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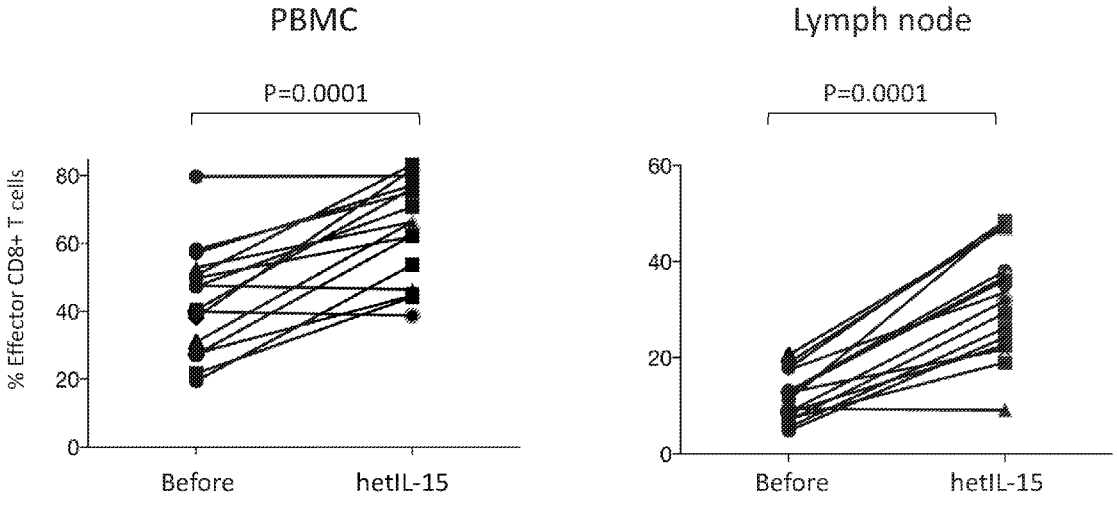
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(54) Title: IL-15/IL-15 RECEPTOR ALPHA TREATMENT REGIMENS AND USE WITH THERAPEUTIC VACCINES

Figure 4



(57) Abstract: The invention provides methods and dose escalation regimens for administering IL-15 to a patient in need thereof.

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[Continued on next page]

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IL-15/IL-15 Receptor alpha treatment regimens and use with therapeutic vaccines

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of U.S. Provisional Application No. 62/362,223, filed July 14, 2016, which is herein incorporated by reference for all purposes.

REFERENCE TO A "SEQUENCE LISTING" SUBMITTED AS AN ASCII TEXT FILE

[0002] The Sequence Listing written in file 077867-629100PC-1051885_SequenceListing.txt created on June 30, 2017, 48,643 bytes, machine format IBM-PC, MS-Windows operating system, in accordance with 37 C.F.R. §§ 1.821- to 1.825, is hereby incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0003] IL-15 plays a pivotal role in modulating the activity of both the innate and adaptive immune system, e.g., expansion and maintenance of the memory T-cell response to invading pathogens, and induction of natural killer ("NK") cell proliferation and cytotoxic activity.

[0004] The IL-15 receptor consists of two polypeptides, the IL-2/IL-15 receptor beta (β) (or CD122), and the gamma chain (γ) (or CD132) that is shared by multiple cytokine receptors. IL-15 signaling has been shown to occur through the heterodimeric complex of IL-15Rβ and IL-15Rγ. A third polypeptide chain binds to IL-15 and was also considered as part of the IL-15 Receptor. Despite existing theories suggesting that IL-15R alpha (referred to herein as IL-15Rα or IL-15Ra) is a receptor for IL-15, an alternative interpretation of the existing data is that IL-15Rα is not a receptor for the IL-15 polypeptide chain. IL-15Rα has evolved very high affinity for IL-15 and is always coexpressed with IL-15 in the same cell. The two molecules form heterodimeric complexes in the endoplasmic reticulum and are transported to the plasma membrane. *See, e.g.*, Bergamaschi *J. Biol. Chem* 283:4189-4199, 2008. This heterodimeric complex can bind to the IL-2/IL-15βγ receptor and activate the cells via the Jak/Stat pathway. Therefore, based upon this interpretation of the data, the IL-15Rα and the soluble form sIL-15Rα are part of the cytokine and not part of the receptor.

[0005] Heterodimeric IL-15 (hetIL-15) in which IL-15 is complexed with IL-15Rα is the form of IL-15 found in the circulation and in human cells. Endogenous hetIL-15 is found in two forms, as a membrane-bound form that is expressed by antigen presenting and stroma cells in various tissues; and as a soluble extracellular complex of IL-15 bound to the soluble IL-15 receptor alpha (IL-15Rα), which is produced by cleavage of the membrane-anchored IL-15Rα by cellular proteases. Although IL-15 mRNA has been reported in cells of both hematopoietic and nonhematopoietic lineage, T cells do not produce IL-15. Instead, IL-15 heterodimers released from the cell surface after cleavage of the membrane-bound heterodimers bind to the IL-15 βγ receptor on lymphocytes.

[0006] Based on its multifaceted role in the immune system, various therapies designed to modulate IL-15-mediated function have been explored. For example, the administration of exogenous IL-15 can enhance the immune function of patients infected with human immunodeficiency virus (HIV). In keeping with its immune enhancing activity, increased expression of endogenous IL-15 is observed in patients with autoimmune diseases, e.g., rheumatoid arthritis, multiple sclerosis, ulcerative colitis, and psoriasis. Because some studies reported that the soluble form of the IL-15Rα (sIL-15Ra) is an antagonist of IL-15-mediated signaling, the sIL-15Ra has been explored for treating autoimmune inflammatory diseases. Nevertheless, recent reports suggest that IL-15, when complexed with the sIL-15Ra, or the sushi domain, maintains its immune enhancing function.

[0007] The present invention provides a further improvement in dosing IL-15 in order to avoid toxicity.

BRIEF SUMMARY OF SOME EMBODIMENTS OF THE DISCLOSURE

[0008] In one aspect, the methods described herein are based, in part, on the discovery of a dose escalation regimen for administering IL-15/IL-15Ra complexes that provides low toxicity. Such regimens can be based on the estimation of consumption of hetIL-15 by human lymphocytes, *e.g.*, using monitoring methods as disclosed herein. In some embodiments, the IL-15 and IL-15Ra contained in the complexes are non-covalently associated. In some embodiments, the IL-15 and IL-15Ra are covalently linked. Any form of IL-15/IL-15Ra complex may be employed, including forms in which one or more of the Il-15 and IL-15Ra has a mutation relative to the naturally occurring forms, complexes in which the IL-15Ra is in the form of a fusio protein, complexes that are modified, *e.g.*, by PEGylation, or any other modification.

[0009] In some aspects, the disclosure is based on the discovery of an unexpected effect associated with the hetIL-15 regimens described herein, namely, the induction of cytotoxic effector cells within the lymph nodes (LN) and especially the B cell follicles and the germinal centers (GCs). Effective hetIL-15 regimens include, but are not limited to, one- and two-week regimens of step-dosing (dose escalation during subsequent doses) where the dose of hetIL-15 was increased with each subsequent dose. B cell areas within the LN are known to be sanctuaries for HIV-infected cells. Infected cells can be detected for many years during the most active antiviral therapies.

[0010] Further, in some aspects, the disclosure is based on the discoveries decribed herein that multiple high dose hetIL-15 step cycles can be delivered in simian immunodeficiency virus (SIV)- or simian/human immunodeficiency virus (SHIV)-infected macaques with or without combination antiretroviral treatment (ART) and that hetIL-15 treatment was well tolerated, safe and effective because it increased the cytotoxic lymphocytes, especially in tissues and LN/GCs.

[0011] In additional aspects, the disclosure is also based on the discovery that hetIL-15 regimens also increased preferentially virus-specific cytotoxic lymphocytes and induce these lymphocytes to increase proliferation and cytotoxic ability measured for example by Granzyme content. For example, a reliable measurement of hetIL-15 activity is the proliferation of lymphocytes and especially NK, CD8 cells as measured in different compartments, *e.g.*, by the proliferation marker Ki67. This can be measured after staining with antibodies by (for example) flow cytometry or immunohistochemistry methods. Other markers and methods measuring cell proliferatio can also be employed. As describe herein, hetIL-15 regimens increased natural killer cells systemically and in lymph nodes and increases NK proliferation and cytotoxic ability measured, for example, by Granzyme content.

[0012] The disclosure is also based, in part on the discovery that hetIL-15 is not equally distributed in the body as determined by *in vivo* measurements in an illustrative macaque model at different anatomical locations after subcutaneous (SC) administration of hetIL-15. The activity on lymphocytes *in vivo* can be measured conveniently and reliably by proliferation markers and also by measuring expansion of hetIL-15 dependent subsets such as NK or effector CD8 cells. These measurements showed that hetIL-15 delivered SC does not affect equally some internal anatomical sites such as the gut lymphoid system and the intraperitoneal LNs, in fact, it takes much higher quantities of cytokine to see some effects in

these tissues. Lymphocytes in these anatomical locations are able to respond to hetIL-15, because they respond normally when removed from the body and treated *ex vivo*.

[0013] The present disclosure also relates to alternative delivery methods for hetIL-15. It was demonstrated that intraperitoneal delivery of hetIL-15 increases the proliferation and activation of lymphocytes in the gut, which can enhance the effects of hetIL-15 on intestinal tumors and on HIV reservoirs located within the gut associated lymphoid tissue. This strategy is important for both cancer and acquired immunodeficiency syndrome (AIDS) immunotherapy approaches. An alternative or additional strategy is the intravenous delivery of hetIL-15 under dose escalation procedures described below.

[0014] In a further aspect, the invention relates to the discovery that hetIL-15 induces expression of chemokine CXCL13 [chemokine (C-X-C motif) Ligand 13], which can be detected in the blood and the lymph nodes and results in decreasing the levels of surface-detected CXCR5 chemokine receptor [chemokine (C-X-C motif) receptor 5] on lymphocytes. CXCL13/CXCR5 can thus be used as markers of hetIL-15 function and to monitor the effects of hetIL-15 in lymphoid tissue, which is not easily accessible in humans. The CXCL13–CXCR5 chemokine axis plays a central role in organizing both B-cell follicles and GCs. CXCL13 has been proposed as a plasma biomarker of germinal center activity (Havenar-Daughton PNAS 2016). CXCL13 can be used as a marker, eventhough its production may be an indirect effect of hetiL-15 administration *in vivo*.

[0015] The effects of hetIL-15 may also be assessed using Ki67 as a marker and/or combinations of markers, including CXCL13 and CCXCR5 on the surface of cells.

[0016] In some aspects of the invention, hetIL-15 is used as a vaccine enhancer and used together with therapeutic vaccination. Thus, hetIL-15 can be administered after therapeutic vaccination to maximize immune response and deliver the immune response to areas of virus reservoirs and sanctuaries. HetIL-15 regimens as a vaccine enhancer is able to activate and multiply antigen-specific cytotoxic cells and also able to deliver cytotoxic cells in areas where they encounter antigen (cancer sites, areas of persistent infection such as lymph nodes in HIV infected individuals). HetIL-15 regimens are synergistic to vaccine effects due to the hetIL-15 properties to affect GC cells through chemokine/cytokine networks (example, CXCR5/CXCL13).

[0017] In some embodiments, the invention thus provides use of hetIL-15 in conjunction with therapeutic vaccination to maximize the immune response.

[0018] In some embodiments, the invention provides methods of monitoring the effects of hetIL-15 in lymphoid tissue. In some embodiments, the method comprises evaluating a blood sample to determine the level of chemokine CXCL13 following hetIL-15 administration. In some embodiments, the method comprises detecting the level of CXCR5 chemokine receptor expressed on the surface of on lymphocytes, *e.g.*, lymphocytes obtained from a blood sample from a patient. In some embodiments, the method comprises evaluating both CXCL13 levels in blood and the levels of CXCR5 expressed on lymphocytes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1. Increase in frequency of Granzyme B+ CD8 T cells in both peripheral blood mononuclear cells (PBMC) and LN after hetIL-15 treatment (3 days after the last IL-15 injection in a 2-week regimen). hetIL-15 treatment triggers a cytotoxic commitment (increased Granzyme B) on CD8+ T cells (including LN)

[0020] Figure 2. Increased T lymphocyte proliferation rate (Ki67+ T cells) in both rectal and vaginal mucosa. Biopsies were obtained before hetIL-15 treatment and at necropsy of the animals (three days after the last macaque hetIL-15 injection). The samples were processed fresh, digested with collagenase and DNase I for one hour at 37°C. Single cell suspensions were obtained by filtering the digested samples through 100 µm cell-strainer, and the lymphocytes were stained with a panel of antibodies including CD3, CD4, CD8, CD95, CD28, CD25, CD127, γ/δ TCR, FoxP3, Granzyme B and Ki67.

[0021] Figure 3. Changes in the frequency of CD8+ T cells with effector phenotype (CD95+CD28low) in PBMC and LN before and after human hetIL-15 treatment (low dose escalation with 2, 4, 8, 16, 32 and 64 µg/Kg of hetIL-15). Data from 15 additional animals are summarized in Figure 4.

[0022] Figure 4. Comparison of the percentage of CD8+ T cells with effector phenotype ($CD95+CD28^{low}$) in both peripheral blood and LN before and after hetIL-15 treatment. Data from 16 macaques. 12 of the animals received low dose escalation (2, 4, 8, 16, 32 and 64 µg/Kg hetIL-15, studies F31a, F31b, F31c, F31d, F31f and F31g); 2 animals received high dose escalation (5, 10, 20, 40, 80, 120 µg/Kg) and two animals received 6 injections with high dose hetIL-15 (50µg/Kg).

[0023] Figure 5. Frequency of proliferating central memory (CM) CD8+ ($CD3+CD8+CD95+CD28^{high}Ki67+$) and effector memory (EM) CD8+

(CD3+CD8+CD95+CD28^{low} Ki67+) in lymph nodes before and after IL-15 treatment. hetIL-15 treatment increases the proliferation rate of CD8+ memory T cells within the LN.

[0024] Figure 6. hetIL-15 treatment results in increased frequency of NK cells in peripheral blood and within the LN. This increase is driven by proliferation. Left panel: Percentage of NK cells (CD16+ or GranzymeB+CD3-) within lymph nodes and PBMC samples before and after hetIL-15 treatment. Right panel: Frequency of proliferating NK cells in the same samples.

[0025] Figure 7. IL-15 treatment increases antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by NK cells. In vitro ADCC assay was performed using as targets the CFSE labeled CEM NKr cell line coated with SIV gp120. Effector cells were human PBMC samples (untreated or treated with human IL-15 at 20ng/ml for 24 hours) and the source of antibodies was plasma from macaque M587 (a macaque vaccinated with DNA+protein co-immunization, study AUP417, group 2), that was an elite controller upon SIV infection). The effector to target ratio was 10:1 and the killing assay was performed for 90 minutes at 37°C. Dead cells were identified by PI staining.

[0026] Figure 8. Frequency of Tregs (CD3+CD4+CD25+) as a percentage of the total CD3+ T cell population is shown in both LN and PBMC before and after IL-15 treatment. hetIL-15 treatment, in contrast to IL-2, does not increase the frequency of Tregs.

[0027] Figure 9. hetIL-15 treatment does not affect the rate of B lymphocyte proliferation. The rate of proliferation (Ki67+) is shown for B lymphocytes (CD20+) in both lymph nodes and PBMC before and after IL-15 treatment.

[0028] Figure 10. hetIL-15 Increases PD-1 expression on CD8 cells in the LN. PD-1 expression data in LN lymphocytes from the 12 macaques included in F31a, b, c, d, f and g. % PD-1 positive cells within CD4, CD8 and Double Negative (DN) subpopulations.

[0029] Figure 11. hetIL-15 treatment induces a significantly higher presence of EM CD8+ T lymphocytes and cytotoxic T cells within the lymph nodes than SIV infection. Frequency of EM CD8+ T cells, Granzyme B+ CD8+ and CD4+ T cells within the lymph nodes of 8 vaccinated macaques (uninfected animals from the AUP490 study), 9 animals infected with SIVsmE660 (AUP417 study) and 12 animals treated with high dose hetIL-15. Samples from the infected macaques were taken 40 weeks after infection.

[0030] Figure 12. hetIL-15 treatment increases SIV-specific T cells within the lymph nodes. Frequency of SIV-specific cells (CM9 Tetramer+ CD8+ T cells) within the axillary

LN obtained before (left panel) or after hetIL-15 treatment (3 days after the last injection, right panel). Animal was treated with human hetIL-15 dose escalation protocol (six doses SC over 2 weeks, days 1, 3, 5, 8, 10, 12 at 2, 4, 8, 16, 32 and 64 μg/Kg of hetIL-15, respectively).

[0031] Figure 13. Increased frequency of CM9-Tetramer+ (Gag specific) CD8 T cells within the lymph nodes (left panels). The antigen-specific CD8+ T cells contain more Granzyme B upon hetIL-15 treatment and are actively dividing (right panels).

[0032] Figure 14. hetIL-15 treatment decreases CXCR5 surface receptor in all lymphocytes within the LN (and increases the levels of CXCL13 chemokine). Two SHIV-infected macaques were treated with 6 SC doses of human hetIL-15 at sequentially increasing doses of 2, 4, 8, 16, 32 and 64 μg/kg. Comparison of the frequency of Tfh cells (defined as CD4+CXCR5+PD-1high) in inguinal LNs obtained before and 3 days after the last injection of hetIL-15.

[0033] Figure 15. Decreased CXCR5 surface expression on B lymphocytes in lymph nodes from macaques treated with either low or high hetIL-15 dose escalation. Left panel: Low Dose hetIL-15 2-64 μg/kg (animal 5726, F31g study). Right panel: High Dose hetIL-15 5-120 μg/kg (animal P941).

[0034] Figure 16. hetIL-15 administration increased CXCL13 plasma levels. CXCL13 levels were measured before and after 2 weeks of hetIL-15 administration. Animals were treated with high dose hetIL-15 for 2 weeks. CXCL13 is the ligand of CXCR5.

[0035] Figure 17. Intraperitoneal delivery of IL-15 increases the CD8+ proliferative responses in the intestine. Intestinal tissue samples and liver samples obtained at necropsy from 10 animals treated with IL-15 subcutaneously (F31b, c, d, f and g) and two macaques (R678 and R680) treated Intraperitoneally with the same 6 dose protocol (Dose escalation 2-64 μg/kg). IP delivery of hetIL-15 increases the proliferative responses in many parts of the intestine.

[0036] Figure 18. Treatment of SIV infected macaques with 3-drug combination cART (Tenofovir (TFV), emtricitabine (FTC) and integase inhibitor Dolutegravir in a single combination, daily SC).

[0037] Figure 19. hetIL-15 Treatment of SIV-infected cART-treated macaques does not increase plasma virus load as monotherapy. Four macaques treated with cART for 7 months were treated with one or two cycles of 2-week high dose hetIL-15 (6 SC injections of 2-64 μg/kg) as indicated. Plasma viral load was measured by a sensitive assay (cutoff 2 RNA

copies/ml). High and effective doses of hetIL-15 can be safely administered with long term ART.

[0038] Figure 20 provides data showing an example of therapeutic vaccination of SIVmac-infected, cART-treated macaques with SIV p27CE pDNA.

[0039] Figure 21A-21C provides data showing DNA (A) and RNA (B) copies of SHIV found in the axilary (AxLN), inguinal (IngLN) lymph node or in PBMC before and after hetIL-15 treatment (6 sc injections , two weeks). (C) Plasma virus load measured at the day of hetIL-15 and one and two weeks later.

DETAILED DESCRIPTION OF ASPECTS OF THE DISCLOSURE

Terminology

[0040] As used herein, the terms "about" and "approximately," when used to modify a numeric value or numeric range, indicate that the numeric value or range as well as reasonable deviations from the value or range, typically 10% or 20% above and 10% or 20%below the value or range, are within the intended meaning of the recited value or range.

[0041] As used herein, the terms "disease" and "disorder" are used interchangeably to refer to a condition, in particular, a pathological condition. In certain embodiments, the terms "disease" and "disorder" are used interchangeably to refer to a disease affected by IL-15 signal transduction.

[0042] As used herein, *e.g.*, in the Examples section, the term "hetIL-15", typically refers to the non-covalently linked IL-15/solubleIL-15 Receptor alpha heterodimeric molecule that is naturally processed similar to the endogenous human cytokine. Although this form is a preferred form as applied in the present disclosure, it is understood that other forms of IL-15 have similar functions and can trigger the same effects when adjusting for the differences in pharmacokinetics and their intrinsic potency. These include, but not limited to, Fc fusions, single chain IL-15, mutant IL-15s, covalently linked and altered IL-15 heterodimers.

[0043] As used herein, the term "peak level" and "peak concentration" refer to the highest levels of free IL-15 in a sample (e.g., a plasma sample) from a subject over a period of time. In certain embodiments, the period of time is the entire period of time between the administration of one dose of IL-15/IL-15Ra complex and another dose of the complex. In some embodiments, the period of time is approximately 24 hours, approximately 48 hours or

approximately 72 hours after the administration of one dose of IL-15/IL-15Ra complex and before the administration of another dose of the complex.

[0044] As used herein, the terms "trough level" and "trough concentration" refer to the lowest levels of free IL-15 in a sample (e.g., a plasma sample) from a subject over a period of time. In certain embodiments, the period of time is the entire period of time between the administration of one dose of IL-15/IL-15Ra complex and another dose of the complex. In some embodiments, the period of time is approximately 24 hours, approximately 48 hours or approximately 72 hours after the administration of one dose of IL-15/IL-15Ra complex and before the administration of another dose of the complex.

[0045] As used herein, the term "normal levels" in the context of the concentration of free IL-15 refers to the concentration of free IL-15 found in a sample obtained or derived from a healthy subject. Basal plasma levels of free IL-15 in healthy subjects are approximately 1 pg/ml in humans and approximately 8-15 pg/ml in monkeys (such as macaques). Normal levels depend on the exact method used for measurement and may vary because of this.

[0046] As used herein, the phase "an effective ratio of IL-15 to lymphocyte cell number" means that the amount of IL-15 available for lymphocytes keeps pace with the number of lymphocytes so that lymphocytes continue proliferating or survive. In a specific embodiment, a trough concentration of approximately 1 pg/ml to 5 pg/ml, approximately 1 pg/ml to 10 pg/ml, approximately 1 pg/ml to 15 pg/ml, approximately 1 pg/ml to 20 pg/ml, approximately 1 to 25 pg/ml, approximately 1 pg/ml to 30 pg/ml, approximately 1 pg/ml to 40 pg/ml, or approximately 1 pg/ml to 50 pg/ml of free IL-15 in a plasma sample from a subject is indicative of "an effective ratio of IL-15 to lymphocyte cell number." In a specific embodiment, a trough concentration of below 50 pg/ml, below 45 pg/ml, below 40 pg/ml, below 35 pg/ml, below 30 pg/ml, below 25 pg/ml, below 20 pg/ml, below 15 pg/ml, below 10 pg/ml, below 5 pg/ml, or below 1 pg/ml of free IL-15 in a plasma sample from a subject is indicative of "an effective ratio of IL-15 to lymphocyte cell number." In another specific embodiment, a trough concentration above 50 pg/ml, 55 pg/ml, 60 pg/ml, 65 pg/ml, 70 pg/ml, 75 pg/ml, 80 pg/ml, 85 pg/ml, 90 pg/ml, 95 pg/ml, or 100 pg/ml of free IL-15 in a plasma sample from a subject is indicative that the ratio of IL-15 to lymphocyte cell number is excessive. In another specific embodiment, a trough concentration 50 pg/ml to 75 pg/ml, 60 pg/ml to 75 pg/ml, 75 pg/ml to 85 pg/ml, 75 pg/ml to 100 pg/ml, 85 pg/ml to 100 pg/ml or 50 pg/ml to 100 pg/ml of free IL-15 in a plasma sample from a subject is indicative that the ratio of IL-15 to lymphocyte cell number is excessive. Any method known to one skilled in the art for measuring free IL-15 concentration in a sample from a subject may be used, such as, e.g.,

an immunoassay. In a specific embodiment, an ELISA is used to measure the free IL-15 concentration in a sample from a subject.

[0047] As used herein, "hetIL-15" refers to a heterodimeric form of IL-15 in which IL-15 is complexed with IL-15Ra. The terms "hetIL-15" and "IL-15/IL-15Ra complex" are used interchangeably in this disclosure.

[0048] As used herein, the terms "native IL-15" and "native interleukin-15" in the context of proteins or polypeptides refer to any naturally occurring mammalian interleukin-15 amino acid sequences, including immature or precursor and mature forms. In the present invention, a native IL-15 is preferably a primate IL-15 sequence and is typically a human IL-15 sequence. Non-limiting examples of GeneBank Accession Nos. for the amino acid sequence of various species of native mammalian interleukin-15 include NP 000576 (human, immature form), CAA62616 (human, immature form), AAB60398 (macaca mulatta, immature form), AAI00964 (human, immature form), and AAH18149 (human). In one embodiment, the amino acid sequence of the immature/precursor form of native human IL-15, which comprises the long signal peptide (underlined) and the mature human native IL-15 (italicized), is provided:
MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEA*NWVNVISDLKKIE DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS* (SEQ ID NO:20). In some embodiments, native IL-15 is the immature or precursor form of a naturally occurring mammalian IL-15. In other embodiments, native IL-15 is the mature form of a naturally occurring mammalian IL-15. In a specific embodiment, native IL-15 is the precursor form of naturally occurring human IL-15. In another embodiment, native IL-15 is the mature form of naturally occurring human IL-15. In one embodiment, the native IL-15 protein/polypeptide is isolated or purified.

[0049] As used herein, the terms "native IL-15" and "native "interleukin-15" in the context of nucleic acids refer to any naturally occurring nucleic acid sequences encoding mammalian interleukin-15, including the immature or precursor and mature forms. Nonlimiting examples of Gene Bank Accession Nos. for the nucleotide sequence of various species of native mammalian IL-15 include NM_000585 (human). In one embodiment, the nucleotide sequence encoding the immature/precursor form of native human IL-15, which comprises the nucleotide sequence encoding the long signal peptide (underlined) and the nucleotide sequence encoding the mature human native IL-15 (italicized), is provided:
atgagaatttcgaaacca catttgagaa gtatttccat ccagtgctac ttgtgtttac ttctaaacag tcatttteta actgaagctg

gcattcatgtcttcattttg ggctgtttca gtgcagggct tcctaaaaca gaagccaact gggtgaatgt aataagtgat ttgaaaaaaattgaagatct tattcaatct atgcatattg atgctacttt atatacggaa agtgatgttc accccagttg caaagtaacagcaatgaagt gctttctctt ggagttacaa gttatttcac ttgagtccgg agatgcaagt attcatgata cagtagaaaa tctgatcatc ctagcaaaca acagtttgtc ttctaatggg aatgtaacag aatctggatg caaagaatgt gaggaactggaggaaaaaaa tattaaagaa tttttgcaga gttttgtaca tattgtccaa atgttcatca acacttcttg a (SEQ ID NO:21). In a specific embodiment, the nucleic acid is an isolated or purified nucleic acid. In some embodiments, nucleic acids encode the immature or precursor form of a naturally occurring mammalian IL-15. In other embodiments, nucleic acids encode the mature form of a naturally occurring mammalian IL-15. In a specific embodiment, nucleic acids encoding native IL-15 encode the precursor form of naturally occurring human IL-15. In another embodiment, nucleic acids encoding native IL-15 encode the mature form of naturally occurring human IL-15.

[0050] As used herein, the terms "IL-15 derivative" and "interleukin-15 derivative" in the context of proteins or polypeptides refer to: (a) a polypeptide that is at least 40%, 45%, 50%, 55%, 60%, 65%, typically at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to a native mammalian IL-15 polypeptide; (b) a polypeptide encoded by a nucleic acid sequence that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical a nucleic acid sequence encoding a native mammalian IL-15 polypeptide; (c) a polypeptide that contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid mutations (i.e., additions, deletions and/or substitutions) relative to a native mammalian IL-15 polypeptide; (d) a polypeptide encoded by nucleic acids that can hybridize under high, moderate or typical stringency hybridization conditions to nucleic acids encoding a native mammalian IL-15 polypeptide; (e) a polypeptide encoded by a nucleic acid sequence that can hybridize under high, moderate or typical stringency hybridization conditions to a nucleic acid sequence encoding a fragment of a native mammalian IL-15 polypeptide of at least 20 contiguous amino acids, at least 30 contiguous amino acids, at least 40 contiguous amino acids, at least 50 contiguous amino acids, at least 100 contiguous amino acids, or at least 150 contiguous amino acids; and/or (f) a fragment of a native mammalian IL-15 polypeptide. IL-15 derivatives also include a polypeptide that comprises the amino acid sequence of a naturally occurring mature form of a mammalian IL-15 polypeptide and a heterologous signal peptide amino acid sequence. In a specific embodiment, an IL-15 derivative is a derivative of a native human IL-15 polypeptide. In another embodiment, an IL-15 derivative is a derivative of an immature or precursor form of naturally occurring human IL-15 polypeptide. In another embodiment, an IL-15 derivative is a derivative of a mature

form of naturally occurring human IL-15 polypeptide. In another embodiment, an IL-15 derivative is the IL-15N72D described in, e.g., Zhu et al., 2009, J. Immunol. 183: 3598 or U.S. Patent No. 8,163,879. In another embodiment, an IL-15 derivative is one of the IL-15 variants described in U.S. Patent No. 8,163,879. In one embodiment, an IL-15 derivative is isolated or purified.

[0051] In a preferred embodiment, IL-15 derivatives retain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,90%, 95%, 98% or 99% of the function of native mammalian IL-15 polypeptide to bind IL-15Ra polypeptide, as measured by assays well known in the art, e.g., ELISA, Biacore, co-immunoprecipitation. In another preferred embodiment, IL-15 derivatives retain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% of the function of native mammalian IL-15 polypeptide to induce IL-15-mediated signal transduction, as measured by assays well-known in the art, e.g., electromobility shift assays, western blots, phosphoprotein analysis, ELISAs and other immunoassays. In a specific embodiment, IL-15 derivatives bind to IL-15Ra and/or IL-15Ra as assessed by, e.g., ligand/receptor binding assays well-known in the art.

[0052] Percent identity can be determined using any method known to one of skill in the art. In a specific embodiment, the percent identity is determined using the "Best Fit" or "Gap" program of the Sequence Analysis Software Package (Version 1 0; Genetics Computer Group, Inc., University of Wisconsin Biotechnology Center, Madison, Wisconsin). In a further specific embodiment, percent identity is determined using the BLAST algorithm. Information regarding hybridization conditions (e.g., high, moderate, and typical stringency conditions) has been described, see, e.g., U.S. Patent Application Publication No. US 2005/0048549 (e.g., paragraphs 72-73).

[0053] As used herein, the terms "IL-15 derivative" and "interleukin-15 derivative" in the context of nucleic acids refer to: (a) a nucleic acid sequence that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to the naturally occurring nucleic acid sequence encoding a mammalian IL-15 polypeptide; (b) a nucleic acid sequence encoding a polypeptide that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical the amino acid sequence of a native mammalian IL-15 polypeptide; (c) a nucleic acid sequence that contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleic acid base mutations (i.e., additions, deletions and/or substitutions) relative to the naturally occurring nucleic acid sequence encoding a mammalian IL-15 polypeptide; (d) a nucleic acid sequence that hybridizes under high, moderate or typical stringency hybridization conditions to a naturally

occurring nucleic acid sequence encoding a mammalian IL-15 polypeptide; (e) a nucleic acid sequence that hybridizes under high, moderate or typical stringency hybridization conditions to a fragment of a naturally occurring nucleic acid sequence encoding a mammalian IL-15 polypeptide; and/or (f) a nucleic acid sequence encoding a fragment of a naturally occurring nucleic acid sequence encoding a mammalian IL-15 polypeptide. In a specific embodiment, an IL-15 derivative in the context of nucleic acids is a derivative of a naturally occurring nucleic acid sequence encoding a human IL-15 polypeptide. In another embodiment, an IL-15 derivative in the context of nucleic acids is a derivative of a naturally occurring nucleic acid sequence encoding an immature or precursor form of a human IL-15 polypeptide. In another embodiment, an IL-15 derivative in the context of nucleic acids is a derivative of a naturally occurring nucleic acid sequence encoding a mature form of a human IL-15 polypeptide. In another embodiment, an IL-15 derivative in the context of nucleic acids is the nucleic acid sequence encoding the IL-15N72D described in, e.g., Zhu et al., 2009, *J. Immunol.* 183: 3598 or U.S. Patent No. 8,163,879. In another embodiment, an IL-15 derivative in the context of nucleic acids is the nucleic acid sequence encoding one of the IL-15 variants described in U.S. Patent No. 8,163,879.

[0054] IL-15 derivative nucleic acid sequences include codon-optimized/RNA-optimized nucleic acid sequences that encode native mammalian IL-15 polypeptide, including mature and immature forms of IL-15 polypeptide. In other embodiments, IL-15 derivative nucleic acids include nucleic acids that encode mammalian IL-15 RNA transcripts containing mutations that eliminate potential splice sites and instability elements (e.g., A/T or A/U rich elements) without affecting the amino acid sequence to increase the stability of the mammalian IL-15 RNA transcripts. In certain embodiments, the IL-15 derivative nucleic acid sequence is codon-optimized.

[0055] In a preferred embodiment, IL-15 derivative nucleic acid sequences encode proteins or polypeptides that retain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% of the function of a native mammalian IL-15 polypeptide to bind IL- 15Ra, as measured by assays well known in the art, e.g., ELISA, Biacore, coimmunoprecipitation or gel electrophoresis. In another preferred embodiment, IL-15 derivative nucleic acid sequences encode proteins or polypeptides that retain at least 50%, 55%,60%,65%,70%,75%, 80%, 85%,90%,95%,98% or 99% of the function of a native mammalian IL-15 polypeptide to induce IL-15-mediated signal transduction, as measured by assays well-known in the art, e.g., electromobility shift assays, ELISAs and other immunoassays. In a specific embodiment, IL-15 derivative nucleic acid sequences encode proteins or polypeptides that

bind to IL-15Ra and/or IL-15R~y as assessed by, e.g., ligand/receptor assays well-known in the art.

[0056] As used herein, the terms "IL-15" and "interleukin-15" refer to a native IL-15, an IL-15 derivative, or a native IL-15 and an IL-15 derivative.

[0057] As used herein, the terms "native IL-15Ra" and "native interleukin-15 receptor alpha" in the context of proteins or polypeptides refer to any naturally occurring mammalian interleukin-15 receptor alpha ("IL-15Ra") amino acid sequence, including immature or precursor and mature forms and naturally occurring isoforms. Non-limiting examples of GeneBank Accession Nos. for the amino acid sequence of various native mammalian IL-15Ra include NP 002180 (human), ABK41438 (Macaca mulatta), and CAI41082 (human). In one embodiment, the amino acid sequence of the immature form of the native full length human IL-15Ra, which comprises the signal peptide (underlined) and the mature human native IL-15Ra (italicized), is provided: MAPRRARGCR TLGLPALLLL LLLRPPATRG *ITCPPPMSVE HADIWVKSYSLYSRERYICN SGFKRKAGTS SLTECVLNKA TNVAHWTTPS LKCIRDPALV HQRPAPPSTVTTAGVTPQPE SLSPSGKEPA ASSPSSNNTA ATTAAIVPGS QLMPSKSPST GTTEISSHESSHGTPSQTTA KNWELTASAS HQPPGVYPQG HSDTTVAIST STVLLCGLSA VSLLACYLKS RQTPPLASVE MEAMEALPVT WGTSSRDEDL ENCSHHL* (SEQ ID NO:22). The amino acid sequence of the immature form of the native soluble human IL-15Ra, which comprises the signal peptide (underlined) and the mature human native soluble IL-15Ra (italicized), is provided: MAPRRARGCR TLGLPALLLL LLLRPPATRG *ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKA GTS SLTECVLNKA TNVAHWTTPS LKCIRDPALV HQRPAPPSTV TTAGVTPQPE SLSPSGKEPA ASSPSSNNTA ATTAAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQG* (SEQ ID NO:23). See below for further discussion regarding the immature and mature forms of human native soluble IL-15Ra. In some embodiments, native IL-15Ra is the immature form of a naturally occurring mammalian IL-15Ra polypeptide. In other embodiments, native IL-15Ra is the mature form of a naturally occurring mammalian IL-15Ra polypeptide. In certain embodiments, native IL-15Ra is the naturally occurring soluble form of mammalian IL-15Ra polypeptide. In other embodiments, native IL-15Ra is the full-length form of a naturally occurring mammalian IL-15Ra polypeptide. In a specific embodiment, native IL-15Ra is the immature form of a naturally occurring human IL-15Ra polypeptide. In another embodiment, native IL-15Ra is the mature form of a naturally occurring human IL-15Ra polypeptide. In certain embodiments, native IL-15Ra is the naturally occurring soluble form of human IL-15Ra polypeptide. In other

embodiments, native IL-15Ra is the full-length form of a naturally occurring human IL-15Ra polypeptide. In one embodiment, a native IL-15Ra protein or polypeptide is isolated or purified.

[0058] As used herein, the terms "native IL-15Ra" and "native interleukin-15 receptor alpha" in the context of nucleic acids refer to any naturally occurring nucleic acid sequences encoding mammalian interleukin-15 receptor alpha, including the immature or precursor and mature forms. Non-limiting examples of GeneBank Accession Nos. for the nucleotide sequence ofvarious species of native mammalian IL-15Ra include NM_002189 (human), and EF033114 (Macaca mulatta). In one embodiment, the nucleotide sequence encoding the immature form of native human IL-15Ra, which comprises the nucleotide sequence encoding the signal peptide (underlined) and the nucleotide sequence encoding the mature human native IL-15Ra (italicized), is provided: atggcccc gcggcgggcg cgcggctgcc ggaccctcgg tctccggccg ctgctactgc tgctgctgct ccggccgccg gcgacgcggg *gcatcacgtg ccctccccct atgtccgtgg aacacgcaga catctgggtc aagagctaca gcttgtactc cagggagcgg tacatttgtaactctggttt caagcgtaaa gccggcacgt ccagcctgac ggagtgcgtg ttgaacaagg ccacgaatgt cgcccactgg acaaccccca gtctcaaatg cattagagac cctgccctgg ttcaccaaag gccagcgcca ccctccacag taacgacggc aggggtgacc ccacagccag agagcctctc ccttctgga aaagagcccg cagcttcatc tcccagctca aacaacacag cggccacaac agcagctatt gtcccgggct cccagctgat gccttcaaaa tcacctttca caggaaccac agagataagc agtcatgagt cctcccacgg cacccccctct cagacaacag ccaagaactg ggaactcaca gcatccgcct cccaccagcc gccaggtgtg tatccacagg gccacagcga caccactgtg gctatctcca cgtccactgt cctgctgtgt gggctgagcg ctgtgtctct cctggcatgc taacttcaagt caaggcaaac tccccgctg gccagcgttg aaatggaagc catggaggct ctgccggtga cttgggggac cagcagcaga gatgaagact tggaaaactg ctctcaccac ctatga* (SEQ ID NO:24). The nucleotide sequence encoding the immature form of native soluble human IL-15Ra protein or polypeptide, which comprises the nucleotide sequence encoding the signal peptide (underlined) and the nucleotide sequence encoding the mature human soluble native IL-15Ra (italicized), is provided: atggcccc gcggcgggcg cgcggctgcc ggaccctcgg tctccggccg ctgctactgc tgctgctgct ccggccgccg gcgacgcggg *gcatcacgtg ccctccccct atgtccgtgg aacacgcaga catctgggtc aagagctaca gcttgtactc cagggagcgg tacatttgta actctggttt caagcgtaaa gccggcacgt ccagcctgac ggagtgcgtg ttgaacaagg ccacgaatgt cgcccactgg acaaccccca gtctcaaatg cattagagac cctgccctgg ttcaccaaag gccagcgcca ccctccacag taacgacggc aggggtgacc ccacagccag agagcctctc ccttctgga aaagagcccg cagcttcatc tcccagctca aacaacacag cggccacaac agcagctatt gtcccgggct cccagctgat gccttcaaaa tcacctttca caggaaccac agagataagc agtcatgagt cctcccacgg cacccccctct cagacaacag ccaagaactg ggaactcaca gcatccgcct cccaccagcc gccaggtgtg tatccacagg gc* (SEQ ID NO:25). In a specificembodiment, the nucleic acid is an isolated or

purified nucleic acid. In some embodiments, naturally occurring nucleic acids encode the immature form of a naturally occurring mammalian IL-15Ra polypeptide. In other embodiments, naturally occurring nucleic acids encode the mature form of a naturally occurring mammalian IL-15Ra polypeptide. In certain embodiments, naturally occurring nucleic acids encode the soluble form of a naturally occurring mammalian IL-15Ra polypeptide. In other embodiments, naturally occurring nucleic acids encode the full-length form of a naturally occurring mammalian IL-15Ra polypeptide. In a specific embodiment, naturally occurring nucleic acids encode the precursor form of naturally occurring human IL-15 polypeptide. In another embodiment, naturally occurring nucleic acids encode the mature of naturally occurring human IL-15 polypeptide. In certain embodiments, naturally occurring nucleic acids encode the soluble form of a naturally occurring human IL-15Ra polypeptide. In other embodiments, naturally occurring nucleic acids encode the full-length form of a naturally occurring human IL-15Ra polypeptide.

[0059] As used herein, the terms "IL-15Ra derivative" and "interleukin-15 receptor alpha derivative" in the context of a protein or polypeptide refer to: (a) a polypeptide that is at least 40%, 45%, 50%, 55%, 60%, 65%, typically at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to a native mammalian IL-15 polypeptide; (b) a polypeptide encoded by a nucleic acid sequence that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical a nucleic acid sequence encoding a native mammalian IL-15Ra polypeptide; (c) a polypeptide that contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid mutations (i.e., additions, deletions and/or substitutions) relative to a native mammalian IL-15Ra polypeptide; (d) a polypeptide encoded by a nucleic acid sequence that can hybridize under high, moderate or typical stringency hybridization conditions to a nucleic acid sequence encoding a native mammalian IL-15Ra polypeptide; (e) a polypeptide encoded by a nucleic acid sequence that can hybridize under high, moderate or typical stringency hybridization conditions to nucleic acid sequences encoding a fragment of a native mammalian IL-15 polypeptide of at least 20 contiguous amino acids, at least 30 contiguous amino acids, at least 40 contiguous amino acids, at least 50 contiguous amino acids, at least 100 contiguous amino acids, or at least 150 contiguous amino acids; (f) a fragment of a native mammalian IL-15Ra polypeptide; and/or (g) a specific IL-15Ra derivative described herein. IL-15Ra derivatives also include a polypeptide that comprises the amino acid sequence of a naturally occurring mature form of mammalian IL-15Ra polypeptide and a heterologous signal peptide amino acid sequence. In a specific embodiment, an IL-15Ra derivative is a derivative of a native human IL-15Ra polypeptide.

In another embodiment, an IL-15Ra derivative is a derivative of an immature form of naturally occurring human IL-15 polypeptide. In another embodiment, an IL-15Ra derivative is a derivative of a mature form of naturally occurring human IL-15 polypeptide. In one embodiment, an IL-15Ra derivative is a soluble form of a native mammalian IL-15Ra polypeptide. In other words, in certain embodiments, an IL-15Ra derivative includes soluble forms of native mammalian IL-15Ra, wherein those soluble forms are not naturally occurring. An example of an amino acid sequence of a truncated, soluble form of an immature form of the native human IL-15Ra comprises the following signal peptide (underlined) and the following truncated form of human native IL-15Ra (italicized): MAPRRARGCR TLGLPALLLL LLLRPPATRG *ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNVAHWTTPS LKCIRDPALV HQRPAPPSTV TTAGVTPQPE SLSPSGKEPA ASSPSSNNTA ATTAAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQG HSDTT* (SEQ ID NO:26). Other examples of IL-15Ra derivatives include the truncated, soluble forms of native human IL-15Ra described herein, or the sushi domain, which is the binding site to IL-15. In a specific embodiment, an IL-15Ra derivative is purified or isolated.

[0060] In a preferred embodiment, IL-15Ra derivatives retain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% of the function of a native mammalian IL-15Ra polypeptide to bind an IL-15 polypeptide, as measured by assays well known in the art, e.g., ELISA, Biacore, co-immunoprecipitation. In another preferred embodiment, IL-15Ra derivatives retain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% of the function of a native mammalian IL-15Ra polypeptide to induce IL-15-mediated signal transduction, as measured by assays well-known in the art, e.g., electromobility shift assays, ELISAs and other immunoassays. In a specific embodiment, IL-15Ra derivatives bind to IL-15 as assessed by methods well-known in the art, such as, e.g., ELISAs.

[0061] As used herein, the terms "IL-15Ra derivative" and "interleukin-15 receptor alpha derivative" in the context of nucleic acids refer to: (a) a nucleic acid sequence that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to the naturally occurring nucleic acid sequence encoding a mammalian IL-15Ra polypeptide; (b) a nucleic acid sequence encoding a polypeptide that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical the amino acid sequence of a native mammalian IL-15Ra polypeptide; (c) a nucleic acid sequence that contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleic acid mutations (i.e.,

additions, deletions and/or substitutions) relative to the naturally occurring nucleic acid sequence encoding a mammalian IL-15Ra polypeptide; (d) a nucleic acid sequence that hybridizes under high, moderate or typical stringency hybridization conditions to a naturally occurring nucleic acid sequence encoding a mammalian IL-15Ra polypeptide; (e) a nucleic acid sequence that hybridizes under high, moderate or typical stringency hybridization conditions to a fragment of a naturally occurring nucleic acid sequence encoding a mammalian IL-15Ra polypeptide; (f) a nucleic acid sequence encoding a fragment of a naturally occurring nucleic acid sequence encoding a mammalian IL-15Ra polypeptide; and/or (g) a nucleic acid sequence encoding a specific IL-15Ra derivative described herein. In a specific embodiment, an IL-15Ra derivative in the context of nucleic acids is a derivative of a naturally occurring nucleic acid sequence encoding a human IL-15Ra polypeptide. In another embodiment, an IL-15Ra derivative in the context of nucleic acids is a derivative of a naturally occurring nucleic acid sequence encoding an immature form of a human IL-15Ra polypeptide. In another embodiment, an IL-15Ra derivative in the context of nucleic acids is a derivative of a naturally occurring nucleic acid sequence encoding a mature form of a human IL-15Ra polypeptide. In one embodiment, an IL-15Ra derivative in the context of nucleic acids refers to a nucleic acid sequence encoding a derivative of mammalian IL-15Ra polypeptide that is soluble. In certain embodiments, an IL-15Ra derivative in context of nucleic acids refers to a nucleic acid sequence encoding a soluble form of native mammalian IL-15Ra, wherein the soluble form is not naturally occurring. In some embodiments, an IL-15Ra derivative in the context of nucleic acids refers to a nucleic acid sequence encoding a derivative of human IL-15Ra, wherein the derivative of the human IL-15Ra is a soluble form of IL-15Ra that is not naturally occurring. An example of an IL-15Ra derivative nucleic acid sequence is the nucleotide sequence encoding the truncated, soluble, immature form of a native human IL-15Ra protein or polypeptide that comprises the following nucleotide sequence encoding the signal peptide (underlined) and the following nucleotide sequence encoding a truncated form of the mature human native IL-15Ra (italicized): <u>atggcccc gcggcgggcg cgcggctgcc ggaccctcgg tctcccggcg ctgctactgc tgctgctgct ccggccgccg gcgacgcggg gc</u>*atcacgtg ccctcccccc atgtccgtgg aacacgcaga catctgggtc aagagctaca gcttgtactc cagggagcgg tacatttgta actctggttt caagcgtaaa gccggcacgt ccagcctgac ggagtgcgtg ttgaacaagg ccacgaatgt cgcccactgg acaaccccca gtctcaaatg cattagagac ctgcccctgg ttcaccaaag gccagcgcca ccctccacag taacgacggc aggggtgacc ccacagccag agagcctctc ccattctgga aaagagcccg cagcttcatc tcccagctca aacaacacag cggccacaac agcagctatt gtcccgggct cccagctgat gccttcaaaa tcacctccca caggaaccac agagataagc agtcatgagt cctcccacgg cacccccttt cagacaacag ccaagaactg ggaactcaca gcatccgcct cccaccagcc gccaggtgtg tatccacagg gccacagcga caccact* (SEQ ID NO:27).

[0062] In specific embodiments, an IL-15Ra derivative nucleic acid sequence is isolated or purified. IL-15Ra derivative nucleic acid sequences include RNA or codon-optimized nucleic acid sequences that encode native IL-15Ra polypeptide, including mature and immature forms of IL-15Ra polypeptide. In other embodiments, IL-15Ra derivative nucleic acids include nucleic acids that encode IL-15Ra RNA transcripts containing mutations that eliminate potential splice sites and instability elements (e.g., A/T or A/U rich elements) without affecting the amino acid sequence to increase the stability of the IL-15Ra RNA transcripts.

[0063] In a preferred embodiment, IL-15Ra derivative nucleic acid sequences encode proteins or polypeptides that retain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% of the function of a native mammalian IL-15Ra polypeptide to bind IL-15, as measured by assays well known in the art, e.g., ELISA, Biacore, coimmunoprecipitation. In another preferred embodiment, IL-15Ra derivative nucleic acid sequnces encode proteins or polypeptides that retain at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% of the function of a native mammalian IL-15Ra to induce IL-15-mediated signal transduction, as measured by assays well-known in the art, e.g., electromobility shift assays, ELISAs and other immunoassays. In a specific embodiment, IL-15Ra derivative nucleic acid sequences encode proteins or polypeptides that bind to IL-15 as assessed by methods well-known in the art, such as, e.g., ELISAs.

[0064] As used herein, the terms "IL-15Ra" and "interleukin-15 receptor alpha" refer to a native IL-15Ra, an IL-15Ra derivative, or a native IL-15Ra and an IL-15Ra derivative.

[0065] As used herein, the term "IL-15/IL-15Ra complex" refers to a complex comprising IL-15 and IL-15Ra covalently or noncovalently bound to each other. In a preferred embodiment, the IL-15Ra has a relatively high affinity for IL-15, e.g., a Kd of 10 to 50 pM as measured by a technique known in the art, e.g., KinEx A assay, plasma surface resonance (e.g., BIAcore assay). In another preferred embodiment, the IL-15/IL-15Ra complex induces IL-15-mediated signal transduction, as measured by assays well-known in the art, e.g., electromobility shift assays, ELISAs and other immunoassays. In some embodiments, the IL-15/IL-15Ra complex retains the ability to specifically bind to the βγ chain. In a specific embodiment, the IL-15/IL-15Ra complex is isolated from a cell.

[0066] As used herein, the terms "subject" and "patient" are used interchangeably and refer to a mammal, such as a non-primate (e.g., cows, pigs, horses, cats, dogs, rats etc.) and a primate (e.g., monkey and human), most preferably a human.

[0067] As used herein, the terms "purified" and "isolated" in the context of a compound or agent (including, e.g., proteinaceous agents) that is chemically synthesized refers to a compound or agent that is substantially free of chemical precursors or other chemicals when chemically synthesized. In a specific embodiment, the compound or agent is 60%, 65%, 75%, 80%, 85%, 90%, 95%, or 99% free (by dry weight) of other, different compounds or agents.

[0068] As used herein, the terms "purified " and "isolated" when used in the context of a compound or agent (including proteinaceous agents such as polypeptides) that can be obtained from a natural source, e.g., cells, refers to a compound or agent which is substantially free of contaminating materials from the natural source, e.g., cellular materials from the natural source, such as but not limited to cell debris, cell wall materials, membranes, organelles, the bulk of the nucleic acids, carbohydrates, proteins, and/or lipids present in cells. The phrase "substantially free of natural source materials" refers to preparations of a compound or agent that has been separated from the material (e.g., cellular components of the cells) from which it is isolated. Thus, a compound or agent that is isolated includes preparations of a compound or agent having less than about 30%, 20%, 10%, 5%, 2%, or 1% (by dry weight) of cellular materials and/or contaminating materials.

[0069] An "isolated" nucleic acid sequence or nucleotide sequence is one which is separated from other nucleic acid molecules which are present in a natural source of the nucleic acid sequence or nucleotide sequence. Moreover, an "isolated", nucleic acid sequence or nucleotide sequence, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or substantially free of chemical precursors when chemically synthesized. In certain embodiments, an "isolated" nucleic acid sequence or nucleotide sequence is a nucleic acid sequence or nucleotide sequence that is recombinantly expressed in a heterologous cell.

[0070] In some embodiments, the terms "nucleic acid", "nucleotide" and "polynucleotide" refer to deoxyribonucleotides, deoxyribonucleic acids, ribonucleotides, and ribonucleic acids, and polymeric forms thereof, and include either single- or double-stranded forms. In certain embodiments, such terms include known analogues of natural nucleotides, for example, peptide nucleic acids ("PNA"s), that have similar binding properties as the reference nucleic acid. In some embodiments, such terms refer to deoxyribonucleic acids (e.g., cDNA or DNA). In other embodiments, such terms refer to ribonucleic acid (e.g., mRNA or RNA).

[0071] As used herein, the terms "protein(s)" and "polypeptide(s)" interchangeably to refer to a chain of amino acids linked together by peptide bonds. In some embodiments, the terms

"protein(s)" and "polypeptide(s)" refer to a macromolecule which comprises amino acids that are linked together by peptide bonds.

[0072] As used herein, the terms "therapies" and "therapy" can refer to any protocol(s), method(s), compositions, formulations, and/or agent(s) that can be used in the prevention, treatment, management, or amelioration of a disease, e.g., cancer, infectious disease, lymphopenia, and immunodeficiencies, or a symptom associated therewith. In certain embodiments, the terms "therapies" and "therapy" refer to biological therapy, supportive therapy, and/or other therapies useful in treatment, management, prevention, or amelioration of a disease or a symptom associated therewith known to one of skill in the art.

[0073] As used herein, the term "in combination" refers to the use of more than one therapy (e.g., one or more prophylactic and/or therapeutic agents). The use of the term "in combination" does not restrict the order in which therapies are administered to a subject with a disease or disorder. A first therapy (e.g., a prophylactic or therapeutic agent) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (e.g., a prophylactic or therapeutic agent) to a subject with a disease or disorder or a symptom thereof.

[0074] As used herein, the term "host cell" refers to any type of cell, e.g., a primary cell or a cell from a cell line. In specific embodiments, the term "host cell" refers a cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0075] As used herein, the terms "treat", "treating" and "treatment" in the context of the administration of a therapy to a subject refer to the beneficial effects that a subject derives from a therapy. Non-limiting examples of such benefits include the reduction or inhibition of the progression, spread and/or duration of a disease or disorder, the reduction or amelioration of the severity of a disease or disorder, amelioration of one or more symptoms of a disease or

disorder, and/or the reduction in the duration of one or more symptom of a disease or disorder resulting from the administration of one or more therapies.

[0076] As used herein, the terms "prevent,"" preventing" and "prevention" in the context of the administration of a therapy to a subject refer to the inhibition of the onset or recurrence of a disease or disorder in a subject.

[0077] As used herein, the terms "manage," "managing," and "management," in the context of the administration of a therapy to a subject, refer to the beneficial effects that a subject derives from a therapy, which does not result in a cure of a disease or disorder. In certain embodiments, a subject is administered one or more therapies to "manage" a disease or disorder so as to prevent the progression or worsening of symptoms associated with a disease or disorder.

[0078] When a dose of an IL-15/IL-15Ra complex is referenced herein, the dose is according to the mass of the single-chain IL-15. The single-chain IL-15 equivalent is calculated from (i) the mass of an IL-15/IL-15Ra complex by amino acid analysis and (ii) the ratio of IL-15 to IL-15Ra (e.g., soluble IL-15Ra) in the specific preparation as determined experimentally by RP-HPLC or by amino acid analysis.

Terminology relating to conserved element vaccines

[0079] A "conserved region" as used herein refers to a protein sequence that is conserved across a protein that has high sequence diversity in nature, e.g., a viral protein such as HIV Gag or HIV Env. A "conserved region" need not have 100% sequence identity across the diversity of naturally occurring sequence of the protein, but the amino acid sequence variability in the naturally occurring conserved region sequences is low, typically 10% or less. A "conserved element" in the context of the present invention is a segment of a conserved region that is usually at least 8 amino acids in length. In some embodiments, a "conserved element" is greater than 8 amino acids in length, e.g., 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 or more amino acids in length. Typically, a conserved element is less than 50 amino acids in length. A "conserved element" need not be 100% conserved across the diversity of sequences, e.g., HIV Gag sequences or HIV Env sequences. As noted above, the sequence variability in the naturally occurring conserved element sequence is low, however, typically 10% or less.

[0080] A "conserved element pair" in the context of this invention as it relates to a conserved element immunogenic composition, e.g., conserved elements of HIV Gag or HIV Env, refers to two versions of a conserved element sequence that have amino acid changes relative to one another such that the two sequence together cover at least 90% of naturally occurring sequences. For example, for HIV Env, the conserved element pair may cover at least 90% of the variants belonging to the HIV-1 M group.

[0081] A "nucleic acid vaccine" as used herein includes both naked nucleic acid vaccines, e.g., plasmid DNA vaccines, and viral vector-based nucleic acid vaccines that are comprised by a viral vector and/or delivered as viral particles.

[0082] An "immunogen" refers to a molecule, typically a protein molecule in the current invention, containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. Normally, an epitope will comprise between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "immunogen" includes isolated immunogens as well as inactivated organisms, such as viruses.

DETAILED DESCRIPTION OF SOME EMBODIMENTS OF THE DISCLOSURE

[0083] In certain aspects, the methods described herein are based, in part, on the discovery of a new dose escalation schedule for the administration of IL-15/IL-15Ra complexes. Such complexes can be used to treat any disorder as described herein. In some embodiments, administration of IL-15/IL-15Ra is in conjunction with other therapeutic agents, such as a conserved element vaccine, *e.g.,* a conserved element vaccine for HIV Gag or HIV Env.

[0084] In a further aspect, the invention provides a method of assessing effects on patient's lymph nodes or other tissues by measuring specific parameters including the number of lymphocytes in specific areas and their properties, specificities and expression characteristics using technologies such as flow and multiplexed confocal imagin (MCI). In certain aspects the effects of hetIL-15 in lymph nodes are measured by measuring the level of CXCR5 on B cells (which express it universally) or T cells locally or in the blood. In certain aspects the effects of hetIL-15 in the LN and tissues are evaluated by measuring the increase of CXCL13 locally or in plasma or serum. Yet in other aspects the function of hetIL-15 is evaluated by measuring increase in IL-18 locally or in the plasma or serum of patient.

IL-15/IL-15Rα complexes

[0085] IL-15/IL-15Ra complexes aministered in accordance with the invention may comprise naturally occurring forms of IL-15Ra and IL-15 or truncated forms of native proteins that retain activity. The IL-15-/Ra and IL-15 polypeptides in the complex may be non-covalently or covalently linked.

IL-15Rα

[0086] IL-15Ra contained in an IL-15/IL-15R complex administered in accordance with the invention may comprise the naturally occurring soluble form of human IL-15Ra or specific IL-15Ra derivatives that are truncated, soluble forms of human IL-15Ra. These specific IL-15Ra derivatives and the naturally occurring soluble form of human IL-15Ra are based, in part, on the identification of the proteolytic cleavage site of human IL-15Ra.

[0087] The proteolytic cleavage of membrane-bound human IL-15Ra takes place between Gly170 and His171 in human IL-15Ra (Chertova et al., 2013, Journal of Biological Chemistry 288(25):18093-103). Thus, the proteolytic cleavage of human IL-15Ra takes place between the residues (i.e., Gly170 and His171) which are shown in bold and underlined in the provided amino acid sequence of the immature form of the native full length human IL-15Ra: MAPRRARGCR TLGLP ALLLL LLLRPP ATRG ITCPPPMSVE HADIWVKSYSL YSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL VHQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT AAIVPGSQLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQ**GH**SDTTV AIST STVLLCGLSA VSLLACYLKS RQTPPLASVE MEAMEALPVT WGTSSRDEDL ENCSHHL (SEQ ID NO:28).

[0088] Accordingly, in one aspect, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra), wherein the amino acid sequence of the soluble form of human IL-15Ra terminates at the site of the proteolytic cleavage of the native membrane-bound human IL-15Ra. In particular, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra), wherein the amino acid sequence of the soluble form of human IL-15Ra terminates with PQG, wherein G is Gly170 of the mature form of human IL-15Ra. In particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: MAPRRARGCR TLGLP ALLLL LLLRPP ATRG ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL VHQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT

AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQG (SEQ ID NO:23). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence and (ii) terminates with the amino acid sequence PQG. In other particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKATNV AHWTTPS LKCIRDPAL VHQRP APPSTV TTAGVTPQPE SLSPSGKEPA ASSPSSNNTA ATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQG (SEQ ID NO:29). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence, and, optionally, wherein the amino acid sequence of the soluble form of the IL-15Ra derivative terminates with PQG.

[0089] In certain embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra), wherein the amino acid sequence of the soluble form of human IL-15Ra terminates with PQGH (SEQ ID NO:30), wherein H is His171 of mature human IL-15Ra. In particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: MAPRRARGCR TLGLPALLLL LLLRPPATRG ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL V HQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQGH (SEQ ID NO:31). In some embodiments, provided herein is a soluble form of IL-15Ra having a sequence ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL V HQRP APPSTV TTAGVTPQPE SLSPSGKEPA ASSPSSNNTA ATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQGH (SEQ ID NO:32). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% to a sequence in this paragraph and (ii) terminates with the amino acid sequence PQGH (SEQ ID NO:30).

[0090] In certain embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra), wherein the amino acid sequence of the soluble form of human IL-15Ra terminates with PQGHS (SEQ ID NO:33), wherein S is Ser172 of mature human IL-15Ra. In particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: MAPRRARGCR TLGLPALLLL LLLRPPATRG ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNVAHWTTPS LKCIRDP AL V HQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWEL TASAS HQPPGVYPQGHS (SEQ ID NO:34). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence; and (ii) terminates with the amino acid sequence PQGHS (SEQ ID NO:33). In other particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL V HQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQGHS (SEQ ID NO:35). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to to this sequence and (ii) terminates with the amino acid sequence PQGHS (SEQ ID NO:33).

[0091] In certain embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra), wherein the amino acid sequence of the soluble form of human IL-15Ra terminates with PQGHSD (SEQ ID NO:36), wherein D is Asp173 of mature human IL-15Ra. In particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: MAPRRARGCR TLGLPALLLL LLLRPPATRG ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNVAHWTTPS LKCIRDP AL V HQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWEL TASAS HQPPGVYPQGHSD (SEQ ID NO:37). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a

polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence; and (ii) terminates with the amino acid sequence PQGHSD (SEQ ID NO:36). In other particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL V HQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQGHSD (SEQ ID NO:38). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence and(ii) terminates with the amino acid sequence PQGHSD (SEQ ID NO:36).

[0092] In certain embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra), wherein the amino acid sequence of the soluble form of human IL-15Ra terminates with PQGHSDT (SEQ ID NO:39), wherein T is Thr174 of mature human IL-15Ra. In particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: MAPRRARGCR TLGLPALLLL LLLRPPATRG ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNVAHWTTPS LKCIRDP AL V HQRP APPSTV TTAGVTPQPE SLSPSGKEPA ASSPSSNNTA ATTAAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWEL TASAS HQPPGVYPQGHSDT (SEQ ID NO:40). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence; and (ii) terminates with the amino acid sequence PQGHSDT (SEQ ID NO:39). In other particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL V HQRP APPSTVTTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATTAAIVPGS QLMPSKSPSTGTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQGHSDT (SEQ ID NO: 41). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%

identical to this sequence; and (ii) terminates with the amino acid sequence PQGHSDT (SEQ ID NO:39).

[0093] In certain embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra), wherein the amino acid sequence of the soluble form of human IL-15Ra terminates with PQGHSDTT (SEQ ID NO:42), wherein T is Thr175 of mature human IL-15Ra. In particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: MAPRRARGCR TLGLPALLLL LLLRPPATRG ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNVAHWTTPS LKCIRDP AL V HQRP APPSTV TTAGVTPQPE SLSPSGKEP A ASSPSSNNTAATT AAIVPGS QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWEL TASASHQPPGVYPQGHSDTT (SEQ ID NO:26). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence; and (ii) terminates with the amino acid sequence PQGHSDTT (SEQ ID NO:42). In other particular embodiments, provided herein is a soluble form of human IL-15Ra (e.g., a purified soluble form of human IL-15Ra) which has the following amino acid sequence: ITCPPPMSVE HADIWVKSYS LYSRERYICN SGFKRKAGTS SLTECVLNKA TNV AHWTTPS LKCIRDPAL V HQRP APPSTVTTAGVTPQPE SLSPSGKEP A ASSPSSNNTA ATT AAIVPGS QLMPSKSPSTGTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQGHSDTT (SEQ ID NO:43). In some embodiments, provided herein is an IL-15Ra derivative (e.g., a purified and/or soluble form of an IL-15Ra derivative), which is a polypeptide that: (i) is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to this sequence; and (ii) terminates with the amino acid sequence PQGHSDTT (SEQ ID NO:42).

[0094] In some embodiments, provided herein is an IL-15Ra derivative of naturally occurring human IL-15Ra, wherein the IL-15Ra derivative is soluble and: (a) the last amino acids at the C-terminal end of the IL-15Ra derivative consist of amino acid residues PQGHSDTT (SEQ ID NO:42), wherein T is at the C-terminal end of the amino acid sequence; (b) the last amino acids at the C-terminal end of the IL-15Ra derivative consist of amino acid residues PQGHSDT (SEQ ID NO:39), wherein Tis at the C-terminal end of the amino acid sequence; (c) the last amino acids at the C-terminal end of the IL-15Ra derivative consist of amino acid residues PQGHSD (SEQ ID NO:36), wherein D is at the C-terminal

end of the amino acid sequence; (d) the last amino acids at the C-terminal end of the IL-15Ra derivative consist of amino acid residues PQGHS (SEQ ID NO:33), wherein S is at the C-terminal end of the amino acid sequence; or (e) the last amino acids at the C-terminal end of the IL-15Ra derivative consist of amino acid residues PQGH (SEQ ID NO:30), wherein H is at the C-terminal end of the amino acid sequence. In some embodiments, provided herein is an IL-15Ra derivative of a naturally occurring human IL-15Ra, wherein the IL-15Ra derivative: is soluble and terminates with the amino acid sequence PQG, wherein G is at the C-terminal end of the amino acid sequence of the IL-15Ra derivative. In some embodiments, these IL-15Ra derivatives are purified.

[0095] Additional IL-15Ra derivative are described in WO2016/018920, each of which is incorporated by referenced herein. Such IL-15Ra derivatives include those in which the cleavage site for an endogenous protease that cleaves native IL-15Ra has been mutated, and various forms of glycosylated IL-15Ra.

IL-15

[0096] IL-15 contained in an IL-15/IL-15Ra complex administered in accordance with the present invention may be any mammalian IL-15, but is preferably human IL-15. Human IL-15 sequences are known, *supra*. IL-15 administered as a component of hetIL-15 comprises the mature protein, *e.g.*, mature native human IL-15: NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDAS IHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:44). Mature IL-15 can be produced using a variety of methods, including, but not limited to, those described in WO2011/020047, WO2007084342, and WO2016/018920, each of which is incorporated by reference.

Therapeutic agents

[0097] The IL-15 dose escalation treatment regimens as described herein comprise administration of IL-15 as an IL-15/IL-15Ra complex. Such complexes bind to the βγ subunits of the IL-15 receptor, induce IL-15 signal transduction (e.g., Jak/Stat signal transduction) and enhance IL-15-mediated immune function, wherein the complexes comprise IL-15 covalently or noncovalently bound to interleukin-15 receptor alpha ("IL-15Ra") ("IL-15/IL-15Ra complexes").

[0098] The IL-15/IL-15Ra complexes may be composed of native IL-15 or an IL-15 derivative and native IL-15Ra or an IL-15Ra derivative. In certain embodiments, an IL-

15/IL-15Ra complex comprises native IL-15 or an IL-15 derivative and an IL-15Ra described herein. In a specific embodiment, an IL-15/IL-15Ra complex comprises native IL-15 or an IL-15 derivative and IL-15Ra with the amino acid sequence of SEQ ID NOS:29, 32, 35, 38, 41, or 43. In another embodiment, an IL-15/IL-15Ra complex comprises native IL-15 or an IL-15 derivative and a glycosylated form of IL-15Ra.

[0099] In a specific embodiment, an IL-15/IL-15Ra complex comprises native IL-15 or an IL-15Ra derivative and native soluble IL-15Ra (e.g., native soluble human IL-15Ra). In another specific embodiment, an IL-15/IL-15Ra complex comprises native IL-15 and native soluble IL-15Ra. In another specific embodiment, an IL-15/IL-15Ra complex is composed of an IL-15 derivative and an IL-15Ra derivative. In another embodiment, an IL-15/IL-15Ra complex is composed of native IL-15 and an IL-15Ra derivative. In one embodiment, the IL-15Ra derivative is a soluble form of IL-15Ra. Specific examples of soluble forms of IL-15Ra are described herein. In a specific embodiment, the soluble form of IL-15Ra lacks the transmembrane domain of native IL-15Ra, and optionally, the intracellular domain of native IL-15Ra. In another embodiment, the IL-15Ra derivative is the extracellular domain of native IL-15Ra or a fragment thereof. In certain embodiments, the IL-15Ra derivative is a fragment of the extracellular domain comprising the sushi domain or exon 2 of native IL-15Ra. In some embodiments, the IL-15Ra derivative comprises a fragment of the extracellular domain comprising the sushi domain or ex on 2 of native IL-15Ra and at least one amino acid that is encoded by ex on 3. In certain embodiments, the IL-15Ra derivative comprises a fragment of the extracellular domain comprising the sushi domain or exon 2 of native IL-15Ra and an IL-15Ra hinge region or a fragment thereof. In certain embodiments, the IL-15Ra comprises the amino acid sequence ofan IL-15ra sequence described herein. In certain embodiments, the IL-15Ra is the native soluble human IL-15Ra.

[0100] In another embodiment, the IL-15Ra derivative comprises a mutation in the extracellular domain cleavage site that inhibits cleavage by an endogenous protease that cleaves native IL-15Ra. As discussed in Section 5.1, supra, the extracellular cleavage site of native IL-15Ra has been identified. In a specific embodiment, the extracellular domain cleavage site of IL-15Ra is replaced with a cleavage site that is recognized and cleaved by a heterologous known protease. Non-limiting examples of such heterologous protease cleavage sites include Arg-X-X-Arg , which is recognized and cleaved by furin protease; and A-B-Pro-Arg-X-Y (A and B are hydrophobic amino acids, and X and Y are nonacidic amino acids) and Gly-Arg-Gly, which are recognized and cleaved by thrombin protease.

[0101] In a specific embodiment, the IL-15Ra is encoded by a nucleic acid sequence optimized to enhance expression of IL-15Ra, e.g., using methods as described in U.S. Provisional Application No. 60/812,566, filed on June 9, 2006; International Patent Application Publication Nos. WO 2007/084342 and WO 2010/020047; and U.S. Patent Nos. 5,965,726; 6,174,666; 6,291,664; 6,414,132; and 6,794,498, which are incorporated by reference herein in their entireties. In another embodiment, the IL-15 is encoded by a nucleic acid sequence optimized to enhance expression of IL-15, e.g., using methods as described in U.S. Provisional Application Nos. 60/812,566, filed on June 9, 2006 and 60/758,819, filed on January 13, 2006, and International Patent Application Publication Nos. WO 2007/084342 and WO 2010/020047; and U.S. Patent Nos. 5,965,726; 6,174,666; 6,291,664; 6,414,132; and 6, 794,498, which are incorporated by reference herein in their entireties.

[0102] In addition to IL-15 and IL-15Ra, the IL-15/IL-15Ra complexes may comprise a heterologous molecule, such as an antigen associated with a disease that one intends to prevent treat and/or manage. Non-limiting examples of viral, bacterial, parasitic, and cancer antigens are described in WO 2016/018920, which examples are incorporated by referenced herein. In other embodiments, the heterologous molecule is an antibody that specifically binds to an antigen associated with a disease that one intends to prevent, treat and/or manage (e.g., an antibody that specifically binds to a viral antigen, bacterial antigen, parasitic antigen, or cancer antigen). Non-limiting examples are described in WO2016 018920, which examples are incorporated by referenced herein. In some embodiments, the heterologous molecule increases protein stability. Non-limiting examples of such molecules include polyethylene glycol (PEG), Fc domain of an IgG immunoglobulin or a fragment thereof, or albumin that increase the half-life of IL-15 or IL-15Ra *in vivo*. In certain embodiments, IL-15Ra is conjugated/fused to the Fc domain of an immunoglobulin (e.g., an IgG 1) or a fragment thereof. In certain embodiments, the heterologous molecule is not an Fc domain of an immunoglobulin molecule or a fragment thereof.

[0103] In those IL-15/IL-15Ra complexes comprising a heterologous molecule, the heterologous molecule may be conjugated to IL-15 and/or IL-15Ra. In one embodiment, the heterologous molecule is conjugated to IL-15Ra. In another embodiment, the heterologous molecule is conjugated to IL-15.

[0104] The components of an IL-15/IL-15Ra complex may be directly fused, using either non-covalent bonds or covalent bonds (e.g., by combining amino acid sequences via peptide bonds), and/or may be combined using one or more linkers. In a specific embodiment, IL-15 and IL-15Ra are directly fused to each other using either non-covalent bonds or covalent

bonds (e.g., by combining amino acid sequences via peptide bonds), and/or may be combined using one or more linkers. In specific embodiments, a polypeptide comprising IL-15 and IL-15Rα directly fused to each other using either non-covalent bonds or covalent bonds is functional (e.g., capable of specifically binding to the IL-15Rα complex and inducing IL-15-mediated signal transduction and/or IL-15-mediated immune function). Linkers suitablefor preparing the IL-15/IL-15Ra complexes comprise peptides, alkyl groups, chemically substituted alkyl groups, polymers, or any other covalently-bonded or non-covalently bonded chemical substance capable of binding together two or more components. Polymer linkers comprise any polymers known in the art, including PEG. In some embodiments, the linker is a peptide that is 1, 2, 3, 4, 5,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids long. In a specific embodiment, the linker is long enough to preserve the ability of IL-15 to bind to the IL-15Ra. In other embodiments, the linker is long enough to preserve the ability of the IL-15/IL-15Ra complex to bind to the βγ receptor complex and to act as an agonist to mediate IL-15 signal transduction. In a specific embodiment, the IL-15/IL-15Ra complex is a fusion protein, such as RLI and ILR, disclosed in U.S. Patent Application Publication No. 2009/0238791 and Mortier et al., 2006, J. Biol. Chern. 281(3):1612-9.

[0105] In particular embodiments, IL-15/IL-15Ra complexes are pre-coupled prior to use in the methods described herein (e.g., prior to contacting cells with the IL-15/IL-15Ra complexes or prior to administering the IL-15/IL-15Ra complexes to a subject). In other embodiments, the IL-15/IL-15Ra complexes are not pre-coupled prior to use in the methods described herein. In specific embodiments, the IL-15/IL-15Ra complex is administered in combination with a vaccine composition to enhance the immune response elicited by the administration of the vaccine composition to a subject. In a specific embodiment, a therapeutic agent comprising IL-15 and IL-15Ra directly fused to each other is administered in combination with a vaccine composition to enhance an immune response elicited by administration of the vaccine composition to a subject.

[0106] In a specific embodiment, a therapeutic agent comprising IL-15 and IL-15Ra enhances or induces immune function in a subject by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the immune function in a subject not administered the therapeutic agent using assays well known in the art, e.g., ELISPOT, ELISA, and cell proliferation assays. In a specific embodiment, the immune function is cytokine release (e.g., interferon-gamma, IL-2, IL-5, IL-10, IL-12, or transforming growth factor (TGF)-beta). In one embodiment, the IL-15

mediated immune function is NK cell proliferation, which can be assayed, e.g., by flow cytometry to detect the number of cells expressing markers of NK cells (e.g., CD56). In another embodiment, the IL-15 mediated immune function is antibody production, which can be assayed, e.g., by ELISA. In some embodiments, the IL-15 mediated immune function is effector function, which can be assayed, e.g., by a cytotoxicity assay or other assays well known in the art.

[0107] In specific embodiments, examples of immune function enhanced by a therapeutic agent comprising IL-15/IL-15Ra include the proliferation/ expansion of lymphocytes (e.g., increase in the number of lymphocytes), inhibition of apoptosis of lymphocytes, activation of dendritic cells (or antigen presenting cells), and antigen presentation. In particular embodiments, an immune function enhanced by the therapeutic agent is proliferation/expansion in the number of or activation of CD4 + T cells, CD8+ T cells (e.g., cytotoxic T lymphocytes, alpha/beta T cells, and gamma/delta T cells), memory T cells, dendritic cells (immature or mature), antigen presenting cells, macrophages, mast cells, tumor-resident T cells, CD122+ T cells, or natural killer cells (NK cells). In one embodiment, the therapeutic agent enhances the proliferation/expansion or number of lymphocyte progenitors. In some embodiments, a therapeutic agent increases the number of CD4+ T cells CD8+ T cells (e.g., cytotoxic T lymphocytes, alpha/beta T cells, and gamma/delta T cells), memory T cells, dendritic cells (immature or mature), antigen presenting cells, macrophages, mast cells, tumor-resident T cells, CD122+ T cells, or natural killer cells (NK cells) by approximately 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 20 fold, or more relative a negative control (e.g., number of the respective cells not treated, cultured, or contacted with a therapeutic agent). In specific embodiments, examples of immune function enhanced by a therapeutic agent comprising IL-15/IL-15Ra include homing of lymphocytes to tissues of interest including but not limited to tumor sites, lymph nodes gut lymphoid tissue.

[0108] IL-15 and IL-15Ra can be expressed and produced as described in WO2016/018920, which sequences, constructs, expression methods, and host cells are incorporated by reference herein.

[0109] The nucleic acid construct(s) encoding IL-15 and/or IL-15Ra can be administered in vivo to a mammal or transfected into primary or immortalized cells in culture. Such a nucleic acid construct(s) can be used to enhance IL-15-mediated function and/or to prevent, treat and/or manage a disease in which enhancement of IL-15-mediated function is beneficial, such as the diseases described herein. The nucleic acid constructs comprising nucleic acids

encoding IL-15 and/or IL-15Ra can be used to generate cells that express IL-15 and/or IL-15Ra. In some embodiments, the cells are primary cells (e.g., tumor cells isolated from a patient). In other embodiments, the cells are mammalian cell lines.

[0110] The host cells chosen for expression of nucleic acids will depend upon the intended use of the cells. Factors such as whether a cell glycosylates similar to cells that endogenously express, e.g., IL-15 and/or IL-15Ra, may be considered in selecting the host cells.

[0111] In one embodiment, cell lines are engineered to express both IL-15 and soluble IL-15Ra, and the purified stable heterodimer of the IL-15 and soluble IL-15Ra, which can be used in vitro or in vivo, e.g., can be administered to a human. In certain embodiments, cell lines are engineered to express both native human IL-15 and native human IL-15Ra, and the stable heterodimer of native human IL-15 and native soluble human IL-15Ra which is formed can be purified, and this purified heterodimer can be used be administered to a human. In one embodiment, the stability of IL-15 is increased when produced from cell lines recombinantly expressing both IL-15 and IL-15Ra.

[0112] In a specific embodiment, the host cell recombinantly expresses IL-15 and the full-length IL-15Ra. In another specific embodiment, the host cell recombinantly expresses IL-15 and the soluble form of IL-15Ra. In another specific embodiment, the host cell recombinantly expresses IL-15 and a membrane-bound form of IL-15Ra which is not cleaved from the surface of the cell and remains cell associated. In some embodiments, the host cell recombinantly expressing IL-15 and/or IL-15Ra (full-length or soluble form) also recombinantly expresses another polypeptide (e.g., a cytokine or fragment thereof).

[0113] The host cells can be used to produce IL-15 and/or IL-15Ra. The recombinant porteins can be purified by any technique. In some embodiments, IL-15 and IL-15Ra are synthesized or recombinantly expressed by different cells and subsequently isolated and combined to form an IL-15/IL-15Ra complex, *in vitro*, prior to administration to a subject. In other embodiments, IL-15 and IL-15Ra are synthesized or recombinantly expressed by different cells and subsequently isolated and simultaneously administered to a subject an IL-15/IL-15Ra complex *in situ* or *in vivo*. In yet other embodiments, IL-15 and IL-15Ra are synthesized or expressed together by the same cell, and the IL-15/IL-15Ra complex formed is isolated.

Therapeutic Compositions

[0114] Provided herein are compositions comprising the therapeutic agents comprising IL-15 and IL-15Ra. The compositions include bulk drug compositions useful in the manufacture of pharmaceutical compositions (e.g., impure or nonsterile compositions) and pharmaceutical compositions (i.e., compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. The compositions (e.g., pharmaceutical compositions) comprise an effective amount of a therapeutic agent or a combination of therapeutic agents and a pharmaceutically acceptable carrier. In specific embodiments, the compositions (e.g., pharmaceutical compositions) comprise an effective amount of one or more therapeutic agents and pharmaceutically acceptable carrier. In some embodiments, the composition further comprises an additional therapeutic agent, including, but not limited to, e.g., anti-cancer agent, anti-viral agent, anti-inflammatory agent, vaccine, or adjuvant.

[0115] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (e.g., Freund's adjuvant (complete and incomplete) or, more preferably, MF59C.1 adjuvant available from Chiron, Emeryville, CA), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. In one embodiment, water is a carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0116] Pharmaceutical compositions may be formulated in any conventional manner using one or more pharmaceutically acceptable carriers or excipients. In a specific embodiment, a

therapeutic agent is administered to a subject in accordance with the methods described herein is administered as a pharmaceutical composition.

[0117] Generally, the components of the pharmaceutical compositions comprising therapeutic agents comprising IL-15 and IL-15Ra as described herein are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the therapeutic agent is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline (e.g., PBS). Where the therapeutic agent is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0118] In some embodiments, therapeutic agents comprising IL-15 and IL-15Ra may be formulated for administration by any method known to one of skill in the art, including but not limited to, parenteral (e.g., subcutaneous, intravenous, intraperitoneal, or intramuscular) and intratumoral administration. In one embodiment, the therapeutic agents are formulated for local or systemic parenteral administration. In a specific embodiment, the therapeutic agents are formulated for subcutaneous, intraperiotoneal, or intravenous administration. In one embodiment, the therapeutic agents are formulated in a pharmaceutically compatible solution.

[0119] The therapeutic agents can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient (i.e., therapeutic agent) may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

Dose Escalation Regimens for Prophylactic and Therapeutic Uses

[0120] In one aspect, provided herein are methods for enhancing IL-15-mediated immune function, comprising administering to a subject agents that induce IL-15 signal transduction and enhance IL-15-mediated immune function in a dose escalation regimen. More specifically, provided herein are methods for enhancing IL-15-mediated immune function, comprising administering to subjects in a dose escalation regimen complexes that bind to the

βγ subunits of the IL-15 receptor, induce IL-15 signal transduction and enhance IL-15-mediated immune function, wherein the complexes comprise IL-15 covalently or noncovalently bound to IL-15Ra (referred to herein as "IL- 15/IL-15Ra complexes"). Since enhancing IL-15-mediated immune function is beneficial for the prevention, treatment and/or management of certain disorders, such as lymphopenia, cancer, and infectious diseases, provided herein are methods for the prevention, treatment and/or management of such disorders comprising administering to a subject in need thereof IL-15/IL-15Ra complexes in a dose escalation regimen. Further, provided herein are methods for eradicating or reducing HIV in HIV-infected cells in a subject comprising administering to a subject in need thereof IL-15/IL-15Ra complexes in a dose escalation regimen.

[0121] In one aspect of the invention, provided herein is a method for preventing, treating and/or managing disorders in a subject, wherein enhancement of IL-15-mediated immune function is beneficial for the prevention, treatment and/or management of such disorders, the method comprising (a) administering at least one initial low dose of an IL-15/IL-15Ra complex to a subject; and (b) administering successively higher doses of the IL-15/IL-15Ra complex to the subject to achieve an effective ratio of IL-15 to lymphocyte cell number. In certain aspects of the present invention, the successively higher doses follow a Fibonacci sequence, which, not to be bound by theory, is in order to avoid accumulation of excess cytokine that may occur upon repeated injections while providing sufficient cytokine for lymphocyte growth and maintenance. Thus, in some embodiments, provided herein is a method for preventing, treating and/or managing lymphocytopenia, cancer or an infectious disease, *e.g.*, HIV, in a subject, method comprising (a) administering at least one initial low dose of an IL-15/IL-15Ra complex to the subject; and (b) administering successively higher doses of the IL-15/IL-15Ra complex to the subject to achieve an effective ratio of IL-15 to lymphocyte cell number, wherein the successively higher doses follow a Fibonacci sequence. In another specific embodiment, provided herein is a method for eradicating or reducing HIV in HIV-infected cells in a subject, the method comprising (a) administering at least one initial low dose of an IL-15/IL-15Ra complex to the subject; and (b) administering successively higher doses of the IL-15/IL-15Ra complex to the subject achieve an effective ratio of IL-15 to lymphocyte cell number, wherein the successively higher doses follow a Fibonacci sequence. In a particular embodiment, the subject is a human subject.

[0122] In a specific embodiment, the initial dose of IL-15/IL-15Ra is about 0.5 μg/kg. The subsequent doses are 1 μg/kg, 2 μg/kg, 4 μg/kg, 8 μg/kg, and 16 μg/kg; or the initial dose may be about 1 μg/kg and subsequent doses are 2 μg/kg, 4 μg/kg, 8 μg/kg, 16 μg/kg, and 32

μg/kg. Thus, in some embodiments, the dose doubles over an injection series. For example, a two-week series of six subcutaneous injections (*e.g.*, MWFMWF):

0.5	1	2	4	8	16
1	2	4	8	16	32.

In some embodiments, the doses are administered intravenously.

[0123] Not to be bound by theory, but in macaques the "rate of use" of hetIL-15 upon subcutaneous injection using a doubling-the-dose protocol results in excess hetIL-15 in plasma at the nadir time point (before the next injection). In humans, application of alternative dose escalation schemes in subsequent doses aims to maximize hetIL-15 availability to lymphocytes and minimize side effects associated with free excess hetIL-15.

[0124] Reference to hetIL-15 is indicative of one form of IL-15/IL-15Ra molecules but similar principles apply and for any other forms of IL-15/IL-15Ra either covalently or non-covalently linked. These forms are included in the concept and must be covered in the claims.

[0125] For humans, a dose escalation based on a Fibonacci sequence dose escalation can be employed. Thus, for example, an initial dose may be about 0.5 μg/kg to about 5 μg/kg as determined based on the mass of hetIL-15, *e.g.*, 1 μg/kg. Subsequent dosages are then administered based on a Fibonacci sequence, *e.g.*, 3 μg/kg, 5 μg/kg, 8 μg/kg, and 13 μg/kg. In some embodiments, an initial dose may be about 2 μg/kg (as determined based on the mass of hetIL-15) and subsequent doses are 3 μg/kg, 5 μg/kg, 8 μg/kg, 13 μg/kg, and 21 μg/kg. For example, a two-week series of six subcutaneous injections (MWFMWF) :

1	2	3	5	8	13
2	3	5	8	13	21

In a second example, the above six subcutaneous injections can be administered in 3 week regimen, *e.g.*, every 3 or 4 days (MThMThMTh), In some embodiments, disease are administered intravenously.

[0126] In some embodiments, hetIL-15 may be administered as a two-week series of four subcutaneous injections (*e.g.*, MThMTh). For example, the following amounts (μg/kg) may be administered subcutaneously:

1	2	4	8
2	4	8	16

4	8	16	32
2	3	5	8
3	5	8	13
2	5	13	34.

In some embodiments, doses are administered intravenously.

[0127] In some embodiment, the initial low dose is in the range of 0.5 μg/kg to 5 μg/kg, as determined based on the mass of hetIL-15, and subsequent doses are increased based on a Fibonacci sequence. In some embodiment, the initial low dose is in the range of 0.1 μg/kg to 5 μg/kg, as determined based on the mass of hetIL-15, and subsequent doses are increased based on a Fibonacci sequence. In some embodiment, the initial low dose is in the range of 0.1 μg/kg to 1 μg/kg, as determined based on the mass of hetIL-15, and subsequent doses are increased based on a Fibonacci sequence. In another embodiment, the initial low dose is about 0.1 μg/kg as determined based on the mass of hetIL-15. In another embodiment, the initial low dose is about 0.2 μg/kg, 0.3 μg/kg, 0.4 μg/kg, 0.5 μg/kg, 0.6 μg/kg, 0.7 μg/kg, 0.8 μg/kg, 0.9 μg/kg, 1.0 μg/kg, 1.1 μg/kg, 1.2 μg/kg, 1.3 μg/kg, 1.4 μg/kg, 1.5 μg/kg, 1.6 μg/kg, 1.7 μg/kg, 1.8 μg/kg , 1.9 μg/kg , or 2 μg/kg, and the subsequent disease are administered based on a Fibonacci series. In certain embodiments, the doses in the dose escalation are administered every day, often every 2 days, or 2 to 3 days and in some embodiments, every 4 days or every week. In some embodiments, the doses are administered over a 1-week or over a 2-week period. In some embodiments, a subject is administered a dose three times per 7-day week (e.g., Monday, Wednesday and Friday). In certain embodiments, the subject is monitored for one, two, or more, or all of the following: (i) signs of an enlarged lymph node(s); (ii) signs of an enlarged spleen; (iii) levels of free IL-15 in a sample (e.g., plasma sample) from the subject; (iv) changes (e.g., increases) in body temperature; (v) changes (e.g., decreases) in blood pressure; (vi) changes (e.g., increases) in cytokines, such as pro-inflammatory cytokines (e.g., IL-l and IL-6) in a sample (e.g., blood sample) from the subject; (vii) elevation of liver enzymes, such as hepatic transaminases (e.g., alanine aminotransferase (ALT) or aspartate aminotransferase (AST)); and/or (viii) adverse events, such as grade 3 or 4 thrombocytopenia, grade 3 or 4 granulocytopenia, grade 3 or 4 leukocytosis (White Blood Cell (WBC) > 100,000 mm3), grade 3 or 4 decreases in WBC, absolute lymphocyte count (ALC) and/or absolute neutrophil count (ANC), lymphocytosis and organ dysfunction (e.g., liver or kidney dysfunction). In specific embodiments, the dose

is not increased if the trough concentration of free IL-15 in a sample (e.g., plasma sample) from the subject is above 50 pg/ml, 55 pg/ml, 60 pg/ml, 65 pg/ml, 70 pg/ml, 75 pg/ml, 80 pg/ml, 85 pg/ml, 90 pg/ml, 95 pg/ml, or 100 pg/ml. In specific embodiments, the dose is not increased if the trough concentration of free IL-15 in a sample (e.g., plasma sample) from the subject is 50 pg/ml to 75 pg/ml, 60 pg/ml to 75 pg/ml, 75 pg/ml to 85 pg/ml, 75 pg/ml to 100 pg/ml, 85 pg/ml to 100 pg/ml or 50 pg/ml to 100 pg/ml.

[0128] In certain embodiments, the dose is not increased and the dose may remain the same, be stopped or reduced if the subject experiences adverse events. In some embodiments, the method further comprises administering a maintenance dose of the IL-15/IL-15Ra complex to the subject, wherein the maintenance dose reaches trough levels of free IL-15 of approximately 1 pg/ml to approximately 5 pg/ml, approximately 2 pg/ml to approximately 5 pg/ml, approximately 2 pg/ml to approximately 10 pg/ml, approximately 5 pg/ml to approximately 10 pg/ml, approximately 10 pg/ml to approximately 15 pg/ml, approximately 10 pg/ml to approximately 20 pg/ml, approximately 20 pg/ml to approximately 30 pg/ml, approximately 30 pg/ml to approximately 40 pg/ml, or approximately 40 pg/ml to approximately 50 pg/ml, or approximately 5 pg/ml to approximately 50 pg/ml in a sample (e.g., a plasma sample) from the subject. In a specific embodiment, the maintenance dose is equal to or less than the highest dose received by the subject during the dose escalation phase of the therapeutic regimen which does not result in one, two, or more adverse events. In a specific embodiment, the maintenance dose reaches trough levels of plasma IL-15 that are close to normal levels (approximately 1 pg/ml plasma). In some embodiments, the maintenance dose is 0.1 μg/kg, 0.5 μg/kg, 1.0 μg/kg, 2 μg/kg, 3 μg/kg, 4 μg/kg, 5 μg/kg, 6 μg/kg, 7 μg/kg, 8 μg/kg, 9 μg/kg, 10 μg/kg, 11 μg/kg, 12 μg/kg, 13 μg/kg, 14 μg/kg, 15 μg/kg, 16 μg/kg, 1 7 μg/kg, 18 μg/kg, 19 μg/kg, or a higher maintenance dose as described in WO2016/018920, which doses are incorporated by reference herein. In other embodiments, the maintenance dose is between 0.1 μg/kg to 5 μg/kg, 0.1 μg/kg to 10 μg/kg, 2 μg/kg to 5 μg/kg, 2 μg/kg to 10 μg/kg, 5 μg/kg to 10 μg/kg, 5 μg/kg to 15 μg/kg, 10 μg/kg g to 15 μg/kg, 0.1 μg/kg to 20 μg/kg, 15 μg/kg to 20 μg/kg, 15 μg/kg to 25 μg/kg. In certain embodiments, the same dose of IL-15/IL-15Ra complex is administered to the subject continuously for a certain period of time (e.g., days, weeks, months, or years) as the maintenance dose. In other embodiments, the dose of IL-15/IL-15Ra complex administered to the subject as the maintenance dose is gradually decreased so that the elevated lymphocytes (in number and activation) in the subject gradually return to physiological conditions.

[0129] In some embodiments, successive doses, *e.g.*, administered in a Fibonacci sequence, are administered if the concentration of free IL-15 in a sample (e.g., a plasma sample) obtained from the subject a certain period of time after the administration of a dose of the IL-15/IL-15Ra complex and before administration of another dose of the IL-15/IL-15Ra complex (e.g., approximately 24 hours to approximately 48 hours, approximately 24 hours to approximately 36 hours, approximately 24 hours to approximately 72 hours, approximately 48 hours to approximately 72 hours, approximately 36 hours to approximately 48 hours, or approximately 48 hours to 60 hours after the administration of a dose of the IL-15/IL-15Ra complex and before the administration of another dose of the IL-15/IL-15Ra complex) is within normal levels or less than normal levels.

[0130] In specific embodiments, in accordance with the methods described herein, a subject may be treated using more than one treatment regimen, i.e., a treatment regimen as described herein may be preceded or follow by a treatment regimen that varies in the dose and/or frequency of administration. For example, in some embodiments, a two-week dose escalation treatment regimen employing dosages based on Fibonacci sequence may be preceded or followed by a different regimen that may involve a single dose or for escalating doses, doses that are doubled or otherwise not based on a Fiboniacci sequence.

[0131] In specific embodiments, the methods described herein are not cyclical in nature. In other words, in specific embodiments, the methods described herein may not include a cyclical administration regimen, wherein the cycle comprises administering a dose of the IL-15/IL-15Ra complex for a certain period of time (e.g., 1 to 4 weeks) followed by another period of time when the subject is not administered a dose of the IL-15/IL-15Ra complex (e.g., 1 week to 2 months) and this cycle is repeated any number of times (e.g, the cycle is repeated 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times).

[0132] In certain embodiments, the IL-15/IL-15Ra complex is administered subcutaneously to a subject in accordance with the methods described herein. In some embodiments, the IL-15/IL-15Ra complex is administered intravaneously or intramuscularly to a subject in accordance with the methods described herein. In certain embodiments, the IL-15/IL-15Ra complex is administered intratumorally to a subject having a tumor in accordance with the methods described herein. In some embodiments, the IL-15/IL-15Ra complex is administered locally to a site (e.g., a tumor or site of infection) in a subject in accordance with the methods described herein. In certain embodiments, the IL-15/IL-15Ra complex is administered intraperiotneally.

[0133] In accordance with the methods described herein, the IL-15/IL-15Ra complex can be administered to a subject in a pharmaceutical composition. In certain embodiments, the IL-15/IL-15Ra complex is sole/single agent administered to the subject. In other embodiments, the IL-15/IL-15Ra complex is administered in combination with one or more other therapies, *e.g.*, antibody that targets a tumor, a checkpoint inhibitor, and the like. Combination therapy includes concurrent and successive administration of an IL-15/IL-15Ra complex and another therapy. As used herein, an IL-15/IL-15Ra complex and another therapy are said to be administered concurrently if they are administered to the patient on the same day, for example, simultaneously, or 1, 2, 3, 4, 5, 6, 7, or 8 hours apart. In contrast, the IL-15/IL-15Ra complex and the therapy are said to be administered successively if they are administered to the patient on the different days, for example, the IL-15/IL-15Ra complex and the therapy can be administered at a 1-day, 2-day, 3-day intervals, or 4-day intervals. Administration of the IL-15/IL-15Ra complex can precede or follow administration of the second therapy. When administered simultaneously, the hetIL-15 and the other therapy can be in the same pharmaceutical composition or in a different pharmaceutical composition

[0134] In specific embodiments, examples of immune function enhanced by the methods described herein include the proliferation/ expansion of lymphocytes (e.g., increase in the number of lymphocytes), inhibition of apoptosis of lymphocytes, activation of dendritic cells(or antigen presenting cells), and antigen presentation. In particular embodiments, an immune function enhanced by the methods described herein is proliferation/expansion in the number of or activation of CD4 + T cells, CD8+ T cells (e.g., cytotoxic T lymphocytes, alpha/beta T cells, and gamma/delta T cells), memory T cells, dendritic cells (immature or mature), antigen presenting cells, macrophages, mast cells, tumor-resident T cells, CD122+ T cells, or natural killer cells (NK cells). In one embodiment, the methods described herein enhance the proliferation/expansion or number of lymphocyte progenitors. In some embodiments, the methods described herein increases the number of CD4+ T cells, CD8+ T cells (e.g., cytotoxic T lymphocytes, alpha/beta T cells, and gamma/delta T cells), memory T cells, dendritic cells (immature or mature), antigen presenting cells, macrophages, mast cells, tumor-resident T cells, CD122+ T cells, or natural killer cells (NK cells) by approximately 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 20 fold, or more relative a negative control (e.g., number of the respective cells not treated, cultured, or contacted with a hetIL-15 therapeutic agent). In one embodiment, an immune function enhanced by the methods described herein is the change in phenotype of T follicular helper cells (Tfh), an important cell type in LN that is persistently infected by HIV/SIV.

[0135] In some embodiments, the methods described herein enhance or induce immune function in a subject by at least 0.2 fold, 0.5 fold, 0.75 fold, 1 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold 9 fold, or at least 10 fold relative to the immune function in a subject not administered the hetIL-15 using assays well known in the art, e.g., ELI SPOT, ELISA, and cell proliferation assays. In a specific embodiment, the methods described herein enhance or induce immune function in a subject by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the immune function in a subject not administered the Therapeutic Agent using assays well known in the art, e.g., ELISPOT, ELISA, and cell proliferation assays. In a specific embodiment, the immune function is cytokine release (e.g., interferon-gamma, IL-2, IL-5, IL-10, IL-12, or transforming growth factor (TGF) -beta). In one embodiment, the IL-15 mediated immune function is NK cell proliferation, which can be assayed, e.g., by flow cytometry to detect the number of cells expressing markers of NK cells (e.g., CD56). In one embodiment, the IL-15 mediated immune function is CD8+ T cell proliferation, which can be assayed, e.g., by flow cytometry. In another embodiment, the IL-15 mediated immune function is antibody production, which can be assayed, e.g., by ELISA. In some embodiments, the IL-15 mediated immune function is effector function, which can be assayed, e.g., by a cytotoxicity assay or other assays. In some embodiments the IL-15 mediated function is change of localization and number of CD8 cells, NK cells CD4 cells, alpha/beta T cells, gamma/delta T cells, B cells (e.g., plasma cells), memory T cells, memory B cells, dendritic cells (immature or mature), antigen presenting cells, macrophages, mast cells, natural killer T cells (NKT cells), tumor-resident T cells, T follicular helper cells, follicular dendritic cells, or CD122+ T cells from a specific anatomic area such as liver, lymph node, intestine, or tumor site. Such changes can be detected by histology, immunohistochemistry, flow cytometry, histo-cytometry in vivo imaging, or other methods.

[0136] The effect of one or more doses of one or more IL-15/IL-15Ra complexes on peripheral blood lymphocyte counts can be monitored/assessed using standard techniques known to one of skill in the art. Peripheral blood lymphocytes counts in a mammal can be determined by, e.g., obtaining a sample of peripheral blood from said mammal, separating the lymphocytes from other components of peripheral blood such as plasma using, e.g., Ficoll-Hypaque (Pharmacia) gradient centrifugation, and counting the lymphocytes using trypan blue. Peripheral blood T -cell counts in mammal can be determined by, e.g., separating the lymphocytes from other components of peripheral blood such as plasma, labeling the T-cells

with an antibody directed to aT-cell antigen such as CD3, CD4, and CD8 which is conjugated to FITC or phycoerythrin, and measuring the number ofT-cells by FACS. Further, the effect on a particular subset of T cells (e.g., CD2+, CD4+, CD8+, CD4+RO+, CD8+RO+, CD4+RA+, or CD8+RA +) or NK cells can be determined using standard techniques known to one of skill in the art such as FACS.

[0137] The plasma levels of IL-15 can be assessed using standard techniques known to one of skill in the art. For example, a plasma can be obtained from a blood sample obtained from a subject and the levels of IL-15 in the plasma can be measured by ELISA.

Monitoring hetIL-15 response

[0138] In some aspects, the disclosure provides methods of monitoring hetIL-15 response. This monitoring step can be performed when hetIL-15 is administered for the treatment of a tumor or a viral infection, such as HIV infection. Monitoring is typically performed routinely during therapy to measure responsiveness of a patient of IL-15.

[0139] In some embodiments, monitoring is performed by evaluating tissue, blood, plasma or serum markers indicative of IL-15 function. In some embodiments, levels of Interleukin-18 (IL-18) or CXCL13 chemokine levels in the blood of a patient that has received hetIL-15 are measured. CXCL13 levels can be assessed in a blood, plasma, or serum sample using any methods. Typically, concentrations are measured by immunoassay. CXCL13 or IL-18 levels are compared to a control, typically levels in the patient prior to hetIL-15 treatment to determine whether CXCL13 or IL-18 levels increase. An increase in IL-18 or CXCL13 level is indicative of hetIL-15 response. In some embodiments, the CXCL3 or IL-18 level is compared to a control value from normal individuals.

[0140] In some embodiments, CXCL13 biomarker is used to test effectiveness of hetIL-15 or to adjust or terminate dosing of hetIL-15. In typical treatment regimens, CXCL13 is increased in treated patients by at least 10%, 20%, 30%, 40%, 50%, 100%, or greater than 100%, relative to normal blood levels or the levels found in the individual patient prior to treatment. Normal levels may be, for example, in the order of 30-40 pg/ml as assessed by a commercially available kit, *e.g.*, a Quantikine kit (R&D Systems) or Thermo Fisher kit, according to the manufacturer's instructions.

[0141] Serum concentrations of soluble CXCL13 protein can be determined using any method, for example, commercially available immunoassay kits. For this illustrative assays, after development of the enzyme-linked immunosorbent assay (ELISA) plates, absorbance is

read at 450 nm. The concentration of CXCL13 in the sera is interpolated from a standard curve, which is generated using the respective recombinant protein.

[0142] In some embodiments, monitoring is performed by evaluating CXCR5 expression levels on lymphocytes, including T lymphocytes or B lymphocytes. The B lymphocytes may be obtained from lymph nodes, but are conveniently obtained from the blood. CXCR5 expression can be determined using any method. In some embodiments, cell surface CXCR5 expression is evaluated, *e.g.*, by an immunoassay such as flow cytometry, and/or a capture assay. In some embodiments, CXCR5 RNA expression in B lymphocytes is determined, *e.g.*, by quantitative PCR. A decrease in CXCR5 level is indicative of hetIL-15 response. In some embodiments, CXCR5 levels are evaluated in comparison to levels expressed on B lymphocytes in the patient prior to hetIL-15 treatment. In some embodiments, levels are determined relative to a normal control value. In some embodiments, CXCR5 levels are determined in conjunction with determining plasma CXCL13 levels.

Administration of hetIL-15 and conserved element vaccines.

[0143] In some embmodiments, hetIL-15 is administered in conjunction with a therapeutic vaccination. An example of a therapeutic vaccine is conserved element vaccine for the treatment of HIV infection. Administration of one or more polypeptides comprising conserved elements, separated by non-naturally occurring linkers and collinearly arranged, from an immunogen of interest, e.g., a viral antigen such as HIV Gag or HIV Env, can provide an enhanced immune response when one or more conserved element nucleic acid constructions is administered to a subject as a prime followed by co-administration to the subject of a nucleic acid construct encoding a full-length antigen, or substantially a full-length antigen, with the conserved element construct(s) as a boost. In typical embodiments, the prime and/or boost components of an immunization protocol are administered as nucleic acids that encode the polypeptides, although in some embodiments, prime and/or boost immunization components are administered as polypeptides.

[0144] hetIL-15 can be administered in conjunction with conserved element vaccines to enhance the immune response. In typical embodiments, HetIl-15 is administered to a human at escalating doses in a Fibonacci series.

[0145] Conserved element vaccines are described, *e.g.*, in WO 2013/131099, which is herein incorporated by reference. In some embodiments, a conserved element vaccine that is administered in accordance with the invention comprises a nucleic acid, *e.g.*, a DNA, encoding a conserved element polypeptide comprising conserved elements that each have a

sequence set forth in SEQ ID NOS:1-7. In some embodiments, a conserved element vaccine that is administered in accordance with the invention comprises a nucleic acid, *e.g.*, a DNA, encoding a conserved element polypeptide comprising conserved elements that each have a sequence set forth in SEQ ID NOS:8-14. In some embodiments, a conserved element vaccine comprises administering two nucleic acid constructs, *e.g.*, DNA constructs, wherein one construct encodes a conserved element polypeptide comprising conserved elements that each have a sequence set forth in SEQ ID NOS:1-7 and the second construct encodes a conserved element polypeptide comprising conserved elements that each have a sequence set forth in SEQ ID NOS:8-14. In some embodiments, the conserved elements vaccines comprise a CE conserved element polynucleotide comprising SEQ ID NO:15 and a conserved element polypeptide comprising SEQ ID NO:16.

[0146] A conserved element nucleic acid construct is employed in an immunization regimen that also employs a nucleic acid encoding full-length protein, *e.g.*, gag or substantially full-length protein, from which the conserved elements are obtained. In the context of the present invention, "substantially full-length" refers to the region of the protein that includes all of the conserved elements, i.e., a sufficient length of a naturally occurring protein is provided that includes all of the conserved elements that are used in the conserved element construct.

[0147] A nucleic acid construct encoding a full-length protein, or substantially full-length protein, is administered following administration of the one or more constructs encoding the CE polypeptide(s), such that the CE polypeptide(s) acts as a prime and the full-length polypeptide, or substantially full-length polypeptide is a boost. In some embodiments, the boost comprises administering the full-length, or substantially full-length polypeptide along with another administration of the CE polypeptide pair. The boost is typically administered anywhere from one, two, three, or four months, one year, or longer, following administration of the initial priming vaccines. Multiple boost vaccinations may be used, and different full-length proteins may be used in a sequence of boosts. A priming vaccination can itself be one or multiple administrations of the polypeptide(s). CE polypeptides and a full-length polypeptide, or substantially full-length polypeptide, is often administered to the host by way of administration of expression constructs that encode the polypeptides, although in some embodiments, the polypeptides are administered in a protein form.

[0148] Nucleic acids encoding multiple CE polypeptides, typically two CE polypeptides, i.e., a conserved element polypeptide pair, are administered in combination. In the context of the current invention, nucleic acid constructs encoding CE polypeptides "administered in

combination" may be administered together or separately. For example, a nucleic acid construct encoding a second CE polypeptide may be administered after (e.g., anywhere from 1 minutes to 60 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, or up to 2 weeks) administration of a first nucleic acid construct encoding a CE polypeptide, but is typically administered at the same time as the first nucleic acid construct. In some embodiments, the nucleic acid construct encoding the second CE polypeptide is administered within 24 hours of administration of the nucleic acid construct encoding the first CE polypeptide.

[0149] Similarly, for co-administration of one or more CE polypeptides with the full-length, or substantially full-length polypeptide, administered as the boost, the co-administration may be formed by administering the constructs together, or they may be administered separately. For example, one or more nucleic acid constructs encoding a CE polypeptide(s) may be administered shortly before (e.g., anywhere from 1 minutes to 60 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, usually within 24 hours) or after, a nucleic acid construct encoding the full-length polypeptide, or the substantially full-length polypeptide.

[0150] In typical embodiments, the one or more CE nucleic acid constructs are co-delivered with a nucleic acid construct encoding a full-length, or substantially full-length protein. In the context of this invention, "co-delivery" refers to administering the nucleic acid constructions together at the same site, e.g., administering them in the same mixture.

[0151] In some embodiments, a nucleic acid immunization regimen in accordance with the invention comprises performing at least two priming administrations with one or more CE nucleic acids constructs, which encode a conserved element pair, either on separate vectors or the same vector, followed by performing at least two boosting administrations of the CE nucleic acid construct(s) co-delivered with the construct encoding the full-length polypeptide or substantially full-length polypeptide. In some embodiments, priming vaccinations can be performed at least two weeks apart. In some embodiments, priming vaccinations are performed at least one month apart or separated by several months. Boost vaccinations are administered at least one month, often at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months; or 1 or more years after the priming vaccinations.

[0152] Nucleic acid constructs may be employed as plasmid expression vectors or may be administered as a virus. In some embodiments, the nucleic acid constructs encoding the

conserved elements and/or full-length polypeptides are one or more purified nucleic acid molecules, for example, one or more DNA plasmid-based vectors ("naked" DNA).

[0153] In some embodiments, hetIL-15 administration is a vaccine enhancer and is administered after therapeutic vaccination to maximize immune response and deliver the immune response to areas of virus reservoirs and sanctuaries. HetIL-15 can be administered at selected intervals as described herein. Examples of therapeutic vaccination and subsequent hetIL-15 treatment is provided in Figures 20, 18, and 19.

Treatment of Cancer

[0154] In some embodiments, hetIL-15 is administered to a subject that has cancer, such as melanoma, renal cancer, colon cancer, or prostate cancer. HetIL-15 may be administered in combination with one or more other anti-cancer agents, cytokines or anti-hormonal agents, to treat and/or manage cancer. In one embodiment, the combination of hetIL-15 and one or more other therapies provides an additive therapeutic effect relative to the therapeutic effects of the IL-15 alone or the one or more other therapies alone. In some embodiments, the combination of hetIL-15 and one or more other therapies provides more than an additive therapeutic effect relative to the therapeutic effects of the hetIL-15 alone or the one or more other therapies alone. In one embodiment, the combination of hetIL-15 and one or more other therapies provides a synergistic therapeutic effect relative to the therapeutic effects of hetIL-15 alone or the one or more other therapies alone.

[0155] Cancers and related disorders that can be prevented, treated, or managed in accordance with the methods described herein include, but are not limited to, the following: Leukemias including, but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome, chronic leukemias such as but not limited to, chronic myelocytic (granulocytic) leukemia, and chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, and non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, non-secretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenstrom' s macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as but not limited to bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas,

angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, and synovial sarcoma; brain tumors including but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, and primary brain lymphoma; breast cancer including, but not limited to, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer, including but not limited to, pheochromocytom and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer, including but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers including but not limited to, Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers including but not limited to, ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; vaginal cancers, including but not limited to, squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer, including but not limited to, squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers including but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers including but not limited to, endometrial carcinoma and uterine sarcoma; ovarian cancers including but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers including but not limited to, squamous cancer, adenocarcinoma, adenoid cyctic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers including but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers including but not limited to hepatocellular carcinoma and hepatoblastoma; gallbladder cancers including but not limited to, adenocarcinoma; cholangiocarcinomas including but not limited to, pappillary, nodular, and diffuse; lung cancers including but not limited to, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers including but not limited to, germinal tumor, seminoma, anaplastic, spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor); prostate cancers including but not limited to, adenocarcinoma,

leiomyosarcoma, and rhabdomyosarcoma; penile cancers; oral cancers including but not limited to, squamous cell carcinoma; basal cancers; salivary gland cancers including but not limited to, adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers including but not limited to, squamous cell cancer, and verrucous; skin cancers including but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, and superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acrallentiginous melanoma; kidney cancers including but not limited to, renal cell cancer, renal cancer, adenocarcinoma, hypernephroma, fibrosarcoma, and transitional cell cancer (renal pelvis and/or uterer); Wilms' tumor; bladder cancers including but not limited to, transitional cell carcinoma, squamous cell cancer, adenocarcinoma, and carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy et al., 1997, Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery, Viking Penguin, Penguin Books U.S.A., Inc., United States of America).

[0156] hetIL-15 can be used in the treatment of pre-malignant as well as malignant conditions. Pre-malignant conditions include hyperplasia, metaplasia, and dysplasia. Treatment of malignant conditions includes the treatment of primary as well as metastatic tumors. In a specific embodiment, the cancer is melanoma, prostate cancer, colon cancer, renal cell carcinoma, or lung cancer (e.g., non-small cell lung cancer). In certain embodiments, the cancer is metastatic melanoma, metastaic colon cancer, metastatic renal cell carcinoma, or metastatic lung cancer (e.g., metastatic non-small cell lung cancer).

[0157] In some embodiments, hetIL-15, or a combination therapy, is administered to refractory patients, such as a patient refractory to a standard anti-cancer therapy. In certain embodiments, a patient with cancer, is refractory to a therapy when the cancer has not significantly been eradicated and/or the symptoms have not been significantly alleviated. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment, using art-accepted meanings of "refractory" in such a context. In various embodiments, a patient with cancer is refractory when a cancerous tumor has not decreased or has increased.

[0158] In some embodiments, hetIL-15, or a combination therapy, is administered to a patient to prevent the onset or reoccurrence of cancer in a patient at risk of developing such cancer. In some embodiments, hetIL-15 is administered to a patient who is susceptible to adverse reactions to conventional therapies.

Treatment of Infectious Diseases

[0159] Infectious diseases that can be treated, prevented, and/or managed using hetIL-15 may be by infectious agents including but not limited to bacteria, fungi, protozae, and viruses.

[0160] Viral diseases that can be prevented, treated and/or managed in accordance with the methods described herein include, but are not limited to, those caused by hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, Epstein-Barr virus, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, small pox virus, Epstein Barr virus, human immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), and agents of viral diseases such as viral meningitis, encephalitis, pneumonia, infectious mononucleosis, hepatitis, mumps, polio, shingles, dengue or small pox. In a specific embodiment, the viral disease is AIDS, meningitis, hepatitis, or pneumonia.

[0161] Bacterial diseases caused by bacteria (e.g., Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecials, Candida albicans, S. pneumonia, Group A streptococcus (Streptococcus pyogenes), Clostridium peifringens, Bacteroidesfragilis, Aeromonas hydrophil, Borrelia burgdorferi, Bacillus antracis, Proteus vulgaris, Staphylococcus viridans, mycobacteria rickettsia, Mycobacterium leprae, Mycobacterium tuberculosis, Clostridium tetani, Neisseria meningitides, Yersinia pestis, and Pseudomonas aeruginosa) that can be prevented, treated and/or managed in accordance with the methods described herein include, but are not limited to, mycoplasma, sepsis, and bubonic plague, Lyme disease, anthrax, tetanus, pertissus, cholera, plague, diptheria, chlamydia, pneumonia, toxic shock syndrome, scarlet fever, leprosy, meningococcal disease, necrotizing disease, *e.g.*, encrotizing fasciitis, tuberculosis, and legionella. In a specific embodiment, the bacterial disease is pneumonia or tuberculosis.

[0162] Protozoal diseases caused by protozoa that can be prevented, treated and/or managed in accordance with the methods described herein include, but are not limited to, leishmania, kokzidioa, trypanosoma or malaria.

[0163] Parasitic diseases caused by parasites that can be prevented, treated and/or managed in accordance with the methods described herein include, but are not limited to, chlamydia and rickettsia.

[0164] In certain embodiments, hetIL-15, or a combination therapy, is administered to a subject with a chronic infection. In a specific embodiment, hetIL-15 is administered to a subject with an infection that persists for weeks, months, years, decades or a lifetime. In certain embodiments, the infection persists for a period of time (e.g., weeks, months, years or decades) without the subject manifesting symptoms.

[0165] Illustrative infectious agents capable of inducing a chronic infection include viruses (e.g., cytomegalovirus, Epstein Barr virus, hepatitis B virus, hepatitis C virus, herpes simplex virus, types I and II, human immunodeficiency virus, types 1 and 2, human papillomavirus, human T lymphotrophic viruses, types 1 and 2, varicella zoster virus and the like), bacteria (e.g., Mycobacterium tuberculosis, Listeria spp., Klebsiella pneumoniae, Streptococcus pneumoniae, Staphylococcus aureus, Borrelia spp., Helicobacter pylori, and the like), protozoan parasites (e.g., Leishmania spp., Plasmodiumfalciparum, Schistosoma spp., Toxoplasma spp., Trypanosoma spp., Taenia carssiceps and the like), and fungi (e.g., Aspergillus spp., Candida albicans, Coccidioides immitis, Histoplasma capsulatum, Pneumocystis carinii and the like). Additional infectious agents include prions or misfolded proteins that affect the brain or neuron structure by further propagating protein misfolding in these tissues, resulting in the formation of amyloid plaques which cause cell death, tissue damage and eventual death.

[0166] In certain embodiments, hetIL-15, or a combination therapy is administered to a subject with a latent infection. In some embodiments, hetIL-15 is administered to a subject with an active infection.

[0167] In some embodiments, a patient is administered hetIL-15, or a combination therapy is administered to refractory patients. In a certain embodiment, refractory patient is a patient refractory to a standard therapy. In certain embodiments, a patient with an infection is refractory to a therapy when the infection has not significantly been eradicated and/or the symptoms have not been significantly alleviated. The determination of whether a patient is refractory can be made either in vivo or in vitro by any method known in the art for assaying the effectiveness of a treatment of infections, using art-accepted meanings of "refractory" in such a context. In various embodiments, a patient with an infection is refractory when replication of the infectious agent has not decreased or has increased.

[0168] In some embodiments, hetIL-15, or a combination therapy, is administered to a patient to prevent the onset or reoccurrence of infections (e.g., viral infections) in a patient at risk of developing such infections. In some embodiments, hetIL-15 is administered to a patient who is susceptible to adverse reactions to conventional therapies.

[0169] In some embodiments, hetIL-15, or a combination therapy, is administered to a patient who has proven refractory to therapies other than Therapeutic Agents, but are no longer on these therapies. In certain embodiments, the patients being managed or treated in accordance with the methods of this invention are patients already being treated with antibiotics, anti-virals, anti-fungals, or other biological therapy/immunotherapy. Among these patients are refractory patients, patients who are too young for conventional therapies, and patients with reoccurring viral infections despite management or treatment with existing therapies.

[0170] In some embodiments, the subject being administered hetIL-15 has not received a therapy for the disease being treated with hetIL-15 prior to the administration of hetIL-15. In other embodiments, hetIL-15 is administered to a subject who has received a therapy for the disease prior to administration of hetIL-15. In some embodiments, the subject administered hetIL-15 is refractory to a prior therapy for the disease or experienced adverse side effects to the prior therapy or the prior therapy was discontinued due to unacceptable levels of toxicity to the subject.

Treatment of Immunodeficiencies and Lymphopenia

[0171] Also provided herein are methods for treating, preventing and/or managing an immunodeficiency or lymphopenia in a dose escalation regimen, comprising administering an effective amount hetIL-15 to a subject in need thereof. In a specific embodiment, the methods described herein do not involve a cyclical administration regimen of hetIL-15.

[0172] In some embodiments, hetIL-15 can be administered in combination with one or more other therapies. In one embodiment, the combination of hetIL-15 and one or more other therapies provides an additive therapeutic effect relative to the therapeutic effects of hetIL-15 alone or the one or more other therapies alone. In one embodiment, the combination hetIL-15 and one or more other therapies provides more than an additive therapeutic effect relative to the therapeutic effects of hetIL-15 alone or the one or more other therapies alone. In one embodiment, the combination of hetIL-15 and one or more other therapies provides a synergistic therapeutic effect relative to the therapeutic effects of hetIL-15 alone or the one or more other therapies alone.

[0173] In certain embodiments, hetIL-15, or a combination therapy, is administered to a subject that has been diagnosed as lymphopenic. The terms "lymphopenia" or "lymphocytopenia" or "lymphocytic leucopenia" interchangeably refer to an abnormally small number of lymphocytes in the circulating blood or in peripheral circulation. Quantitatively, lymphopenia can be described by various cutoffs. In some embodiments, a patient is suffering from lymphopenia when their circulating blood total lymphocyte count falls below about 600/mm^3. In some embodiments, a patient suffering from lymphopenia has less than about 2000/μL total circulating lymphocytes at birth, less than about 4500/μL total circulating lymphocytes at about age 9 months, or less than about 1000 /μL total circulating lymphocytes patients older than about 9 months.

[0174] Lymphocytopenia has a wide range of possible causes, including viral (e.g., HIV or hepatitis infection), bacterial (e.g., active tuberculosis infection), and fungal infections; chronic failure of the right ventricle of the heart, Hodgkin's disease and cancers of the lymphatic system, leukemia, a leak or rupture in the thoracic duct, side effects of prescription medications including anticancer agents, antiviral agents, and glucocorticoids, malnutrition resulting from diets that are low in protein, radiation therapy, uremia, autoimmune disorders, immune deficiency syndromes, high stress levels, and trauma. Lymphopenia may also be of unknown etiology (i.e., idiopathic lymphopenia). Peripheral circulation of all types of lymphocytes or subpopulations of lymphocytes (e.g., CD4+ T cells) may be depleted or abnormally low in a patient suffering from lymphopenia. See, e.g., Lymphopenia Description, The Merck Manual (18th Edition, 2006, Merck & Co.).

[0175] In some embodiments, hetIL-15, or a combination therapy, is administered to refractory patients. In a certain embodiment, refractory patient is a patient refractory to a standard therapy.

[0176] In some embodiments, hetIL-15 or a combination therapy is administered to a patient to prevent the onset or reoccurrence of an immunodeficiency or lymphopenia in a patient at risk of developing such infections. In some embodiments, hetIL-15 is administered to a patient who is susceptible to adverse reactions to conventional therapies. In some embodiments, htIL-15 is administered to a patient who has proven refractory to therapies other than hetIL-15, but are no longer on these therapies.

[0177] In some embodiments, the subject being administered hetIL-15 has not received a therapy prior to the administration of hetIL-15. In other embodiments, hetIL-15 is administered to a subject who has received a therapy prior to administration of hetIL-15. In

some embodiments, the subject to whom hetIL-15 is administered is refractory to a prior therapy or experienced adverse side effects to the prior therapy or the prior therapy was discontinued due to unacceptable levels of toxicity to the subject.

EXAMPLES

Example 1. Administration of hetIL-15 with CE vaccines

[0178] IL-15 heterodimer was purified form stable human HEK293-derivede cell lines that were modified to express IL-15 and IL-15Ra and thereby produce, process, and secrete hetIL-15. Purified complex was tested upon subcutaneous (SC) administration to macaques. Phenotype and functional changes in lymphocyte subsets were monitored by flow cytometry and multiplexed confocal imaging (MCI).

[0179] To overcome HIV sequence diversity and to address potential "decoy" epitopes preventing efficacy, we developed DNA vaccines targeting the highly conserved (CE) in Gag and vaccine-induced cellular immunity was analyzed. These were administered to macaques.

[0180] Treatment with hetIL-15 resulted in a significant increase of circulating CD8+ effector T cells and NK cells with activated cytotoxic phenotype (Granzyme$^+$). This expanded T lymphocyte population was also present in secondary lymphoid organs where an increased frequency of Ag-specific effector CD8 T cells could be observed by both flow cytometry and MCI. A subset of CD8 T cells present in lymph nodes expresses CXCR5, indicating ability to migrate into germinal centers where chronically infected CD4+Tfh reside. MCI confirmed their presence in germinal centers and showed that these cells are cytotoxic (GrzmB$^+$) and actively proliferating (Ki67$^+$) in response to hetIL-15.

[0181] Contrary to macaques administered gag DNA only, all CE DNA-vaccinated macaques developed robust cytotoxic T cell responses targeting the conserved epitopes. Interestingly, vaccination with intact Gag efficiently boosted magnitude and breadth of preexisting CE responses indicating a change in epitope hierarchy. The induced T cell responses rapidly disseminated into secondary lymphoid organs and effector mucosal sites. hetIL-15 is therefore enhancing the effects of therapeutic vaccination by inducing the number of cytotoxic cells and by inducing their localization to areas of virus propagation and persistence.

[0182] This example demonstrates that hetIL-15 in combination with pDNA vaccine targeting the "Achilles' heel" of the virus, i.e., the highly-conserved regions (CE), in virus

sanctuary areas (germinal centers) thus provides an HIV treatment strategy to reduce or eliminate HIV infection in a patient.

Summary: This example explored the potential of hetIL-15 as a viral reservoir reducing agent in ART-treated SIV infected macaques therapeutically vaccinated with DNA. Effective levels of hetIL-15 can be delivered without side effects. hetIL-15 treatment in combination with DNA vaccination enhances access to virus sanctuary areas (germinal centers).

Example 2. Subcutaneous administration of hetIL-15 in macaques

[0183] Methods: Heterodimeric IL-15 (hetIL-15) was purified and evaluated in macaques upon subcutaneous (SC) administration. Human and macaque purified molecules showed similar effects. Phenotype and functional changes in lymphocyte subsets were monitored by flow cytometry and multiplexed confocal imaging (MCI). Blood and tissue samples were tested including complete blood counts and chemistry.

[0184] Results: Treatment with hetIL-15 resulted in a significant increase of CD8+ effector T cells and NK cells with activated cytotoxic phenotype (Granzyme+). This expanded T lymphocyte population was distributed in the tissues and was also present in secondary lymphoid organs where an increased frequency of Ag-specific effector and total effector CD8 T cells could be observed by both flow cytometry and MCI. A subset of CD8 T cells present in lymph nodes expresses CXCR5, indicating ability to migrate into germinal centers where chronically infected CD4+Tfh cells reside. MCI confirmed the presence of effector CD8 in germinal centers and showed that these cells are cytotoxic (GrzmB+) and actively proliferating (Ki67+) in response to hetIL-15.

[0185] Using the 2 week hetIL-15 protocol of six increasing doses (2, 4, 8, 16, 32, 64 μg/kg of IL-15 calculated as mass of single chain IL-15), it was shown that no adverse effects were detected in macaques. There was no decrease in blood pressure, fever, or any other effects associated with high dose hetIL-15 administration. The animals were not different in any blood parameters compared to controls injected with saline solution instead of hetIL-15. The only detected difference at the last measurement in 2 week in hetIL-15 animals was lower Albumin serum levels, indicative of some vascular leak, a function associated with hetIL-15. Animals recovered to normal levels rapidly. This shows that the dose escalation protocol can achieve a very high amplification of cytotoxic lymphocytes and their accumulation to tissues but it has low toxicity compared to other methods of delivery.

Example 3. Intraperitoneal administration of hetIL-15 in macaques

[0186] Methods: Heterodimeric IL-15 (hetIL-15) was purified and tested in macaques upon intraperitoneal administration (IP). Dosing was every two-three days for 2 weeks (MWF MWF) in 1 ml of saline solution. Increasing doses were 2, 4, 8, 16, 32, 64 μg/kg calculated as single chain IL-15 polypeptide mass. Phenotype and functional changes in lymphocyte subsets were monitored by flow cytometry and multiplexed confocal imaging (MCI). Blood and tissue samples were tested including complete blood counts and chemistry.

[0187] Comparison of IP-treated animals to SC-treated animals with the same dosage and schedule (Figure 17) showed that IP hetIL-15 increased lymphocyte accumulation and proliferation in intestinal sites. Therefore, IP administration and other methods of local administration increase preferentially hetIL-15 effects on lymphocyte numbers and activation. This is important for maximizing effects of hetIL-15 on intestinal tumors or virus reservoirs associated with the intestine.

Example 4. Treatment by hetIL-15 of chronically SHIV infected macaques decreases SHIV-infected cells in LN.

[0188] Infected animals were treated by increasing doses of hetIL-15 sc for 2 weeks (2, 4, 8, 16, 32, 64 μg/kg) as detailed previously. Lymph nodes and blood were removed before the first injection and on day 15 (day 3 after last injection). DNA and RNA were extracted and the SHIV copies were measured after quantitative PCR amplification. Results were expressed as copies per 10^6 cell equivalents, by normalizing to amplified CCR5 DNA in the same samples.

[0189] The results of these measurements on the axillary or inguinal LN of two macaques are shown in Figure 21 and Table A. The results indicate that both viral DNA and RNA are significantly reduced upon hetIL-15 treatment. The decrease in RNA was significantly higher than the DNA, indicating that hetIL-15 results in the true decrease in virus producing cells.

TABLE A. Quantification of DNA and RNA reduction and of the difference in RNA/DNA ratio for the specific LN and Blood of two animals after 2 week hetIL-15 treatment (6 sc injections MWFMWF).

FOLD change after IL-15 treatment:

a. DNA Before/After

	AxLN	IngLN	PBMC
T416	22.6	5.0	0.9
T422	6.8	1.3	3.4

b. RNA Before/After

	AxLN	IngLN	PBMC
T416	6097	127	63
T422	938	12	40

c. RNA/DNA Ratio

	AxLN	IngLN	PBMC
T416 B	289	292	73
T416 A	1	11	1
T422 B	415	124	8
T422 A	3	14	1

FOLD Drop:

AxLN	IngLN	PBMC	
270	25	73	T416
137	9	12	T422

[0190] These results suggest that hetIL-15 can be used to disrupt LN and to decrease virus-producing cells known to persist in the LN.

[0191] Certain aspects of the disclosure are illustrated in Figures 1-20.

[0192] Figure 1 provides data showing that hetIL-15 treatment triggered a cytotoxic commitment (increased Granzym B) on CD8+ T cells including lymph node (LN).

[0193] Figure 2 provides data showing that there was an increased frequency of proliferating T lymphocytes in mucosal effector sites after treatment with het IL-15.

[0194] Figure 3 provides data showing that IL-15 treatment results in increased frequency of effector memory CD8+ T cells in secondary lymphoid organs.

[0195] Figure 4 provides data showing that het IL-15 increased effector memory CD8+ T cells in lymph nodes.

[0196] Figure 5 provides data showing that het IL-15 treatment increased the proliferation rate of CD8+ memory T cells within the LN.

[0197] Figure 6 provides data showing that hetIL-15 treatment resulted in increased frequency of NK cells in peripheral blood and within the LN. This increase is driven by proliferation.

[0198] Figure 7 provides data showing that IL-15 treatment increased ADCC mediated by NK cells.

[0199] Figure 8 provides data showing that hetIL-15 treatment, in contrast to IL-2, does not affect the frequency of Tregs (CD4+CD25+).

[0200] Figure 9 provides data showing that hetIL-15 treatment did not affect the rate of B lymphocyte proliferation.

[0201] Figure 10 provides data showing that hetIL-15 increased PD-1 expression on CD8+ T cells in the LN.

[0202] Figure 11 provides data showing that hetIL-15 treatment induced a significantly higher presence of EM CD8+ T lymphocytes and cytotoxic T cells within the lymph nodes than SIV infection.

[0203] Figure 12 provides data showing that hetIL-15 treatment increased SIV-specific T cells within the lymph nodes.

[0204] Figure 13 provides data showing that hetIL-15 induced preferential expansion of LN CM9 tetramer^{+} lymphocytes with increased cytotoxic potential (GranzB+).

[0205] Figure 14 provides data showing that hetIL-15 treatment (dose escalation) reduced the frequency of $CD4^{+}CXCR5^{+}PD\text{-}1^{high}$ (Tfh) cells in peripheral LN (inguinal).

[0206] Figure 15 provides data showing that hetIL-15 treatment down-regulated CXCR5 also in B lymphocytes in LN.

[0207] Figure 16 provides data showing that hetIL-15 administration increased CXCL13 plasma levels.

[0208] Figure 17 provides data showing that IP delivery of IL-15 increased the CD8+ proliferative responses in the intestine.

[0209] Figure 18 provides data showing efficient long-term control of viremia in SIV_{mac251}-infected macaques under cART treatment for > 7 months.

[0210] Figure 19 provides data showing that hetIL-15 treatment of SIV-infected cART-treated macaques does not increase plasma virus load.

[0211] Figure 20 provides data showing an example of therapeutic vaccination of SIVmac-infected, cART-treated macaques with SIV p27CE pDNA. This example thus combines methods of therapeutic vaccination that induce broad and non-escaped immunity to infected animals during cART treatment with hetIL-15 as a vaccine enhancer. The results showed that the vaccine induces robust T cell responses.

[0212] These data thus demonstrated that heterodimeric IL-15 increases cytotoxic CD8 effector cells in Lymph Nodes and Germinal Centers, a known HIV/SIV reservoir/sanctuary; that hetIL-15 affects the organization of germinal centers; that high and effective doses of hetIL-15 can be safely administered with long term ART; and that hetIL-15 does not significantly increase virus load after *in vivo* administration in ART treated macaques.

Methods

Immune phenotyping and flow cytometry:

[0213] The following fluorophore conjugated monoclonal antibodies were used in this study:

From BD: CD3 (clone SP34-2), CD4 (clone L200), CD95 (clone DX2), Ki-67 (clone B56), CD25 (clone M-A251), CD16 (clone 3G8), g/d TCR, CCR6 (clone 11A9), CXCR3 (clone 1C6), CD20 (clone 2H7), HLA-DR (clone TU39) and CCR5 (clone 3A9).
From Biolegend: CD28 (clone CD28.2), CD127 (clone A019D5) and PD-1 (clone EH12.2H7).
From e-Bioscience: CXCR5 (clone MU5UBEE) and FoxP3.
From Life Technologies: CD8 (clone 3B5) and Granzyme B (clone GB12).
From R&D: CCR7 (clone 150503)

[0214] Macaque samples were collected and processed as follows: Lymph nodes and rectal pinches were collected from macaques under anesthesia before and after hetIL-15 dosing. Three days after the last hetIL-15 injection, some animals were sacrificed and the following samples were collected for flow cytometric analysis and immunohistochemistry; peripheral blood, axillary, inguinal, mediastinal and mesenteric lymph nodes, bone marrow, liver, spleen, tonsils, vagina, duodenum, jejunum, ileum, colon and rectum. PBMC were obtained from blood samples by gradient centrifugation as previously described. Single lymphocyte cell suspensions were obtained from lymph nodes, tonsils and spleen by gently squeezing the samples through 100μm strainers. Single cell suspensions from solid tissues were obtained after mincing the samples and enzymatic digestion for 1 hour at 37°C in RPMI 1640 medium supplemented with FBS, antibiotics, collagenase (125 units/ml) and DNAse I (10 units/ml).

[0215] For Figure 20: Six animals infected by SIV were put in cART as described for Figure 18 using 3-drug combination (Tenofovir (TFV), emtricitabine (FTC), Dolutegravir in a single combination (sc daily)). Animals were vaccinated during cART treatment by DNA electroporation using SIV p27CE conserved element vaccine (4 animals) or sham DNA as negative control (2 animals). The four vaccinated animals developed high responses measured as antigen specific cells in the blood by standard flow cytometry procedures (intracellular IFN-gamma production upon p27CEgag peptide stimulation). Two weeks after vaccination the animals were treated with hetIL-15 (2-week cycle of 6 increasing doses of 2-64 μg/kg IL-15 calculated as single chain IL-15 mass) as indicated for the animals in Figure 19.

[0216] All publications, accession numbers, patents, and patent applications cited in this specification are herein incorporated by reference as if each was specifically and individually indicated to be incorporated by reference.

Table 1. Illustrative HIV Gag Conserved Element Sequences

p24 Gag conserved elements for p24CE1 vaccine ("also referred to as "Core1"):

SEQ ID NO:1 conserved element 1 (CE1)
ISPRTLNAWVKV

SEQ ID NO:2 conserved element 2 (CE2)
VIPMFSALSEGATPQDLN

SEQ ID NO:3 conserved element 3 (CE3)
VGGHQAAMQMLKDTINEEAAEWDR

SEQ ID NO:4 conserved element 4 (CE4)
PRGSDIAGTTSTLQEQIGW

SEQ ID NO:5 conserved element 5 (CE5)
KRWIILGLNKIVRMYSPTSI

SEQ ID NO:6 conserved element 6 (CE6)
YVDRFYKTLRAEQA

SEQ ID NO:7 conserved element 7 (CE7)
LEEMMTACQGVGGPGHK

p24 Gag conserved elements for p24CE2 vaccine ("also referred to as "Core2"):

SEQ ID NO:8 conserved element 1 (CE1)
LSPRTLNAWVKV

SEQ ID NO:9 conserved element 2 (CE2)
VIPMFTALSEGATPQDLN

SEQ ID NO:10 conserved element 3 (CE3)
VGGHQAAMQMLKETINEEAAEWDR

SEQ ID NO:11 conserved element 4 (CE4)

PRGSDIAGTTSTLQEQIAW

SEQ ID NO:12 conserved element 5 (CE5)

KRWIILGLNKIVRMYSPVSI

SEQ ID NO:13 conserved element 6 (CE6)

YVDRFFKTLRAEQA

SEQ ID NO:14 conserved element 7 (CE7)

LEEMMTACQGVGGPSHK

SEQ ID NO:15 p24 Gag conserved elements for p24CE1 vaccine ("also referred to as "Core1"):

VIPMFSALSEGATPQDLNAAVGGHQAAMQMLKDTINEEAAEWDRAAAEPRGSDIAG
TTSTLQEQIGWAAAKRWIILGLNKIVRMYSPTSIAAKYVDRFYKTLRAEQAAGLEEM
MTACQGVGGPGHKAAISPRTLNAWVKV

SEQ ID NO:16 p24 Gag conserved elements for p24CE2 vaccine ("also referred to as "Core2"):

VIPMFTALSEGATPQDLNAAVGGHQAAMQMLKETINEEAAEWDRAAAEPRGSDIAG
TTSTLQEQIAWAAAKRWIILGLNKIVRMYSPVSIAAKYVDRFFKTLRAEQAAGLEEM
MTACQGVGGPSHKAALSPRTLNAWVKV

SEQ ID NO: 17 Nucleic acid construct encoding Conserved Element1 plus Conserved Element 2 (p24CE1+p24CE2) (306H) (genes underlined)

CCTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCA
TGTCCAACATTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATC
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GCTATTGGCATTATGCC

SEQ ID NO:18 p24CE1 encoded by SEQ ID NO:17 (includes a GM-CSF signal peptide)
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RFYKTLRAEQAAGLEEMMTACQGVGGPGHKAAISPRTLNAWVKV

SEQ ID NO:19 p24CE2 encoded by SEQ ID NO:17 (includes a GM-CSF signal peptide)
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RFFKTLRAEQAAGLEEMMTACQGVGGPSHKAALSPRTLNAWVKV

WHAT IS CLAIMED IS:

1. A method for treating lymphocytopenia, cancer, or an infectious disease in a human subject, the method comprising administering an initial dose of IL-15/IL-15Ra complex to the subject and administering successively higher doses of IL-15/IL-15Ra complex in a Fibonacci series.

2. The method of claim 1, wherein the initial dose of IL-15/IL-15Ra complex is between 0.1 μg/kg to 5 μg/kg as determined based on the mass of IL-15/IL-15Ra complex.

3. The method of claim 1or 2, wherein the IL-15 and IL-15Ra comprised by the complex are non-covalently linked.

4. The method of claim 1, 2, or 3, wherein the IL-15Ra is soluble IL-15Ra.

5. The method of claim 1, 2, or 3, wherein the IL-15Ra comprises a soluble IL-15Ra-Fc fusion protein.

6. The method of any one of claims 1 to 5, wherein the IL-15/IL15Ra is administered subcutaneously.

7. The method of any one of claims 1 to 5, wherein the IL-15/IL15Ra is administered intraperitoneally.

8. The method of any one of claims 1 to 5, wherein the IL-15/IL15Ra is administered intravenously.

9. The method of any one of claims 1 to 7, wherein the initial dose and at least one successively higher dose is administered within one week.

10. The method of any one of claims 1 to 7, wherein the initial dose and at least one successively higher dose is administered within two weeks.

11. The method of any one of claims 1 to 10, wherein the patient is infected with HIV.

12. The method of claim 11, wherein the patient is undergoing treatment anti-retroviral therapy.

13. The method of claim 11 or 12, wherein the patient has been administered a CE immunogen prior to the administration of the IL-15/IL-15Ra complex.

14. The method of claim 13, wherein the IL-15/IL-15Ra complex is administered about 4 days, about 5 days, about 6 days, or about 1 week following the administration of the CE immunogen; or at least 1, 2, 3, 4, 8, or 12 weeks following the administration of the CE immunogen.

15. The method of any one of claims 1 to 10, wherein the patient has cancer.

16. A method for treating lymphocytopenia, cancer, or an infectious disease in a human subject, the method comprising administering an initial dose of a cytokine-containing complex to the subject, wherein the cytokine complex binds IL-2/IL-15 Receptor beta-gamma and dos not trigger Treg preferential amplification;
and administering successively higher doses of IL-15/IL-15Ra complex in a Fibonacci series

17. A method of monitoring treatment of a patient with IL-15, the method comprising determining the levels of expression of CXCR5 on the surface of lymphocytes, or blood levels of CXCL13 or IL-18 in a patient following administration of an IL-15/IL-15Ra complex.

18. A method of monitoring treatment of a patient with IL-15, the method comprising determining the proliferation of lymphocytes as indicated by Ki67 levels.

19. The method of claim 18, further comprising monitoring the levels of one or more selecting markers selected from the group consisting of CXCR5, CXCL13, abd IL-18.

Figure 1

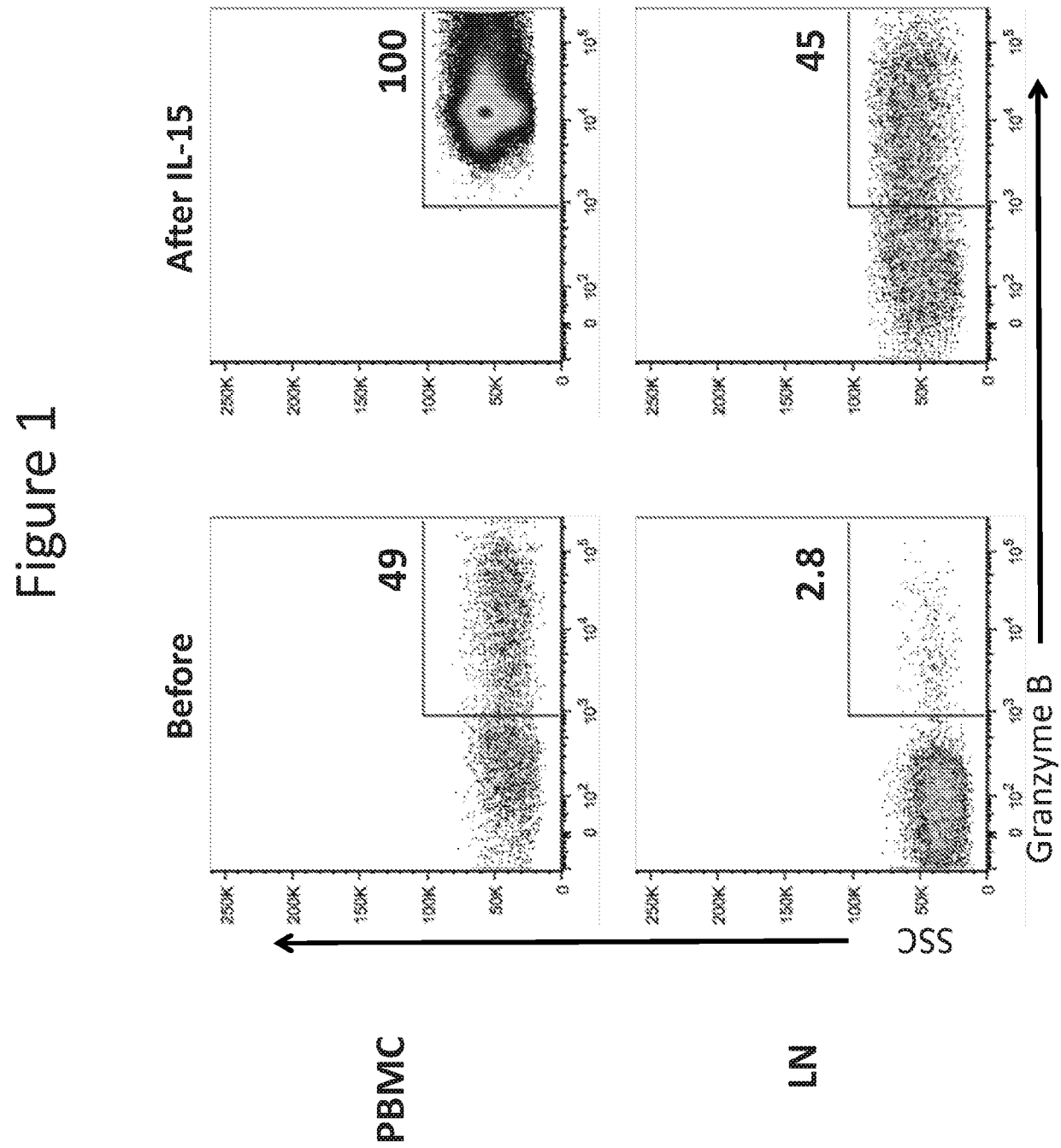


Figure 2

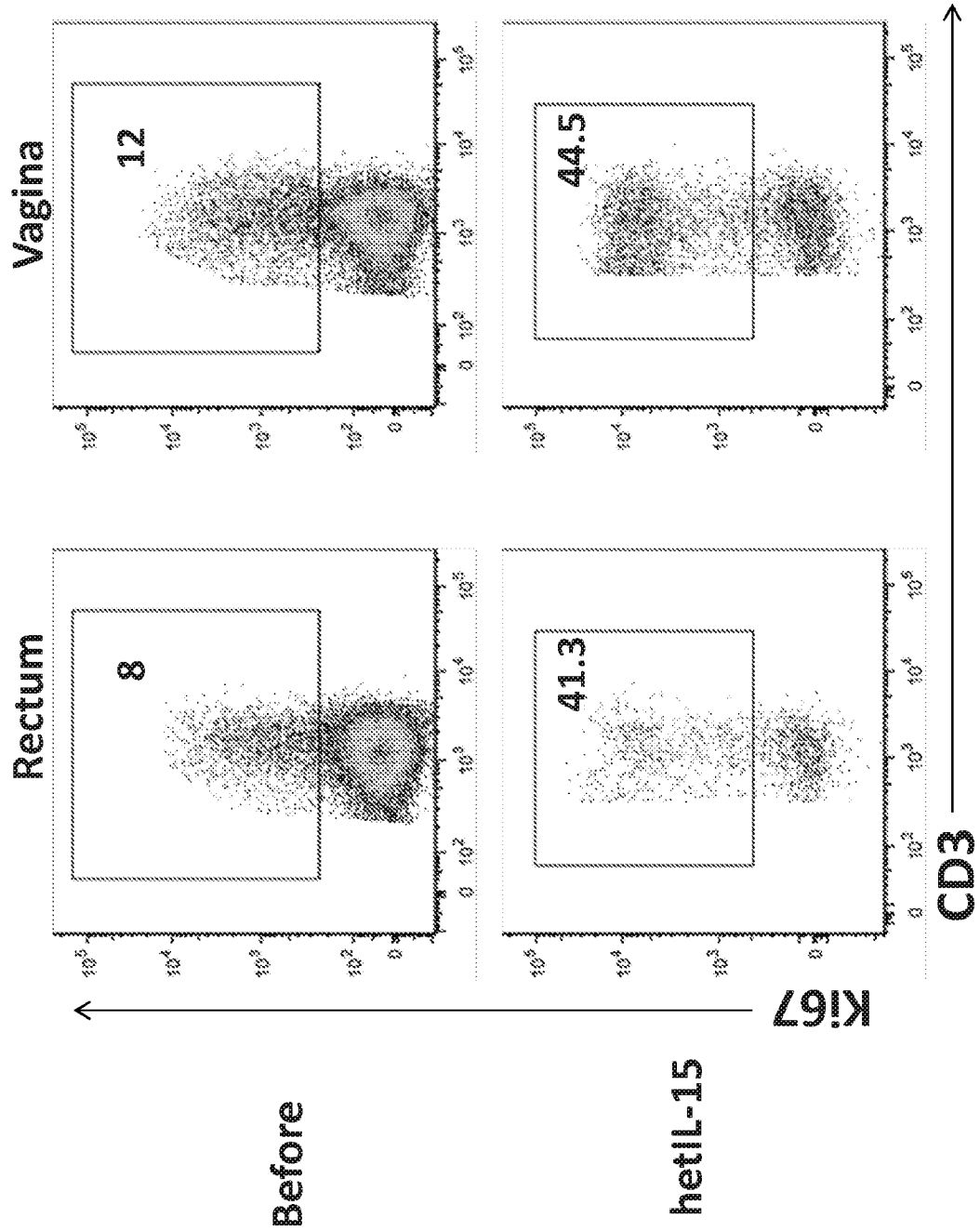


Figure 3

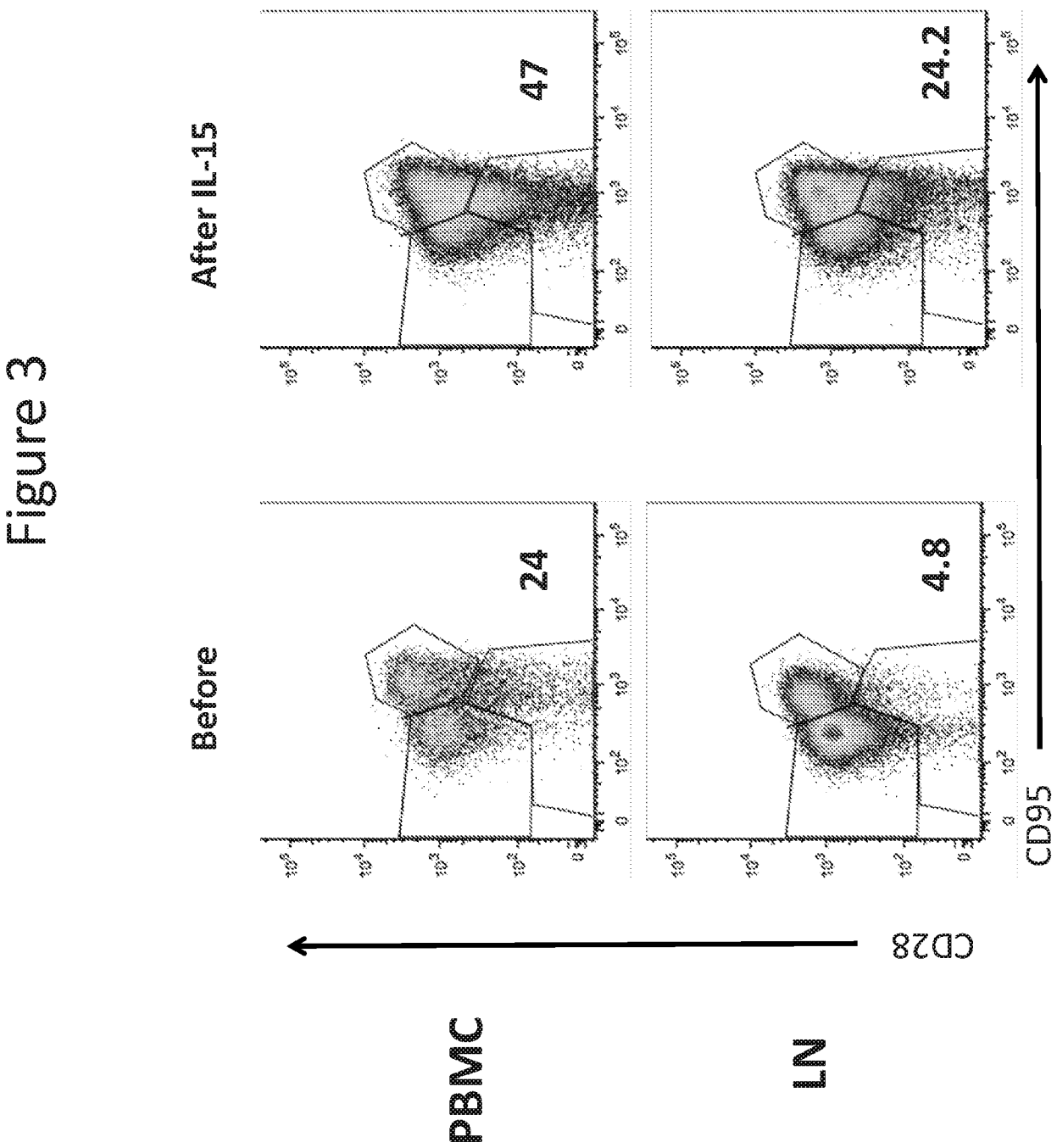


Figure 4

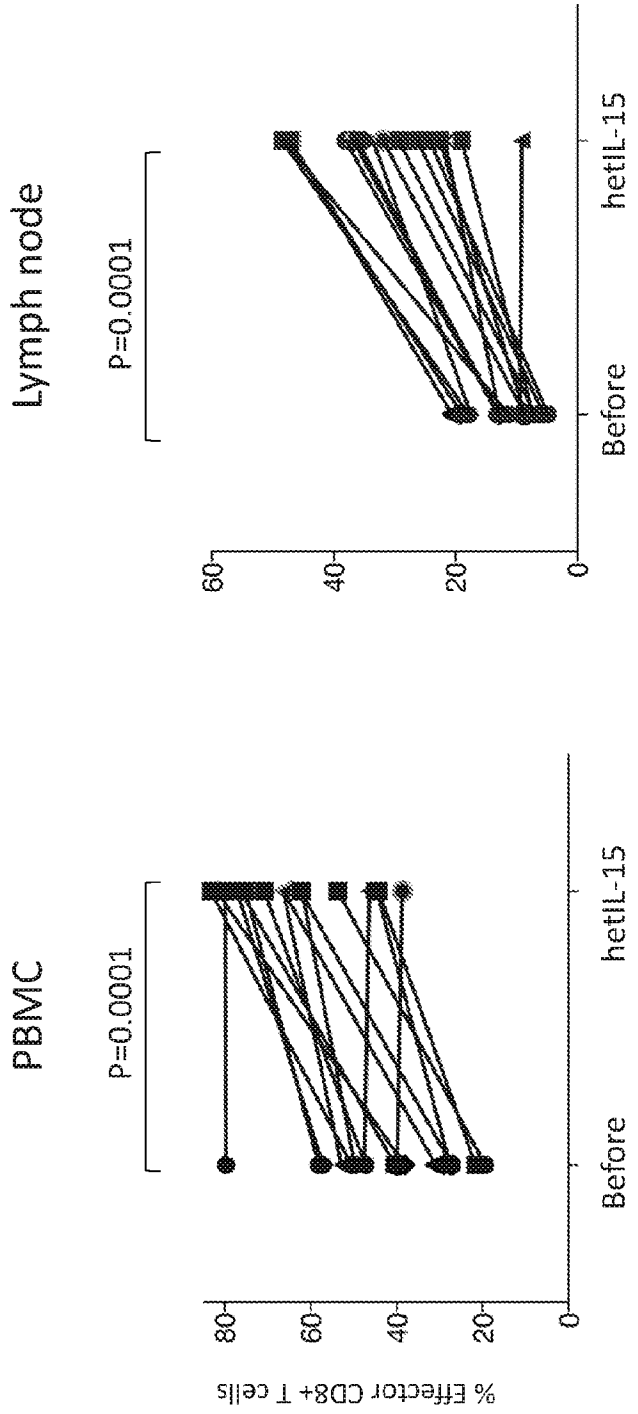

PBMC
P=0.0001
Lymph node
P=0.0001
% Effector CD8+ T cells
0
20
40
60
80
Before
hetIL-15

Figure 5

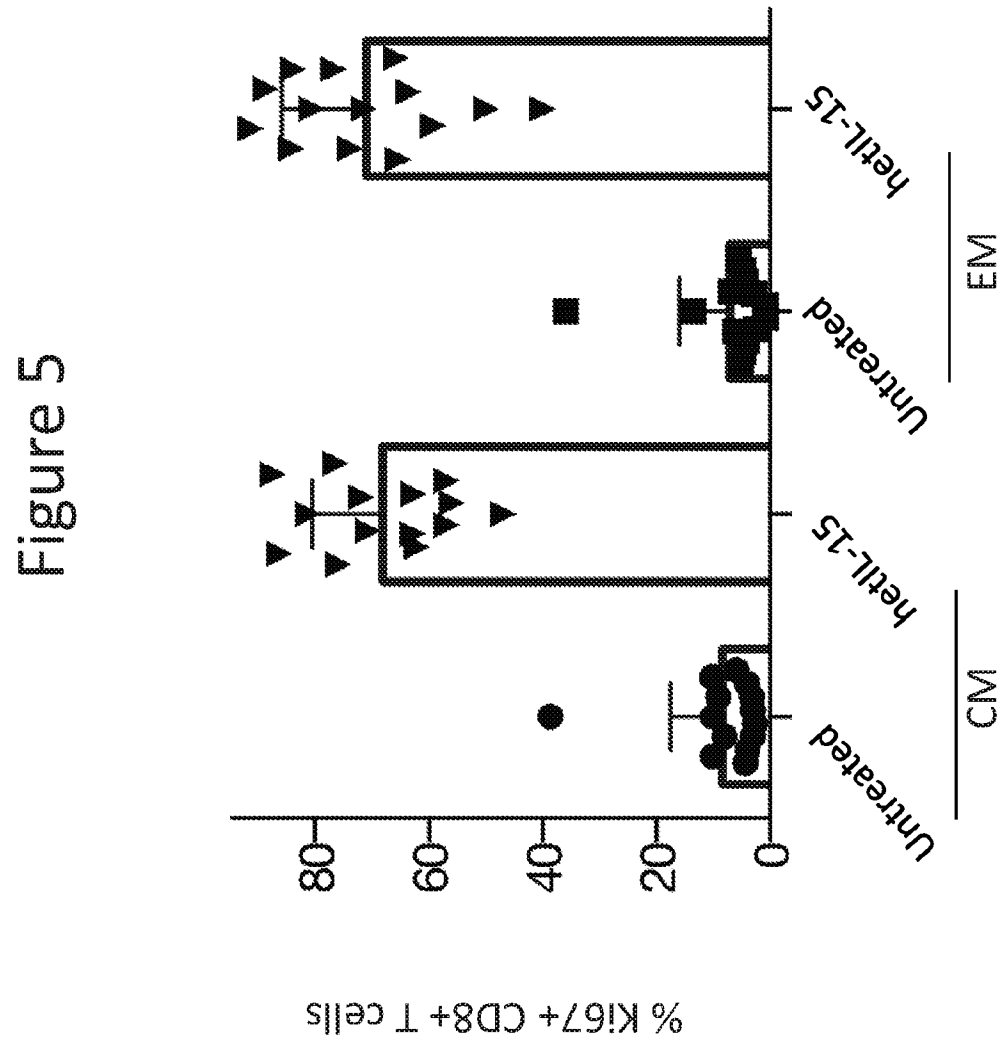

% Ki67+ CD8+ T cells
0
20
40
60
80
Untreated
hetIL-15
Untreated
hetIL-15
CM
EM

Figure 6

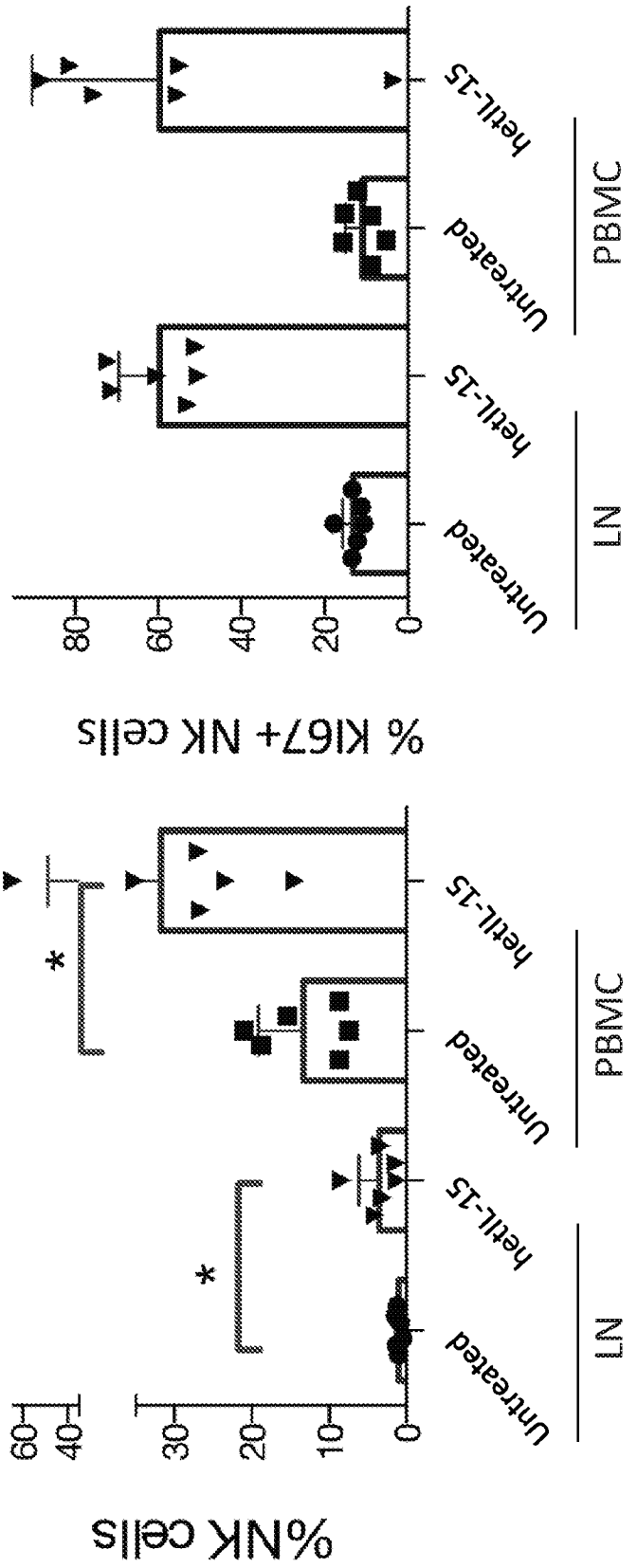

%NK cells
60
40
30
20
10
0
*
*
Untreated
hetIL-15
Untreated
hetIL-15
LN
PBMC
% KI67+ NK cells
80
60
40
20
0
Untreated
hetIL-15
Untreated
hetIL-15
LN
PBMC

Figure 7

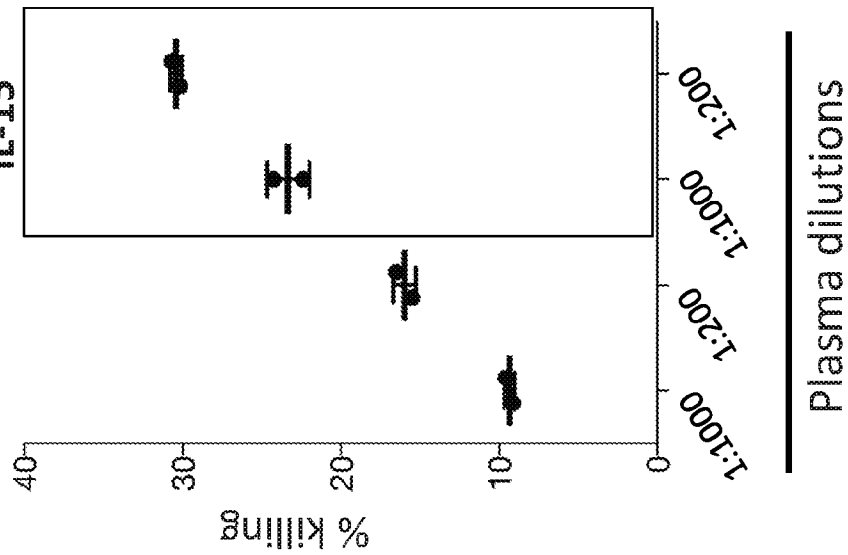

IL-15
% killing
0
10
20
30
40
1:1000
1:200
1:1000
1:200
Plasma dilutions

Figure 8

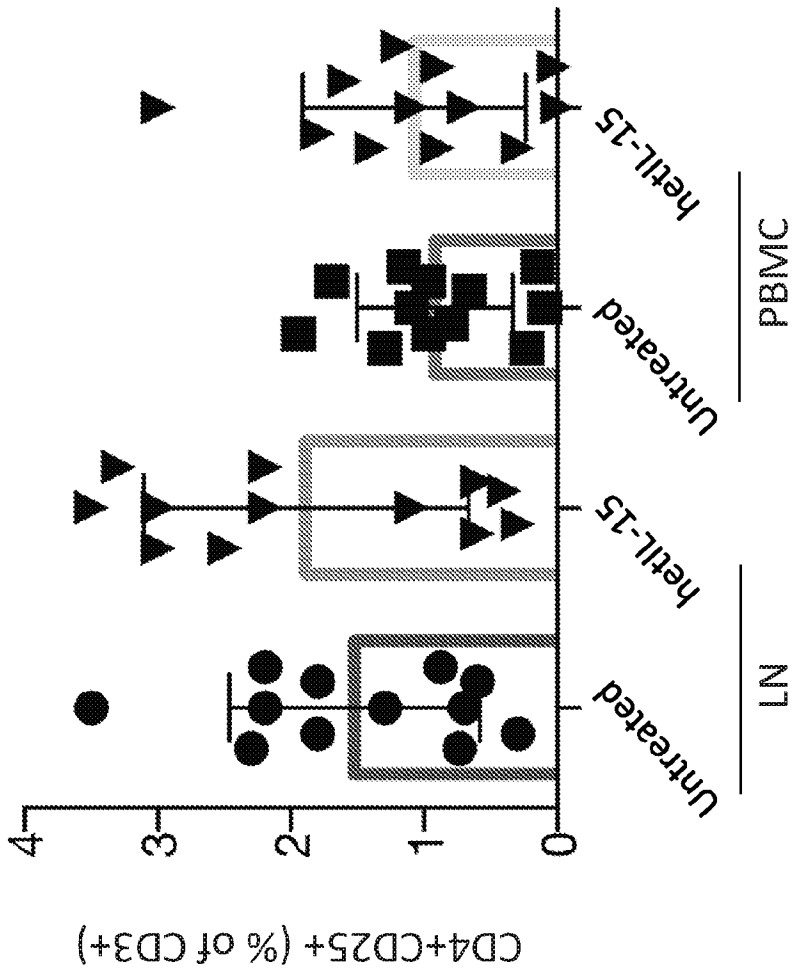

CD4+CD25+ (% of CD3+)
0
1
2
3
4
Untreated
hetIL-15
Untreated
hetIL-15
LN
PBMC

Figure 9

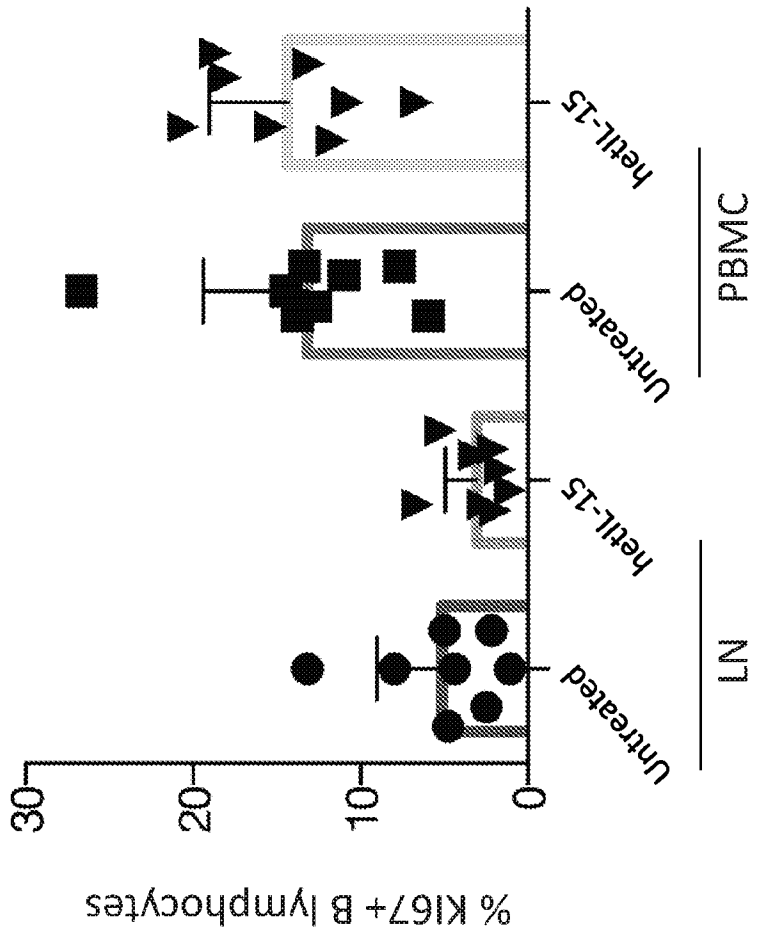

% Ki67+ B lymphocytes
30
20
10
0
Untreated
hetIL-15
Untreated
hetIL-15
LN
PBMC

Figure 10

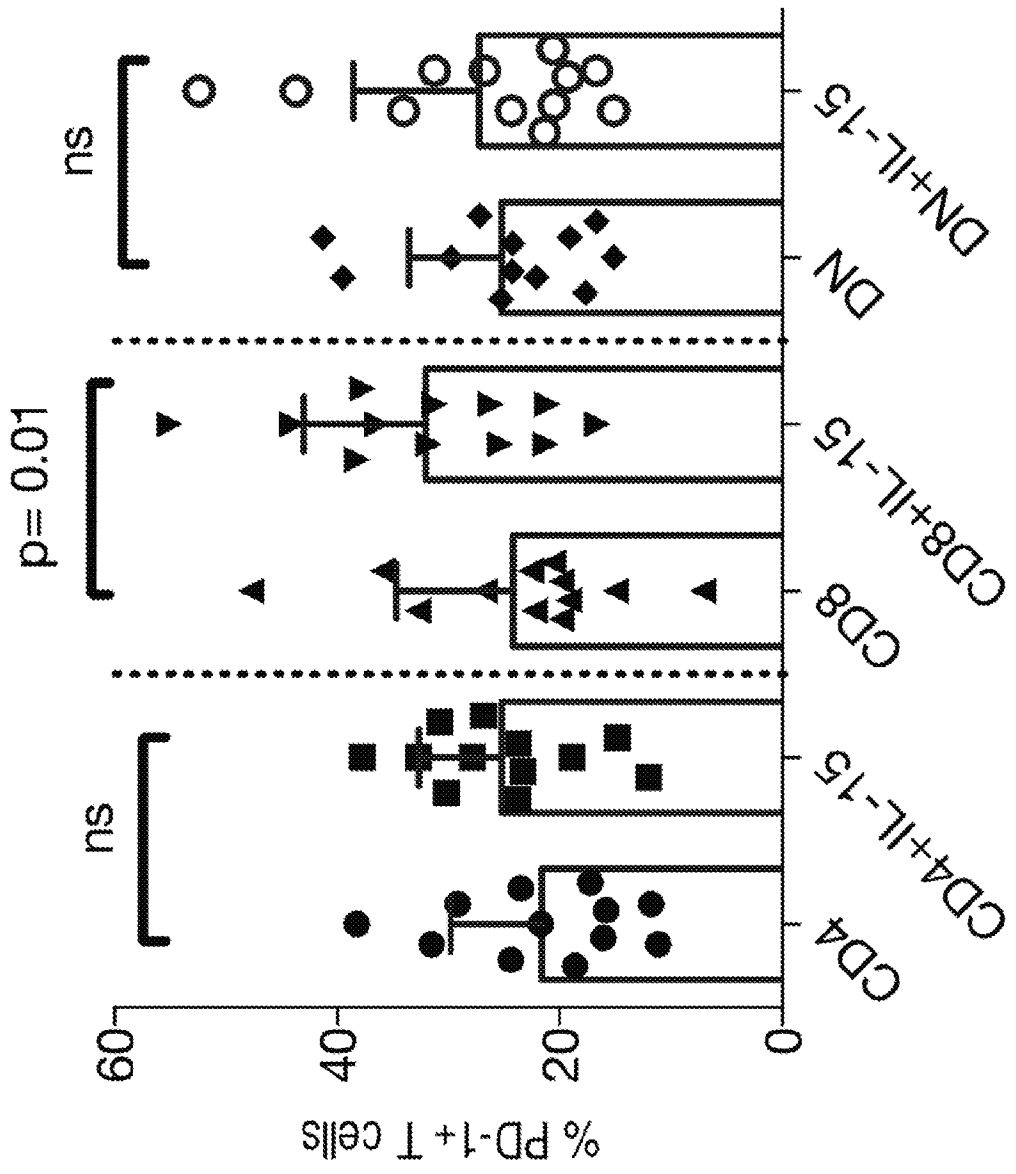

% PD-1+ T cells
60
40
20
0
CD4
CD4+IL-15
CD8
CD8+IL-15
DN
DN+IL-15
ns
ns
p= 0.01

Figure 11

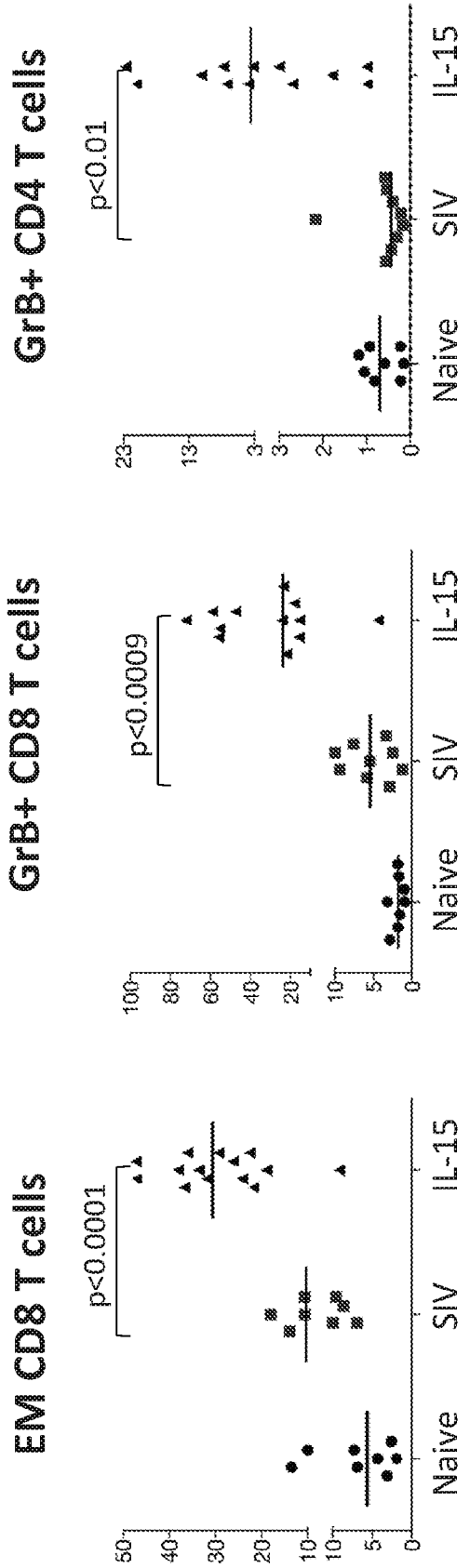

EM CD8 T cells
p<0.0001
50
40
30
20
10
10
5
0
Naive
SIV
IL-15
GrB+ CD8 T cells
p<0.0009
100
80
60
40
20
10
5
0
Naive
SIV
IL-15
GrB+ CD4 T cells
p<0.01
23
13
3
3
2
1
0
Naive
SIV
IL-15

Figure 12

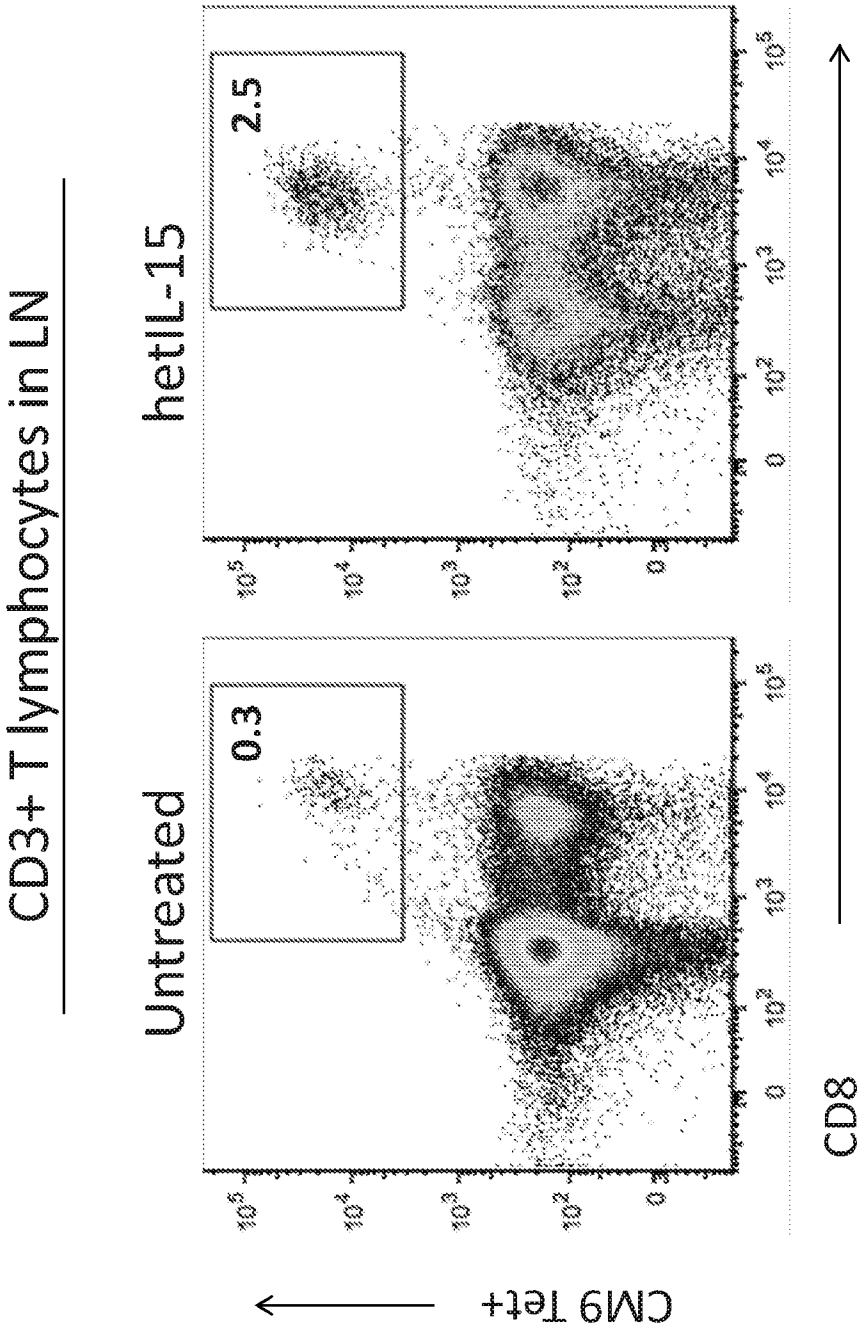


Figure 13

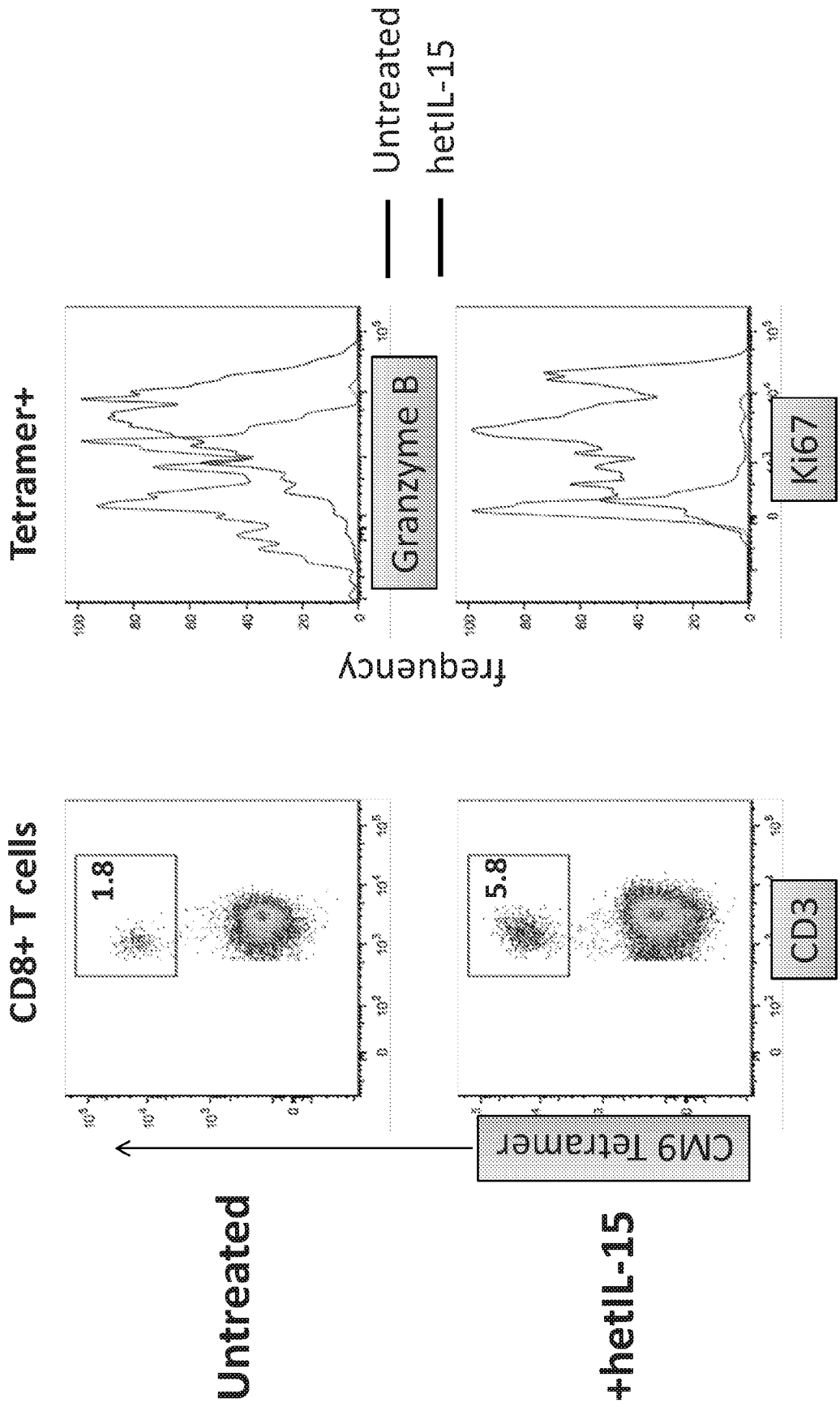

Untreated
hetIL-15
Tetramer+
Granzyme B
Ki67
frequency
CD8+ T cells
1.8
5.8
CD3
CMV9 Tetramer
Untreated
+hetIL-15

Figure 14

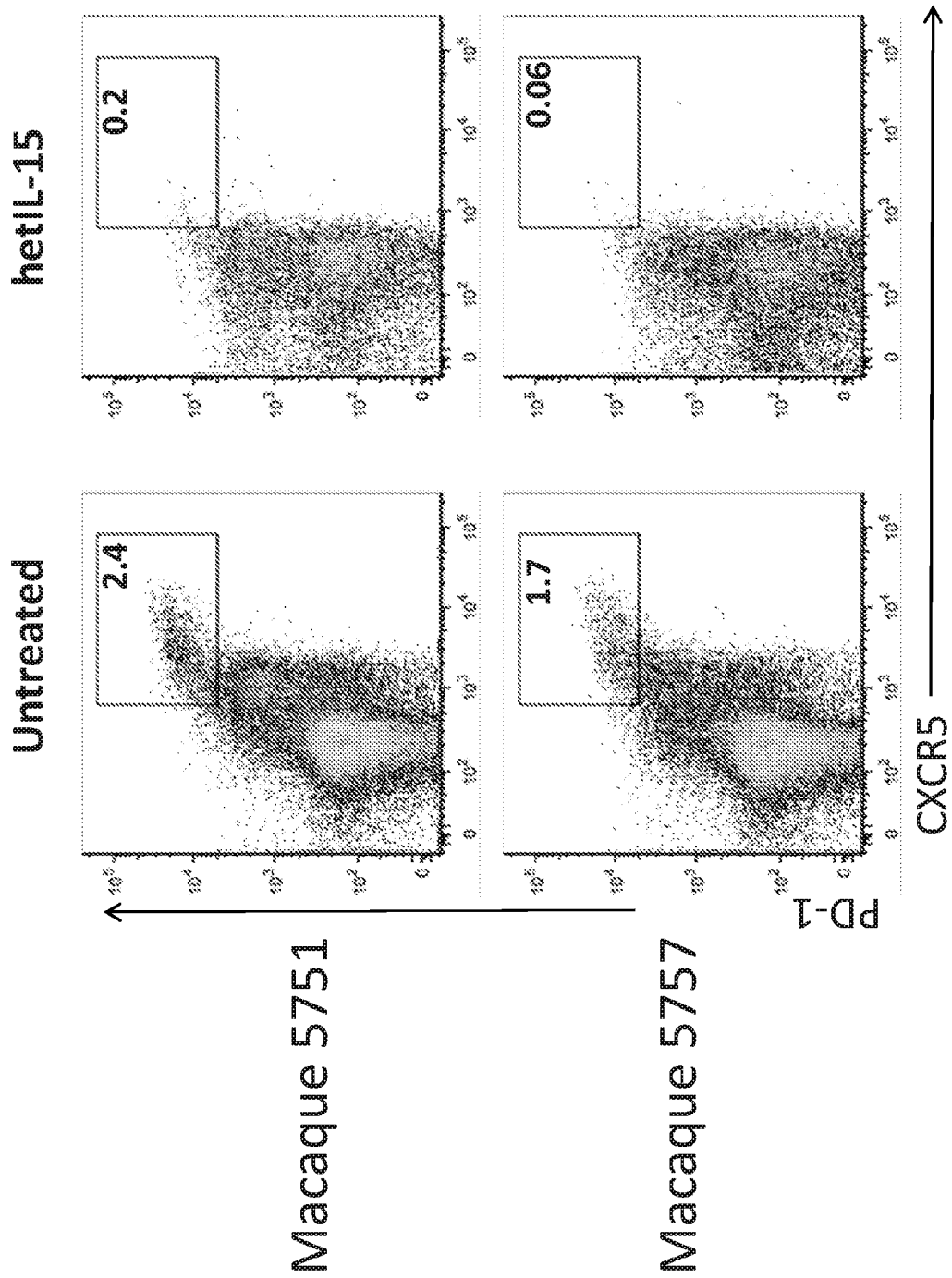

Untreated
hetIL-15
2.4
0.2
1.7
0.06
Macaque 5751
Macaque 5757
PD-1
CXCR5

Figure 15

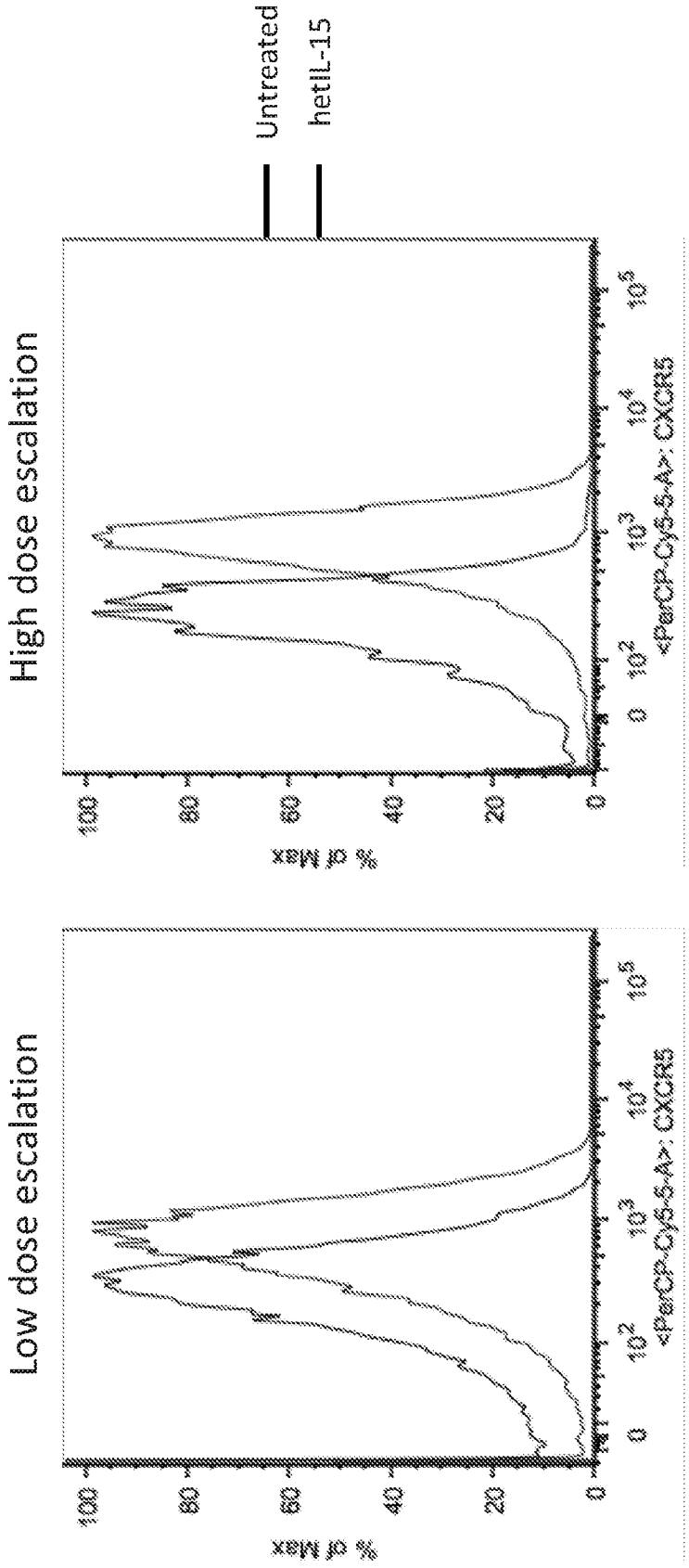
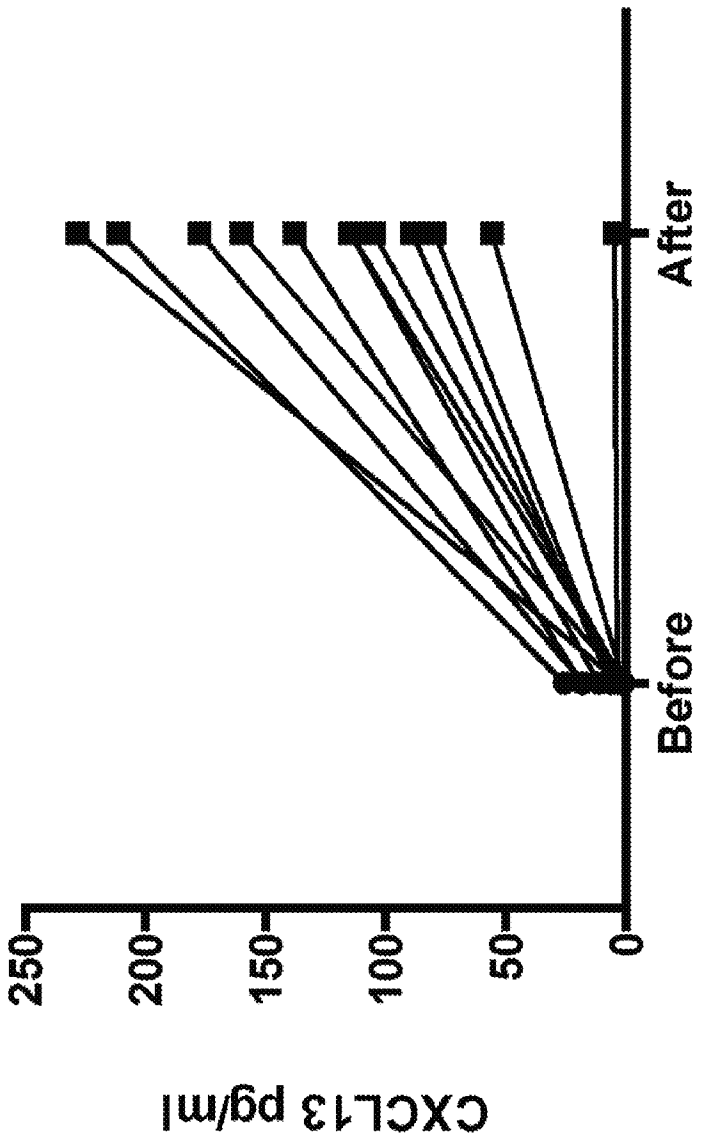

Untreated
hetIL-15
High dose escalation
Low dose escalation
% of Max
<PerCP-Cy5-5-A>: CXCR5
0
20
40
60
80
100
0
10^2
10^3
10^4
10^5

Figure 16



Average % CD8 Proliferation	SC, n=10	IP, n=2
Liver	79.6	67.1
Jejunum	9.5	11.8
Ileum	12.4	53.9
Colon	16.2	41.2
Rectum	24.1	61.3

Figure 17

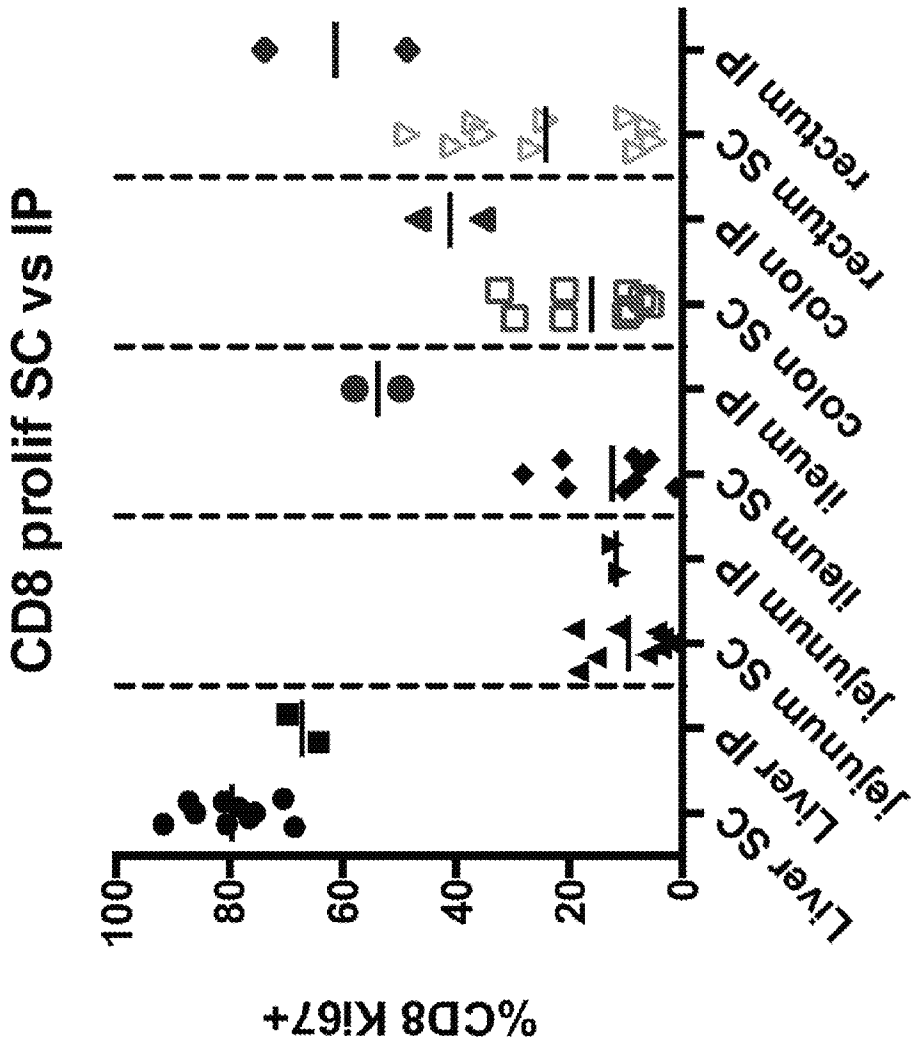


Figure 18

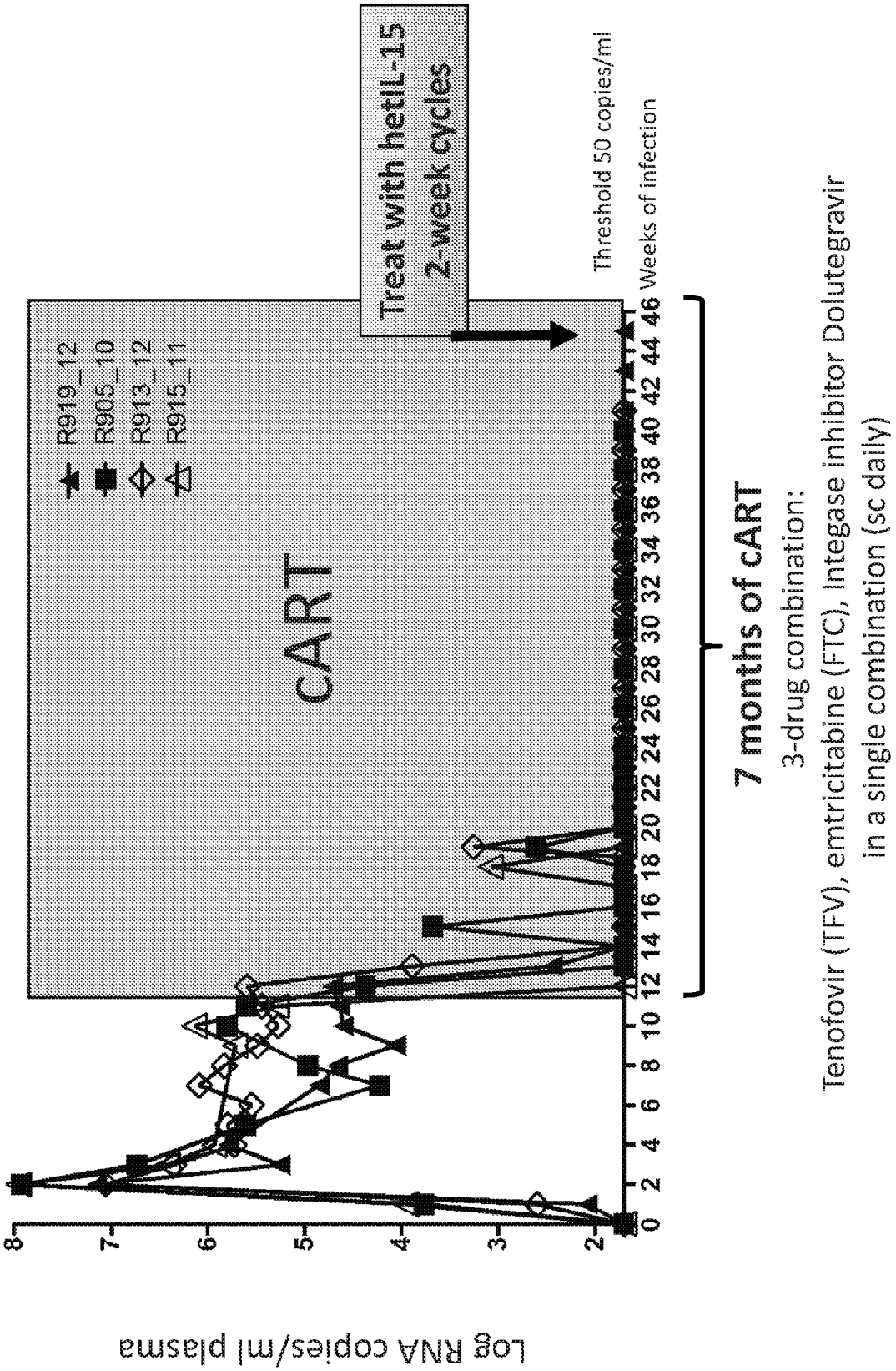

Log RNA copies/ml plasma
8
7
6
5
4
3
2
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Weeks of infection
Threshold 50 copies/ml
R919_12
R905_10
R913_12
R915_11
cART
Treat with hetIL-15
2-week cycles
7 months of cART
3-drug combination:
Tenofovir (TFV), emtricitabine (FTC), Integase inhibitor Dolutegravir
in a single combination (sc daily)

Figure 19

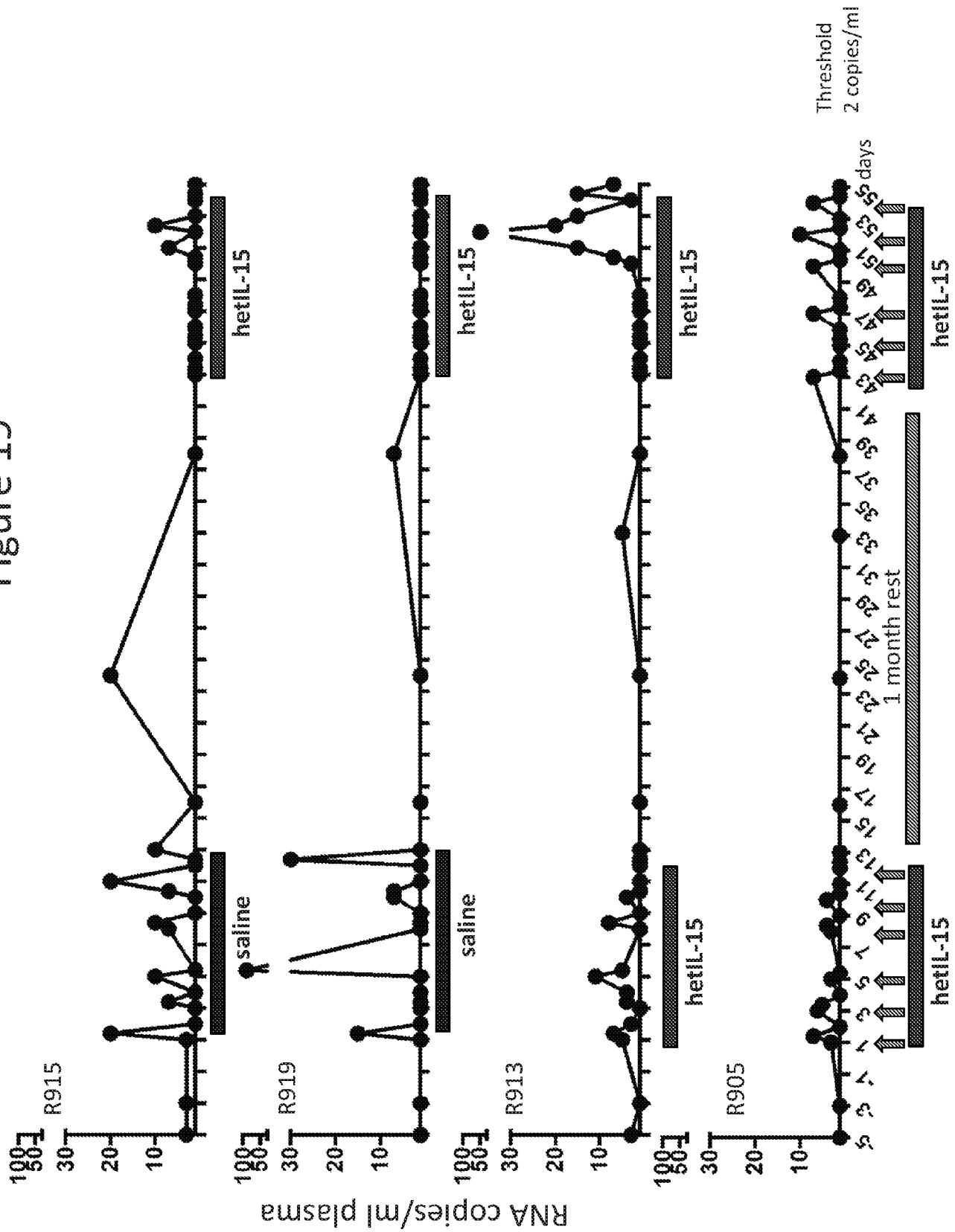

RNA copies/ml plasma
R915
R919
R913
R905
saline
hetIL-15
1 month rest
Threshold
2 copies/ml
days

Figure 20

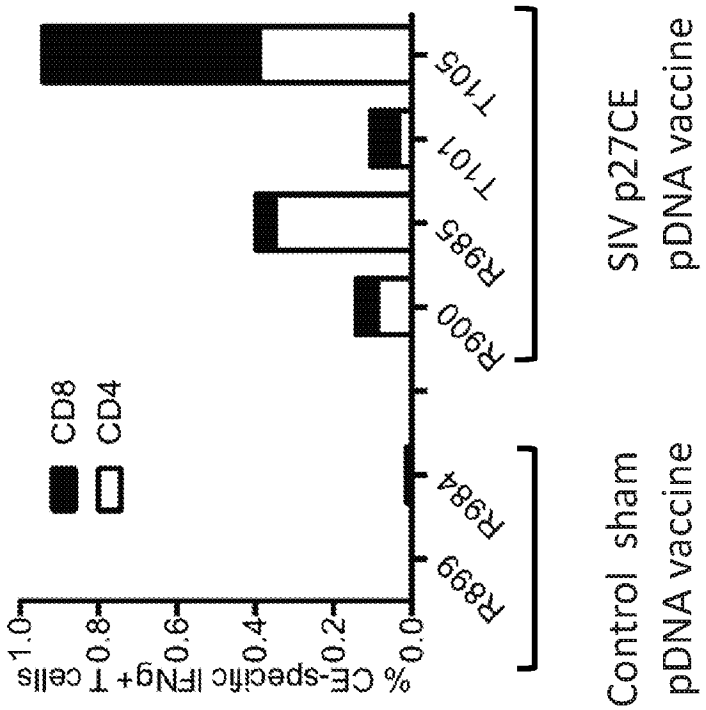

% CE-specific IFNg+ T cells
1.0
0.8
0.6
0.4
0.2
0.0
CD8
CD4
R899
R984
R900
R985
T101
T105
Control sham
pDNA vaccine
SIV p27CE
pDNA vaccine

Figure 21A-21C

