). Abbreviations: CTL: control; Hu-NSG: humanized nod/SCID/IL2r γ null; IL-7R α : interleukin-7 receptor alpha; MFI: mean fluorescence intensity; PBS: phosphate buffered saline; pSTAT5: phosphorylated signal transducer and activator of transcription 5.

[0028] FIGs. 6A and 6B show data after administration of single ascending dose (SAD; 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 2 mg/kg and 4 mg/kg) and multiple doses (MAD; 1 mg/kg) in phase 1 clinical data in human (see Example 8). FIG. 6A shows percentage of IL-7 receptor occupancy (RO) in CD3+ T cells, and FIG. 6B shows percentage of inhibition of IL-7 signaling via phosphorylated STAT5 (pSTAT5) in T cells.

[0029] FIG. 7A shows data (Mean $\pm$ SD) on serum concentration profile in patients with atopic dermatitis (AD) after subcutaneous administration of COMPOUND A at doses of 2 or 3 mg/kg every 2 weeks. SD: standard deviation. 5 μ g/mL represents the concentration with predicted maximum receptor occupancy in skin.

[0030] FIG. 7B shows preliminary data (mean $\pm$ SD) on IL-7R α receptor occupancy (RO) on circulating CD3+ T cells in patients with atopic dermatitis (AD) after subcutaneous administration of COMPOUND A at doses of 2 or 3 mg/kg every 2 weeks. RO: receptor occupancy; SD: standard deviation. Dashed vertical lines represent the dosing days.

DETAILED DESCRIPTION OF THE INVENTION

[0031] Provided is a method of treating a hair-loss disorder (such as alopecia areata (AA)) or reducing hair loss in said hair-loss disorder (such as reducing hair loss associated with AA, including autoimmune driven AA), in a mammalian (*e.g.*, human) subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises: (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 sequence comprising the sequence of SEQ ID NO: 1 (DHAMH) (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2 (GISWNSRGIGYADSVKG), and a VH CDR3 sequence of SEQ ID NO: 3

(DEYSRGYYVLDV); and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4 (RASQGISSALA), a VL CDR2 sequence of SEQ ID NO: 5 (DASSLES), and a VL CDR3 sequence of SEQ ID NO: 6 (QQFNSYPLWIT), such that the hair-loss disorder (*e.g.*, AA) is treated and/or the hair loss is reduced in the mammalian (*e.g.*, human) subject.

[0032] Also provided herein is a composition for use in treating a hair-loss disorder (such as alopecia areata (AA), including autoimmune-driven AA), or for use in reducing hair loss in the hair-loss disorder (such as reducing hair loss associated with AA), in a mammalian (*e.g.*, human) subject in need thereof, wherein the composition comprises an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 sequence comprising the sequence of SEQ ID NO: 1 (DHAMH), a VH CDR2 sequence of SEQ ID NO: 2 (GISWNSRGIGYADSVKG), and a VH CDR3 sequence of SEQ ID NO: 3 (DEYSRGYYVLDV); and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4 (RASQGISSALA), a VL CDR2 sequence of SEQ ID NO: 5 (DASSLES), and a VL CDR3 sequence of SEQ ID NO: 6 (QQFNSYPLWIT).

[0033] In some embodiments, the hair-loss disorder comprises androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, hypotrichosis, or hereditary hypotrichosis simplex.

[0034] Also provided herein is a method of inducing hair growth in a mammalian (*e.g.*, human) subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2, and a VH CDR3 sequence of SEQ ID NO: 3; and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4, a VL CDR2 sequence of SEQ ID NO: 5, and a VL CDR3 sequence of SEQ ID NO: 6, thereby inducing hair growth in the mammalian (*e.g.*, human) subject.

[0035] Also provided herein is a composition for use in inducing hair growth in a mammalian (*e.g.*, human) subject in need thereof, wherein the composition comprises an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2, and a VH CDR3 sequence of SEQ ID NO: 3; and (b) a light chain variable region (LCVR) that comprises a

VL CDR1 sequence of SEQ ID NO: 4, a VL CDR2 sequence of SEQ ID NO: 5, and a VL CDR3 sequence of SEQ ID NO: 6.

[0036] In some embodiments, the method is a cosmetic or non-therapeutic method.

[0037] Also provided herein is a composition for use in a therapeutic method of inducing hair growth in a mammalian (*e.g.*, human) subject in need thereof, wherein the composition comprises an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2, and a VH CDR3 sequence of SEQ ID NO: 3; and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4, a VL CDR2 sequence of SEQ ID NO: 5, and a VL CDR3 sequence of SEQ ID NO: 6. In some embodiments, the subject (*e.g.*, human) has or is suffering from a hair-loss disorder.

[0038] In some embodiments, the subject suffers from a hair-loss disorder, wherein the hair-loss disorder is selected from: androgenetic alopecia, telogen effluvium, alopecia areata, tinea capitis, hypotrichosis, cutaneous lupus such as discoid lupus erythematosus, folliculitis decalvans, frontal fibrosing alopecia, and hereditary hypotrichosis simplex.

[0039] In some embodiments, the hair-loss disorder is associated with an autoimmune condition / disease, such as alopecia areata, alopecia totalis, systemic lupus erythematosus (SLE), discoid lupus; hypothyroidism such as Hashimoto's thyroiditis; hyperthyroidism such as Graves' disease; dermatomyositis; scleroderma; and certain autoimmune disorders of the skin (like pemphigus vulgaris).

[0040] In some embodiments, the hair-loss disorder is androgenetic alopecia.

[0041] In some embodiments, the hair-loss disorder is alopecia areata.

[0042] In some embodiments, the alopecia areata is alopecia totalis or alopecia universalis.

[0043] In some embodiments, the antibody has a heavy chain sequence of SEQ ID NO: 23 and a light chain sequence of SEQ ID NO: 24.

[0044] In some embodiments, the antibody is administered to the subject subcutaneously (*s.c.*).

[0045] In some embodiments, the antibody is administered once a week (Q1W), once every two weeks (Q2W), once every three weeks (Q3W), once every four weeks (Q4W), once every eight weeks (Q8W), once every twelve weeks (Q12W), or any intermittent PRN (*pro re nata*).

[0046] In some embodiments, each administration, starting from the second dose, is separated from the immediate prior administration by the same number of days, preferably around the same time of the day of the administration.

[0047] In some embodiments, the antibody is administered once every two weeks, preferably, each administration starting from the second dose is separated by 14 days from the immediate prior administration, preferably around the same time of the day of the administration.

[0048] In some embodiments, the antibody is administered for 10-15 (*e.g.*, 11, 12, or 13) consecutive doses. In some embodiments, the antibody is administered for 10 consecutive doses. In some embodiments, the antibody is administered for 11 consecutive doses. In some embodiments, the antibody is administered for 12 consecutive doses. In some embodiments, the antibody is administered for 13 consecutive doses. In some embodiments, the antibody is administered for 14 consecutive doses. In some embodiments, the antibody is administered for 15 consecutive doses.

[0049] In some embodiments, the antibody is administered for a period of up to 10 weeks, 13 weeks, 26 weeks, 52 weeks, 2 years, 3 years, 5 years, 10 years, or lifetime / permanently. In some embodiments, the antibody is administered as needed when there is hair loss.

[0050] In certain embodiments, the antibody has a heavy chain sequence of SEQ ID NO: 23 and a light chain sequence of SEQ ID NO: 24, and is administered at about 200 mg per dose to the mammalian (*e.g.*, human) subject subcutaneously (*s.c.*) once every two weeks (Q2W), for 10-15 (*e.g.*, 11, 12, or 13) consecutive doses.

[0051] In some embodiments, the subject has moderate-to-severe AA, or severe to very severe AA (*e.g.*, with $\geq 50\%$ total scalp hair loss as defined by the SALT score).

[0052] As used herein, “moderate AA” has a SALT score of between 21-49. “Severe AA” has a SALT score of between 50-94. “Very severe AA” has a SALT score of 95-100. “Moderate-to-severe AA” has a SALT score of 21-94. “Severe to very severe AA” has SALT score of 50-100.

[0053] In some embodiments, the hair loss in the subject is assessed by mean relative percent change in Severity of Alopecia Tool (SALT) Score at 12 weeks (Week 12), 18 weeks (Week 18), 24 weeks (Week 24), 26 weeks (Week 26), 48 weeks (Week 48), and/or 52 weeks (Week 52), after the first dose at Week 0, as compared with a Baseline SALT Score obtained just prior to the first dose at Week 0. As used herein, “just prior to” refers to a Baseline

SALT Score assessment obtained within one day, 12 hrs, 6 hours, or 1 hour prior to the first dose at Week 0.

[0054] In some embodiments, $\geq 50\%$ relative reduction in SALT score is achieved in the subject at 12 weeks (Week 12), 18 weeks (Week 18), and/or 24 weeks (Week 24) after the first dose at Week 0.

[0055] In some embodiments, absolute SALT score of the subject is ≤ 5 , 10, 20, 30, or 50 at 12 weeks (Week 12), 18 weeks (Week 18), and/or 24 weeks (Week 24) after the first dose at Week 0.

[0056] In some embodiments, the subject has $\geq 50\%$ relative reduction in SALT score at Week 36 from Baseline and/or Week 24.

[0057] In some embodiments, absolute SALT score of the subject is ≤ 5 , ≤ 10 , ≤ 20 , ≤ 30 , or ≤ 50 at Week 36.

[0058] In some embodiments, hair loss comprises eyelash loss and/or eyebrow loss.

[0059] In some embodiments, reduction in eyebrow loss is based on ClinRO Measure for Eyebrow Hair Loss scores of 0 or 1 (full coverage or minimal gaps) with a ≥ 2 -point improvement from Baseline at Week 24 and/or 36.

[0060] In some embodiments, reduction in eyelash loss is based on ClinRO Measure for Eyelash Hair Loss scores of 0 or 1 (full coverage or minimal gaps) with a ≥ 2 -point improvement from Baseline at Week 24 and/or 36.

[0061] In some embodiments, the patient has improved quality of life (QOL) following treatment, as measured by improvement in SKINDEX-16 AA compared to that of Baseline at Week 12, 18, 24, and/or 36.

[0062] In some embodiments, the antibody is formulated as 100 mg/mL solution in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0, with an optional 0.05 mM pentetic acid.

[0063] In some embodiments, the antibody is administered to the subject based on a weight-based dosing regimen.

[0064] In some embodiments, the antibody is administered to the subject at about 0.1 mg/kg – about 10 mg/kg per dose, about 0.2 mg/kg – about 8 mg/kg per dose, about 0.5 mg/kg – about 5 mg/kg per dose, about 1 mg/kg – about 4 mg/kg per dose, or about 2 mg/kg – about 3 mg/kg per dose.

[0065] In some embodiments, the antibody is administered to the subject at about 0.2 mg/kg per dose, about 0.5 mg/kg per dose, about 1 mg/kg per dose, about 2 mg/kg per dose, about 3 mg/kg per dose, about 4 mg/kg per dose, about 5 mg/kg per dose, about 6 mg/kg per

dose, about 7 mg/kg per dose, about 8 mg/kg per dose, about 9 mg/kg per dose, or about 10 mg/kg per dose.

[0066] In some embodiments, the antibody is administered to the subject based on a body surface area (BSA)-based dosing regimen. For example, the dose for an adult male patient of any weight (in kg) and height (in cm) can be calculated / converted, based on a BSA Base Dose used for a standard male weight of 91 kg and height of 175 cm, to reach a dose of about 10 mg – about 800 mg per dose, about 20 mg – about 500 mg per dose, about 50 mg – about 300 mg per dose, about 100 mg – about 250 mg per dose.

[0067] In some embodiments, the dose for an adult male patient of any weight (in kg) and height (in cm) can be calculated / converted, based on a BSA Base Dose used for a standard male weight of 91 kg and height of 175 cm, to reach a dose of about 25 mg per dose, about 50 mg per dose, about 100 mg per dose, about 150 mg per dose, about 200 mg per dose, about 250 mg per dose, about 300 mg per dose, or about 350 mg per dose.

[0068] In some embodiments, the dose for an adult female patient of any weight (in kg) and height (in cm) can be calculated / converted, based on a BSA Base Dose used for a standard female weight of 77.5 kg and height of 160 cm, to reach a dose of about 10 mg – about 800 mg per dose, about 20 mg – about 500 mg per dose, about 50 mg – about 300 mg per dose, or about 100 mg – about 250 mg per dose.

[0069] In some embodiments, the dose for an adult female patient of any weight (in kg) and height (in cm) can be calculated / converted, based on a BSA Base Dose used for a standard female weight of 77.5 kg and height of 160 cm, to reach a dose of about 25 mg per dose, about 50 mg per dose, about 100 mg per dose, about 150 mg per dose, about 200 mg per dose, about 250 mg per dose, about 300 mg per dose, or about 350 mg per dose.

[0070] In some embodiments, the BSA dosing is based on Dubois Formula for BSA Dosing, in which $\text{Dose} = \text{BSA Based Dose} \times 0.007184 \times \text{Height (cm)}^{0.725} \times \text{Weight (kg)}^{0.425}$.

[0071] In some embodiments, the BSA dosing is based on Monteller Formula for BSA Dosing, in which $\text{Dose} = \text{BSA Based Dose} \times \text{square root} [(\text{Height (cm)} \times \text{Weight (kg)}) / 3600]$.

[0072] In some embodiments, the antibody is administered to the subject based on a fixed / flat dosing regimen.

[0073] In some embodiments, the antibody is administered to the subject at about 10 mg – about 800 mg per dose, about 20 mg – about 500 mg per dose, about 50 mg – about 300 mg per dose, or about 100 mg – about 250 mg per dose.

[0074] In some embodiments, the antibody is administered to the subject at about 25 mg per dose, about 50 mg per dose, about 100 mg per dose, about 150 mg per dose, about 200 mg per dose, about 250 mg per dose, about 300 mg per dose, or about 350 mg per dose.

[0075] In some embodiments, the antibody is administered to the subject based on a weight-banded dosing regimen, in which patients within certain ranges of weights (weight-bands) are dosed a fixed amount for that specific weight band. In some embodiments, patients with a body weight of about 10-25 kg are grouped in the same body weight band and given the same fixed dose. In some embodiments, patients with a body weight of about 25-50 kg are grouped in the same body weight band and given the same fixed dose. In some embodiments, patients with a body weight of about 50-75 kg are grouped in the same body weight band and given the same fixed dose. In some embodiments, patients with a body weight of about 75-100 kg are grouped in the same body weight band and given the same fixed dose. In some embodiments, the fixed dose for each weight band is based on the average weight in the weight band (*e.g.*, all patients in the weight band of 50-75 kg are dosed a fixed dose based on the dose for the middle weight – *i.e.*, 62.5 kg for this weight band). In some embodiments, the fixed dose for the weight band 50-75 kg is about 150 mg, about 200 mg, about 250 mg, about 300 mg, or about 350 mg. In some embodiments, the fixed dose for a weight band is proportional to the fixed dose for the 50-75 kg weight band (*e.g.*, the fixed dose for the weight band 10-25 kg is based on the middle weight of 17.5 kg, which is $17.5 / 62.5 = 28\%$ of the fixed dose for the 50-75 kg weight band).

[0076] In some embodiments, the antibody is administered to the subject based on a dosing regimen comprising a loading dose or an accelerated initial dosing schedule, followed by a maintenance dose, wherein the maintenance dose is administered according to any of the preceding weight-based dosing regimens, body surface area (BSA)-based dosing regimens, or fixed dose dosing regimens. In some embodiments, the loading dose comprises twice the dose of the maintenance dose (*e.g.*, if the maintenance doses are administered once every two weeks (Q2W) as 200 mg fixed dose, the loading dose of 400 mg fixed dose may be administered once every two weeks (Q2W)). In some embodiments, an initial accelerated dosing schedule is used (*e.g.*, if the 200 mg maintenance doses are administered once every two weeks (Q2W), the initial accelerated dosing schedule includes administering 200 mg once a week (Q1W) for two weeks).

[0077] In some embodiments, the maintenance dose comprises infinite number of doses, which may be useful, for example, for management of a chronic condition such as alopecia areata. In some embodiments, the maintenance dose is administered intermittently (prn).

[0078] In some embodiments, the subject has impaired renal function (*e.g.*, mildly impaired renal function (such as a glomerular filtration rate <60 mL/min and/or the presence of albuminuria >30 mg/d), moderately impaired renal function (such as a glomerular filtration rate <45 mL/min), or severely impaired renal function (such as a glomerular filtration rate <30 mL/min). In other embodiments, the subject is normal, *e.g.*, normal with respect to renal function.

[0079] In some embodiments, the subject is an adult (*e.g.*, 18 years and older).

[0080] In some embodiments, the subject is not an adult, or is a pediatric patient or an adolescent patient (*e.g.*, a patient of under 18-year old, under 16-year old, under 14-year old, under 12-year old, under 10-year old, under 5-year old, under 3-year old, under 2-year old, under 1-year old, under 6-month old, or under 3-month old).

[0081] In some embodiments, the subject is a Caucasian. In some embodiments, the subject is Asian. In some embodiments, the subject is African. In some embodiments, the subject is native American. In some embodiments, the subject is of mixed race or ethnic group. In some embodiments, the subject is biologic male. In some embodiments, the subject is biologic female.

[0082] In some embodiments, the subject is further being treated by or has been treated by a second therapeutic agent effective to treat alopecia areata. In some embodiments, the second therapeutic agent comprises a glucocorticoid, Minoxidil, Anthralin, Bimatoprost, Methotrexate, a DMARD, dupilumab, and/or a JAK inhibitor (*e.g.*, tofacitinib, ruxolitinib, upadacitinib, abrocitinib, and baricitinib).

[0083] In some embodiments, the antibody is administered to the subject at about 25, 50, 100, 150, 200, or 250 mg per dose; optionally, the doses of the antibody are administered to the subject once every two weeks, for a total of 10, 11, 12, 13, 14, or 15 doses.

[0084] In some embodiments, the method reduces the measurement of an AA-associated biomarker after treatment (compared to before treatment).

[0085] In some embodiments, the AA-associated biomarker is a gene expression signature that is an Alopecia Areata Disease Activity Index (ALADIN).

[0086] In some embodiments, the AA-associated biomarker is an Alopecia Areata Gene Signature (AAGS) comprising one or more genes set forth below.

NCBI GenBank ID	Official Gene Name
	T cell activation
596	B-cell CLL/lymphoma 2
914	CD2 molecule
915	CD3d molecule, delta (CD3-TCR complex)
920	CD4 molecule
972	CD74 molecule, major histocompatibility complex, class II invariant chain
942	CD86 molecule
925	CD8a molecule
926	CD8b molecule
10320	IKAROS family zinc finger 1 (Ikaros)
8440	NCK adaptor protein 2
1499	catenin (cadherin-associated protein), beta 1, 88 kDa
55636	chromodomain helicase DNA binding protein 7
8320	leomesodermin homolog (Xenopus laevis)

3683	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
3684	integrin, alpha M (complement component 3 receptor 3 subunit)
3659	interferon regulatory factor 1
3600	interleukin 15
3936	lymphocyte cytosolic protein 1 (L-plastin)
3932	lymphocyte-specific protein tyrosine kinase
3108	major histocompatibility complex, class II, DM alpha
6693	sialophorin
387357	thymocyte selection pathway associated
	immune response
596	B-cell CLL/lymphoma 2
29760	B-cell linker
23601	C-type lectin domain family 5, member A
929	CD14 molecule
9332	CD163 molecule
100133941	CD24 molecule; CD24 molecule-like 4
972	CD74 molecule, major histocompatibility complex, class II invariant chain
9308	CD83 molecule
8832	CD84 molecule
50848	F11 receptor
2268	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog
10866	HLA complex P5
150372	NFAT activating protein with ITAM motif 1
25939	SAM domain and HD domain 1
50852	T cell receptor associated transmembrane adaptor 1
7078	TIMP metalloproteinase inhibitor 3
7305	TYRO protein tyrosine kinase binding protein
11326	V-set and immunoglobulin domain containing 4
84632	actin filament associated protein 1-like 2
90	activin A receptor, type I
199	allograft inflammatory factor 1
197	alpha-2-HS-glycoprotein
60489	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G
80833	apolipoprotein L, 3
650	bone morphogenetic protein 2
9435	carbohydrate (N-acetylglucosamine-6-O)-sulfotransferase 2
1508	cathepsin B

3683	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
3684	integrin, alpha M (complement component 3 receptor 3 subunit)
3659	interferon regulatory factor 1
3600	interleukin 15
3936	lymphocyte cytosolic protein 1 (L-plastin)
3932	lymphocyte-specific protein tyrosine kinase
3108	major histocompatibility complex, class II, DM alpha
6693	sialophorin
387357	thymocyte selection pathway associated
	immune response
596	B-cell CLL/lymphoma 2
29760	B-cell linker
23601	C-type lectin domain family 5, member A
929	CD14 molecule
9332	CD163 molecule
100133941	CD24 molecule; CD24 molecule-like 4
972	CD74 molecule, major histocompatibility complex, class II invariant chain
9308	CD83 molecule
8832	CD84 molecule
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7305	TYRO protein tyrosine kinase binding protein
11326	V-set and immunoglobulin domain containing 4
84632	actin filament associated protein 1-like 2
90	activin A receptor, type I
199	allograft inflammatory factor 1
197	alpha-2-HS-glycoprotein
60489	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G
80833	apolipoprotein L, 3
650	bone morphogenetic protein 2
9435	carbohydrate (N-acetylglucosamine-6-O)-sulfotransferase 2
1508	cathepsin B

6357	chemokine (C-C motif) ligand 13
6362	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
6351	chemokine (C-C motif) ligand 4
6352	chemokine (C-C motif) ligand 5
6355	chemokine (C-C motif) ligand 8
1230	chemokine (C-C motif) receptor 1
1234	chemokine (C-C motif) receptor 5
3627	chemokine (C-X-C motif) ligand 10
4283	chemokine (C-X-C motif) ligand 9
4261	class II, major histocompatibility complex, transactivator
713	complement component 1, q subcomponent, B chain
714	complement component 1, q subcomponent, C chain
1755	deleted in malignant brain tumors 1
8456	forkhead box N1
3055	hemopoietic cell kinase
8347, 8343,	histone cluster 1, H2bi, histone cluster 1, H2bg, histone
8346, 8344,	cluster 1, H2be; histone cluster 1, H2bf; histone cluster 1,
8339	H2be
3399	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
3683	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1, alpha polypeptide)
3689	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
3694	integrin, beta 6
64135	interferon induced with helicase C domain 1
338376	interferon, epsilon
3600	interleukin 15
9235	interleukin 32
3579	interleukin 8 receptor, beta
3822	killer cell lectin-like receptor subfamily C, member 2
3988	lipase A, lysosomal acid, cholesterol esterase
58530	lymphocyte antigen 6 complex, locus G6F; lymphocyte antigen 6 complex, locus G6D
3107, 3106	major histocompatibility complex, class I, C; major histocompatibility complex, class I, B
4332	myeloid cell nuclear differentiation antigen
4542	myosin IF

5551	perforin 1 (pore forming protein)
30814	phospholipase A2, group IIE
5341	pleckstrin
10544	protein C receptor, endothelial (EPCR)
5265	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
12	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
6614	sialic acid binding Ig-like lectin 1, sialoadhesin
6693	sialophorin
6469	sonic hedgehog homolog (Drosophila)
7057	thrombospondin 1
7096	toll-like receptor 1
7042	transforming growth factor, beta 2
6890	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
7133	tumor necrosis factor receptor superfamily, member 1B
7534	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide
	plasma membrane
8909	26 serine protease
417	ADP-ribosyltransferase 1
24	ATP-binding cassette, sub-family A (ABC1), member 4
5243	ATP-binding cassette, sub-family B (MDR/TAP), member 1
5244	ATP-binding cassette, sub-family B (MDR/TAP), member 4
9619	ATP-binding cassette, sub-family G (WHITE), member 1
29760	B-cell linker
598	BCL2-like 1
23601	C-type lectin domain family 5, member A
160365	C-type lectin-like 1
135228	CD109 molecule
929	CD14 molecule
9332	CD163 molecule
911	CD1c molecule
914	CD2 molecule
30835	CD209 molecule
100133941	CD24 molecule; CD24 molecule-like 4
915	CD3d molecule, delta (CD3-TCR complex)
920	CD4 molecule

1043	CD52 molecule
963	CD53 molecule
972	CD74 molecule, major histocompatibility complex, class II invariant chain
3732	CD82 molecule
9308	CD83 molecule
8832	CD84 molecule
942	CD86 molecule
925	CD8a molecule
926	CD8b molecule
10225	CD96 molecule
56882	CDC42 small effector 1
30845	EH-domain containing 3
1969	EPH receptor A2
2048	EPH receptor B2
50848	F11 receptor
22844	FERM and PDZ domain containing 1
2205	Fc fragment of IgE, high affinity I, receptor for, alpha polypeptide
2212	Fc fragment of IgG, low affinity IIa, receptor (CD32)
2213	Fc fragment of IgG, low affinity IIb, receptor (CD32), Fc fragment of IgG, low affinity IIc, receptor for (CD32)
166647	G protein-coupled receptor 125
23432	G protein-coupled receptor 161
1880	G protein-coupled receptor 183
55507	G protein-coupled receptor, family C, group 5, member D
3927	LIM and SH3 protein 1
130576	LY6/PLAUR domain containing 6B
65108	MARCKS-like 1
3071	NCK-associated protein 1-like
150372	NFAT activating protein with ITAM motif 1
4864	Niemann-Pick disease, type C1
5754	PTK7 protein tyrosine kinase 7
376267	RAB15, member RAS oncogene family
22931	RAB18, member RAS oncogene family
285613	RELT-like 2
56963	RGM domain family, member A
23504	RIMS binding protein 2
10900	RUN domain containing 3A
6016	Ras-like without CAAX 1
51458	Rh family, C glycoprotein
30011	SH3-domain kinase binding protein 1

4092	SMAD family member 7
8869	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
6461	Src homology 2 domain containing adaptor protein B
50852	T cell receptor associated transmembrane adaptor 1
28639	T cell receptor beta variable 19; T cell receptor beta constant 1
6967	T cell receptor gamma locus; T cell receptor gamma constant 2
445347	TCR gamma alternate reading frame protein; T cell receptor gamma variable 9; T cell receptor gamma constant 1
7305	TYRO protein tyrosine kinase binding protein
11326	V-set and immunoglobulin domain containing 4
65266	WNK lysine deficient protein kinase 4
10152	abl interactor 2
90	activin A receptor, type I
120425	adhesion molecule, interacts with CXADR antigen 1
199	allograft inflammatory factor 1
83543	allograft inflammatory factor 1-like
351	amyloid beta (A4) precursor protein
56899	ankyrin repeat and sterile alpha motif domain containing 1B
83464	anterior pharynx defective 1 homolog B (C. elegans)
54796	basonuclein 2
144453	bestrophin 3
685	Betacellulin
1952	cadherin, EGF LAG__seven-pass G-type receptor 2 (flamingo homolog, Drosophila)
776	calcium channel, voltage-dependent, L type, alpha 1D subunit
8913	calcium channel, voltage-dependent, T type, alpha 1G subunit
27092	calcium channel, voltage-dependent, gamma subunit 4
800	caldesmon 1
768	carbonic anhydrase IX
1499	catenin (cadherin-associated protein), beta 1, 88 kDa
1500	catenin (cadherin-associated protein), delta 1
1501	catenin (cadherin-associated protein), delta 2 (neural plakophilin-related arm-repeat protein)
1508	cathepsin B
1230	chemokine (C-C motif) receptor 1
1234	chemokine (C-C motif) receptor 5

25932	chloride intracellular channel 4
1464	chondroitin sulfate proteoglycan 4
23562	claudin 14
1436	colony stimulating factor 1 receptor
594855	complexin 3
1525	coxsackie virus and adenovirus receptor pseudogene 2; coxsackie virus and adenovirus receptor
26999	cytoplasmic FMR1 interacting protein 2
1824	desmocollin 2
1830	desmoglein 3 (pemphigus vulgaris antigen)
147409	desmoglein 4
55740	enabled homolog (Drosophila)
30816	endogenous retroviral family W, env(C7), member 1
1946	ephrin-A5
2099	estrogen receptor 1
2260	fibroblast growth factor receptor 1
23767	fibronectin leucine rich transmembrane protein 3
54751	filamin binding LIM protein 1
2323	fms-related tyrosine kinase 3 ligand
2350	folate receptor 2 (fetal)
342184	formin 1
7976	frizzled homolog 3 (Drosophila)
8323	frizzled homolog 6 (Drosophila)
8324	frizzled homolog 7 (Drosophila)
2523	fucosyltransferase 1 (galactoside 2-alpha-L-fucosyltransferase, H blood group)
2554	gamma-aminobutyric acid (GABA) A receptor, alpha 1
2561	gamma-aminobutyric acid (GABA) A receptor, beta 2
2700	gap junction protein, alpha 3, 46 kDa
2706	gap junction protein, beta 2, 26 kDa
10804	gap junction protein, beta 6, 30 kDa
125111	gap junction protein, delta 3, 31.9 kDa
342035	Gliomedin
2892	glutamate receptor, ionotropic, AMPA 3
3001	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)
3002	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)
2774	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type
2782	guanine nucleotide binding protein (G protein), beta polypeptide 1

115362	guanylate binding protein 5
64399	hedgehog interacting protein
9456	homer homolog 1 (Drosophila)
9455	homer homolog 2 (Drosophila)
3683	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
3684	integrin, alpha M (complement component 3 receptor 3 subunit)
3687	integrin, alpha X (complement component 3 receptor 4 subunit)
3689	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
3694	integrin, beta 6
3587	interleukin 10 receptor, alpha
3594	interleukin 12 receptor, beta 1
3600	interleukin 15
3561	interleukin 2 receptor, gamma (severe combined immunodeficiency)
3579	interleukin 8 receptor, beta
182	jagged 1 (Alagille syndrome)
58494	junctional adhesion molecule 2
3821	killer cell lectin-like receptor subfamily C, member 1
3822	killer cell lectin-like receptor subfamily C, member 2
22914	killer cell lectin-like receptor subfamily K, member 1
8549	leucine-rich repeat-containing G protein-coupled receptor 5
3977	leukemia inhibitory factor receptor alpha
58530	lymphocyte antigen 6 complex, locus G6F; lymphocyte antigen 6 complex, locus G6D
3936	lymphocyte cytosolic protein 1 (L-plastin)
3932	lymphocyte-specific protein tyrosine kinase
4033	lymphoid-restricted membrane protein
9170	lysophosphatidic acid receptor 2
23566	lysophosphatidic acid receptor 3
3107, 3106	major histocompatibility complex, class I, C; major histocompatibility complex, class I, B
3134	major histocompatibility complex, class I, F
3108	major histocompatibility complex, class II, DM alpha
3109	major histocompatibility complex, class II, DM beta
3111	major histocompatibility complex, class II, DO alpha
3113	major histocompatibility complex, class II, DP alpha 1

3119	major histocompatibility complex, class II, DQ beta 1; similar to major histocompatibility complex, class II, DQ beta 1
4118	mal, T-cell differentiation protein
4360	mannose receptor, C type 1
55686	Melanoregulin
154043, 9223	membrane associated guanylate kinase, WW and PDZ domain containing 1; CNKSR family member 3
23499	microtubule-actin crosslinking factor 1
9053	microtubule-associated protein 7
4128	monoamine oxidase A
4155	myelin basic protein
8828	neuropilin 2
4846	nitric oxide synthase 3 (endothelial cell)
123264	organic solute transporter beta
29780	parvin, beta
64098	parvin, gamma
5551	perforin 1 (pore forming protein)
5141	phosphodiesterase 4A, cAMP-specific (phosphodiesterase E2 dunce homolog, Drosophila)
27445	piccolo (presynaptic cytomatrix protein)
5317	plakophilin 1 (ectodermal dysplasia/skin fragility syndrome); similar to plakophilin 1 isoform 1a
11187	plakophilin 3
5341	Pleckstrin
23362	pleckstrin and Sec7 domain containing 3
5362	plexin A2
5818	poliovirus receptor-related 1 (herpesvirus entry mediator C)
81607	poliovirus receptor-related 4
200845	potassium channel tetramerisation domain containing 6
3784	potassium voltage-gated channel, KQT-like subfamily, member 1
56937	prostate transmembrane protein, androgen induced 1
10544	protein C receptor, endothelial (EPCR)
5579	protein kinase C, beta
5587	protein kinase D1
26051	protein phosphatase 1, regulatory (inhibitor) subunit 16B
5099	protocadherin 7
53829	purinergic receptor P2Y, G-protein coupled, 13
9934	purinergic receptor P2Y, G-protein coupled, 14
54509	ras homolog gene family, member F (in filopodia)
9699	regulating synaptic membrane exocytosis 2

9783	regulating synaptic membrane exocytosis 3
6248	regulatory solute carrier protein, family 1, member 1
22800	related RAS viral (r-ras) oncogene homolog 2; similar to related RAS viral (r-ras) oncogene homolog 2
6404	selectin P ligand
64218	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A
6614	sialic acid binding Ig-like lectin 1, sialoadhesin
89790	sialic acid binding Ig-like lectin 10
6693	sialophorin
140885	signal-regulatory protein alpha
55423	signal-regulatory protein gamma
6504	signaling lymphocytic activation molecule family member 1
4301	similar to Afadin (Protein AF-6), myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4
57228	small trans-membrane and glycosylated protein
6509	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
6511	solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6
10723	solute carrier family 12 (potassium/chloride transporters), member 7
9120	solute carrier family 16, member 6 (monocarboxylic acid transporter 7); similar to solute carrier family 16, member 6
220963	solute carrier family 16, member 9 (monocarboxylic acid transporter 9)
6575	solute carrier family 20 (phosphate transporter), member 2
28965	solute carrier family 27 (fatty acid transporter), member 6
10991	solute carrier family 38, member 3
30061	solute carrier family 40 (iron-regulated transporter), member 1
200010	solute carrier family 5 (sodium/glucose cotransporter), member 9
11254	solute carrier family 6 (amino acid transporter), member 14
55117	solute carrier family 6 (neutral amino acid transporter), member 15
23428	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 8

23657	solute carrier family 7, (cationic amino acid transporter, y ⁺ system) member 11
6751	somatostatin receptor 1
6752	somatostatin receptor 2
6469	sonic hedgehog homolog (Drosophila)
6272	sortilin 1
124460	sorting nexin 20
2040	stomatin
6854	synapsin 11
54843	synaptotagmin-like 2
117178	synovial sarcoma, X breakpoint 2 interacting protein
7057	thrombospondin 1
6915	thromboxane A2 receptor
7096	toll-like receptor 1
7039	transforming growth factor, alpha
7053	transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)
140803	transient receptor potential cation channel, subfamily M, member 6
4071	transmembrane 4 L six family member 1
6890	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
10381	tubulin, beta 3; melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)
8795	tumor necrosis factor receptor superfamily, member 10b
51330	tumor necrosis factor receptor superfamily, member 12A
7133	tumor necrosis factor receptor superfamily, member 1B
7126	tumor necrosis factor, alpha-induced protein 1 (endothelial)
94015	twenty homolog 2 (Drosophila)
5412	ubiquitin-like 3
673	v-raf murine sarcoma viral oncogene homolog B1
6843	vesicle-associated membrane protein 1 (synaptobrevin 1)
	antigen presentation
920	CD4 molecule
972	CD74 molecule, major histocompatibility complex, class II invariant chain
925	CD8a molecule
926	CD8b molecule
1508	cathepsin B
1520	cathepsin S
4261	class II, major histocompatibility complex, transactivator

3306	heat shock 70 kDa protein 2
3821	killer cell lectin-like receptor subfamily C, member 1
3822	killer cell lectin-like receptor subfamily C, member 2
3107, 3106	major histocompatibility complex, class I, C; major histocompatibility complex, class I, B
3134	major histocompatibility complex, class I, F
3108	major histocompatibility complex, class II, DM alpha
3109	major histocompatibility complex, class II, DM beta
3111	major histocompatibility complex, class II, DO alpha
3113	major histocompatibility complex, class II, DP alpha 1
3119	major histocompatibility complex, class II, DQ beta 1; similar to major histocompatibility complex, class II, DQ beta 1
2923	protein disulfide isomerase family A, member 3
5993	regulatory factor X, 5 (influences HLA class II expression)
6890	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
hair cycle/hair follicle dev/epidermis dev	
596	B-cell CLL/lymphoma 2
8538	BARX homeobox 2
2001	E74-like factor 5 (ets domain transcription factor)
646	hasonuclin 1
1499	catenin (cadherin-associated protein), beta 1, 88 kDa
1474	cystatin ELM
2068	excision repair cross-complementing rodent repair deficiency, complementation group 2
2171	fatty acid binding protein 5-like 2; fatty acid binding protein 5 (psoriasis-associated); fatty acid binding protein 5-like 8; fatty acid binding protein 5-like 7; fatty acid binding protein 5-like 9
2304	forkhead box E1 (thyroid transcription factor 2)
8456	forkhead box N1
3229	homeobox C13
182	jagged 1 (Alagille syndrome)
3868	keratin 16; keratin type 16-like
342574	keratin 27
3881	keratin 31
3882	keratin 32
3885	keratin 34
3854	keratin 6B
3889	keratin 83
3891	keratin 85

3846	keratin associated protein 5-9
51176	lymphoid enhancer-binding factor 1
55686	melanoregulin
5017	ovo-like 1(Drosophila)
864	runt-related transcription factor 3
6469	sonic hedgehog homolog (Drosophila)
7042	transforming growth factor, beta 2
7053	transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)

[0087] In some embodiments, the method further comprises detecting in the individual an AA-associated biomarker, either before or after treatment, or both.

[0088] In some embodiments, the AA-associated biomarker is indicative of severity of alopecia areata.

[0089] In some embodiments, the AA-associated biomarker is indicative of the propensity of the individual to respond to treatment with the subject anti-IL-7R α antibody, antigen binding fragment thereof, or a pharmaceutically acceptable salt thereof.

[0090] In some embodiments, prior to administering the subject anti-IL-7R α antibody, antigen binding fragment thereof, or a pharmaceutically acceptable salt thereof, to the individual, the method further comprises selecting the individual based on a level of an AA-associated biomarker.

[0091] In some embodiments, the administration of the subject anti-IL-7R α antibody, antigen binding fragment thereof, or a pharmaceutically acceptable salt thereof, results in a change in a level of an AA-associated biomarker in the individual.

[0092] In some embodiments, the subject in need thereof satisfies one or more criteria in the inclusion criteria specified in Example 1. In some embodiments, the subject in need thereof satisfies all criteria in the inclusion criteria specified in Example 1.

[0093] In some embodiments, the subject in need thereof does not satisfy (*i.e.*, is not excluded by) any one criteria in the exclusion criteria specified in Example 1.

[0094] In some embodiments, the subject has had alopecia areata for over 6 months and less than 10 years prior to administering the antibody.

[0095] In a related aspect, also provided is a method of treating alopecia areata (AA) in a mammalian subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (DHAMH, such as any one of SEQ ID NOs: 7-22), a VH CDR2

sequence of SEQ ID NO: 2 (GISWNSRGIGYADSVKG), and a VH CDR3 sequence of SEQ ID NO: 3 (DEYSRGYYVLDV); and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4 (RASQGISSALA), a VL CDR2 sequence of SEQ ID NO: 5 (DASSLES), and a VL CDR3 sequence of SEQ ID NO: 6 (QQFNSYPLWIT); wherein the subject has $\geq 50\%$ total scalp hair loss as defined by a baseline SALT score, wherein the baseline SALT score is determined up to 24 hours prior to administering the antibody; wherein the antibody comprises an effectorless IgG1 Fc, and wherein 200 mg of the antibody is administered subcutaneously every 2 weeks.

[0096] In some embodiments, the VH CDR1 comprises SEQ ID NO: 7 (GFTFDDHAMH).

[0097] In some embodiments, the subject has had alopecia areata for over 6 months and less than 10 years prior to administering the antibody.

[0098] In some embodiments, the composition comprises 100 mg/ml of the antibody in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0, with an optional 0.05 mM pentetic acid.

[0099] In some embodiments, the subject has an absolute lymphocyte count (ALC) of ≥ 800 per microL.

[0100] In some embodiments, administration of the antibody is stopped when the subject's ALC is < 500 per microL.

[0101] In some embodiments, the composition comprising the antibody is administered to the subject to maintain serum concentration of the antibody at ≥ 5 $\mu\text{g/mL}$ throughout dosing.

[0102] Serum concentration of the antibody can be measured using any methods known in the art. For example, the level of the anti-IL-7R α antibody in serum can be determined using a target capture approach. Specifically, the serum is flowed through a surface (*e.g.*, column) pre-coated with a capture agent specific for the anti-IL-7R α antibody (*e.g.*, human IL-7R extracellular domain), and the antibody in the serum is captured onto the surface. After washing off unbound materials, a labeled secondary antibody that specifically binds to the anti-IL-7R α antibody but does not compete for binding with the capture agent (*e.g.*, a labeled secondary antibody which binds to the Fc region of the anti-IL-7R α antibody) is added to detect the presence of the anti-IL-7R α antibody captured by the pre-coated substrate. Signal intensity from the labeled secondary antibody is proportional to the concentration of the anti-IL-7R α antibody in the serum, and serum concentration of the antibody is determined by comparing its measured signal to signals produced by standards with known concentrations of the antibody.

[0103] In a non-limiting example, serum concentration of the antibody can be measured using a Gyrolab[®] system which is a flow through immunoassay platform. In this fit-for-purpose assay, the antibody present in serum is captured on the columns in the Gyrolab[®] CD, which is pre-coated with biotinylated recombinant human IL-7R extracellular domain (target). The bound anti-IL-7R α antibody is detected with AlexaFluor[®]-647 labeled Cyno anti-human IgG Fc mAb (Pur Clone 10C7). The immunofluorescence measured using the Gyrolab[®]'s laser-induced fluorescence (LIF) detector is proportional to the concentration of the anti-IL-7R α antibody in samples, standards, and controls.

[0104] In another non-limiting example, serum concentration of the antibody can be measured using a Gyrolab[®] system, which is a flow through immunoassay platform. In this validated assay, the antibody present in serum is captured on the columns in the Gyrolab[®] CD which is pre-coated with biotinylated anti-idiotypic monoclonal antibody (mAb) that specifically bind to the anti-IL-7R α antibody. The bound anti-IL-7R α antibody is detected with AlexaFluor[®]-647 labeled mAb which is a non-competing clone but also binds to anti-IL-7R α antibody. The immunofluorescence measured using the Gyrolab[®]'s LIF detector is proportional to the concentration of the anti-IL-7R α antibody in samples, standards, and controls.

[0105] In some embodiments, the antibody is administered to the subject at about 200 mg per dose.

[0106] In some embodiments, the antibody is administered once every two weeks (Q2W).

[0107] In some embodiments, the antibody is administered for at least about 13 doses.

[0108] In some embodiments, the antibody comprises an effectorless IgG1 Fc.

[0109] In certain embodiments, the antibody has a heavy chain sequence of SEQ ID NO: 23 and a light chain sequence of SEQ ID NO: 24, and is administered at about 200 mg per dose to the mammalian (*e.g.*, human) subject subcutaneously (*s.c.*) once every two weeks (Q2W), for 10-15 (*e.g.*, 11, 12, or 13) consecutive doses.

[0110] Also provided is a pharmaceutical composition comprising an antibody having two heavy chains and two light chains, wherein each said heavy chain has the amino acid sequence of SEQ ID NO: 23, and each said light chain has the amino acid sequence of SEQ ID NO: 24; and wherein the antibody is formulated in a pharmaceutically acceptable solution comprising about 50-200 mg/mL (*e.g.*, about 100 mg/mL) of the antibody in about 15-25 mM (*e.g.*, about 20 mM) histidine, 200-300 mM (*e.g.*, about 260 mM) sucrose, and about 0.03-0.07% (w/v) (*e.g.*, about 0.05% (w/v)) polysorbate 80 at pH 6.0.

[0111] In some embodiments, the solution further comprises about 0.01-0.10 mM (*e.g.*, about 0.05 mM) of pentetic acid.

[0112] In some embodiments, the pharmaceutical composition comprises, consists essentially of, or consists of the antibody formulated as 100 mg/mL of the antibody in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0.

[0113] In some embodiments, the pharmaceutical composition comprises, consists essentially of, or consists of the antibody formulated as 100 mg/mL of the antibody in 20 mM histidine, 260 mM sucrose, 0.05 mM of pentetic acid, and 0.05% (w/v) polysorbate 80 at pH 6.0.

[0114] Also provided is a container (*e.g.* a polycarbonate bottle) containing about 2 mL of extractable volume of a pharmaceutical composition described herein.

[0115] In some embodiments, the container comprises or contains about 2.26 mL of a pharmaceutical composition described herein.

[0116] The definitions and methods provided define the present disclosure and guide those of ordinary skill in the art in the practice of the present disclosure. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0117] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.”

[0118] As used herein, the term “about” is construed to include all values less than a full increment and greater than a full decrement of the least significant digit claimed. In other words, the term “about,” as used herein, is intended to qualify the numerical values which it modifies, denoting such a value as variable within a margin of error. When no particular margin of error, such as a standard deviation to a mean value given in a chart or table of data, is recited, the term “about” should be understood to mean that range which would encompass the recited value and the range which would be included by rounding up or down to that figure as well, taking into account significant figures.

[0119] As used herein, “alopecia areata” or “AA” is a chronic T-cell mediated autoimmune skin disease leading to hair loss. Hair may be lost more diffusely over the whole scalp, in which case the condition is called diffuse alopecia areata. Alopecia areata monolocularis describes baldness in only one spot. It may occur anywhere on the head. Alopecia areata multilocularis refers to multiple areas of hair loss. Ophiasis refers to hair loss in the shape of a wave at the circumference of the head. The disease may be limited only to

the beard, in which case it is called alopecia areata barbae. If the person loses all the hair on the scalp, the disease is then called alopecia areata totalis. If all body hair is lost, the diagnosis then becomes alopecia areata universalis. Severe alopecia areata refers to 50% or more involvement of the entire scalp, alopecia totalis, and alopecia universalis. Moderate alopecia areata refers to at least 30% and up to 50% involvement of the entire scalp.

[0120] As used herein, “SALT score” refers to the Severity of Alopecia Tool which is a tool for determining degree of hair loss based on the percentage of scalp surface area involved on the top, back, and each side of the scalp. The SALT II score is an updated tool which includes smaller increments of scalp coverage to facilitate the assessment of hair loss where small patches of hair loss predominate. The SALT score, using either the original SALT I or SALT II, is determined by adding the percentage hair loss in the various areas of the scalp.

[0121] As used herein, with respect to alopecia areata, “clinical response” refers to achieving 50% or greater hair re-growth from baseline, based on the SALT score at end of treatment.

[0122] As used herein, the “Alopecia Areata Disease Activity Index” or “ALADIN” is a three-dimensional quantitative composite gene expression score for use as a biomarker for tracking disease severity and response to treatment.

[0123] As used herein “AASIS” or “alopecia areata symptom impact scale (AASIS),” can refer to a 13-item, disease-specific measure that asks AA patients about symptoms related to AA and how these symptoms interfere with daily functioning. Patients can be asked to rate how severe each of the following 7 symptoms pertaining to AA symptoms have been in the past week using an 11-point scale ranging from 0 “not present” to 10 “as bad as you can imagine”: 1) scalp hair loss, 2) body or eye lashes hair loss, 3) tingling/numbness of the scalp, 4) itchy or painful skin, 5) irritated skin, 6) feeling anxious or worry, or 7) feeling sad. Patients can also be asked to rate how severe each of the following 6 areas of daily functioning are interfered with by AA in the past week using an 11-point scale ranging from 0 = “did not interfere” to 10 “interfered completely”: work, enjoyment of life, interaction with others, daily activities, sexual relationships, and quality of life. The overall scoring system ranges from 0 to 130 with high scores indicative of greater AA symptom impact.

[0124] As used herein the Alopecia Areata-Related Quality of Life Index (AA-QLI) refers to a disease-specific questionnaire developed to evaluate the impact of AA on quality of life. The results are presented on a scale varying between 0 and 84; a score of 0 represents the best quality of life, while a score of 84 represents the poorest quality of life outcomes.

The AA-QLI consists of questions covering 3 areas of daily life: subjective symptoms, relationships, and objective signs.

[0125] As used herein, the term “AA-associated biomarker” means any biological response, cell type, parameter, protein, polypeptide, enzyme, enzyme activity, metabolite, nucleic acid, carbohydrate, or other biomolecule which is present or detectable in an AA patient at a level or amount that is different from (*e.g.*, greater than or less than) the level or amount of the marker present or detectable in a non-AA patient. The term “AA-associated biomarker” also includes a gene or gene probe known in the art which is differentially expressed in a subject with AA as compared to a subject without A. Alternatively, “AA-associated biomarker” also includes genes which are down regulated due to AA.

[0126] In some embodiments, the biomarker is assessed using histology. In some embodiments, the biomarker is assessed using RNAseq. In some embodiments, the biomarker is assessed using proteomic analysis. In some embodiments, the biomarker is assessed using enzyme-linked immunosorbent assay. In some embodiments, the biomarker is assessed using mass spectrometry. In some embodiments, the biomarker is assessed using a blood sample. In some embodiments, the biomarker will be assessed using a serum sample. In some embodiments, the biomarker will be assessed using a plasma sample. In some embodiments, the biomarker is assessed using a tissue sample. In some embodiments, the biomarker will be assessed using a punch biopsy.

[0127] In some embodiments, the biomarker is selected from Th2/IL-13, Th22/IL-22, Th1/IFN- γ , and Th17/IL-17A. In some embodiments, the biomarker is selected from IFN- γ , IL-2, IL-12, IL-13, IL-10, and IL-17. In some embodiments, the biomarker is selected from at least one of IL-2, IL-10, IL-12, IL-13, IL-17, IL-17A, IL-22, and IFN- γ . In some embodiments, the biomarker is IL-2. In some embodiments, the biomarker is IL-10. In some embodiments, the biomarker is IL-12. In some embodiments, the biomarker is IL-13. In some embodiments, the biomarker is IL-17. In some embodiments, the biomarker is IL-17A. In some embodiments, the biomarker is IL-22. In some embodiments, the biomarker is IFN- γ .

[0128] In some embodiments, the biomarker is a gene expression signature. In some embodiments, the gene expression signature comprises gene expression information of one or more of the following groups of genes: hair keratin (KRT) associated genes, cytotoxic T lymphocyte infiltration (CTL) associated genes, and interferon (IFN) associated genes. In some embodiments, the KRT-associated genes comprise DSG4, HOXC31, KRT31, KRT32, KRT33B, KRT82, PKP1 and/or PKP2. In some embodiments, the CTL-associated genes

comprise CD8A, GZMB, ICOS and/or PRF1. In some embodiments, the IFN-associated genes comprise CXCL9, CXCL10, CXCL11, STAT1 and/or MX1. In some embodiments, the AA-associated biomarker is chosen from IL-15, CCL2, CCL3, CXCL10, IL-13, CCL13, CCL17, CCL22, CCL26, CCL4, and CCL11 and the level of the biomarker is increased in the sera from individuals with AA as compared with sera from healthy patients. In some embodiments, the AA-associated biomarker is chosen from IL-15 and eotaxin/CCL11 and the level of the biomarker is associated with SALT score.

[0129] In some embodiments, the AA-associated biomarker is chosen from scalp TH2-related markers (CCL13 and IL-13) and serum T-cell/NK-cell activation marker (IL-15).

[0130] Also provided are biomarkers for monitoring disease reversal with the administration of the subject antibody, or a pharmaceutically acceptable formulation thereof. Methods for detecting and/or quantifying such AA-associated biomarkers are known in the art; kits for measuring such AA-associated biomarkers are available from various commercial sources; and various commercial diagnostic laboratories offer services which provide measurements of such biomarkers as well.

[0131] As used herein, "TSLP" refers to a growth factor that closely resembles IL-7 and plays a role in the maturation and activation of myeloid cells (*e.g.*, monocytes and dendritic cells). TSLP is produced by various cell types, such as fibroblasts, epithelial cells, and stromal cells. Elevated levels of TSLP have been associated with diseases, such as asthma, atopic dermatitis, and inflammatory arthritis, which are also known to be associated with abnormal regulation of IL-7.

[0132] As used herein, the term "antibody" refers to a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (abbreviated herein as CH). In certain antibodies, *e.g.*, naturally occurring IgG antibodies, the heavy chain constant region is comprised of a hinge and three domains, CH1, CH2 and CH3. In certain antibodies, *e.g.*, naturally occurring IgG antibodies, each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain (abbreviated herein as CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of

the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system. A heavy chain may have the C-terminal lysine or not. Unless specified otherwise herein, the amino acids in the variable regions are numbered using the Kabat numbering system and those in the constant regions are numbered using the EU system. “Antibody” includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal and polyclonal antibodies; chimeric and humanized antibodies; human and nonhuman antibodies and wholly synthetic antibodies.

[0133] An “IgG antibody”, *e.g.*, a human IgG1, IgG2, IgG3 and IgG4 antibody, as used herein, has, in some embodiments, the structure of a naturally occurring IgG antibody, *i.e.*, it has the same number of heavy and light chains and disulfide bonds as a naturally occurring IgG antibody of the same subclass. For example, an anti-IL-7R IgG1, IgG2, IgG3 or IgG4 antibody consists of two heavy chains (HCs) and two light chains (LCs), wherein the two heavy chains and light chains are linked by the same number and location of disulfide bridges that occur in naturally occurring IgG1, IgG2, IgG3 and IgG4 antibodies, respectively (unless the antibody has been mutated to modify the disulfide bridges).

[0134] Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 10^{-5} to 10^{-11} M or less. Any K_D greater than about 10^{-4} M is generally considered to indicate nonspecific binding.

[0135] In some embodiments, the anti-IL-7R α antibody used or useful for the method described herein specifically binds to IL-7R α , such as binding specifically with a K_D of 10^{-5} to 10^{-11} M or less (*e.g.*, 10^{-5} M or less, 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, or 10^{-11} M or less).

[0136] In some embodiments, the anti-IL-7R α antibody used or useful for the method described herein binds specifically to IL-7R α (such as human IL-7R α) with a K_D of 10^{-7} M or less, 10^{-8} M or less, 5×10^{-9} M or less, or between 10^{-8} M and 10^{-10} M or less, but does not bind with high affinity to unrelated antigens.

[0137] An antigen is “substantially identical” to a given antigen if it exhibits a high degree of sequence identity to the given antigen, for example, if it exhibits at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% sequence identity to the sequence of the given antigen. By way of example, an antibody that binds specifically to human IL-7R α can, in some embodiments, also have cross-reactivity with IL-7R α antigens from certain primate

species (*e.g.*, cynomolgus IL-7Ra), but cannot cross-react with IL-7Ra antigens from other species or with an antigen other than IL-7Ra.

[0138] As used herein, “isotype” refers to the antibody class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE antibody) that is encoded by the heavy chain constant region genes. The IgG isotype is divided in subclasses in certain species: IgG1, IgG2, IgG3 and IgG4 in humans, and IgG1, IgG2a, IgG2b and IgG3 in mice. In some embodiments, the anti-IL-7R antibodies described herein are of the IgG1 isotype. Immunoglobulins, *e.g.*, IgG1, exist in several allotypes, which differ from each other in at most a few amino acids.

[0139] As used herein, the term “allotype” refers to naturally occurring variants within a specific isotype group, wherein the variants differ in a few amino acids. Anti-IL-7R antibodies described herein can be of any allotype. As used herein, antibodies referred to as “IgG1f,” “IgG1. If,” or “IgG1.3f” isotype are IgG1, effectorless IgG1.1, and effectorless IgG1.3 antibodies, respectively, of the allotype “f,” *i.e.* having 214R, 356E and 358M according to the EU index as in Kabat, as shown, *e.g.*, in SEQ ID NO: 23.

[0140] The term “antigen-binding portion” of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, human IL-7Ra). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antigen-binding portion” of an antibody, *e.g.*, an anti-IL-7R antibody described herein, include (i) a Fab fragment (fragment from papain cleavage) or a similar monovalent fragment consisting of the VL, VH, LC and CH1 domains; (ii) a F(ab')₂ fragment (fragment from pepsin cleavage) or a similar bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a F_d fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment which consists of a VH domain; (vi) an isolated complementarity determining region (CDR) and (vii) a combination of two or more isolated CDRs which can optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv)). Such single chain antibodies are also intended to be encompassed within the term “antigen-binding portion” of an antibody.

[0141] These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are

intact antibodies. Antigen-binding portions can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

[0142] The term “monoclonal antibody,” as used herein, refers to an antibody from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprised in the population are substantially similar and bind the same epitope(s) (*e.g.*, the antibodies display a single binding specificity and affinity), except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

[0143] The term “human monoclonal antibody” refers to an antibody from a population of substantially homogeneous antibodies that display(s) a single binding specificity and which has variable and optional constant regions derived from human germline immunoglobulin sequences. In one embodiment, human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

[0144] The term “recombinant human antibody,” as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, *e.g.*, from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies comprise variable and constant regions that utilize particular human germline immunoglobulin sequences are encoded by the germline genes, but include subsequent rearrangements and mutations which occur, for example, during antibody maturation. As known in the art, the variable region contains the antigen binding domain, which is encoded by various genes that rearrange to form an antibody specific for a foreign antigen. In addition to rearrangement, the variable region can be further modified by multiple single amino acid changes (referred to as somatic mutation or hypermutation) to increase the affinity of the antibody to the foreign antigen. The constant region will change in further response to an antigen (*i.e.*, isotype switch). Therefore, the rearranged and somatically mutated nucleic acid

molecules that encode the light chain and heavy chain immunoglobulin polypeptides in response to an antigen cannot have sequence identity with the original nucleic acid molecules, but instead will be substantially identical or similar (*i.e.*, have at least 80% identity).

[0145] A “human” antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The anti-IL-7R antibodies described herein can include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term “human antibody”, as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms “human” antibodies and “fully human” antibodies are used synonymously.

[0146] A “humanized” antibody refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a particular antigen. A “humanized” antibody retains an antigenic specificity similar to that of the original antibody.

[0147] A “chimeric antibody” refers to an antibody in which the variable regions are derived from one species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody.

[0148] The phrases “an antibody recognizing an antigen” and “an antibody specific for an antigen” are used interchangeably herein with the term “an antibody which binds specifically to an antigen.”

[0149] An “isolated antibody,” as used herein, is intended to refer to an antibody which is substantially free of other proteins and cellular material.

[0150] As used herein, an antibody that “inhibits binding of IL-7 to IL-7R α ” is intended to refer to an antibody that inhibits the binding of IL-7R α to its ligand, *e.g.*, interleukin-7 (IL-7), *e.g.*, in binding assays using T cells from whole blood that express IL-7R α , with an EC₅₀ of about 1 pg/mL or less, such as about 0.9 pg/mL or less, about 0.85 pg/mL or less, about 0.8 pg/mL or less, about 0.75 pg/mL or less, about 0.7 pg/mL or less, about 0.65 pg/mL or less, about 0.6 pg/mL or less, about 0.55 pg/mL or less, about 0.5 pg/mL or less, about 0.45 pg/mL or less, about 0.4 pg/mL or less, about 0.35 pg/mL or less, about 0.3 pg/mL or less, about 0.25 pg/mL or less, about 0.2 pg/mL or less, about 0.15 pg/mL or less, about 0.1 pg/mL or less, or about 0.05 pg/mL or less, in art-recognized methods, *e.g.*, the FACS-based binding assays described herein.

[0151] An “effector function” refers to the interaction of an antibody Fc region with an Fc receptor or ligand, or a biochemical event that results therefrom. Exemplary “effector functions” include Clq binding, complement dependent cytotoxicity (CDC), Fc receptor binding, Fc γ R-mediated effector functions such as ADCC and antibody dependent cell-mediated phagocytosis (ADCP), and downregulation of a cell surface receptor (*e.g.*, the B cell receptor; BCR). Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.*, an antibody variable domain).

[0152] An “Fc receptor” or “FcR” is a receptor that binds to the Fc region of an immunoglobulin. FcRs that bind to an IgG antibody comprise receptors of the Fc γ R family, including allelic variants and alternatively spliced forms of these receptors. The Fc γ R family consists of three activating (Fc γ RI, Fc γ RIII, and Fc γ RIV in mice; FcRIA, FcRIIA, and FcRIIIA in humans) and one inhibitory (Fc γ RIIB) receptor. Various properties of human Fc γ Rs are known in the art. The majority of innate effector cell types coexpress one or more activating Fc γ R and the inhibitory Fc γ RIIB, whereas natural killer (NK) cells selectively express one activating Fc receptor (Fc γ RIII in mice and Fc γ RIIIA in humans) but not the inhibitory Fc γ RIIB in mice and humans. Human IgG1 binds to most human Fc receptors and is considered equivalent to murine IgG2a with respect to the types of activating Fc receptors that it binds to.

[0153] An “Fc region” (fragment crystallizable region) or “Fc domain” or “Fc” refers to the C-terminal region of the heavy chain of an antibody that mediates the binding of the immunoglobulin to host tissues or factors, including binding to Fc receptors located on various cells of the immune system (*e.g.*, effector cells) or to the first component (Clq) of the classical complement system. Thus, an Fc region comprises the constant region of an antibody excluding the first constant region immunoglobulin domain (*e.g.*, CHI or CF). In

IgG, IgA and IgD antibody isotypes, the Fc region comprises two identical protein fragments, derived from the second (CH2) and third (CH3) constant domains of the antibody's two heavy chains; IgM and IgE Fc regions comprise three heavy chain constant domains (CH domains 2-4) in each polypeptide chain. For IgG, the Fc region comprises immunoglobulin domains CH2 and CH3 and the hinge between CH1 and CH2 domains. Although the definition of the boundaries of the Fc region of an immunoglobulin heavy chain might vary, as defined herein, the human IgG heavy chain Fc region is defined to stretch from an amino acid residue D221 for IgG1, V222 for IgG2, F221 for IgG3 and P224 for IgG4 to the carboxy-terminus of the heavy chain, wherein the numbering is according to the EU index as in Kabat. The CH2 domain of a human IgG Fc region extends from amino acid 237 to amino acid 340, and the CH3 domain is positioned on C-terminal side of a CH2 domain in an Fc region, *i.e.*, it extends from amino acid 341 to amino acid 447 or 446 (if the C-terminal lysine residue is absent) or 445 (if the C-terminal glycine and lysine residues are absent) of an IgG. As used herein, the Fc region can be a native sequence Fc, including any allotypic variant, or a variant Fc (*e.g.*, a non-naturally occurring Fc). Fc can also refer to this region in isolation or in the context of an Fc-comprising protein polypeptide such as a "binding protein comprising an Fc region," also referred to as an "Fc fusion protein" (*e.g.*, an antibody or immunoadhesion).

[0154] A "native sequence Fc region" or "native sequence Fc" comprises an amino acid sequence that is identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region; native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof. Native sequence Fc include the various allotypes of Fcs.

[0155] The term "epitope" or "antigenic determinant" refers to a site on an antigen (*e.g.*, IF-7R α) to which an immunoglobulin or antibody specifically binds, *e.g.*, as defined by the specific method used to identify it. Epitopes can be formed both from contiguous amino acids (usually a linear epitope) or noncontiguous amino acids juxtaposed by tertiary folding of a protein (usually a conformational epitope). Epitopes formed from contiguous amino acids are typically, but not always, retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods for determining what epitopes are bound by a given antibody (*i.e.*, epitope mapping) are well known in the art and include, for example, immunoblotting and immunoprecipitation assays, wherein overlapping or contiguous peptides from (*e.g.*, from

IF-7R α) are tested for reactivity with a given antibody (*e.g.*, anti-IF-7R antibody). Methods of determining spatial conformation of epitopes include techniques in the art and those described herein, for example, x-ray crystallography, antigen mutational analysis, 2-dimensional nuclear magnetic resonance and HDX-MS.

[0156] The term “ k_{assoc} ” or “ k_a ”, as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term “ k_{dis} ” or “ k_d ”, as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term “ K_D ”, as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of k_d to k_a (*i.e.*, k_d/k_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. Available methods for determining the K_D of an antibody include surface plasmon resonance, a biosensor system such as a BIAcore® system or flow cytometry and Scatchard analysis.

[0157] As used herein, the term “high affinity” for an IgG antibody refers to an antibody having a K_D of 10^{-8} M or less, 10^{-9} M or less, or 10^{-10} M or less for a target antigen. However, “high affinity” binding can vary for other antibody isotypes. For example, “high affinity” binding for an IgM isotype refers to an antibody having a K_D of 10^{-10} M or less, or 10^{-8} M or less.

[0158] The term “EC50” in the context of an *in vitro* or *in vivo* assay using an antibody or antigen binding fragment thereof, refers to the concentration of an antibody or an antigen-binding portion thereof that induces a response that is 50% of the maximal response, *i.e.*, halfway between the maximal response and the baseline.

[0159] As used herein, the term “autoimmune disease” refers to a disease or disorder in which the immune system produces an immune response (*e.g.*, a B cell or a T cell response) against an antigen that is part of the normal host (*i.e.*, an autoantigen), with consequent injury to tissues. An autoantigen may be derived from a host cell, or may be derived from a commensal organism such as the micro-organisms (known as commensal organisms) that normally colonize mucosal surfaces.

[0160] The term “inflammation” or an “inflammatory process,” as used herein, refers to a complex series of events, including dilatation of arterioles, capillaries and venules, with increased permeability and blood flow, exudation of fluids, including plasma proteins and leukocyte migration into the inflammatory focus. Inflammation may be measured by many methods well known in the art, such as the number of leukocytes, the number of polymorphonuclear neutrophils (PMN), a measure of the degree of PMN activation, such as

luminal enhanced-chemiluminescence, or a measure of the amount of proinflammatory cytokines (*e.g.*, IL-6 or TNF- α) present.

[0161] As used herein, the term “regulatory T cells” (Tregs) refer to a population of T cells with the ability to reduce or suppress the induction and proliferation of effector T cells, and thereby, modulate an immune response. In some embodiments, Tregs can suppress an immune response by secreting anti-inflammatory cytokines, such as IL-10, TGF- β , and IL-35, which can interfere with the activation and differentiation of naive T cells into effector T cells. In some embodiments, Tregs can also produce cytolytic molecules, such as Granzyme B, which can induce the apoptosis of effector T cells. In some embodiments, the regulatory T cells are natural regulatory T cells (nTregs) (*i.e.*, developed within the thymus). In some embodiments, the regulatory T cells are induced regulatory T cells (iTregs) (*i.e.*, naive T cells that differentiate into Tregs in the peripheral tissue upon exposure to certain stimuli). Methods for identifying Tregs are known in the art. For example, Tregs express certain phenotypic markers (*e.g.*, CD25, Foxp3, or CD39) that can be measured using flow cytometry. In some embodiments, the Tregs are CD45RA⁻ CD39⁺ T cells.

[0162] As used herein, “administering” refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Different routes of administration for the anti-IL-7R antibodies described herein include intravenous, intraperitoneal, intramuscular, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase “parenteral administration” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intraperitoneal, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion, as well as *in vivo* electroporation. Alternatively, an antibody described herein can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0163] As used herein, the terms “inhibits” or “blocks” (*e.g.*, referring to inhibition/blocking of binding of IL-7 to IL-7R α on cells) are used interchangeably and encompass both partial and complete inhibition/blocking. In some embodiments, the anti-IL-

7R antibody inhibits binding of IL-7 to IL-7Ra by at least about 50%, for example, about 60%, 70%, 80%, 90%, 95%, 99%, or 100%, determined, *e.g.*, as further described herein.

[0164] The terms “treat,” “treating,” and “treatment,” as used herein, refer to any type of intervention or process performed on, or administering an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, or slowing down or preventing the progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease or enhancing overall survival. These terms do not include prophylactic intervention.

[0165] The term “prophylactic intervention” refers to treating a subject who does not yet have a disease for preventive purpose.

[0166] The term “effective dose” or “effective dosage” is defined as an amount sufficient to achieve or at least partially achieve a desired effect.

[0167] A “therapeutically effective amount” or “therapeutically effective dosage” of a drug or therapeutic agent (*e.g.*, the subject anti-IL-7Ra antibody) is any amount of the drug that, when used alone or in combination with another therapeutic agent, promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. A therapeutically effective amount or dosage of a drug includes a “prophylactically effective amount” or a “prophylactically effective dosage,” which is any amount of the drug that, when administered alone or in combination with another therapeutic agent to a subject at risk of developing a disease or of suffering a recurrence of disease, inhibits the development or recurrence of the disease. The ability of a therapeutic agent to promote disease regression or inhibit the development or recurrence of the disease can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials (including the methods described in the examples), in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0168] The term “patient” includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment. In some embodiments, the patient is a human.

[0169] As used herein, the term “subject” includes any human or non-human animal. For example, the methods and compositions described herein can be used to treat a subject having cancer. The term “non-human animal” includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, *etc.*

[0170] The term “weight based” dose or dosing as referred to herein means that a dose that is administered to a patient is calculated based on the weight of the patient. For example, when a patient with 60 kg body weight requires 3 mg/kg of an anti-IL-7R antibody, one can calculate and use the appropriate amount of the anti-IL-7Ra antibody (*i.e.*, 180 mg) for administration.

[0171] Provided are methods for the treatment of alopecia areata, the method comprising administering to a subject in need thereof an isolated antibody or antigen binding portion thereof, which specifically binds to an alpha-chain of a human IL-7 receptor (“anti-IL-7R α antibody,” or “anti-IL-7R antibody” for short), comprising a heavy chain CDR1, CDR2, and CDR3, and a light chain CDR1, CDR2, and CDR3, respectively, wherein the antibody

(a) is capable of binding to T cells (CD4⁺CD45RA⁺, CD4⁺CD45RA⁻, CD8⁺CD45RA⁺, and/or CD8⁺CD45RA⁻) in whole blood with an EC₅₀ of about 5 nM or less (*e.g.*, less than about 3 nM);

(b) is not capable of binding to non-T cells in whole blood;

(c) does not agonize IL-7 receptor signaling upon binding to the IL-7 receptor, *e.g.*, minimal pSTAT5 activation; or

(d) any combination thereof.

[0172] In some embodiments, the heavy chain CDR3 of the subject anti-IL-7R α antibody (*e.g.*, disclosed herein) comprises the amino acid sequence set forth in SEQ ID NO: 3 (DEYSRGYYVLDV).

[0173] In some embodiments, the anti-IL-7R α antibody has one or more properties selected from the group consisting of:

(a) is capable of selectively binding to an alpha-chain of a human and cynomolgus IL-7 receptor (IL-7R);

(b) is capable of binding to an alpha-chain of soluble and membrane bound IL-7R;

(c) is capable of blocking an expansion and/or survival of pathogenic T cells when administered to a subject in need thereof;

(d) is capable of restoring a T regulatory cell (Treg) function and/or promoting a Treg survival when administered to a subject in need thereof;

(e) is capable of maintaining a drug free remission longer than that by CTLA4-Ig (ORENCIA®);

(f) is capable of blocking inflammation and mucosal damage, *e.g.*, induced by pathogenic T cells, within an intestinal tissue of a subject in need thereof;

(g) is capable of decreasing a frequency of T effector cells in the mesenteric lymph nodes (MLN) and/or lamina propria (LP) in a subject in need thereof;

(h) is capable of reducing or inhibiting IL-7 mediated pSTAT activation in T cells (*e.g.*, CD4⁺CD45RA⁺);

(i) is capable of blocking expansion of IL-17 and/or IFN-gamma producing cells;

(j) is capable of treating a subject with an inflammatory disease (*e.g.*, inflammatory bowel disease); and

(k) any combination thereof.

[0174] In some embodiments, the subject anti-IL-7R α antibody comprises a heavy chain CDR1, wherein the heavy chain CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 1 (DHAMH), such as any one of amino acid sequences set forth in SEQ ID NOs: 7 to 22. In some embodiments, an anti-IL-7R α antibody comprises a heavy chain CDR2, wherein the heavy chain CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 2 (GISWNSRGIGYADSVKG). In some embodiments, an anti-IL-7R α antibody comprises a heavy chain CDR3, wherein the heavy chain CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 3 (DEYSRGYYVLDV). In some embodiments, an anti-IL-7R α antibody comprises a light chain CDR1, wherein the light chain CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 4 (RASQGISSALA). In some embodiments, an anti-IL-7R α antibody comprises a light chain CDR2, wherein the light chain CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 5 (DASSLES). In some embodiments, an anti-IL-7R α antibody comprises a light chain CDR3, wherein the light chain CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 6 (QQFNSYPLWIT).

[0175] In some embodiments, the subject anti-IL-7R α antibody comprises a heavy chain CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 1 (*e.g.*, any one of SEQ ID NOs: 7-22, such as SEQ ID NO: 7 (GFTFDDHAMH)); a heavy chain CDR2 of the amino acid sequence set forth in SEQ ID NO: 2 (GISWNSRGIGYADSVKG); a heavy chain CDR3 of the amino acid sequence set forth in SEQ ID NO: 3 (DEYSRGYYVLDV); a light chain CDR1 of the amino acid sequence set forth in SEQ ID NO: 4 (RASQGISSALA); a light chain CDR2 of the amino acid sequence set forth in SEQ ID NO: 5 (DASSLES); and a light chain CDR3 of the amino acid sequence set forth in SEQ ID NO: 6 (QQFNSYPLWIT).

[0176] In some embodiments, the subject anti-IL-7R α antibody comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises an amino acid sequence which is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NO: 25. In some embodiments, the VL comprises an amino acid sequence which is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence set forth in SEQ ID NO: 26.

[0177] In some embodiments, the subject anti-IL-7R α antibody disclosed herein specifically binds to the alpha-chain of the human IL-7 receptor at an epitope selected from the group consisting of: ²⁴SQLEVNGSQHSLTCAF³⁹ (SEQ ID NO: 27); ⁷³FIETKKFLLIGKSNIC⁸⁸ (SEQ ID NO: 28); ⁸⁹VKVGEKSLTCKKIDLT¹⁰⁵ (SEQ ID NO: 29); ¹³⁶QKKYVKVLMHDVAY¹⁴⁹ (SEQ ID NO: 30); ¹⁸¹YEIKVRSIPDHYFKGF¹⁹⁶ (SEQ ID NO: 31); and combinations thereof.

[0178] In some embodiments, an anti-IL-7R α antibody specifically binds to the alpha-chain of the human IL-7 receptor at an epitope comprising one or more amino acid residues selected from the group consisting of H33, E75, F79, 182, K84, M144, R186, H191, Y192, and combinations thereof.

[0179] In some embodiments, an anti-IL-7R antibody is selected from the group consisting of an IgG1, IgG2, IgG3, IgG4, and a variant thereof.

[0180] In some embodiments, an IL-7R antibody is an IgG1 antibody. In some embodiments, an anti-IL-7R antibody comprises an effectorless IgG1 Fc.

[0181] In some embodiments, the subject anti-IL-7R α antibody or antigen binding portion thereof comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises the amino acid sequence set forth in SEQ ID NO: 25 and the VL comprises the amino acid sequence set forth in SEQ ID NO: 26.

[0182] In some embodiments, the subject anti-IL-7R α antibody or antigen binding portion thereof comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence set forth in SEQ ID NO: 23 and wherein the light chain comprises the amino acid sequence set forth in SEQ ID NO: 24.

[0183] In some embodiments, an anti-IL-7R α antibody binds to the alpha-chain of the human IL-7 receptor with a K_D of less than 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM (*e.g.*, 1.3 nM), as measured by surface plasmon resonance. In some

embodiments, an anti-IL-7R α antibody binds to the alpha-chain of the cynomolgus IL-7 receptor with a K_D of less than 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM (*e.g.*, 1.7 nM), as measured by surface plasmon resonance. In some embodiments, the binding to the alpha-chain of the human IL-7 receptor or the alpha-chain of the cynomolgus IL-7 receptor is pH-dependent. In some embodiments, an anti-IL-7R α antibody binds to the alpha-chain of the human IL-7 receptor with a K_D of about 1.3 nM at pH 7.4 and with a K_D of about 5.3 nM at pH 6. In some embodiments, an anti-IL-7R α antibody binds to the alpha-chain of the cynomolgus IL-7 receptor with a K_D of about 1.7 nM at pH 7.4 and with a K_D of about 7.0 nM at pH 6.

[0184] In some embodiments, the isolated antibody or antigen binding portion thereof, which specifically binds to an alpha-chain of a human IL-7 receptor (“anti-IL-7R α antibody”) comprising a heavy chain (HC) CDR1, CDR2, and CDR3, and a light chain (LC) CDR1, CDR2, and CDR3, wherein: (i) the heavy chain CDR1 comprises an amino acid sequence set forth in any one of SEQ ID NOs: 7 to 22; (ii) the heavy chain CDR2 comprises an amino acid sequence set forth in SEQ ID NO: 2 (GISWNSRGIGYADSVKG); (iii) the heavy chain CDR3 comprises an amino acid sequence set forth in SEQ ID NO: 3 (DEYSRGYYVLVDV); (iv) the light chain CDR1 comprises an amino acid sequence set forth in SEQ ID NO: 4 (RASQGISSALA); (v) the light chain CDR2 comprises an amino acid sequence set forth in SEQ ID NO: 5 (DASSLES); and (vi) the light chain CDR3 comprises an amino acid sequence set forth in SEQ ID NO: 6 (QQFNSYPLWIT). In certain aspects, the heavy chain CDR1 comprises an amino acid sequence set forth in SEQ ID NO: 8 (GYTFDDHAMH), SEQ ID NO: 19 (GFDFFDDHAMH), or SEQ ID NO: 20 (GFEFDDHAMH).

[0185] The relevant sequences of the exemplary antibodies are provided below.

[0186] **Table 1: Amino acid sequences of anti- IL-7R α antibodies**

SEQ ID NO	DESCRIPTION	SEQUENCE
1	VH CDR1	DHAMH
7	VH CDR1 (18B1)	GFTFDDHAMH
8	VHCDR1 (F27Y)	GYTFDDHAMH
9	VH CDR1 (T28P)	GFPFDDHAMH
10	VH CDR1 (T28A)	GFAFDDHAMH
11	VH CDR1 (T28V)	GFVFDDHAMH
12	VH CDR1 (T28L)	GFLFDDHAMH
13	VH CDR1 (T28I)	GFIFDDHAMH
14	VH CDR1 (T28M)	GFMFDDHAMH
15	VH CDR1 (T28H)	GFHFDDHAMH
16	VH CDR1 (T28F)	GFFFDDHAMH
17	VH CDR1 (T28Y)	GFYFDDHAMH

18	VH CDR1 (T28N)	GFNFDDHAMH
19	VH CDR1 (T28D)	GFDFDDHAMH
20	VH CDR1 (T28E)	GFEFDDHAMH
21	VH CDR1 (T28Q)	GFQFDDHAMH
22	VH CDR1 (M34L)	GFTFDDHALH
2	VH CDR2	GISWNSRGIGYADSVKG
3	VH CDR3	DEYSRGYYVLDV
4	VL CDR1	RASQGISSALA
5	VL CDR2	DASSLES
6	VL CDR3	QQFNSYPLWIT
25	Heavy Chain Variable Region, VH	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDH AMHWVRQAPGKGLEWVSGISWNSRGIGYAD SVKGRFTIFRDNAKNSLYLQMNSLRAEDTALY YCAKDEYSRGYYVLDVWGQGTTVTVSS
26	Light Chain Variable Region, VL	AIQLTQSPSSLSASVGDRVTITCRASQGISSALA WYQQKPGKAPKLLIYDASSLESGVPSRFSGSG SGTDFTLTISLQPEDFATYYCQQFNSYPLWIT FGQGTRLEIK
23	Heavy Chain	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDH AMHWVRQAPGKGLEWVSGISWNSRGIGYAD SVKGRFTIFRDNAKNSLYLQMNSLRAEDTALY YCAKDEYSRGYYVLDVWGQGTTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVKDRVEPK SCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTL MISRTPEVTCVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFCFSVMHEALHNHYTQK SLSLSPG
24	Light Chain	AIQLTQSPSSLSASVGDRVTITCRASQGISSALA WYQQKPGKAPKLLIYDASSLESGVPSRFSGSG SGTDFTLTISLQPEDFATYYCQQFNSYPLWIT FGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDYSLSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC

[0187] In some embodiments, the anti-IL-7R α antibody binds to the alpha-chain of the human IL-7 7R α with a K_D of less than 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM (*e.g.*, 1.3 nM), as measured by surface plasmon resonance. In some embodiments, an anti-IL-7R α antibody binds to the alpha-chain of the cynomolgus IL-7 receptor with a K_D of less than 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM (*e.g.*, 1.7 nM), as measured by surface plasmon resonance. In some embodiments, the

binding to the alpha-chain of the human IL-7 receptor or the alpha-chain of the cynomolgus IL-7 receptor is pH-dependent. In some embodiments, an anti-IL-7R α antibody binds to the alpha-chain of the human IL-7 receptor with a K_D of about 1.3 nM at pH 7.4 and with a K_D of about 5.3 nM at pH 6. In some embodiments, an anti-IL-7R antibody binds to the alpha-chain of the cynomolgus IL-7 receptor with a K_D of about 1.7 nM at pH 7.4 and with a K_D of about 7.0 nM at pH 6.

[0188] In some embodiments, an anti-IL-7R α antibody is formulated for administration to the subject at a flat dose or a body weight-based dose. In some embodiments, an anti-IL-7R α antibody is formulated for administration to the subject at a body weight-based dose (*e.g.*, mg/kg). In some embodiments, an anti-IL-7R α antibody is formulated for administration intravenously, subcutaneously, intramuscularly, intradermally, or intraperitoneally. In some embodiments, an anti-IL-7R α antibody is formulated for administration intravenously (*i.v.*) or subcutaneously (*s.c.*).

[0189] Provided herein are compositions comprising an anti-IL-7R α antibody or antigen-binding portion thereof described herein having the desired degree of purity in a physiologically acceptable carrier, excipient or stabilizer. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG).

[0190] In a specific embodiment, pharmaceutical compositions comprise an antibody or antigen binding portion thereof, a bispecific molecule, or an immunoconjugate described herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In a specific embodiment, pharmaceutical compositions comprise an effective amount of an antibody or antigen-binding portion thereof described

herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In some embodiments, the antibody is the only active ingredient included in the pharmaceutical composition. Pharmaceutical compositions described herein can be useful in modulating (*e.g.*, reducing or inhibiting) IL-7 activity in a T cell (*e.g.*, pathogenic T cell) and treating a disease or disorder, such as an inflammatory disease, *e.g.*, inflammatory bowel disease.

[0191] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In some embodiments, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, immunoconjugate, or bispecific molecule, can be coated in a material to protect the compound from the action of acids and other natural conditions that can inactivate the compound.

[0192] Also provided is a pharmaceutical formulation, which improves the stability of the anti-IL-7R α antibodies and thus, allows for their long-term storage. In some embodiments, the pharmaceutical formulation disclosed herein comprises: (a) an anti-IL-7R antibody; (b) a buffering agent; (c) a stabilizing agent; (d) a salt; (e) a bulking agent; and/or (f) a surfactant. In some embodiments, the pharmaceutical formulation is stable for at least 1 month, at least 2 months, at least 3 months, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 5 years or more. In some embodiments, the formulation is stable when stored at 4°C, 25°C, or 40°C.

[0193] Buffering agents can be a weak acid or base used to maintain the acidity (pH) of a solution near a chosen value after the addition of another acid or base. Suitable buffering agents can maximize the stability of the pharmaceutical formulations by maintaining pH control of the formulation. Suitable buffering agents can also ensure physiological compatibility or optimize solubility. Rheology, viscosity and other properties can also depend on the pH of the formulation. Common buffering agents include, but are not limited to, histidine, citrate, succinate, acetate and phosphate. In some embodiments, a buffering agent comprises histidine (*e.g.*, L-histidine) with isotonicity agents and potentially pH adjustment with an acid or a base known in the art. In some embodiments, the buffering agent is L-histidine. In some embodiments, the pH of the formulation is maintained between about 2 and about 10, or between about 4 and about 8.

[0194] Stabilizing agents are added to a pharmaceutical product in order to stabilize that product. Such agents can stabilize proteins in a number of different ways. Common stabilizing agents include, but are not limited to, amino acids such as glycine, alanine, lysine, arginine, or threonine, carbohydrates such as glucose, sucrose, trehalose, raffinose, or maltose, polyols such as glycerol, mannitol, sorbitol, cyclodextrins or dextrans of any kind and molecular weight, or PEG. In some embodiments, the stabilizing agent is chosen in order to maximize the stability of FIX polypeptide in lyophilized preparations. In some embodiments, the stabilizing agent is sucrose and/or arginine.

[0195] Bulking agents can be added to a pharmaceutical product in order to add volume and mass to the product, thereby facilitating precise metering and handling thereof. Common bulking agents include, but are not limited to, lactose, sucrose, glucose, mannitol, sorbitol, calcium carbonate, or magnesium stearate.

[0196] Surfactants are amphipathic substances with lyophilic and lyophobic groups. A surfactant can be anionic, cationic, zwitterionic, or nonionic. Examples of nonionic surfactants include, but are not limited to, alkyl ethoxylate, nonylphenol ethoxylate, amine ethoxylate, polyethylene oxide, polypropylene oxide, fatty alcohols such as cetyl alcohol or oleyl alcohol, cocamide MEA, cocamide DEA, polysorbates, or dodecyl dimethylamine oxide. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80.

[0197] In some embodiments, the pharmaceutical formulation comprises: (a) about 0.25 mg/mL to 250 mg/mL (*e.g.*, 10 to 200 mg/mL) of an anti-IL-7R α antibody; (b) about 20 mM histidine; (c) about 260 mM sucrose; (d) about 0.5 mM DTPA; and (e) about 0.05% Tween-80.

[0198] In some embodiments, the pharmaceutical formulation comprises about 100 mg/mL of an anti-IL-7R α antibody in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0, with an optional 0.05 mM pentetic acid.

[0199] The formulation can further comprise one or more of a buffer system, a preservative, a tonicity agent, a chelating agent, a stabilizer and/or a surfactant, as well as various combinations thereof. The use of preservatives, isotonic agents, chelating agents, stabilizers and surfactants in pharmaceutical compositions is well-known to the skilled person.

[0200] In some embodiments, the pharmaceutical formulation is an aqueous formulation. Such a formulation is typically a solution or a suspension, but may also include colloids, dispersions, emulsions, and multi-phase materials. The term "aqueous formulation" is defined as a formulation comprising at least 50% w/w water. Likewise, the term "aqueous solution" is

defined as a solution comprising at least 50 % w/w water, and the term "aqueous suspension" is defined as a suspension comprising at least 50 % w/w water.

[0201] In some embodiments, the pharmaceutical formulation is a freeze-dried formulation, to which the physician or the patient adds solvents and/or diluents prior to use.

[0202] Pharmaceutical compositions described herein also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include an IL-7R antibody described herein combined with at least one other therapeutic agent. Examples of therapeutic agents that can be used in combination therapy can include other compounds, drugs, and/or agents used for the treatment of a disease or disorder (*e.g.*, an inflammatory disorder). Such compounds, drugs, and/or agents can include, for example, anti-inflammatory drugs or antibodies that block or reduce the production of inflammatory cytokines. In some embodiments, therapeutic agents can include an anti-IP-10 antibody, an anti-TNF- α antibody (*e.g.*, adalimumab (HUMIRA®), golimumab (SIMPONI®), infliximab (REMICADE®), certolizumab pegol (CIMZIA®)), interferon beta-1a (*e.g.*, AVONEX®, REBIF®), interferon beta-1b (*e.g.*, BETASERON®, EXTAVIA®), glatiramer acetate (*e.g.*, COPAXONE®, GLATOPA®), mitoxantrone (*e.g.*, NOVANTRONE®), non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, corticosteroids, and combinations thereof. In some embodiments, therapeutic agents can include compounds, drugs, and/or agents that can induce the generation of regulatory T cells (*e.g.*, induced regulatory T cells). Non-limiting examples of such therapeutic agents include TGF- β , IL-10, IL-2, and combinations thereof.

[0203] The pharmaceutical compounds described herein can include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects. Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl- substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0204] A pharmaceutical composition described herein can also include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable

antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[0205] Examples of suitable aqueous and nonaqueous carriers that can be employed in the pharmaceutical compositions described herein include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0206] These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms can be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It can also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0207] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions described herein is contemplated. A pharmaceutical composition can comprise a preservative or can be devoid of a preservative. Supplementary active compounds can be incorporated into the compositions.

[0208] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and

suitable mixtures thereof. The proper fluidity can 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. In many cases, the compositions can include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0209] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, some methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0210] The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 percent to about ninety-nine percent of active ingredient, from about 0.1 percent to about 70 percent, or from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

[0211] Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions 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 subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms described herein are dictated by and directly dependent on (a) the unique

characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0212] In some embodiments, the anti-IL-7R α antibody is administered at a flat dose (flat dose regimen).

[0213] In some embodiments, for administration of an anti-IL-7R α antibody, *e.g.*, described herein, the dosage range for each dose is from about 10 mg – about 800 mg per dose, about 20 mg – about 500 mg per dose, about 50 mg – about 300 mg per dose, or about 100 mg – about 250 mg per dose.

[0214] In some embodiments, the anti-IL-7R α antibody is administered to the subject at about 25, 50, 100, 150, 200, 250, 300, or 350 mg per dose.

[0215] In some embodiments, the anti-IL-7R α antibody is administered at a dose based on body weight.

[0216] In some embodiments, for administration of an anti-IL-7R α antibody, *e.g.*, described herein, the dosage ranges from about 0.1 mg/kg – about 10 mg/kg per dose, about 0.2 mg/kg – about 8 mg/kg per dose, about 0.5 mg/kg – about 5 mg/kg per dose, about 1 mg/kg – about 4 mg/kg per dose, or about 2 mg/kg – about 3 mg/kg per dose.

[0217] In some embodiments, the anti-IL-7R α antibody is administered to the subject at about about 0.2 mg/kg per dose, about 0.5 mg/kg per dose, about 1 mg/kg per dose, about 2 mg/kg per dose, about 3 mg/kg per dose, about 4 mg/kg per dose, about 5 mg/kg per dose, about 6 mg/kg per dose, about 7 mg/kg per dose, about 8 mg/kg per dose, about 9 mg/kg per dose, or about 10 mg/kg per dose.

[0218] In some embodiments, the anti-IL-7R α antibody is administered at a fixed dose with another antibody.

[0219] Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, bi-weekly (*i.e.*, once every two weeks), monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient.

[0220] An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months.

[0221] In some embodiments, the anti-IL-7R α antibody, *e.g.*, those described herein, is administered once a week.

[0222] In some embodiments, the anti-IL-7R α antibody, *e.g.*, those described herein, is administered once every two weeks.

[0223] In some embodiments, the treatment comprises 10-15 (*e.g.*, 11, 12, or 13) consecutive doses, administered once every two weeks. For example, the treatment may comprise 10, 11, 12, 13, 14, or 15 consecutive doses, with each dose administered every two weeks apart.

[0224] In some embodiments, the antibody is administered for a period of up to 10 weeks, 13 weeks, 26 weeks, 52 weeks, 2 years, 3 years, 5 years, 10 years, or lifetime / permanently. In some embodiments, the antibody is administered as needed when there is hair loss.

[0225] In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

[0226] In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 0.1 – about 10 $\mu\text{g/mL}$, about 0.2 – about 9 $\mu\text{g/mL}$, about 0.5 – about 8 $\mu\text{g/mL}$, about 1 – about 7 $\mu\text{g/mL}$, about 2 – about 6 $\mu\text{g/mL}$, about 4 – about 5 $\mu\text{g/mL}$.

[0227] In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 2 $\mu\text{g/mL}$, about 3 $\mu\text{g/mL}$, about 4 $\mu\text{g/mL}$, about 5 $\mu\text{g/mL}$, about 6 $\mu\text{g/mL}$, about 7 $\mu\text{g/mL}$, or about 8 $\mu\text{g/mL}$.

[0228] An antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a maintenance regime.

[0229] Actual dosage levels of the active ingredients in the pharmaceutical compositions described herein can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will

depend upon a variety of pharmacokinetic factors including the activity of the particular compositions described herein employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, 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.

[0230] A composition described herein can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Routes of administration for the anti-IL-7R antibodies described herein can include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion.

[0231] Alternatively, an antibody described herein could potentially be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[0232] The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and poly lactic acid. Many methods for the preparation of such formulations are generally known to those skilled in the art.

[0233] Therapeutic compositions can be administered with medical devices known in the art. For example, in a particular embodiment, a therapeutic composition described herein can be administered with a needleless hypodermic injection device. Examples of well-known implants and modules for use with anti-IL-7R antibodies described herein include an implantable micro-infusion pump for dispensing medication at a controlled rate; a therapeutic device for administering medicaments through the skin; a medication infusion pump for

delivering medication at a precise infusion rate; a variable flow implantable infusion apparatus for continuous drug delivery; an osmotic drug delivery system having multi-chamber compartments; and an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art.

Additional Treatable Disease Indications

[0234] Although the examples relate to the treatment of alopecia areata (AA), the invention described herein is not so limited as to the hair loss disorders treatable by the compounds and compositions described herein. Non-limiting examples of other hair loss disorders treatable according to the methods described herein: androgenetic alopecia, Alopecia areata, telogen effluvium, alopecia areata, tinea capitis, alopecia totalis, hypotrichosis, hereditary hypotrichosis simplex, and alopecia universalis.

Combination Therapy

[0235] In some embodiments, a second / additional therapeutic agent effective for treating the hair-loss disorder is administered to the subject.

[0236] In some embodiments, the second / additional therapeutic agent comprises a Corticosteroid. The corticosteroid may be formulated as, for example, a topical formulation or a local injection formulation (*e.g.*, injection into the bald spot / diseases skin area).

[0237] In some embodiments, the second / additional therapeutic agent comprises Minoxidil (marketed by the brand name ROGAINE®).

[0238] In some embodiments, the second / additional therapeutic agent comprises anthralin.

[0239] In some embodiments, the second / additional therapeutic agent comprises Methotrexate.

[0240] In some embodiments, the hair loss comprises eyelash loss, and the second / additional therapeutic agent comprises Bimatoprost (or a similar medication).

[0241] In some embodiments, the second / additional therapeutic agent comprises an inhibitor of a protein tyrosine kinase (PTK) involved in cytokine signaling. In some embodiments, the inhibitor is a Jak/Stat inhibitor. In a further embodiment, the inhibitor is INCB 018424. In some embodiments, the Jak3 inhibitor is an antibody that specifically binds to a Jak3 protein or a fragment thereof; an antisense RNA or antisense DNA that decreases expression of the gene that encodes the Jak3 protein; an antisense RNA or antisense DNA that decreases expression of the Jak3 protein; a siRNA that specifically targets the Jak3 gene;

a small molecule; or a combination thereof. In some embodiments, the inhibitor is a Jak3 inhibitor. In a further embodiment, the inhibitor is tofacitinib (CP690550). In a further embodiment, the small molecule is Janex 1 (WHI-P131), PF-956980, WHI-P154, VX-509, JAK3 Inhibitor IV, NSC114792, or R348. In some embodiments, the Stat inhibitor is a Stat 1 inhibitor, such as an antibody or antibody fragment that is directed to Stat 1. In some embodiments, the Stat inhibitor is a Stat 2 inhibitor, such as an antibody or antibody fragment that is directed to Stat2.

[0242] Non-limiting examples of Jak1/Jak2 inhibitors include: baricitinib, tofacitinib, ruxolitinib, baricitinib, upadacitinib, abrocitinib, pacritinib, fedratinib, AG490; CYT387; SB1518; LY3009104 (INCB28050); TG101348; and BMS-911543.

[0243] JAK1/2 inhibitors in clinical development, which can also be the second therapeutic agent include a) INCB018424, topical and oral; 5 nM activity (Incyte); b) CEP-701 (Cephalon); and c) TG101348.

[0244] In some embodiments, the second / additional therapeutic agent comprises a JAK3 inhibitor. Non-limiting examples of Jak3 inhibitors include: Janex 1 (WHI-P131), PF-956980, WHI-P154, VX-509, JAK3 Inhibitor IV (ZM-39923), NSC114792, tofacitinib (CP690550), and R348.

[0245] Non-limiting JAK3 inhibitors include a) Janex 1, oral and topical; b) PF-956980, i.v. infusion; c) WHI-P154; d) ZM-39923; e) NSC114792; f) tofacitinib (CP690550), oral.

[0246] Other non-limiting examples of JAK inhibitors useful as the second therapeutics include, for example, Type I and Type II Jak Inhibitors.

[0247] Non-limiting examples of Stat inhibitors that can be used as second therapeutics include: WP-1034, fludarabine, epigallocatechin-3-gallate (EGCG), and Hyperforin.

[0248] Additional second therapeutics that can be used in the methods described herein include any and all RTK inhibitors such as JAK/STAT inhibitors.

EXAMPLES

Example 1 A Phase 2a, Randomized, Double-blind, Placebo-Controlled, Trial of COMPOUND A for the Treatment of Severe Alopecia Areata

[0249] COMPOUND A (the antibody having the heavy chain amino acid sequence of SEQ ID NO: 23 and the light chain amino acid sequence of SEQ ID NO: 24) is a fully human, high affinity anti-interleukin-7 receptor α (IL-7R α) antagonist monoclonal antibody (mAb) developed from human immunoglobulin transgenic mice. It exhibits high affinity as

an anti-interleukin-7 receptor α (IL-7R α) and reduced affinity to Fc γ R (Type I, II, or III), the receptors that mediate effector functions, and to complement component 1 (C1q), the mediator of complement cell lysis. COMPOUND A is demonstrated here as an effective treatment for alopecia areata.

[0250] Alopecia areata is an autoimmune condition that affects hair follicles and leads to hair loss. This condition may develop at any age and in both genders, and the incidence of this disease has been estimated to be 2% of the population worldwide. Some patients recover spontaneously, while approximately 25% of patients progress to alopecia totalis (total scalp hair loss) or alopecia universalis (total body hair loss). Although the pathophysiology has not been fully delineated, development of the condition is mediated by inflammatory mechanisms.

[0251] Onset of hair loss is thought to originate from genetic (*i.e.*, inherited predisposition) and environmental factors (*e.g.*, discordance between identical twins; toxic agent mediating AA cases). The cascade of inflammatory events involves the recruitment of autoreactive CD8+ T cells in the periphery of the hair follicles of AA skin lesions. These CD8+ T cells get activated locally by the protein MICA binding on surface NKG2D and presented by ectopic MHC I molecules in the immune privileged-hair follicle. Cytotoxic T cell-related and IFN γ -related gene expression signatures were identified in lesions compared to normal individual skin reflected local T cell activation, that will subsequently induce the recruitment of other immune cells types (CD4+ Th1 cells, macrophages, NK cells and eosinophils) leading to the collapse of hair follicle immune privilege.

[0252] Nonclinical pharmacology studies confirm IL-7R α receptor occupancy (RO) and inhibition of IL-7R α -mediated pharmacodynamic (PD) responses and T-cell mediated biology. Both interleukin (IL)-7 and thymic stromal lymphopoietin (TSLP) receptors are heterodimeric, and share the common IL-7R α subunit.

[0253] In a Phase 1 study in healthy participants, COMPOUND A single doses up to 4 mg/kg and repeat doses of 1 mg/kg every two weeks were safe and well tolerated. Results from that Phase 1 study demonstrated that doses of COMPOUND A that achieved $\geq 95\%$ receptor occupancy were associated with $>90\%$ inhibition of IL-7 signaling via phosphorylation of signal transducer and activator of transcription-5 (STAT5). Repeat administration of COMPOUND A at 1 mg/kg demonstrated durable inhibition of IL-7R α after the 2nd dose over the 2-week dose regimen. In addition, the lymphocyte subsets and T cell immune function assessments exhibited modest, dose-dependent effects on these biomarkers. Based on the available nonclinical and Phase 1 safety data, no expected adverse

events have been identified. Thus, the Phase 1 study has shown that COMPOUND A have acceptable tolerability and safety, has confirmed the expected pharmacology, and has allowed for the generation of a predictive pharmacokinetic (PK)-PD model, supporting initiation of the present Phase 2a study.

[0254] Mechanistically, COMPOUND A is a high affinity effector-minimized anti-IL-7R α antagonist antibody that blocks the IL-7 cytokine binding to human IL-7R α (CD127) and inhibits IL-7 receptor (IL-7R) (CD127/CD132) mediated intracellular signaling pathways.

[0255] This example demonstrates that the anti-IL7Ra antibody COMPOUND A is effective to reduce hair loss in patients with severe AA, at least by Week 22 and/or 24 after commencement of treatment based on the dosing regimen described herein. The primary objective of the trial is to show the effect of COMPOUND A vs placebo on reducing hair loss at Week 24 after the commencement of the treatment, in participants with AA. This primary objective is assessed based on the primary endpoint of mean relative percent change in Severity of Alopecia Tool (SALT) Score at 24 weeks compared with Baseline.

[0256] The primary efficacy endpoint of proportion of participants with the relative change in SALT score compared to Baseline at Week 24 is an established measure of severity of alopecia and is clinically relevant for measuring the efficacy of COMPOUND A in this patient population.

[0257] A secondary objective of the trial is to show the effect of COMPOUND A vs placebo on reducing hair loss through 24 weeks of treatment in participants with AA. This secondary objective is assessed through a analyzing number of secondary endpoints, including:

- Mean relative percent change in SALT Score compared with Baseline at Weeks 12 and 18.
- Mean relative percent change in SALT Score at 24 weeks compared to treatment nadir.
- Proportion of participants who achieve $\geq 30\%$ and $\geq 50\%$ relative reduction in SALT score compared with Baseline at Weeks 12, 18, and 24.
- Proportion of participants with absolute SALT score $\leq 5, 10, 20, 30$, and 50 at Weeks 12, 18, and 24.

[0258] Patients receiving treatment (*e.g.*, baseline COMPOUND A group) have statistically significant improvement over matched control (*e.g.*, patients receiving placebo) in mean relative percent change in SALT Score at Week 12, 18, 22, and/or 24.

[0259] Further, more than 50% of the patients receiving treatment have statistically significant improvement over matched control (*e.g.*, patients receiving placebo) in mean relative percent change in SALT Score at Week 12, 18, 22, or 24.

[0260] Further, a significant proportion of participants have absolute SALT score of ≤ 5 , 10, 20, 30, or 50, at Week 12, 18, 22, and/or 24.

[0261] The treatment also has post-treatment duration effect on reducing hair loss in participants with AA, in that a significant percentage of patients receiving treatment (1) maintain relative percent change from baseline in SALT score at Week 36 compared with Baseline and Week 24, (2) achieve $\geq 50\%$ relative reduction in SALT score from baseline, at Week 36 compared with Baseline and Week 24, and/or (3) proportion of participants achieving absolute SALT score ≤ 5 , 10, 20, 30, and 50, at Week 36.

[0262] Pharmacokinetics (PK) and immunogenicity of COMPOUND A in treated patients are also characterized to be desirable, based on assays including serum COMPOUND A concentrations based on Schedule of Assessments (SoA), and anti-drug antibody (ADA) rate.

[0263] Finally, the safety and tolerability of COMPOUND A are evaluated, including acceptable numbers or percentages of adverse events (AEs), serious adverse events (SAEs), and adverse events of special interest (AESIs) including injection site tolerability, in view of laboratory evaluations, physical examinations, vital signs, and 12-lead electrocardiogram (ECG).

[0264] The trial described herein also includes several exploratory objectives.

[0265] One of the exploratory objectives includes evaluating the effect of COMPOUND A vs placebo on reducing eyelash and eyebrow loss in participants with AA. Such exploratory objectives are evaluated by assessing one or more of the following exploratory endpoints:

- Change from Baseline in Clinician Reported Outcome (ClinRO) for Eyebrow Hair Loss at Weeks 12, 18, 24, and 36;
- Change from Baseline in ClinRO for Eyelash Hair Loss at Weeks 12, 18, 24, and 36;
- Proportion of participants achieving ClinRO for Eyebrow Hair Loss scores of 0 or

1 (full coverage or minimal gaps) with a ≥ 2 -point improvement from Baseline at 24 and 36 weeks (among those with Baseline scores ≥ 2 [significant gaps to no notable hair]);

- Proportion of participants achieving ClinRO for Eyelash Hair Loss scores of 0 or 1 (full coverage or minimal gaps) with a ≥ 2 -point improvement from Baseline at 24 and 36 weeks (among those with Baseline scores ≥ 2 [significant gaps to no notable hair]).

[0266] Another exploratory objective includes evaluating the impact on patient QOL (Quality of Life), which can be assessed through measuring improvements in SKINDEX-16 AA compared with Baseline at Weeks 12, 18, 24, and 36.

[0267] Yet another exploratory objectives includes investigating COMPOUND A PK, immunogenicity, receptor occupancy (RO), and pharmacodynamics (PD) in subjects with AA. These investigations are facilitated by evaluating:

- Serum PK profile of SC-administered COMPOUND A, as assessed by population PK analysis using nonlinear mixed-effects modeling
- Incidence and titer of serum anti-COMPOUND A antibodies and associated effects on PK, PD, efficacy, and safety
- Changes in whole blood IL-7 receptor (IL-7R)- α RO on circulating CD3⁺ T cells
- Changes in T cell subsets
- Serum, plasma, peripheral blood mononuclear cells (PBMC), and whole blood RNA and DNA (DNA is optional) will be collected and stored for potential future assessment of biomarkers related to COMPOUND A treatment responses and/or AA disease biomarkers
- Any relationships between PK, RO, PD, and AE profiles, exploratory biomarkers, clinical laboratory data, and clinical activity of COMPOUND A may be explored.

Trial Design:

[0268] The clinical study was designed as a Phase 2a, multicenter, placebo controlled, proof-of-concept trial to assess the preliminary efficacy, safety, tolerability, PK, and PD of COMPOUND A in participants with severe-to-very severe AA, with $\geq 50\%$ total scalp hair loss as defined by the SALT score at Day 1. Approximately 40 participants with alopecia

areata (AA) are enrolled. A participant is considered to have completed the study if the participant has completed all periods of the study including the last visit.

[0269] Participants will be between 18 and 75 years of age (inclusive), with a current episode of severe hair loss of >6 months and <10 years, along with investigator's assessment that hair regrowth is possible, no evidence of regrowth present at baseline, no known history of significant regrowth during the last 3 months, and no more than 10% regrowth over the past 6 months. Approximately 40 participants will be enrolled and randomly assigned (3:1) to receive 200 mg COMPOUND A or matching placebo administered subcutaneously every 2 weeks for 24 weeks (*i.e.*, SC Q2W for 24 weeks, for a total of 13 doses). Randomization is stratified based on SALT score at Day 1 (≥ 50 to <95 , ≥ 95 to 100). Enrollment of participants in the ≥ 95 to 100 SALT score stratum is limited to approximately $n=14$. See FIG. 1.

[0270] The trial will comprise a Screening period of up to 30 days with enrollment on Day 1 (baseline). Participants are treated with COMPOUND A or placebo starting on Day 1 (Baseline) and every 2 weeks (Q2W) through Week 24. Based on prior studies, trough exposures at Week 24 remains in the projected therapeutic range. The primary endpoint assessment occurs at the Week 24 visit. All participants will have a follow-up period of 12 weeks after the last administered dose (through Week 36) to evaluate long-term safety and to observe the duration of efficacy after the treatment period.

[0271] Efficacy is evaluated by assessment of SALT scores, ClinRO Measure for Eyebrow Hair Loss™, and ClinRO Measure for Eyelash Hair Loss™, PRO measure for eyebrow hair loss, PRO measure for eyelash hair loss, and SKINDEX16-AA.

[0272] Blood samples are collected from all participants to characterize the PK, PD, and immunogenicity of COMPOUND A. Safety is assessed by collecting AEs, SAEs, AESIs, performing local injection site tolerability assessment, recording vital signs, performing physical examinations and ECGs, and evaluating clinical laboratory results.

[0273] Thus, the overall duration of the trial is approximately 40 weeks, including up to 30 days for the Screening period, 24 weeks for the treatment period where participants receive COMPOUND A or matching placebo administered subcutaneously every 2 weeks, and approximately 12 weeks for the follow-up period, though the treatment period is only about 24 weeks.

Dose and Frequency (Dosing Regimen)

[0274] Participants are administered either COMPOUND A or matching placebo.

[0275] COMPOUND A is formulated as 100 mg/mL drug product solution for *s.c.* injection, provided in aseptic filled, sterile, 2R borosilicate glass vials with a Flurotec serum stopper and a matte cap flip-off seal. Each vial contains an extractable volume of 2 mL COMPOUND A. That is, each dose of COMPOUND A contains 200 mg of COMPOUND A antibody, which is administered as a flat dose once every two weeks according to the Schedule of Activities (SoA) detailed below (also see FIG. 1), for a total of 13 doses. This dosing regimen (*i.e.*, 200 mg of COMPOUND A administered *s.c.* Q2W) is expected to result in steady-state serum trough concentrations >5 $\mu\text{g/mL}$, and maximum RO on circulating T cells in $>90\%$ of subjects throughout dosing. Additionally, exposures after 200 mg are predicted to fall within the range of previously tested exposures at 3 mg/kg, and is expected to be safe and well tolerated.

[0276] Preliminary PK and RO data supports this assessment. Predicted median maximum concentration (C_{max}) at steady state (29.5 $\mu\text{g/mL}$) and AUC_{ss} (8531 $\mu\text{g}\cdot\text{h/mL}$) in patients with AA after a dose of 200 mg SC Q2W are >100 -fold and >50 -fold lower, respectively, than exposures at the NOAEL of 150 mg/kg/week SC COMPOUND A in cynomolgus monkeys in the 6-month GLP study (C_{max} of 3260 $\mu\text{g/mL}$ and AUC of 466,000 $\mu\text{g}\cdot\text{h/mL}$ for non-sexually mature animals, and C_{max} of 3780 $\mu\text{g/mL}$ and AUC of 540,000 $\mu\text{g}\cdot\text{h/mL}$ for sexually mature animals).

[0277] The placebo is identically formulated with volume matched to active dose, and is identical in appearance to COMPOUND A. The formulations are stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with limited access to the authorized personnel until use.

[0278] TEAEs experienced by subjects, standard safety laboratory data and systematic assessment of ADAs are routinely monitored to ensure subject safety and to identify the occurrence of events that meet the criteria for IP discontinuation (see below).

Stopping Criteria

Event	Criteria for IP Discontinuation in Any Subject
Any TEAE	Drug-related TEAE that is Grade ≥ 3
Immunological TEAE	Grade ≥ 3 or serious immunological event of any severity

Lymphocyte counts	Absolute lymphocyte count $\leq 500/\mu\text{L}$ sustained for 2 weeks on repeat testing
Viral reactivation or infection	$\geq$ Grade 3 viral reactivation or serious infection

[0279] The Sponsor conducts blinded periodic safety reviews as the study progresses described in a Safety Monitoring Plan.

Patient Inclusion Criteria

[0280] Participants meeting all of the following criteria are eligible for trial participation at the screening and/or Day 1 visits, as noted in the criterion:

1. Adult men and women, aged 18 to 75 years, inclusive, at the time of informed consent.
2. Clinical diagnosis of severe to very severe AA, defined as the presence of $\geq 50\%$ total scalp hair loss at screening and baseline (Day 1) as measured by the SALT score.
3. Duration of current episode of hair loss > 6 months and < 10 years, with no spontaneous improvement over the past 6 months, along with investigator's assessment that hair loss has been stable for at least 3 months and regrowth is possible, at the screening visit.
4. No evidence of active regrowth present at baseline and no known history of significant regrowth, as per investigator's judgement, over the last 3 months.
5. Willing to keep the same hair style, including length and color (*e.g.*, hair products, process, and timing for hair appointments), at the direction of the investigator, for the duration of the trial.

Patient Exclusion Criteria:

[0281] Participants are ineligible for trial participation if there is any of the following criteria at the screening and/or Day 1 visits, as applicable:

1. Body weight < 48 kg or > 120 kg at screening.
2. Active forms of other inflammatory skin disease(s) or evidence of other skin conditions (*e.g.*, psoriasis, seborrheic dermatitis, lupus) at the time of screening and through Day 1 that, in the opinion of the investigator, may interfere with evaluation of AA and the assessment of the disease activity measures.
3. History of or diagnosis at screening of another form of alopecia based on assessment by investigator (except for androgenic alopecia).

4. History of male or female pattern hair loss of Hamilton stage >III or Ludwig stage >II.
5. History (lifetime) or presence of hair transplants.
6. History (lifetime) or presence of micropigmentation of the scalp (Note: Microblading of the eyebrows is permitted).
7. Systemic, topical, or device-based therapy for AA, or immunotherapy required for any other condition, except as noted:

- a. Use of steroids (systemic and intralesional), anthralin, squaric acid, diphenylcyclopropenone, dinitrochlorobenzene, tacrolimus, minoxidil, or any other medication that, in the opinion of the investigator, may affect hair regrowth within 4 weeks of the Day 1 visit.

Note: Intranasal and inhaled corticosteroids are allowed; eye and ear drops containing corticosteroids are also allowed.

- b. Use of platelet-rich plasma injections in the 12 weeks before Day 1.
- c. Use of topical medicated treatment that could affect AA including, but not limited to, topical corticosteroids, minoxidil, calcineurin inhibitors, antimicrobials, and medical devices within 2 weeks before the Day 1 visit.

Note: Topical corticosteroids are permitted outside of the scalp, eyebrows, and eyelids.

- d. Previous treatment with any biologic B-cell–depleting therapy (*e.g.*, rituximab, ocrelizumab, or ofatumumab) or other B-cell–targeting therapy (*e.g.*, belimumab) within 12 months before Day 1.
- e. History (lifetime) of plasmacytoid dendritic cell–inhibiting therapies (*e.g.*, anti-ILT7 [immunoglobulin-like transcript 7], anti-BDCA2 [blood dendritic cell antigen 2]).
- f. Use of any conventional disease-modifying antirheumatic drugs, immunosuppressants (*e.g.*, cyclosporine, methotrexate, or azathioprine), or JAK inhibitors (*e.g.*, baricitinib), if last dose was taken: (i) within 8 weeks before Day 1, or (ii) within the drug-specific 5 half-lives elimination period (if longer than 8 weeks).
- g. Received any marketed or investigational biological agent within 12 weeks or 5 half-lives (whichever is longer) before Day 1.

- h. Currently receiving a nonbiologic IP or device or has received one within 4 weeks before Day 1 or within 5 published half-lives, whichever is longer.
 - i. Received any ultraviolet (UV)-B phototherapy (including tanning beds), has had psoralen-UV-A treatment, or excimer laser within 4 weeks before Day 1.
 - j. Allergen immunotherapy started within 4 weeks before randomization; patients on a stable allergy immunotherapy regimen may be eligible provided the therapy is continued unchanged throughout the study duration.
8. History of, recent, or current clinically serious viral, bacterial, fungal, or parasitic infection or mycobacterial infection or at risk of serious infection:
- a. History of organ or bone marrow transplant.
 - b. History of a primary immunodeficiency disorder.
 - c. History of infected joint prosthesis or other implanted device remaining in place.
 - d. History of opportunistic infection, eg, pneumonia.
 - e. History of disseminated *Staphylococcus aureus* or eczema herpeticum infection.
9. Recent (within 2 months of informed consent except as noted) or current clinically serious viral, bacterial, fungal, or parasitic infection or mycobacterial infection including but not limited to:
- a. Symptomatic herpes zoster infection within 12 weeks before screening or any history of disseminated/complicated herpes zoster (*e.g.*, multidermatomal involvement, ophthalmic zoster, central nervous system involvement, postherpetic neuralgia).
 - b. Active herpes simplex virus type 1 or 2 at the time of screening or during the screening period up to Day 1. This includes individuals who have clinically active disease and are asymptomatic on suppressive therapy.
 - c. Positive serology for HIV-1 or HIV-2 at screening.
 - d. Evidence of active hepatitis A virus (HAV). Participants with a positive HAV total antibody should have HAV immunoglobulin (Ig)M reflexively performed. Participants with positive HAV IgM are excluded.

- d. Evidence of active hepatitis A virus (HAV). Participants with a positive HAV total antibody should have HAV immunoglobulin (Ig)M reflexively performed. Participants with positive HAV IgM are excluded. If HAV IgM is not available at the time of randomization, randomization may proceed if there is a reliable history of vaccination to HAV or the participant has no clinical or laboratory evidence of HAV infection including normal liver function tests.
 - e. Evidence of active hepatitis C virus (HCV) infection. Participants with a positive HCV antibody require reflexive HCV RNA testing. Participants with positive HCV RNA are excluded. Participants previously treated for HCV, with positive HCV antibody but negative HCV RNA, may be enrolled with ongoing monitoring to ensure that they remain negative.
 - f. Evidence of active hepatitis B virus (HBV) infection. Participants with positive hepatitis B surface antigen (HBsAg) are excluded. Participants with positive hepatitis B core antibody and negative HBsAg require reflexive testing for HBV DNA. Participants with positive HBV DNA are excluded.
 - g. Evidence of active cytomegalovirus (CMV) or Epstein-Barr virus infection by polymerase chain reaction. PCR must be available and negative prior to enrollment only if clinical CMV/EBV infection is suspected by the investigator.
 - h. On any therapy for chronic infection, eg, CMV, herpes zoster, atypical mycobacterium.
10. A positive QuantiFERON-TB Gold test at screening or history of tuberculosis (TB), unless the participant has a negative chest x-ray and has been treated with 9 months of isoniazid or appropriate therapeutic regimen per World Health Organization or national guidelines. It is the responsibility of the investigator to verify medical records and document the adequacy of previous anti-TB treatment. The QuantiFERON-TB Gold test may be repeated one time for participants with an indeterminate test, and participants with 2 indeterminate tests may be enrolled after consultation with the medical monitor and sponsor.
11. Exposed to a live vaccine within 12 weeks before planned randomization or expected to receive a live vaccine during the study.
12. Received a vaccine for COVID-19 (*e.g.*, RNA-based vaccines, protein-based vaccines, and nonreplicating viral vector-based vaccines) within 4 weeks before Day 1.

13. Screening laboratory results at the central laboratory that meet any of the following criteria. These may be repeated once before declaring the participant a screen failure. In addition, no participant with a clinically significant laboratory test value based on the investigator's judgement, or with a laboratory abnormality value greater than Grade 1 per the modified National Cancer Institute Common Terminology Criteria for Adverse Events v5.0, may be enrolled.

- a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) $>1.5\times$ upper limit of normal (ULN).
- b. Bilirubin $\geq 1.5\times$ ULN (isolated bilirubin $\geq 1.5\times$ ULN is acceptable if bilirubin is fractionated and direct bilirubin $<35\%$).
- c. Hemoglobin <10 g/dL.
- d. White blood cell count $<3.0\times 10^9/L$.
- e. Platelet count $<150\times 10^9/L$.
- f. Absolute lymphocyte count $<LLN$.

14. Evidence of poorly controlled metabolic disease per investigator's discretion, including Type 2 diabetes as evidenced by hemoglobin A1c >8 , independent of treatment, or associated nonalcoholic steatohepatitis (NASH)/nonalcoholic fatty liver disease (NAFLD) based on previous histologic assessment or noninvasive assessments including transient elastography, magnetic resonance imaging proton density fat fractionation, Fibrosis-4 index, AST/ALT ratio, or AST/platelet ratio.

15. Heart rate-corrected QT interval (Fridericia method) >470 milliseconds (ms) for women and >450 ms for men at screening.

16. Other screening laboratory values outside the reference range of the central laboratory for the population or investigative study site that, in the opinion of the investigator, pose an unacceptable risk for the individual's participation in the study.

17. History or the presence of other cardiovascular, respiratory, hepatic, gastrointestinal, endocrine, hematologic, immunologic, neurologic, or psychiatric disorder, or any other serious and/or uncontrolled disease that, in the opinion of the investigator, could constitute a risk when taking IP or could interfere with the interpretation of data.

18. Current or history of renal disease or individual estimated glomerular filtration rate calculated by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation of <60 mL/min at screening.

19. Serious concomitant illness that could require the use of systemic corticosteroids or otherwise interfere with study participation or require active frequent monitoring.

20. Any history of malignancy except for non-melanomatous skin cancer that, in the opinion of the investigator, has been appropriately treated without recurrence; or cervical carcinoma *in situ* that has been resected.

21. Other active autoimmune disease other than those above that would make it difficult to appropriately assess AA disease activity or would pose a risk to the individual's participation in the trial.

Efficacy Analyses

SALT Assessment

[0282] To participate in this study, a subject must have a SALT score of ≥ 50 at screening and baseline (Day 1).

[0283] The SALT score is a well-validated metric and widely used tool for determining the degree of hair loss based on the percentage of scalp surface area involved on the top, back, and each side of the scalp for AA. The investigator will determine the percentage of scalp hair loss in a given quadrant and record the finding in the Canfield software program. The software program will multiply this value by the total scalp area and sum the resultant numbers for each quadrant to give the total percentage of scalp hair loss, with a maximum possible score of 100. See Olson *et al.* (*J Am Acad Dermatol.* 2004;51(3):440-447. doi: 10.1016/j.jaad.2003.09.032, incorporated herein by reference). The SALT assessments are scheduled at Weeks 0 (first injection at Day 1), 6, 12, 18, 24 (end of treatment (EOT), the 13th and the last injection), 26 (first post treatment follow-up visit), 30, and Week 36 (end of study or EOS). The SALT assessment tool is provided in FIG. 2, according to the criteria below:

Left Quadrant Raw Score (LRS)	Top Quadrant Raw Score (TRS)
Right Quadrant Raw Score (RRS)	Back Quadrant Raw Score (BRS)
Total SALT Score $[(LRS \times 0.18) + (RRS \times 0.18) + (TRS \times 0.40) + (BRS \times 0.24)]$	

Eyelash /Eyebrow Assessment (ClinRO)

[0284] The ClinRO Measure for Eyebrow Hair Loss and the ClinRO Measure for Eyelash Hair Loss are to be assessed by the investigator and comprise single-item, 4-point, Likert-type response scales of 0 to 3 to assess incremental severity of each ClinRO measure.

Responses range from 0 = normal appearance/no hair loss to 3 = severe appearance/severe hair loss. See Wyrwich *et al.* (*Am J Clin Dermatol.* 2020; 21(5):725-732. doi: 10.1007/s40257-020-00545-9, incorporated herein by reference). The ClinROs assessments are scheduled at Weeks 0 (first injection at Day 1), 6, 12, 18, 24 (end of treatment (EOT), the 13th and the last injection), 26 (first post treatment follow-up visit), 30, and Week 36 (end of study or EOS). The ClinROs are summarized below.

ClinRO AND PRO FOR EYEBROW HAIR LOSS AND EYELASH HAIR LOSS

ClinRO Measure for Eyebrow Hair Loss™ Examine both eyebrows from two feet away and select the best response below. ☐ The eyebrows have full coverage and no areas of hair loss ☐ There are minimal gaps in eyebrow hair and distribution is even ☐ There are significant gaps in eyebrow hair or distribution is not even ☐ No notable eyebrow hair	PRO Measure for Eyebrows™ Look at the hair in both of your eyebrows. Please rate your eyebrows, as they look today. This question asks about gap(s) in your eyebrows or thinning in your eyebrows. If you have gap(s) in your eyebrows and thinning in your eyebrows, please choose your answer based on the type of hair loss that bothers you the most. Please select one answer. ☐ I have full eyebrows on each eye ☐ I have a minimal gap(s) or a minimal amount of thinning in at least one of my eyebrows ☐ I have a large gap(s) or a large amount of thinning in at least one of my eyebrows ☐ I have no or barely any eyebrow hairs
ClinRO Measure for Eyelash Hair Loss™ Examine the upper and lower eyelashes of both eyes and select the best response option below. ☐ The eyelashes form a continuous line along the eyelid on both eyes ☐ There are minimal gaps and the eyelashes are evenly spaced along the eyelids on both eyes ☐ There are significant gaps along the eyelids or the eyelashes are not evenly spaced along the eyelids ☐ No notable eyelashes	PRO Measure for Eyelashes™ Look at your upper and lower eyelashes on both your eyes. Please rate your eyelashes, as they look today. Please select one answer. ☐ I have full eyelashes on each eyelid ☐ I have a minimal gap or minimal gaps along the eyelids ☐ I have a large gap or large gaps along the eyelids ☐ I have no or barely any eyelash hair

The ClinRO Measure for Eyebrow Hair Loss™, ClinRO Measure of Eyelash Hair Loss™, PRO Measure for Eyebrows™, PRO Measure for Eyelashes™ and accompanying photo-guides (ESM 10) are subject to copyright owned by Eli Lilly and Company. Permission to use is granted under Creative Commons attribution-No derivatives 4.0 International License. Contact Eli Lilly and Company in the event of any proposed modification

ClinRO clinician-reported outcome, PRO patient-reported outcome

Eyelash/ Eyebrow Assessment (PRO)

[0285] The PRO Measure for Eyebrows and PRO Measure for Eyelashes were developed to provide valid and clinically meaningful measures that reflect patients’ perspectives of AA. These measures have severity gradations ranging from 0 to 3, where 0 corresponds to full eyebrows / eyelashes, and 3 corresponds to no or barely any eyebrow / eyelash hairs. See

Wyrwich *et al.* (*Am J Clin Dermatol.* 2020; 21(5):725-732. doi: 10.1007/s40257-020-00545-9, incorporated herein by reference). The PRO Measure for Eyebrows and PRO Measure for Eyelashes are scheduled at Weeks 0 (first injection at Day 1), 6, 12, 18, 24 (end of treatment (EOT), the 13th and the last injection), 26 (first post treatment follow-up visit), 30, and Week 36 (end of study or EOS). The PRO Measure for Eyebrows and PRO Measure for Eyelashes assessment questionnaires are provided in the table above.

SkinDex 16-AA (psychosocial measure)

[0286] The Skindex-16 is a PRO instrument designed to assess how bothersome the effects of skin disease are in 3 areas of patient quality of life: symptoms, emotions, and functioning. The Skindex-16 is general in nature and can be used for patients regardless of specific skin disease. The Skindex-16 for AA was adapted for use among adults with AA. Using a Likert scale of 0 (Never) to 6 (Always), the patient rates how often they have been bothered by their condition during the past week. The Skindex-16 for AA provides a single score for each area (symptoms, emotions, and functioning) as well as a total score overall. The Skindex-16 for AA are scheduled at Weeks 0 (first injection at Day 1), 6, 12, 18, 24 (end of treatment (EOT), the 13th and the last injection), 26 (first post treatment follow-up visit), 30, and Week 36 (end of study or EOS). The Skindex-16 for AA is provided in FIG. 3.

Time Period and Frequency for Collecting AE and SAE Information

[0287] An AE is defined as any untoward medical occurrence in a clinical study participant, temporally associated with the use of IP, whether or not considered related to the IP. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of IP.

[0288] Events meeting the AE definition include:

- Conditions newly detected or diagnosed after the signing of the ICF, including conditions that may have been present but undetected prior to the start of the study
- Conditions known to have been present prior to the start of the study that worsen after the signing of the ICF
- Signs, symptoms, or the clinical sequelae of a suspected drug interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either IP or a concomitant medication (overdose per se should not be reported as an AE)

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from Baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease, or more severe than expected for the participant's condition)

- Lack of efficacy or failure of expected pharmacological action per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events not meeting the AE definition include:

- Any abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Medical occurrences that begin before the start of IP but after obtaining informed consent will be recorded as medical history/current medical conditions, not as AEs.

An SAE is defined as any untoward medical occurrence that, at any dose, meets one or more of the criteria listed:

- Results in death
- Is life threatening

[0289] The term life threatening in the definition of serious refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization
 - ☐ In general, hospitalization signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, consult the Sponsor's Medical Monitor.
 - ☐ Hospitalization for elective treatment of a pre-existing condition that did not worsen from Baseline is not considered an AE.
- Results in persistent or significant disability/incapacity
 - ☐ The term disability means a substantial disruption of a person's ability to conduct normal life functions.
 - ☐ This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect
- Other situations:
 - ☐ Medical or scientific judgment should be exercised by the investigator in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
 - o Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, convulsions not resulting in hospitalization, or development of intervention dependency or intervention abuse. If in doubt as to whether or not an event qualifies as an important medical event, consult the Sponsor's Medical Monitor.

Adverse Events of Special Interest

[0290] An AESI (serious or nonserious) is defined as an AE or SAE of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor could be appropriate.

[0291] Following events are considered AESIs for this study and are reported using the same process as for AEs:

- Injection site reactions (will also be recorded on the injection site reaction CRF).
- Infection AEs including viral reactivation infections.
- Lymphopenia CTCAE Grade ≥ 3 ($<500 \mu\text{L}$).

Biomarkers

[0292] Blood samples are collected to assess the PD properties of COMPOUND A, which may include, but are not limited to, changes in immune cell subsets, and the treatment effect of COMPOUND A on AA disease biomarkers (including serum CCL17 (TARC)). Immune cell subsets may include, but are not limited to total T cells, helper T cells, cytotoxic T cells, total natural killer cells, and total B cells. Additional T-cell subsets are evaluated, which may include, but are not limited to regulatory, naïve, memory, and regulatory T-cell subsets. Blood samples collected in this study may be used to measure other biomarkers that may be identified in the future and to assist in any biomarker assay development and validation relevant to this study.

[0293] The following exploratory biomarker collections are performed:

- Serum and plasma samples will be collected and stored for potential testing of exploratory markers related to COMPOUND A treatment response. Exploratory markers may include, but will not be limited to, soluble IL-7R α , chemokines, cytokines, and/or other markers of inflammation, COMPOUND A activity, or AA disease biomarkers. Samples will be analyzed at the Sponsor's discretion.
- Peripheral blood mononuclear cells will be separated from whole blood samples and frozen for testing at a later time for potential evaluation of additional immune cell subsets associated with AA disease and/or immune function related to ADX914 treatment responses, *e.g.*, inhibition of IL-7 and TSLP-mediated effects. Samples will be tested at the Sponsor's discretion.

- Whole blood cell samples will be collected (*i.e.*, PAXgene® tubes) and stored for potential ribonucleic acid (RNA) expression profiling of COMPOUND A treatment response. The purpose of RNA collection is to potentially evaluate the effect of COMPOUND A on transcripts related to the IL-7R α pathway (*e.g.*, immune cell signatures, IL7/TSLP pathway signatures, *etc.*). The RNA samples may be utilized for genome-wide or candidate gene expression studies. Samples will be tested at the Sponsor's discretion.

Genetics

[0294] A deoxyribonucleic acid (DNA) sample may be collected in the form of whole blood cells, for exploratory research related to AA disease activity and/or the response to ADX 914, to be analyzed per the Sponsor's discretion.

[0295] Genetic polymorphism in genes encoding drug targets or the downstream pathways may affect the efficacy and safety of the study treatment. In the event of an unusual response or observation of unexplained AEs, DNA samples may be used to determine if there are any pharmacogenetic associations with drug response. In the future, as the understanding of the role of IL-7 and TSLP pathways in AA increases, additional genetic analyses may be warranted to refine the knowledge of the molecular basis of the disease, drug response, and to advance the development of novel therapeutics. Notably, but not restricted to, sequencing of T-cell receptor beta chain could identify the impact of COMPOUND A on the T cell compartment clonality.

[0296] Genetic polymorphism study is a one-time required blood collection as T cell clonality study requires pre- and post-treatment blood collections listed in the Schedule of Activities and governed by the main ICF for the study. In addition, where allowed by local, regional, and national regulatory authorities and ethics committees, subjects are offered the option for residual DNA samples to be retained for future exploratory unspecified genetic research that may be used to understand the biology of other diseases and traits of interest to the Sponsor and/or to develop diagnostic and analytical tests. Subjects who opt for samples to be retained for future unspecified use will be required to sign a separate ICF.

Example 2 Stability of COMPOUND A Formulation

[0297] COMPOUND A (the antibody having the heavy chain amino acid sequence of SEQ ID NO: 23 and the light chain amino acid sequence of SEQ ID NO: 24) was formulated as 100 mg/mL drug product in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0. Based on ongoing long-term stability studies, this formulation has a shelf life of about 24 months when stored at -20°C ($\pm 5^\circ\text{C}$) away from direct light.

[0298] Formulation characterization studies were performed on the COMPOUND A bulk drug substance (BDS) excipients and formulation including photostability, thermal, agitation, and freeze-thaw in 2R glass vials. The studies were performed in 2R glass vials to generate formulation data in drug product container closures.

[0299] The photostability of COMPOUND A was evaluated in 2R glass vials at 10 and 60 mg/mL in the BDS formulation of 20 mM histidine, 260 mM sucrose, 0.05% (w/v) polysorbate 80, and 0.05 mM pentetic acid at pH 6.0 for up to five days at room temperature under room light. When compared to samples held at room temperature away from direct light, the appearance, protein concentration by A280, SE-HPLC, cIEF, pH, and subvisible particulates counts were similar, indicating that COMPOUND A BDS at these concentrations and formulation is stable under these light stress conditions.

[0300] The buffer histidine and excipient sucrose were shown to have good conformational stability in comparison to other buffers such as citrate, phosphate and excipients arginine and sodium chloride at pH ranges of 5.5 to 6.5. Conformation stability assessment utilized differential scanning fluorimetry to evaluate unfolding temperature and dynamic light scattering to evaluate hydrodynamic radius.

[0301] The addition of polysorbate 80 as a stabilizing surfactant in the COMPOUND A BDS was evaluated in agitation and freeze thaw studies at a protein concentration of 60 mg/mL. The study showed that agitation stress up to one week had no observable effect on aggregation or significant increase of subvisible particulates in both formulations with and without polysorbate 80. Up to 5 freeze thaw cycles at -80°C of COMPOUND A BDS at 60 mg/mL was also evaluated in formulations with and without polysorbate 80. In the freeze thaw study, an increase in subvisible particulates by micro-flow imaging (MFI) was observed in the formulation without polysorbate 80. This indicated that polysorbate 80 is an important excipient for the freeze stability of COMPOUND A.

[0302] Development stability studies of COMPOUND A in the BDS formulation at pH 5.5, 6.0, 6.5 at 10 and 60 mg/mL over 12 weeks were performed at 2-8°C, 25°C, and 40°C. The pH, SE-HPLC, cIEF, CE-SDS, and particle analysis by MFI particle counts of the samples were trended. The data shows that the samples showed similar trends across all stability conditions.

[0303] The formulation of COMPOUND A 100 mg/mL rBDS was determined by various screening studies. This formulation development was intended to explore the design space of COMPOUND A 70 mg/mL BDS formulation without implementing major changes to pH and the buffering system. Therefore, histidine buffer at pH 6.0 was tested with several

excipient combinations targeting a protein concentration of 100 mg/mL. Formulation combinations tested sucrose, trehalose, mannitol, sorbitol, sodium chloride and arginine. Pentetic acid, a chelator that was part of the BDS formulation, was also evaluated and compared to ethylenediaminetetraacetic acid (EDTA). Polysorbate 80 was included with each formulation combination. Based on the results of the initial studies, it was determined that histidine buffer with sucrose/pentetic acid in polysorbate 80 had the capability to stabilize COMPOUND A at 100 mg/mL.

[0304] The formulation combination and role of pentetic acid were evaluated further. An accelerated stability study was performed comparing COMPOUND A 100 mg/mL formulations with and without pentetic acid at 40°C for 14 days and at 50°C for 7 days. The data from these studies are shown below in Tables 2-1, 2-2, 2-3, and 2-4.

Table 2-1: Accelerated stability study for COMPOUND A reformulated BDS without pentetic acid Formulation ID (100 mg/mL COMPOUND A, 20mM Histidine, 260 mM Sucrose, 0.05 % polysorbate 80, pH 6.0) at 40°C

Analytical Test	T ₀	T1d	T2d	T3d	T5d	T7d	T14d
Appearance (visible particles)	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles
% Monomer (SE-HPLC)	97%	97%	97%	97%	97%	97%	95%
% HMW	2.8%	3.0%	3.2%	3.3%	2.8%	3.2%	3.8%
% LMW	ND	ND	ND	ND	ND	ND	0.9%
% Acidic species (CEX)	41.6%	41.1%	41.0%	35.3%	38.9%	36.6%	36.4%
% Main peak (CEX)	55.0%	55.4%	53.7%	64.7%	55.9%	54.1%	46.1%
% Basic species (CEX)	3.4%	3.5%	5.3%	ND	5.2%	9.2%	17.5%
% Purity CE-SDS (NR)	98.2%	98.6%	98.8%	99.3%	99.2%	96.3%	99.1%
% Sum of minor peaks > LOQ CE-SDS (NR)	1.8%	1.4%	1.2%	0.7%	0.8%	3.7%	0.9%
% Purity CE-SDS (R)	99.4%	99.7%	99.9%	99.3%	99.1%	98.6%	99.0%

Analytical Test	T ₀	T1d	T2d	T3d	T5d	T7d	T14d
% Sum of minor peaks > LOQ CE-SDS (R)	0.6%	0.3%	0.1%	0.7%	0.9%	1.4%	1.0%
Particle size (DLS) nm	12.7	12.9	12.9	12.8	13.0	12.9	13.5
Particle size (DLS) PDI	0.26	0.26	0.25	0.26	0.27	0.26	0.26

Table 2-2: Accelerated stability study for COMPOUND A reformulated BDS with pentetic acid Formulation ID (100 mg/mL COMPOUND A, 20 mM Histidine, 260 mM Sucrose, 0.05 mM DTPA, 0.05% polysorbate 80, pH 6.0) at 40°C

Analytical Test	T ₀	T1d	T2d	T3d	T5d	T7d	T14d
Appearance (visible particles)	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles
% Monomer (SE-HPLC)	97%	97%	97%	97%	97%	97%	95%
% HMW	2.8%	3.1%	3.3%	3.4%	3.0%	3.2%	3.9%
% LMW	ND	ND	ND	ND	ND	ND	0.9
% Acidic species (CEX)	41.4%	41.4%	41.4%	36.0%	39.0%	37.5%	37.5%
% Main peak (CEX)	56.1%	54.5%	53.3%	64.0%	56.2%	53.5%	43.2%
% Basic species (CEX)	2.5%	4.1%	5.3%	ND	4.8%	9.0%	19.3%
CE-SDS (NR)	98.7%	98.3%	98.3%	99.3%	98.5%	96.1%	98.4%

Analytical Test	T ₀	T1d	T2d	T3d	T5d	T7d	T14d
% Sum of minor peaks > LOQ CE-SDS (NR)	1.3%	1.7%	1.8%	0.7%	1.5%	3.9%	1.6%
CE-SDS (R)	99.3%	98.0%	96.5%	99.3%	98.2%	98.9%	99.0%
% Sum of minor peaks > LOQ CE-SDS (R)	0.7%	2.0%	3.5%	0.7%	1.8%	1.1%	1.0%
Particle size (DLS)	14.8%	15.2%	15.1%	15.2%	15.3%	15.4%	15.5%
Particle size (DLS) PDI	0.29	0.31	0.30	0.31	0.31	0.32	0.31

Table 2-3: Accelerated stability study for COMPOUND A reformulated BDS without pentetic acid Formulation ID (100 mg/mL COMPOUND A, 20 mM Histidine, 260 mM Sucrose, 0.05 % polysorbate 80, pH 6.0) at 50°C

Analytical Test	T ₀	T1d	T2d	T3d	T5d	T7d
Appearance (visible particles)	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles
% Monomer (SE-HPLC)	97%	97%	96%	95%	97%	96%
% HMW	2.8%	3.0%	3.2%	3.4%	2.1%	2.9%
% LMW	ND	ND	1.0%	1.2%	1.3%	1.5%
% Acidic species (CEX)	41.6%	41.0%	41.5%	37.7%	43.5%	45.3%
% Main peak (CEX)	55.0%	51.0%	49.1%	49.1%	44.6%	41.2%
% Basic species (CEX)	3.4%	7.9%	9.4%	13.2%	11.9%	13.6%
% Purity CE-SDS (NR)	98.2%	98.2%	98.4%	98.9%	98.8%	92.5%
% Sum of minor peaks > LOQ CE-SDS (NR)	1.8%	1.8%	1.6%	1.1%	1.2%	7.5%
% Purity CE-SDS (R)	99.4%	99.2%	98.9%	98.5%	97.1%	96.6%
% Sum of minor peaks > LOQ CE-SDS (R)	0.6%	0.8%	1.1%	1.5%	2.9%	3.4%
Particle size (DLS) nm	12.7	13.1	13.2	13.6	14.1	15.6

Analytical Test	T ₀	T1d	T2d	T3d	T5d	T7d
Particle size (DLS) PDI	0.26	0.26	0.28	0.29	0.30	0.33

Table 2-4: Accelerated stability study for COMPOUND A reformulated BDS with pentetic acid Formulation ID (100 mg/mL COMPOUND A, 20 mM Histidine, 260 mM Sucrose, 0.05mM DTPA, 0.05% polysorbate 80, pH 6.0) at 50°C

Analytical Test	T ₀	T1d	T2d	T3d	T5d	T7d
Appearance (visible particles)	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles
% Monomer (SE-HPLC)	97%	97%	96%	95%	96%	95%
% HMW	2.8%	3.1%	3.4%	3.6%	2.3%	3.3%
% LMW	ND	ND	1.0%	1.2%	1.6%	1.9%
% Acidic species (CEX)	41.4%	41.7%	42.3%	38.4%	45.6%	48.1%
% Main peak (CEX)	56.1%	50.9%	48.5%	49.4%	42.3%	37.9%
% Basic species (CEX)	2.5%	7.4%	9.1%	12.2%	12.2%	14.1%
CE-SDS (NR)	98.7%	98.8%	98.5%	98.2%	98.6%	91.3%
% Sum of minor peaks > LOQ CE-SDS (NR)	1.3%	1.7%	1.5%	1.8%	1.4%	8.7%
CE-SDS (R)	99.3%	99.8%	99.6%	97.2%	96.4%	95.9%
% Sum of minor peaks > LOQ CE-SDS (R)	0.7%	0.2%	0.4%	2.8%	3.6%	4.1%
Particle size (DLS)	14.8	15.2	15.3	15.4	18.1	19.9
Particle size (DLS) PDI	0.29	0.30	0.30	0.30	0.46	0.53

[0305] Due to instrument availability, a CEX method was utilized in the accelerated stability studies in place of the cIEF method that was utilized in long-term stability studies. The number of assays was also reduced to focus on assays believed to be the most stability indicating. For accelerated stability studies, protein concentration by A280 and potency by binding ELISA were not monitored whereas these tests would be conducted for all timepoints and conditions during long-term stability studies. Additionally, particle size and Polydispersity Index (PDI) by dynamic light scattering (DLS) were monitored for accelerated stability studies as the growth in particle size and/or PDI could be indicative of protein aggregation especially during accelerated stability conditions. Particle size by DLS and PDI are not part of release and stability testing panel for long term stability testing.

[0306] The presence of pentetic acid did not impact stability positively or negatively in accelerated stability studies for a period of 7 days at 50°C and for 14 days at 40°C. In addition, the increased concentration of COMPOUND A to 100 mg/mL from 70 mg/mL did not have any impact on accelerated stability over a period of 7 days at 50°C and for 14 days at 40°C.

[0307] A freeze-thaw stability study was also performed comparing COMPOUND A 25 mg/mL formulations with and without pentetic acid for up to 5 freeze-thaw cycles. Appearance and purity testing by SEC were evaluated. The data from these studies are shown below in

[0308] Table 2-5 and 2-6.

Table 2-5: Freeze-thaw stability study for COMPOUND A reformulated BDS without pentetic acid Formulation ID (125 mg/mL COMPOUND A, 20 mM Histidine, 260 mM Sucrose, 0.05mM DTPA, 0.05% polysorbate 80, pH 6.0)

Analytical Test	T ₀	1 F/T	3 F/T	5 F/T
Appearance (visible particles)	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles
% Monomer (SE-HPLC)	96.8%	96.0%	96.0%	95.8%
% HMW	3.2%	3.2%	3.2%	3.4%
% LMW	ND	0.76%	0.76%	0.77%

Table 2-6: Freeze-thaw stability study for COMPOUND A reformulated BDS with pentetic acid Formulation ID (125 mg/mL COMPOUND A, 20 mM Histidine, 260 mM Sucrose, 0.05mM DTPA, 0.05 % polysorbate 80, pH 6.0)

Analytical Test	T ₀	1 F/T	3 F/T	5 F/T
Appearance (visible particles)	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles	Clear and Colorless with no visible particles
% Monomer (SE-HPLC)	96.8%	95.8%	96.0%	96.0%
% HMW	3.2%	3.4%	3.2%	3.2%
% LMW	ND	0.79%	0.76%	0.78%

[0309] No instability was observed across all freeze-thaw cycles for formulations with and without pentetic acid. Both formulations at 125 mg/mL were considered stable up to 5 freeze-thaw cycles.

[0310] Based on the results from the formulation process development, pentetic acid was determined to be optional in the formulation. The formulation of COMPOUND A 100 mg/mL rBDS was determined to be 20 mM histidine, 260 mM sucrose, 0.05% polysorbate 80 at pH 6.0. The concentrated 100 mg/mL rBDS without the chelator is expected to be stable at the intended storage temperature of $-20 \pm 5^{\circ}\text{C}$ for the intended duration of storage.

[0311] Furthermore, an in-use clinical compatibility study was performed during development to verify the stability of the above COMPOUND A drug product across the range of potential doses over 4 hours at ambient room temperature without light protection in the clinical presentation (polypropylene syringe and stainless-steel needles). The drug product was demonstrated to be compatible and stable with the administration materials over a 4-hour period by enzyme-linked immunosorbent assay (ELISA) analysis of binding potency, protein concentration, and size-exclusion high-performance liquid chromatography for antibody purity.

Example 3: Binding properties of COMPOUND A

SPR analysis of binding to FcγR by a COMPOUND A precursor

[0312] Fcγ receptors are found on hematopoietic cells and mediate antibody-antigen-induced effector functions. These include antibody-dependent cell-mediated cytotoxicity and antibody dependent cellular phagocytosis. The binding of specific non-variable regions of

IgG1 with Fc γ Rs expressed on immune cells mediates effector functions of the antigen-antibody complexes.

[0313] The precursor of COMPOUND A (C1GM/2hIgG1) was tested for its binding to human and monkey Fc γ receptors (types I, II, and III) by surface plasma resonance (SPR). The Fc γ Rs screened included Fc γ RI (hCD64 and cynoCD64), Fc γ RII (hCD32a-H131 and hCD32a-R131, hCD32b, cynoCD32a, and cynoCD32b), and Fc γ RIII (hCD16a-V158, hCD16b-NA2, and cynoCD16) subtypes. The C1GM/2hIgG1 antibody demonstrated the expected binding profiles for human and cynomolgus monkey Fc γ RI, Fc γ RII and Fc γ RIII (data not shown)

Lack of binding by COMPOUND A to cynomolgus monkey and human Fc γ receptors

[0314] Three amino acid substitutions were engineered into the Fc chain of the precursor C1GM/2hIgG1 to attenuate effector function, arriving at COMPOUND A. The mutations included a leucine to alanine substitution at position 234 (L234A), a leucine to glutamic acid substitution at position 235 (L235E), and a glycine to alanine substitution at position 237 (G237A). COMPOUND A was expressed in HEK293 and CHO cells.

[0315] COMPOUND A (1 μ M) was purified from cell supernatants and assessed for binding to human and cynomolgus monkey Fc γ Rs by SPR. The Fc γ Rs screened included Fc γ RI (hCD64 and cynoCD64), Fc γ RII (hCD32a-H131, hCD32a-R131, hCD32b, cynoCD32a, and cynoCD32b), and Fc γ RIII (hCD16a-V158, hCD16b-NA2, and cynoCD16) subtypes.

[0316] The binding of COMPOUND A to all Fc γ Rs was below detectable limits when tested at a high concentration (1 μ M) (data not shown). In contrast, an IgG1-positive control antibody showed the expected high degree of binding to human Fc γ RI, with moderate binding to both Fc γ RII and Fc γ RIII (data not shown).

[0317] Taken together, these data confirm that the combined L234A, L235E, and G237A substitutions engineered into the Fc domain of COMPOUND A effectively minimize potential effector function.

Lack of binding by COMPOUND A to C1q

[0318] C1q binding to the Fc domain of IgG1 antibodies can mediate complement-mediated cell lysis. The ability of COMPOUND A to bind C1q was evaluated using an ELISA assay. Briefly, microtiter plates were coated with human IL-7R α , and COMPOUND A from 2 different lots were added at varying concentrations to the coated plates, followed by

a saturating concentration of C1q purified from human serum. Bound C1q was detected with a sheep anti-human C1q antibody. Positive control included human IgG1. Positive control IgG1 showed the expected affinity for C1q; however, neither lot of COMPOUND A showed any measurable binding to C1q at concentrations of up to 1000 ng/mL (6.7 nM).

[0319] This data shows that COMPOUND A can evade C1q-mediated complement-mediated cell lysis when administered to human.

Binding properties of COMPOUND A to human and cynomolgus monkey FcRn at pH 6.0 and 7.4

[0320] Neonatal Fc receptor (FcRn), expressed on endothelial and bone marrow-derived cells, extends the half-life of IgGs by facilitating their transport out of epithelial cell endosomes (pH 6.0) where they would otherwise be degraded, and back into the bloodstream as functional immunoglobulins (pH 7.4). Endosomal recycling is facilitated by a pH shift (tight binding at pH 6.0 and low binding/high dissociation at pH 7.4) in the IgG-FcRn interaction.

[0321] The binding of COMPOUND A to a panel of human and cynomolgus neonatal Fc receptors (hFcRn and cFcRn, respectively) were assessed using a single cycle Biacore analysis. The K_d of COMPOUND A binding to human and cynomolgus monkey FcRn was 686 and 866 nM, respectively, at pH 6.0. COMPOUND A did not bind to FcRn from either human or monkey at pH 7.4. This pH-dependent shift in antigen affinity facilitates the release of complexed COMPOUND A in lysosome from lysosomal degradation. Irrelevant human IgG1 and IgG4 wild-type antibodies were run as controls.

[0322] These findings suggest that endosomal recycling is expected to enhance the biostability/half-life of COMPOUND A in vivo.

SPR analysis of binding to human and cynomolgus IL-7Rα at pH 6.0 and 7.4

[0323] COMPOUND A was evaluated for binding to purified recombinant human and cynomolgus monkey IL-7Rα via SPR. The K_d of COMPOUND A for human IL-7Rα is 1.3 nM and for cynomolgus monkey IL-7Rα is 1.7 nM at pH 7.4. The binding affinity of COMPOUND A for both human and cynomolgus monkey IL-7Rα was 4-fold weaker at pH 6.0, with K_d values of 5.3 nM for human IL-7Rα (hIL-7Rα), and 7.0 nM for cynomolgus monkey IL-7Rα (cIL-7Rα) (see Table 3 below).

Table 3: Binding Affinity of COMPOUND A to Human and Cynomolgus Monkey IL-7R α

Target	Temperature	pH	Kd (nM)
hIL-7R α	37°C	7.4	1.3
		6.0	5.3
cIL-7R α	37°C	7.4	1.7
		6.0	7.0

Kd: receptor binding affinity dissociation constant; SPR: surface plasmon resonance.

[0324] These data demonstrate that COMPOUND A bound with equivalent high affinity to human and cynomolgus monkey IL-7R α at pH 6.0 and 7.4. For both species the binding affinity was roughly 4-fold higher at pH 7.4 compared with pH 6.0. This pH-dependent shift in antigen affinity may further facilitate the release of complexed COMPOUND A from the lysosomal to avoid lysosomal degradation of COMPOUND A.

Example 4: In vitro assessment of efficacies of COMPOUND A

Receptor Occupancy and Inhibition of IL-7 Stimulated Phosphorylation of STAT5 (pSTAT5) in CD4 T Cells From Healthy Volunteers

[0325] The effect of COMPOUND A on IL-7–induced pSTAT5 expression was evaluated in CD4⁺ T cells in healthy human volunteers (n = 3). Anticoagulated whole blood from healthy volunteers was incubated with increasing concentrations of COMPOUND A in the presence of recombinant human IL-7 (10 ng/mL). The percentage of pSTAT5 in CD4⁺ T cells was measured by flow cytometry using fluorescent probes for CD3⁺, CD4⁺, CD45RA⁺, and pSTAT5.

[0326] COMPOUND A showed potent inhibition of pSTAT5 on naïve CD4⁺ T cells from all 3 healthy volunteers (n = 3) with mean IC₅₀ values of 0.16 to 0.27 nM (23.7 to 40.4 ng/mL, respectively).

Inhibition of pSTAT5 in Naïve CD4⁺ and CD8 T-Cells From Healthy Volunteers

[0327] COMPOUND A was evaluated for target engagement and inhibition of IL-7 induced IL-7R signaling on human T cells from normal healthy volunteers. Freshly drawn blood was incubated ex vivo with IL-7 (100 ng/mL) for 15 minutes, and pSTAT5 levels were measured by flow cytometry.

[0328] As shown in Table 4, COMPOUND A potently inhibited IL-7–induced phosphorylation of STAT5 in naïve (CD45RA⁺) and memory (CD45RA⁻) T cells (both CD4

and CD8 phenotypes) from normal healthy volunteers with IC₅₀ values ranging between 0.38 and 0.54 nM (equivalent to about 57 to 81 ng/mL).

Table 4: Inhibition of IL-7–Induced pSTAT5 in T Cells by COMPOUND A (IC₅₀ in nM)

	IC ₅₀ (nM)			
	CD4 ⁺ CD45RA ⁺	CD4 ⁺ CD45RA ⁻	CD8 ⁺ CD45RA ^{±a}	CD8 ⁺ CD45RA ^{-a}
NHV (n = 19)	0.54±0.32	0.52±0.35	0.42±0.29	0.38±0.30

Abbreviations: IC₅₀: half maximal inhibitory concentration; NHV: normal healthy volunteers; CD45RO: marker of naïve CD3⁺ T cells.

^a IC₅₀ values were calculated only when CD8 T cells responded to IL-7.

COMPOUND A is an IL-7R antagonist antibody with no partial agonist activity

[0329] To determine if COMPOUND A had any partial agonist capabilities, whole blood or purified peripheral blood monocyte cells (PBMCs) were collected from healthy volunteers (n = 3). COMPOUND A was incubated in each matrix at 8 concentrations ranging from 1 to 100 nM. Signal transduction was assessed by AlphaLISA® SureFire®Ultra™ pSTAT5 to detect pSTAT5. Negative control used a non-targeting IgG isotype, and the positive control was IL-7 (2 nM). IL-7 increased pSTAT5 levels by an average of 4.7-fold (±1.6) and 21.1-fold (±2.8) in whole blood and PBMCs, respectively. No significant induction of pSTAT5 was observed at any concentration of COMPOUND A (1, 2, 3, 6, 13, 25, 50, or 100 nM) in either whole blood or PBMCs, demonstrating that COMPOUND A is a full antagonist at the IL-7R with no partial agonist activity. These data are presented in Table 5.

Table 5. COMPOUND A is an Antagonist With no Intrinsic Partial Agonist Activity

COMPOUND A (nM)	Peripheral blood mononuclear cells					
	Donor 1		Donor 2		Donor 3	
	pSTAT5 AlphaLISA units	pSTAT5 % control (IL-7)	pSTAT5 AlphaLISA units	pSTAT5 % control (IL-7)	pSTAT5 AlphaLISA units	pSTAT5 % control (IL-7)
100	786	1.73	1836	6.32	1083	1.53
50	868	2.48	915	0.47	1386	3.16
25	894	2.72	1072	1.46	1333	2.88
13	1038	4.03	1060	1.39	1373	3.09
6	941	3.14	1031	1.21	1476	3.65
3	938	3.12	1467	3.98	1711	4.91
2	896	2.73	1075	1.49	1582	4.22
1	1174	5.28	1467	3.98	1787	5.32
0	597	0.00	841	0.00	797	0.00
Isotype ctrl	934	3.09	1174	2.12	1325	2.84
IL-7 (2 nM)	11520	100	16575	100.00	19406	100.00
COMPOUND A (nM)	Whole blood					
	Donor 4		Donor 5		Donor 6	
	pSTAT5 AlphaLISA units	pSTAT5 % control (IL-7)	pSTAT5 AlphaLISA units	pSTAT5 % control (IL-7)	pSTAT5 AlphaLISA units	pSTAT5 % control (IL-7)
100	14704	-5.39	1055	-1.99	8328	-9.41
50	17982	-0.55	1001	-2.87	9524	-4.13
25	16463	-2.79	1140	-0.62	11526	4.71
13	15041	-4.90	1031	-2.37	11212	3.33
6	19004	0.97	976	-3.26	10891	1.91
3	15281	-4.54	958	-3.55	9132	-5.86
2	21046	3.99	1016	-2.62	14779	19.08
1	17489	-1.27	1045	-2.16	10224	-1.04
0	18350	0.00	1178	0.00	10459	0.00
Isotype ctrl	18648	0.44	952	-3.65	14024	15.74
IL-7 (2 nM)	85954	100	7371	100	33102	100.00

COMPOUND A Inhibits TSLP-Induced TARC Production by PBMCs

[0330] Thymus-activated regulated chemokine (TARC, also known as CCL17) is an important chemokine released from monocytes and dendritic cells in response to thymic stromal lymphopoietin (TSLP) stimulation. TARC drives the maturation of dendritic cells and their production of IL-23 as well as the proliferation of CD4⁺ T cells. TSLP is highly expressed in atopic dermatitis (AD) skin lesions. Signaling through IL-7R α /TLSPR and IL-7R α /CD132 has been demonstrated to produce additive inflammatory effects.

[0331] COMPOUND A binds to the IL-7R α subunit that is common to both the IL-7 receptor (IL-7R α /CD132) and the TSLP receptor complex (IL-7R α /TSLPR). COMPOUND A

inhibited TSLP-induced production of TARC from PBMCs enriched for monocytes (3 independent assays with 1-2 donors) with an average IC₅₀ of 2.9 nM (428 ng/mL).

[0332] Thus, in addition to blocking IL-7-induced signaling, COMPOUND A also inhibits TSLP induced signaling at low nM concentrations.

Example 5: Assessment of efficacies of COMPOUND A in humanized mouse model

Efficacy of COMPOUND A in a Humanized Mouse Graft versus Host Disease Model

[0333] Graft-versus-host disease (GvHD) is a systemic disease, and skin is often one of the target organs.

[0334] COMPOUND A was evaluated with prophylactic treatment in a humanized non-obese diabetic severe combined immune deficiency-mice with a humanized immune system (NOD-SCID-IL2 γ^{null} [Hu-NSG]) mouse model of GvHD. NSG mice are immunodeficient due to the lack of mature murine lymphocytes and natural killer (NK) cells, which allows human peripheral blood mononuclear cells (PBMCs) to engraft with high efficiency. NSG mice were injected intravenously (IV) with human PBMCs that engrafted and led to the development of a robust xenogeneic-GvHD response, reproducing many aspects of the human disease.

[0335] A reference IL-7R α antagonist antibody (A3312F, which epitope residues appear to include all epitope residues of COMPOUND A) was evaluated in 2 treatment regimens (prophylactic and therapeutic modes), and the data from the prophylactic regimen was presented herein, with A3312F serving as a positive control for COMPOUND A in this model of GvHD.

[0336] In the prophylactic treatment study, COMPOUND A, administered subcutaneously twice weekly (5 mg/kg) for 3 weeks starting on Day (-1) (the day before adoptive PBMC transfer) to NSG mice (n = 7 females), showed robust inhibition of GvHD as demonstrated by a reduction in body weight loss, inflammatory cytokine expression, and immune cell proliferation (see FIGs. 4A-4C). A3312F, tested in the same study, showed comparable efficacy to COMPOUND A.

[0337] In a separate experiment, target engagement was assessed by evaluating the level of pSTAT5 in cells from whole blood and spleens. COMPOUND A treatment (0.2, 1, or 5 mg/kg 2x/week SC) was initiated 5 days after human PBMC transfer to NSG mice. Whole blood and spleens were collected 72 and 120 hours, respectively, after the fourth dose of COMPOUND A. Cells were collected and stimulated with human IL-7 for 15 minutes, and pSTAT5 levels were analyzed in human CD4⁺ and CD8⁺ T cells by flow cytometry.

COMPOUND A demonstrated complete target engagement and inhibition of IL-7–induced pSTAT5 at 1 and 5 mg/kg treatments (see FIGs. 5A-5B). This confirms that inhibition of IL-7R α signaling with COMPOUND A is protective in a humanized mouse model of GvHD and that complete target engagement can be achieved.

[0338] In several related experiments, a mouse surrogate antibody (SB14) was used to further assess the role of IL-7R α inhibition to treat other disease indications in mouse models of such disease indications (COMPOUND A was not used in these experiments partly because COMPOUND A does not cross-react with the mouse IL-7R α (data not shown)). Collectively, these additional *in vivo* studies demonstrated that short-term inhibition of IL-7R α signaling provides a durable, efficacious response, and suggested the potential for an infrequent dosing paradigm to sustain clinical efficacy. Furthermore, it demonstrated the importance of IL-7R signaling in driving T cell-mediated disease processes.

[0339] Interestingly, in at least one such disease model, a different inhibitor of T cell activation, CTLA4-Ig, did not exhibit efficacy in that disease model using the same treatment strategy (data not shown), thus demonstrating a clear differentiation between anti-IL-7R α mAb and CTLA4-Ig treatment, and highlighting the potential for a durable, drug-free response with IL-7R α mAb treatment.

Example 6: Pharmacokinetics/Pharmacodynamics (PK/PD) in Cynomolgus Monkeys

Single-dose intravenous pharmacokinetics and pharmacodynamics

[0340] This study was conducted to compare the PK and PD profiles of low doses of COMPOUND A with a reference positive control IL-7R α antagonist antibody (A3312F) when administered to cynomolgus monkeys, and to provide data to support the use of COMPOUND A in humans. COMPOUND A and A3312F were administered by IV injection at doses of 0 (vehicle), 0.1 (low dose), 0.5 (mid dose), or 3.0 (high dose) mg/kg to groups of 1 or 2 monkeys per sex. All doses were administered at 1 mL/kg in a vehicle/carrier consisting of 20 mM histidine, 260 mM sucrose, 0.05 mM diethylenetriaminepentaacetic acid (DTPA), and 0.05% Tween 80, pH 6.0.

[0341] Criteria for PD evaluation included total and soluble IL-7R levels in blood and plasma, inhibition of IL-7–induced pSTAT5, receptor occupancy (RO), and peripheral lymphocyte phenotyping. Pharmacokinetic endpoints included AUC, C_{max}, and T_{max} and immunogenicity endpoint included anti-drug antibody (ADA).

[0342] On Day 1, 100% RO was achieved at 4 hours after dosing and maintained on Days 2-4 at all doses for both COMPOUND A and A3312F. The vehicle control group consistently

exhibited no RO for the duration of the study. RO decreased for the remainder of the study, starting with the low dose of A3312F falling below 95% RO on Day 4, while the other drug-treated groups maintained 100% RO. On Day 7, the RO of both low dose groups fell below 50%. On Day 10, the RO of low dose of A3312F returned to vehicle control levels, the mid dose below 50% and the high dose below 90%. The low dose of COMPOUND A also returned to vehicle control, while the mid dose averaged below 50% (with variability), and the high dose remained at 100% RO. On Day 14, all A3312F dose groups returned to vehicle control levels (no RO), whereas the COMPOUND A low and mid doses both returned to vehicle levels but the high dose of COMPOUND A maintained >50% RO. All doses for both COMPOUND A and A3312F were comparable to vehicle control levels by Day 17.

[0343] Cynomolgus monkey IL-7-induced pSTAT5 was strongly inhibited by both COMPOUND A and A3312F. The low dose of 0.1 mg/kg showed notable inhibition of pSTAT5 ($\geq 80\%$) at early time points (Days 1-3) with COMPOUND A being slightly more effective than A3312F. The intermediate dose of 0.5 mg/kg COMPOUND A was more effective than A3312F at maintaining complete inhibition of pSTAT5 through Day 5, with an approximate 4-fold higher suppression on Day 5. At the high dose of 3 mg/kg, complete inhibition of pSTAT5 activity was sustained through Day 8 with COMPOUND A treatment, while A3312F treatment demonstrated lesser inhibition of pSTAT5 over the same time period. Moreover, COMPOUND A at Day 11 continued to substantially inhibit IL-7 induced pSTAT5 while A3312F exhibited a complete loss of this inhibitory activity.

[0344] Both mean total (blood) and soluble (plasma) IL-7R concentrations, which encompass both free and bound IL-7R, increased after administration of COMPOUND A and maximum levels were generally reached by 8 to 11 days. Mean soluble (plasma) IL-7R concentrations increased to a greater extent compared to total (blood) IL-7R levels. The cell surface IL-7R levels measured using flow cytometry did not significantly change after COMPOUND A or A3312F administration, suggesting that decreased elimination of soluble IL-7R– COMPOUND A complexes is the cause for increase in total IL-7R levels in blood.

[0345] On Day 8, there was a trend of reduction in peripheral lymphocytes as compared to baseline in both COMPOUND A and A3312F treated animals, which was most prominent at the highest dose (3 mg/kg). However, no overt differences in peripheral lymphocyte subsets were observed for either COMPOUND A or A3312F during the study. There was variability in lymphocyte populations among animals in the same group, however, suggesting that the variability may be due to stress (*i.e.*, handling of the monkeys, *etc.*) and the study's small sample size per dose group ($n = 3$).

[0346] COMPOUND A exposure was evaluated after single IV doses of ≤ 3 mg/kg and compared to A3312F. Mean exposures (AUCs) at 3 mg/kg in the first week, before anti-drug antibody (ADA) formation, were higher for COMPOUND A compared to A3312F with a mean AUC from 0 extrapolated to infinity (AUC_{inf}) ≤ 287 $\mu\text{g}\cdot\text{day/mL}$ and $AUC_{inf} \leq 135$ $\mu\text{g}\cdot\text{day/mL}$, respectively) (see Table 6). ADA formation was accompanied by concomitant accelerated decline of serum concentrations, RO, and inhibition of pSTAT5 for COMPOUND A and A3312F.

Table 6. Mean Serum COMPOUND A and A3312F Pharmacokinetic Parameters

Parameter	COMPOUND A IV dose (mg/kg)			A3312F-IV dose (mg/kg)		
	0.1	0.5	3	0.1	0.5	3
C_{max} ($\mu\text{g/mL}$) ^a	3.1	12.4	79.1	2.61	12.5	71
T_{max} (h) ^a	0.5	0.5	0.5	0.5	0.5	0.5
AUC_{inf} ($\mu\text{g}\cdot\text{day/mL}$) ^b	3.7	33.3	287.2	2.4	19.4	134.9
CL (mL/h/kg) ^a	1.15	0.64	0.44	1.76	1.16	0.94

Abbreviations: AUC_{inf} : area under the concentration-time curve from 0 extrapolated to infinity; C_{max} : maximum plasma concentration; CL: clearance; IV: intravenous; PK: pharmacokinetic; T_{max} : time of maximum plasma concentration.

[0347] ADAs were detected in all monkeys dosed with COMPOUND A. ADAs were detected in 3, 3, and 2 of 3 monkeys dosed with A3312F at 0.1, 0.5, and 3 mg/kg, respectively, by Day 11. The presence of ADAs led to generally lower serum COMPOUND A and A3312F concentrations and lower individual COMPOUND A and A3312F systemic exposures on and after Day 11 (240 hours post dose) in all monkeys.

[0348] In summary, COMPOUND A and A3312F were well tolerated by monkeys after single IV doses of up to 3 mg/kg. There were no drug-related clinical observations or effects on body weight observed during the study. Good correlation was observed between the inhibition pSTAT5 in the CD4+CD45RA+ T cell population and serum concentration of anti-human IL-7R antibodies. More than 95% RO resulted in 90% inhibition of pSTAT5. The inhibition of pSTAT5 levels, a proximal biomarker of IL-7R signaling, by COMPOUND A was similar but more prolonged than that by A3312F and suggests that COMPOUND A exhibits improved PK, but similar *in vivo* potency compared to A3312F. No significant change in lymphocyte phenotypes was observed for either COMPOUND A or A3312F after a single-dose administration. Despite the impact of ADAs on COMPOUND A's concentration, meaningful exposure and evaluation of toxicological endpoints were achieved.

Single-dose subcutaneous pharmacokinetics and pharmacodynamics

[0349] Systemic toxicokinetic profile, tolerability, RO, and PD changes as a measure of target engagement, assess immune responses was determined for subcutaneous (SC) administration of COMPOUND A at doses of 0 (vehicle), 3, and 30 mg/kg to groups of 2 male and 2 female cynomolgus monkeys. Criteria for PD evaluation included assessments of IL-7–induced pSTAT5, IL-7 RO, peripheral blood lymphocyte phenotyping (T cell, B cell, and NK cell [T/B/NK]), T cell-dependent antibody response (TDAR) to keyhole limpet hemocyanin (KLH) immunization, and total and soluble IL-7R levels.

[0350] In the 3 mg/kg SC dose group, the RO was 100% through Day 8, 98% on Day 11, 16% on Day 15, 12% on Day 22, and below the limit of quantitation (BLQ) on Day 36. In the 30 mg/kg SC dose group, RO was 100% through Day 11, 70% on Day 15, and BLQ on Day 22.

[0351] Inhibition of IL-7–induced pSTAT5 on CD3+ T cells was observed in all dose groups, 4 hours post dose (between 92% and 99% mean inhibition), indicating significant PD activity. In the 3 mg/kg SC group, the mean % pSTAT5 levels were 100% through Day 8, 79% on Day 11, and returned to baseline levels on Day 22. In the 30 mg/kg SC dose group the % pSTAT5 inhibition was 100% through Day 11, 30% on Day 15, and returned to baseline on Day 22. Inhibition of pSTAT5 generally correlated with the degree of IL-7 RO and systemic exposure of COMPOUND A that was decreased due to the presence of ADAs in all monkeys by Day 11.

[0352] Mean exposures (AUC_{0-336h} and C_{max}) generally increased approximately dose-proportionally by SC injection between 3 and 30 mg/kg. No substantial sex differences were noted. The systemic exposure after a single SC dose of 3 mg/kg was comparable to that after a single IV dose of 3 mg/kg, and the bioavailability was 78% by calculating with the first week data.

[0353] Overall, COMPOUND A was well tolerated by monkeys after a single SC dose ≤ 30 mg/kg ($AUC_{0-336h} \leq 61,314 \mu g \cdot h/mL$). COMPOUND A–related PD activity was noted at all doses, as demonstrated by inhibition of IL-7–induced pSTAT5, and generally correlated with IL-7 RO and COMPOUND A exposure, which was decreased due to the presence of ADAs in all monkeys by Day 11. Decreased lymphocyte subpopulations observed as early as on Day 8 was consistent with hematology findings of decreased lymphocytes (at ≥ 3 mg/kg SC). Despite the impact of ADAs on COMPOUND A's concentration, meaningful exposure and evaluation of toxicological endpoints were achieved.

Six-week intermittent-dose (QW) SC toxicity study with an 8-week recovery period

[0354] COMPOUND A was administered SC once weekly at doses of 0 (vehicle), 2, 10, or 50 mg/kg to groups of 5 monkeys per sex. Scheduled necropsies were conducted after 6 weeks of dosing (7 doses) (3/sex/group) and after an 8-week recovery period (2/sex/group).

[0355] At all doses near-complete (>95%) inhibition of IL-7 induced pSTAT5 on CD3+ T cells (PD activity) and near-complete ($\geq 98\%$) IL-7 RO on CD3+ T cells (target engagement) were observed at 4 hours post dose on Day 1 through Day 8. Recovery of pSTAT5 and loss of IL-7 RO were noted on Day 22 and correlated with decreased systemic exposures to COMPOUND A due to the presence of higher levels of ADAs. Overall, target engagement and PD activity correlated with sustained systemic exposures in monkeys with minimal or no ADA responses.

[0356] At all doses, there were decreases in total T (as low as 0.51 $\times$ relative to vehicle control group mean), helper T (CD4+, as low as 0.51 $\times$), and cytotoxic T (CD8+, as low as 0.48 $\times$) cells on Day 8 consistent with the hematology findings of a decrease in total lymphocyte counts on Day 8, but no decrease in B and NK cells. Due to reduced systemic exposures of COMPOUND A as a result of ADA, T cell subset counts returned to baseline levels by Day 22 with the exception of the highest dose group of 50 mg/kg in which drug levels remained in circulation. In the 50 mg/kg dose group, the decreases in T cell subsets were maintained through the end of the recovery period (Day 97/99) and consistent with drug maintained in circulation.

[0357] After doses up to 50 mg/kg, there were no COMPOUND A-related changes in qualitative and quantitative electrocardiographic evaluations. Additional examinations demonstrated no COMPOUND A-related changes in neurological endpoints, respiratory rates, body temperatures, evaluations of lung sounds and mucus membrane color, and arterial oxygen saturation determinations.

[0358] Overall, COMPOUND A was well tolerated by monkeys for 6 weeks at SC, once weekly doses of 2, 10, or 50 mg/kg. SC toxiketic profile was approximately dose-proportional on Day 1 across the dose range tested but was later affected by the development of ADA in most animals. High-dose animals maintained exposure, albeit variable, allowing for meaningful interpretation of toxicological endpoints and the definition of a NOAEL. At all doses, PD activity (inhibition of IL-7 induced pSTAT5) generally correlated with IL-7 RO (target engagement) and COMPOUND A exposure and was decreased due to high levels of ADAs (more prominently at the lower doses).

Three-month repeat dose toxicity study of COMPOUND A by subcutaneous injection with a 6-month recovery period

[0359] COMPOUND A was administered SC once weekly at doses of 0 (vehicle), 10, 50, and 150 mg/kg to groups of 5 monkeys per sex.

[0360] During Week 1 of the ECG evaluations, 7 ventricular premature complexes (VPCs) were observed in 1 male monkey over a 10-second interval in the high dose group (150 mg/kg QW). The relationship of the VPCs to COMPOUND A is unclear for the following reasons: VPCs were observed in only 1 of 30 animals in this study; and VPCs can be an incidental finding in naïve cynomolgus monkeys (~8% of animals with 1 minute ECGs performed 2 weeks apart) as well as spontaneous incidence over time can be quite variable and their occurrence sporadic when evaluated over 24 hours. Additionally, there were no histologic correlations or QT/QTc interval changes to predispose this animal to ventricular arrhythmias. The presence of VPCs in this animal was not associated with any clinical signs and hence the arrhythmia was not considered adverse.

[0361] Overall, administration of COMPOUND A to cynomolgus monkeys, administered by weekly subcutaneous injection for 3 months was well tolerated at doses of 10, 50, and 150 mg/kg. Greater than 95% RO was maintained at 50 mg/kg (7/10 animals) and at 150 mg/kg (10/10 animals) through the 3-month dosing phase. Loss of >95% RO at 10 mg/kg (9/10 animals) and 50 mg/kg (3/10 animals) was observed on Day 29 or Day 57 and correlated with detectable ADA. A decline in CD4+ and CD8+ T cell populations were observed, with the largest impact on naïve and CM T-cells at doses of COMPOUND A ≥ 50 mg/kg. These reductions are related to the pharmacological effects of COMPOUND A and were generally correlated with observed RO. Total T lymphocyte counts, T cell subsets and B cells were partially or completely recovered to baseline in the males, but decreases in T lymphocyte counts, T cell subsets and B cells were maintained with minimal or no recovery in female monkeys, which are generally correlated with observed durability of RO. There were no histological correlations with the decreased lymphocytes at end of the recovery phase.

[0362] Exposure of COMPOUND A was maintained $\geq 60,800$ $\mu\text{g}\cdot\text{h}/\text{mL}$ through the dosing phase in 7/10 animals at 50 mg/kg and 10/10 animals at 150 mg/kg despite detectable ADA. Based on the non-adverse nature of the COMPOUND A-related pathology changes at the end of the dosing period, the no-observed-adverse-effect level (NOAEL) was considered to be 150 mg/kg, the highest dose tested. At the phase NOAEL, the mean C_{max} and AUC sex combined values were 3570 $\mu\text{g}/\text{mL}$ and 314,000 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively.

Six-month study of COMPOUND A by subcutaneous injection in sexually mature and not sexually mature cynomolgus monkeys with an 8-month recovery period (ongoing)

[0363] COMPOUND A was administered SC weekly at doses of 0 (vehicle), 25, 50, and 150 mg/kg to groups of sexually mature and not sexually mature monkeys. Scheduled necropsy was conducted after 6 months of dosing (27 doses) (2-4/sex/group) and after an 8-month recovery period (1-2/sex/group). All doses were administered at 1 mL/kg in a vehicle/carrier consisting of 20 mM histidine, 260 mM sucrose, and 0.05% PS80, pH 6, which is consistent with the clinical formulation.

[0364] In the dosing phase, low signals of anti-COMPOUND A antibody (most below 10,000 mean signal responses) were detected in 10 of 12 animals in the control groups as observed in the control animals in the 3-month GLP study. Similarly, it was determined that the bridging method used to detect the presence of ADAs in serum can detect ADA against all parts of the COMPOUND A molecule, including the human IgG1 Fc region and the complementarity determining regions. No adverse clinical signs were observed in the 10 control animals with detectable ADA. In addition, COMPOUND A exposure was not detected in the control animals; thus, the reason for the detected ADA in control animals was determined to be unlikely related to COMPOUND A exposure but rather possibly due to generic reactivity to IgG of unknown etiology.

[0365] In the thymus, decreased lymphoid cellularity was observed in the thymus of sexually mature males administered ≥ 25 mg/kg (mild to moderate), and sexually mature females and not sexually mature males and females administered ≥ 50 mg/kg (moderate to marked), correlating with decreased thymus weights (absolute and relative to body and brain weights), small thymus size noted macroscopically, and decreases in lymphocyte counts. The COMPOUND A-related decreased lymphoid cellularity observed in the thymus and associated hematology effects during the dosing phase of the study were considered non-adverse because of the low incidence and severity of the changes. There were no significant differences between the findings noted in not sexually mature and sexually mature animals. To date, no effects on reproductive organs have been observed in general toxicology studies.

[0366] Systemic exposure of COMPOUND A was mostly independent of sex or sexual maturity. At 25 mg/kg, exposure disparities were observed, likely due to ADA presence. Systemic exposure (AUC_{0-t} and C_{max} values) generally increased with increasing dose in an approximately dose-proportional manner with a mean accumulation ratio ranging approximately 2- to 5-fold from Day 1 to the end of the dosing phase. Exposure of

COMPOUND A was maintained at $\geq 141,000 \mu\text{g}\cdot\text{h}/\text{mL}$ on Day 92 throughout the dosing phase in 22 of 28 animals at $\geq 50 \text{ mg}/\text{kg}$ despite detectable ADA.

[0367] In summary, weekly SC administration of COMPOUND A to not sexually mature and sexually mature cynomolgus monkeys for 6 months at dose levels of 25, 50, and 150 mg/kg was well tolerated. The NOAEL was considered to be 150 $\text{mg}/\text{kg}/\text{week}$. At the NOAEL, the mean C_{max} and AUC_{0-t} values for not sexually mature animals (sex combined) were 3260 $\mu\text{g}/\text{mL}$ and 466,125 $\mu\text{g}\cdot\text{h}/\text{mL}$, and for sexually mature animals (sex combined) were 3780 $\mu\text{g}/\text{mL}$ and 540,200 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively, after 6 months of SC weekly dosing. This corresponds to >50 -fold greater than the projected steady-state C_{max} ($C_{\text{max,ss}}$) and steady-state AUC over the dosing interval ($\text{AUC}_{\text{tau,ss}}$) of the proposed dose of 200 mg COMPOUND A for Phase 2 studies. The preliminary data reported here are considered sufficient to support the dose frequency, dosing duration, and dose regimen in Phase 2 studies.

Example 7 Phase 1, double-blind, placebo-controlled, single and multiple ascending dose study to assess the safety, PK, and PD of COMPOUND A after SC administration in healthy subjects

[0368] The example demonstrates that COMPOUND A is safe and well tolerated in healthy human subjects (male and female subjects aged 18 to 50 years old). Further, PK studies based on the data collected in these healthy human subjects show that COMPOUND A can be administered at relatively low doses and still achieve high receptor occupancy (RO) with respect to the IL-7R receptor. Additional analysis revealed that COMPOUND A administration in healthy human subjects led to minimal ADA host response due to the low immunogenicity of COMPOUND A.

[0369] For Part 1, SAD (single ascending dose) study, the protocol defined up to 6 cohorts of 8 subjects per cohort. In each cohort, subjects were randomly assigned in a 3:1 ratio to receive either COMPOUND A or matching placebo. SAD cohort 6 was optional and was not utilized. A minimum of 7 dosed subjects evaluable for safety were required for each cohort in order to escalate to the subsequent cohort. The 5 SAD cohorts dosed in the study were 0.1 mg/kg , 0.3 mg/kg , 1 mg/kg , 2 mg/kg and 4 mg/kg .

[0370] For Part 2, MAD (multiple ascending dose) study, the protocol defined up to 3 cohorts of 8 subjects per cohort. In each cohort, subjects were to be randomly assigned in a 3:1 ratio to receive either COMPOUND A or matching placebo. In the end, MAD Cohorts 2

and 3 were not enrolled per termination decision due to COVID-19 caseload and COVID-19 operational concerns where the study was being conducted.

[0371] Overall, the PD analyses demonstrated that COMPOUND treatment at doses that achieved $\geq 95\%$ RO also demonstrated $>90\%$ inhibition of IL-7 signaling via phosphorylation of STAT5 (FIGs. 6A-6B). Single doses of COMPOUND A administered at 2 mg/kg or higher demonstrated sustained full RO for at least 2 weeks. Repeat administration of COMPOUND A at 1 mg/kg demonstrated durable saturation of IL-7R α after the second dose (see FIG. 6A). In addition, the lymphocyte subsets and T cell immune function assessments demonstrated modest, dose-dependent effects on these biomarkers, consistent with the expected pharmacology when COMPOUND A fully occupies IL-7R α . An attenuation of T-dependent antibody responses to a neo-antigen was observed at dose levels where full RO was maintained over the evaluation period while the impact of COMPOUND A treatment on delayed-type hypersensitivity (DTH) responses to a recall antigen was more variable.

[0372] The presence of ADA against COMPOUND A in human serum samples from treated subjects in phase 1 was measured using a 3-tier approach (screen, confirm, and titer) using a validated assay. After single and multiple SC administrations of COMPOUND A, generally, there was an increase in the incidence of positive ADA with time for all cohorts. While most ADA-positive samples had low titers near the sensitivity threshold of the assay, there was an increase in ADA titer with time and dose level. Based on the surrogate antibody positive control for the ADA assay, titer values ≤ 80 ng/mL reflect ADA detectable below the US FDA recommended sensitivity guideline of 100 ng/mL. Most subjects had ADA levels below this 100 ng/mL threshold, and only 8 of 34 (23.5%) subjects had titer values >80 ng/mL during the observation period.

[0373] In the context of repeat dosing with COMPOUND A in MAD Cohort 1, all 6 subjects treated with active drug developed treatment-emergent ADA responses with titers that increased to 40, 80, or 320 by Day 85, but then a decrease in ADA titer was observed upon reaching the end of the study (Day 127).

[0374] Given the ability to detect low titer antibodies in the presence of circulating drug, the assay can detect both clinically relevant and irrelevant anti-COMPOUND A antibodies. The impact of ADA on COMPOUND A exposure and PD activity was also evaluated.

[0375] Where evaluable, no impact of ADA on PK or RO was observed since the PK and RO profiles for treatment-emergent ADA-positive subjects fell within the range of observed PK profiles for ADA-negative subjects. Where the impact of ADA on PK profiles compared

with ADA-negative subjects was not evaluable, concentrations generally fell within the range of expected exposures and no accelerated clearance was observed.

[0376] Across cohorts, there were no associations between the timing of positivity and any observations in the PK profiles. Many treatment-emergent ADA-positive samples occurred at or beyond Day 50 when COMPOUND A concentrations were BLQ.

[0377] Similarly, there was no apparent impact on RO, substantiating that the low titers of ADA observed in the study are unlikely to be clinically relevant. No ADA-related AEs were observed in the study. Immunogenicity and its potential clinical impact will continue to be evaluated throughout the clinical development of COMPOUND A.

[0378] In Part 1 study (SAD), while 85.7% of the subjects receiving COMPOUND 1 and 70% of the subjects receiving placebo reported treatment-emergent adverse events (TEAEs), all TEAEs were mild or moderate in intensity. 57.1% of subjects who received COMPOUND A and 30.0% subjects who received placebo reported at least one injection site reaction (ISR) event; no ISRs were reported as severe. Transient reductions in lymphocyte counts were observed after COMPOUND A treatment centered on the Day 3 time point, corresponding to C_{max} , with a more prolonged time course for the highest dose (2 mg/kg and 4 mg/kg). None of the lymphocyte count reduction events were considered clinically significant.

[0379] In Part 2 study (MAD), similarly, all TEAEs were mild or moderate in intensity and no ISR were reported as severe. Reductions in absolute lymphocyte counts were observed after COMPOUND A treatment, but none of the lymphocyte count events were considered clinically significant. A total lymphocyte count of $<0.5 \times 10^9/L$, a prespecified protocol stopping criterion, was not observed in any of the subjects during the study. There were no cases of lymphopenia as defined by the CTCAE guidance.

[0380] Overall, Phase 1 data indicates that dose up to 4 mg/kg is well tolerated in healthy human volunteers, with no observable impact on pharmacology or safety by ADA. Doses less than 3 mg/kg SC was observed to achieve full RO, and multiple administration of COMPOUND A at 1 mg/kg SC every two weeks also achieved full RO and pSTAT5 inhibition. A total lymphocyte count of $<0.5 \times 10^9/L$, a prespecified protocol stopping criterion, was not observed in any of the study subjects during the study. There were no cases of lymphopenia as defined by the Common Terminology Criteria for Adverse Events (CTCAE) guidance.

Example 8: Preliminary Phase 2 Clinical Data in Human

Assessment of Antibody Serum Concentration and Receptor Occupancy (RO) by COMPOUND A in AD patients

[0381] Serum PK and blood IL-7R α RO of COMPOUND A were evaluated in subjects dosed with 2 mg/kg (N = 5) or 3 mg/kg (N = 5) subcutaneous administration. The interim cutoff date for this analysis resulted in inclusion of data of at least 98 days (14 weeks) or 14 days (2 weeks) postdose for PK. Analyses were performed by an external unblinded pharmacometrician and results were reblinded for the Sponsor. To maintain blinding, time points or groups with N <2 were not included in the analysis. Nominal doses and nominal times of dosing and sampling were used in the analyses.

[0382] The preliminary mean serum concentration versus time profiles of free COMPOUND A after 2 and 3 mg/kg SC administration every 2 weeks are shown in FIG. 7A. After SC dosing of 2 or 3 mg/kg COMPOUND A, apparent dose-dependent exposures were observed over the first 2 weeks post-dose, with mean Day 15 trough concentrations (at pre-dose) of 5.8 and 7.76 μ g/mL, respectively. With continued dosing of 2 mg/kg every 2 weeks, 60% to 80% of patients maintained trough concentrations above 5 μ g/mL, the target therapeutic concentration. Trough serum concentrations demonstrated generally high variability, with CV% ranging from approximately 40% to 90%.

[0383] Preliminary RO (mean $\pm$ standard deviation [SD]) versus time profiles after dosing of 2 and 3 mg/kg COMPOUND A SC Q2W are presented in FIG. 7B. The preliminary mean IL-7R α RO on circulating CD3+ T cells reached complete saturation (ie, >90% RO) by Day 3 after a dose of 2 or 3 mg/kg COMPOUND A. Upon repeat subcutaneous administration of 2 mg/kg COMPOUND A Q2W, 100% of patients maintained complete RO at all time points except for one outlier at Day 43. After administration of 3 mg/kg COMPOUND A SC Q2W, 100% patients maintained complete RO above 90% up to Study Day 15.

[0384] COMPOUND A has an acceptable safety profile at doses up to 3 mg/kg every 2 weeks in subjects with AD. All adverse events (AEs) were assessed as mild or moderate, and there were no reported severe AEs. Reductions in lymphocyte count below 1000/ μ L, consistent with the mechanism of action, were observed in 5 subjects across both cohorts. The minimum lymphocyte count observed was 750/ μ L and no adverse sequelae were reported; the subject's lymphocyte count returned to normal upon retesting approximately 1 week later with no further reduction below normal values observed. No dose-dependent reduction in lymphocyte count was observed. The lymphocyte count reductions were

generally transient and trended to within normal limits at follow-up visits. No CTCAE Grade ≥ 3 lymphopenia or associated AEs or infections were observed. No doses were delayed due to TEAEs or due to a subject's absolute lymphocyte count (ALC) being $< 800/\mu\text{L}$ (as per protocol). No other clinically significant findings in laboratory values related to COMPOUND A have been observed.

[0385] In summary, both 2 and 3 mg/kg dose groups maintained target PK threshold $> 5 \mu\text{g/mL}$ (dose expected to achieve full target engagement in tissue). Maximal RO ($> 90\%$) was achieved in all subjects of both dose groups by day 3 and maintained through the entire dosing period. Safety profile is also acceptable at these two doses; particularly, no lymphopenia-associated AEs, including viral infections, have been observed.

[0386] Based on these preliminary data, a dose of 200 mg ($\sim 2.7 \text{ mg/kg}$) administered SC Q2W is expected to result in steady-state serum trough concentrations $> 5 \mu\text{g/mL}$ and maximum RO on circulating T cells in $> 90\%$ of subjects throughout dosing, while expected to cause no or only minimal lymphopenia. In patients with AA, predicted median $C_{\text{max,ss}}$ and $\text{AUC}_{\text{tau,ss}}$ after a dose of 200 mg SC administered Q2W are > 100 -fold and > 50 -fold lower, respectively, than exposures at the NOAEL of 150 mg/kg/week SC in cynomolgus monkeys from the 6-month GLP toxicology study (Example 6).

[0387] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications, and publications to provide yet further embodiments.

[0388] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A method of treating alopecia areata (AA) or reducing hair loss associated with AA, in a mammalian (*e.g.*, human) subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (DHAMH, such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2 (GISWNSRGIGYADSVKG), and a VH CDR3 sequence of SEQ ID NO: 3 (DEYSRGYYVLDV); and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4 (RASQGISSALA), a VL CDR2 sequence of SEQ ID NO: 5 (DASSLES), and a VL CDR3 sequence of SEQ ID NO: 6 (QQFNSYPLWIT), such that AA is treated and/or the hair loss is reduced in the mammalian subject.
2. A method of inducing hair growth in a mammalian (*e.g.*, human) subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises:
 - (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2, and a VH CDR3 sequence of SEQ ID NO: 3; and
 - (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4, a VL CDR2 sequence of SEQ ID NO: 5, and a VL CDR3 sequence of SEQ ID NO: 6,thereby inducing hair growth in the mammalian subject.
3. The method of claim 2, wherein the subject suffers from a hair-loss disorder such as alopecia areata.
4. The method of claim 1 or 3, wherein the hair-loss disorder is alopecia areata.
5. The method of claim 4, wherein the alopecia areata is alopecia totalis or alopecia universalis.
6. The method of any one of the preceding claims, wherein the antibody is administered to the subject subcutaneously (*s.c.*).

7. The method of any one of the preceding claims, wherein the antibody is administered once a week (Q1W), once every two weeks (Q2W), once every three weeks (Q3W), once every four weeks (Q4W), once every eight weeks (Q8W), once every twelve weeks (Q12W), or any intermittent PRN (*pro re nata*).
8. The method of any one of the preceding claims, wherein the antibody is administered for 10-15 (*e.g.*, 11, 12, or 13) consecutive doses.
9. The method of any one of the preceding claims, wherein the antibody has a heavy chain sequence of SEQ ID NO: 23 and a light chain sequence of SEQ ID NO: 24.
10. The method of any one of the preceding claims, wherein the antibody has a heavy chain sequence of SEQ ID NO: 23 and a light chain sequence of SEQ ID NO: 24, and is administered at about 200 mg per dose to the mammalian (*e.g.*, human) subject subcutaneously (*s.c.*) once every two weeks (Q2W), for 10-15 (*e.g.*, 11, 12, or 13) consecutive doses.
11. The method of any one of the preceding claims, wherein the subject has moderate-to-severe AA, or severe-to-very severe AA (*e.g.*, with $\geq 50\%$ total scalp hair loss as defined by the SALT score).
12. The method of any one of the preceding claims, wherein hair loss in the subject is assessed by mean relative percent change in Severity of Alopecia Tool (SALT) Score at 12 weeks (Week 12), 18 weeks (Week 18), and/or 24 weeks (Week 24) after the first dose at Week 0, as compared with a Baseline SALT Score obtained just prior to the first dose.
13. The method of claim 12, wherein $\geq 50\%$ relative reduction in SALT score is achieved in the subject at 12 weeks (Week 12), 18 weeks (Week 18), and/or 24 weeks (Week 24) after the first dose at Week 0.
14. The method of claim 12 or 13, wherein absolute SALT score of the subject is ≤ 5 , 10, 20, 30, or 50 at 12 weeks (Week 12), 18 weeks (Week 18), and/or 24 weeks (Week 24) after the first dose at Week 0.
15. The method of any one of claims 12 to 14 wherein the subject has $\geq 50\%$ relative reduction in SALT score at Week 36 from Baseline and/or Week 24.
16. The method of any one of claims 12 to 15, wherein absolute SALT score of the subject is ≤ 5 , ≤ 10 , ≤ 20 , ≤ 30 , or ≤ 50 at Week 36.

17. The method of any one of the preceding claims, wherein hair loss comprises eyelash loss and/or eyebrow loss.
18. The method of claim 17, wherein reduction in eyebrow loss is based on ClinRO Measure for Eyebrow Hair Loss scores of 0 or 1 (full coverage or minimal gaps) with a ≥ 2 -point improvement from Baseline at Week 24 and/or Week 36.
19. The method of claim 17, wherein reduction in eyelash loss is based on ClinRO Measure for Eyelash Hair Loss scores of 0 or 1 (full coverage or minimal gaps) with a ≥ 2 -point improvement from Baseline at Week 24 and/or Week 36.
20. The method of any one of the preceding claims, wherein the patient has improved quality of life (QOL) following treatment, as measured by improvement in SKINDEX-16 AA compared to that of Baseline at Week 12, 18, 24, and/or 36.
21. The method of any one of the preceding claims, wherein the antibody is formulated as 100 mg/mL solution in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0, with an optional 0.05 mM pentetic acid.
22. The method of any one of the preceding claims, wherein the antibody is administered to the subject at about 0.1 mg/kg – about 10 mg/kg per dose, about 0.2 mg/kg – about 8 mg/kg per dose, about 0.5 mg/kg – about 5 mg/kg per dose, about 1 mg/kg – about 4 mg/kg per dose, about 2 mg/kg – about 3 mg/kg per dose, about 0.2 mg/kg per dose, about 0.5 mg/kg per dose, about 1 mg/kg per dose, about 2 mg/kg per dose, about 3 mg/kg per dose, about 4 mg/kg per dose, about 5 mg/kg per dose, about 6 mg/kg per dose, about 7 mg/kg per dose, about 8 mg/kg per dose, about 9 mg/kg per dose, or about 10 mg/kg per dose.
23. The method of any one of the preceding claims, wherein the antibody is administered to the subject at about 10 mg – about 800 mg per dose, about 20 mg – about 500 mg per dose, about 50 mg – about 300 mg per dose, about 100 mg – about 250 mg per dose, about 25 mg per dose, about 50 mg per dose, about 100 mg per dose, about 150 mg per dose, about 200 mg per dose, about 250 mg per dose, about 300 mg per dose, or about 350 mg per dose.
24. The method of any one of the preceding claims, wherein the subject has impaired renal function (*e.g.*, mildly impaired renal function (such as a glomerular filtration rate < 60 mL/min and/or the presence of albuminuria > 30 mg/d), moderately impaired

renal function (such as a glomerular filtration rate <45 mL/min), or severely impaired renal function (such as a glomerular filtration rate <30 mL/min).

25. The method of any one of the preceding claims, wherein the subject is further being treated by or has been treated by a second therapeutic agent effective to treat alopecia areata.
26. The method of claim 25, wherein the second therapeutic agent comprises a glucocorticoid, Minoxidil, Anthralin, Bimatoprost, Methotrexate, a DMARD, dupilumab, and/or a JAK inhibitor (*e.g.*, tofacitinib, ruxolitinib, upadacitinib, abrocitinib, and baricitinib).
27. The method of any preceding claims wherein the subject has had alopecia areata for over 6 months and less than 10 years prior to administering the antibody.
28. A method of treating alopecia areata (AA) in a mammalian (*e.g.*, human) subject in need thereof, the method comprising administering an effective amount of a composition comprising an antibody for IL-7R α , wherein the antibody comprises (a) a heavy chain variable region (HCVR) that comprises a VH CDR1 comprising the sequence of SEQ ID NO: 1 (DHAMH, such as any one of SEQ ID NOs: 7-22), a VH CDR2 sequence of SEQ ID NO: 2 (GISWNSRGIGYADSVKG), and a VH CDR3 sequence of SEQ ID NO: 3 (DEYSRGYYVLDV); and (b) a light chain variable region (LCVR) that comprises a VL CDR1 sequence of SEQ ID NO: 4 (RASQGISSALA), a VL CDR2 sequence of SEQ ID NO: 5 (DASSLES), and a VL CDR3 sequence of SEQ ID NO: 6 (QQFNSYPLWIT);
wherein the subject has $\geq 50\%$ total scalp hair loss as defined by a baseline SALT score, wherein the baseline SALT score is determined up to 24 hours prior to administering the antibody;
wherein the antibody comprises an effectorless IgG1 Fc, and
wherein 200 mg of the antibody is administered subcutaneously every 2 weeks.
29. The method of claim 28, wherein the VH CDR1 comprises SEQ ID NO: 7 (GFTFDDHAMH).
30. The method of claim 28 or 29, wherein the subject has had alopecia areata for over 6 months and less than 10 years prior to administering the antibody.

31. The method of any of claims 28-30, wherein the composition comprises 100 mg/ml of the antibody in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0, with an optional 0.05 mM pentetic acid.
32. The method of any of claims 28-31, wherein the subject has an absolute lymphocyte count (ALC) of ≥ 800 per microL.
33. The method of any of claims 28-32, wherein administration of the antibody is stopped when the subject's ALC is < 500 per microL.
34. The method of any of claims 28-33, wherein the composition comprising the antibody is administered to the subject to maintain serum concentration of the antibody at ≥ 5 $\mu\text{g/mL}$ throughout dosing.
35. The method of any preceding claims, wherein the antibody is administered to the subject at about 200 mg per dose.
36. The method of any preceding claims, wherein the antibody is administered once every two weeks (Q2W).
37. The method of any preceding claims, wherein the antibody is administered for at least about 13 doses.
38. The method of any preceding claims, wherein the antibody comprises an effectorless IgG1 Fc.
39. The method of any one of the preceding claims, wherein the antibody has a heavy chain sequence of SEQ ID NO: 23 and a light chain sequence of SEQ ID NO: 24, and is administered at about 200 mg per dose to the mammalian (*e.g.*, human) subject subcutaneously (*s.c.*) once every two weeks (Q2W), for 10-15 (*e.g.*, 11, 12, or 13) consecutive doses.
40. A pharmaceutical composition comprising an antibody having two heavy chains and two light chains, wherein each said heavy chain has the amino acid sequence of SEQ ID NO: 23, and each said light chain has the amino acid sequence of SEQ ID NO: 24; and wherein the antibody is formulated in a pharmaceutically acceptable solution comprising about 50-200 mg/mL (*e.g.*, about 100 mg/mL) of the antibody in about 15-25 mM (*e.g.*, about 20 mM) histidine, 200-300 mM (*e.g.*, about 260 mM) sucrose, and about 0.03-0.07% (w/v) (*e.g.*, about 0.05% (w/v)) polysorbate 80 at pH 6.0.
41. The pharmaceutical composition of claim 40, wherein the solution further comprises about 0.01-0.10 mM (*e.g.*, about 0.05 mM) of pentetic acid.

42. The pharmaceutical composition of claim 40, comprising, consisting essentially of, or consisting of the antibody formulated as 100 mg/mL of the antibody in 20 mM histidine, 260 mM sucrose, and 0.05% (w/v) polysorbate 80 at pH 6.0.
43. The pharmaceutical composition of claim 40, comprising, consisting essentially of, or consisting of the antibody formulated as 100 mg/mL of the antibody in 20 mM histidine, 260 mM sucrose, 0.05 mM of pentetic acid, and 0.05% (w/v) polysorbate 80 at pH 6.0.
44. A container (*e.g.* a polycarbonate bottle) containing about 2 mL of extractable volume of the pharmaceutical composition of claim 42 or 43.
45. The container of claim 44, comprising about 2.26 mL of the pharmaceutical composition of claim 42 or 43.