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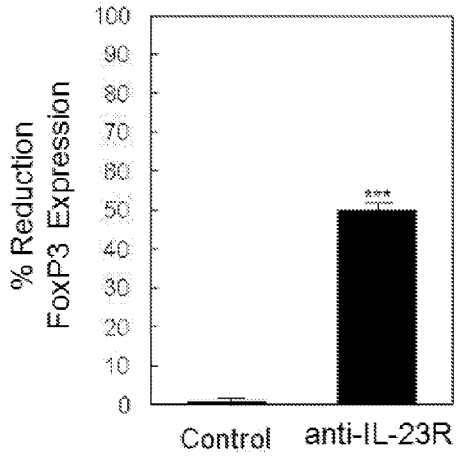


FIG. 11A

(57) Abstract: Provided by the disclosure are compositions and methods for modulating differentiation of regulatory T cells. In some embodiments, methods include selectively decreasing IL-23R activity and/or IL-23R expression without significantly decreasing IL-12RP activity and/or IL-12RP expression.

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IL-23R ANTAGONISTS TO REPROGRAM INTRATUMORAL T REGULATORY CELLS INTO EFFECTOR CELLS

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 62/500,443, filed on May 2, 2017, entitled "IL-23R ANTAGONISTS TO REPROGRAM INTRATUMORAL T REGULATORY CELLS INTO EFFECTOR CELLS," the entire contents of which are incorporated by reference herein.

FEDERALLY SPONSORED RESEARCH

This invention was made with government support under R01AI37562 and 5T32AI074549-07 awarded by the National Institute of Allergy and Infectious Diseases. The government has certain rights in the invention.

BACKGROUND OF INVENTION

Regulatory T cells (Treg cells) are critically important for maintenance of a balanced immune system, in part because they help to dampen excessive immune responses and prevent autoimmunity. However, immune suppression exerted by Treg cells can impede anti-tumor immune responses and promote cancer growth. The efficacy of several successful immunotherapies has been associated with depletion or blockade of CD4 Treg cells (Mahoney et al., Nat Rev Drug Discov 14:561-584).

SUMMARY OF INVENTION

The disclosure relates to selective induction of Treg cell phenotypic instability and selective conversion of intratumoroal, but not systemic, Treg cells into T effector cells (Teff cells). The disclosure relates to compositions for modulating the differentiation of Treg cells. The disclosure is based, in part, on the surprising discovery that the IL-23R cytokine receptor subunit is expressed on Treg cells and that inhibition of IL-23R induces differentiation of Treg cells (e.g., to Teff cells). Inducing Treg cell differentiation is particularly useful, for example, in the context of treating cancer.

In one aspect, disclosed herein are methods for treating cancer in a human subject. The methods involve administering to a human subject in need thereof an agent that decreases IL-23R activity and does not decrease IL-12Rβ activity, in an amount effective to

treat the cancer. In embodiments, the agent is a polypeptide, nucleic acid, or small molecule. In some embodiments, the agent binds the extracellular domain of IL-23R.

In some embodiments, the agent is a human or humanized monoclonal antibody. In some embodiments, the antibody binds IL-23R and does not bind IL-12Rβ. In some embodiments, the antibody has 100-fold, 1,000-fold, or 10,000-fold greater binding affinity for IL-23R than for IL-12Rβ. In some embodiments, the antibody is free of antibody-dependent cellular cytotoxicity (ADCC) activity. In some embodiments, the antibody has an IgG4 heavy chain immunoglobulin constant domain.

In some embodiments, the agent binds a nucleic acid coding for or expressing IL-23R.

In some embodiments, the methods further comprise administering to the human subject an immunomodulatory agent. In some embodiments, the immunomodulatory agent is an immune checkpoint inhibitor. In some embodiments, immune checkpoint inhibitor is an antagonist of a molecule selected from the group consisting of PD-1, TIM-3, TIGIT, VISTA, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR and LAG3. In some embodiments, the immunomodulatory agent in an antagonist of inhibitory macrophages and/or dendritic cells.

In some embodiments, the method further comprises administering an agent that inhibits cancer cell growth.

In another aspect, disclosed herein are methods for inducing differentiation of a Treg cell to a Teff cell. The methods involve contacting the Treg cell in vitro with an agent that decreases IL-23R activity and does not decrease IL-12Rβ activity. In some embodiments, the agent is a polypeptide, nucleic acid, or small molecule.

In some embodiments, the agent binds the extracellular domain of IL-23R. In some embodiments, the agent is a human or humanized monoclonal antibody. In some embodiments, the antibody binds IL-23R and does not bind IL-12Rβ. In some embodiments, the antibody has 100-fold, 1,000-fold, or 10,000-fold greater binding affinity for IL-23R than for IL-12Rβ. In some embodiments, the antibody is free of antibody-dependent cellular cytotoxicity (ADCC) activity. In some embodiments, the antibody has an IgG4 heavy chain immunoglobulin constant domain.

In some embodiments, the agent binds a nucleic acid coding for or expressing IL-23R.

In some embodiments, the Treg cell is a CD4+ Treg cell, a CD8+ Treg cell, a Helios+ Treg cell, or a CD25+ and FoxP3+ Treg cell.

In some embodiments, the method further comprises contacting the Treg cell in the presence of one or more inflammatory cytokines. In some embodiments, the one or more

inflammatory cytokines is selected from the group consisting of IL-2, IL-4, and combination thereof.

In another aspect, disclosed herein are methods for identifying a compound that will convert a Treg cell into a Teff cell. The methods comprise:

contacting a Treg cell with a test compound;

measuring IL-23R activity in the cell; and

measuring IL-12Rβ activity in the cell;

wherein the test compound is a compound for converting a Treg cell into an effector cell if:

(i) the IL-23R activity is decreased relative to a control cell that has been treated with a compound known to not decrease IL-23R activity and IL-12Rβ activity; and

(ii) the IL-12Rβ activity is not decreased relative to the control cell.

In some embodiments, the methods further comprise measuring FoxP3 activity in the cell.

In some embodiments, the methods further comprise measuring an effector cytokine produced by the cell. In some embodiments, the effector cytokine is TNF-α, IFN-γ, IL-17, IL-10, or IL-2.

In some embodiments, the test compound is selected from the group consisting of a polypeptide, a nucleic acid and a small molecule.

BRIEF DESCRIPTION OF DRAWINGS

The accompanying drawings are not intended to be drawn to scale. In the drawings, each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

FIGs. 1A-1B show that Treg specific Helios$^{-/-}$ mice exhibit elevated antitumor immunity**.** Treg specific Helios$^{+/+}$ and Helios$^{-/-}$ mice (Helios^{WT} and Helios^{KO} respectively) were inoculated with B16/F10 or MC38 cancer cell lines and assessed for tumor progression and cytotoxic T cell activity. **FIG. 1A** shows tumor volume (mm^3) of subcutaneously injected B16/F10 or MC38 cells. **FIG. 1B** shows intratumoral CD8 and IFN-γ expression. Representative FACS plots from triplicate experiments are shown. *P value:* <0.05.*

FIGs. 2A-2B. show Helios binding motifs in the first intron of *STAT5b* using ChiP-Seq analysis of Helios binding and modified histones at *STAT5b* in CD4 nTregs. The start

sites of each gene locus are indicated. The vertical lines in the gene diagrams (bottom) indicate exons. **FIG. 2A** shows the Helios binding site in the first intron of the *STAT5b* gene locus. **FIG. 2B** shows the Helios binding motifs from genome wide analysis of Helios binding sites.

FIGs. 3A-3C show isolated function of Helios deficient CD4 Treg in antitumor immunity. nTregs were isolated from $Rag2^{-/-}$ mice ($2x10^6$ CD4: $1x10^6$ CD8: $0.5x10^6$ $Helios^{WT}$ or $Helios^{KO}$ nTreg) B16/F10 tumor. Flow cytometry was gated on $CD45.2^+FoxP3^+$ cells. **FIG. 3A** shows the B16/F10 tumor growth (volume mm^3) curve beginning with first appearance of tumor (day 10). **FIG. 3B** shows the proportion of splenic and tumor infiltrating nTregs positive for FoxP3 expression**. FIG. 3C** shows the proportion of splenic and tumor infiltrating nTregs positive for INF-γ expression. Representative FACS plots from triplicate experiments are shown.

FIGs. 4A-4B show that Treg specific $Helios^{KO}$ mice present a reduced anergic phenotype. Anergic phenotype was determined in Treg specific $Helios^{WT}$ and $Helios^{KO}$ spleen and tumor infiltrate (MC38 inoculated mice) using co-expression of surface markers CD73 and FR4 in $FoxP3^+$ Treg. **FIG. 4A** shows expression of FoxP3 and **FIG. 4B** shows expression of $CD73^{high}FR4^{high}$ cells. Both **FIGs. 4A-4B** show representative FACS plots from triplicate experiments. *P value:* * *<0.05,* **, *<0.01.*

FIGs. 5A-5B show that $Helios^+$ nTreg lose surface receptor expression co-cultured with IL-4. nTreg were co-cultured with anti-CD3/anti-CD28 coated beads as well as steady state cytokine IL-2 and inflammatory cytokine IL-4. **FIG. 5A** shows a loss of Helios expression upon addition of IL-4. **FIG. 5B** shows the dose dependent IL-4-mediated shift in $Helios^+TIGIT^+$ nTreg proportion. The cells were gated on $TCR^+CD4^+CD25^+FoxP3^+$.

FIG. 6 shows a relevant Treg pathways in the inflammatory tumor microenvironment. Treg stabilize their suppressive phenotype by 1) enhanced IL-2 responsiveness via a Helios dependent genetic program and/or 2) enhanced STAT5 activation by IL-23R signaling that is uniquely operative within the tumor microenvironment. The blockade of either signaling pathway induces Treg conversion.

FIGs. 7A-7D shows that ligation of specific interleukin receptors induces Treg conversion *in vitro*. Receptors of interest were screened for loss of FoxP3 (**FIG. 7A**) and gain on IFN-γ expression (**FIG. 7B**). Anti-IL-23R, which resulted in the highest IFN-γ expression, was selected for further analysis. The kinetics of anti-IL-23R ligation on Treg conversion was assessed at 24 and 120 hours (5 days). A significant decrease in FoxP3 (**FIG. 7C**) and subsequent increase in IFN-γ (**FIG. 7D**) was observed when anti-IL-23R was co-cultured

with Treg after 24 hours of stimulation was observed. ANOVA: * p<0. 5 ** p<0.01 *** p<0.001 **** p<0.0001.

FIG. 8 shows that FoxP3 and IFN-γ expression after engagement of GITR induces Helios downregulation in CD4 Treg. FoxP3$^+$ CD4 Treg were isolated from spleens of wild type B6 mice and cultured in the anti-CD3/ CD28 coated wells in the presence of IL-4, increasing doses of IL-2, and anti-GITR (DTA-1) or isotype antibodies. After 5 days, the cells were analyzed for the levels of FoxP3 expression and IFN-γ production.

FIGs. 9A-9B provide confirmation of the *in vitro* conversion assay using a known TNF receptor super family member GITR agonist. Sorted C57BL/6 (WT) Treg (>90% pure CD4$^+$CD25$^+$) were cultured with plate bound anti-CD3/anti-CD28 in the presence of IL-2 and IL-4 for 24 hours or 5 days, and then assessed for conversion defined as loss of FoxP3 and gain of IFN-γ expression. The representative dot plots for Treg (gated on TCR$^+$CD4$^+$ cells) (**FIG. 9A**) and IFN-γ$^+$ cells (gated on FoxP3$^+$ cells) (**FIG. 9B**) with graphical representation of change from control (no antibody) are shown. ANOVA: ** p≤0.01 **** p≤0.0001.

FIGs. 10A-10C show that treatment with GITR agonist induces Treg conversion in TME. Wild type (WT) mice were inoculated with B16/F10 subcutaneously and treated with an intraperitoneal injection prophylactically on days 3, 6 and 9 or therapeutically on days 10, 12, 14 and 16). **FIG. 10A** shows tumor growth curves. Intratumoral and splenic Treg were assessed for IFN-γ (gated on FoxP3$^+$ cells) (**FIG. 10B**) and FoxP3 (gated on CD4$^+$CD25$^+$ cells) (**FIG. 10C**) expression. Student's T test: *** p≤0.001.

FIGs. 11A-11B show *in vitro* screen results for Treg conversion by anti-IL-23R antibody. Naïve peripheral Treg were sorted at >90% purity and cultured for 5 days in the presence of plate bound anti-CD3 and anti-CD28 as well as soluble IL-2 (20ng/mL) and IL-4 (50ng/mL). **FIG. 11A** shows the expression of FoxP3 by Treg (% reduction) and **FIG. 11B** shows the fold-increase IFN-γ$^+$ Treg cells after co-culture with IL-23 associated large molecules. Average ± SEM-ANOVA: ***p<0.001 ***p<0.0001.

FIG. 12 shows FoxP3 and CD25 expression in sorted Treg plated with plate bound anti-CD3/anti-CD28 and cultured for 5 days in the presence of IL-2 (20ng/mL) and IL-4 (50ng/mL). Antibodies were added at 10ng/mL on Day 0 of culture.

FIG. 13 shows that FoxP3^{hi} expression is lost with anti-IL-23R dose response. Sorted Treg were plated with plate bound anti-CD3/anti-CD28 and cultured for 5 days. In the presence of IL2 (20ng/mL) and IL4 (50ng/mL), anti-IL-23R antibodies were added at 1, 10 and 25 ng/mL incremental doses on Day 0 of culture. The reduced ratio of FoxP3^{hi} to FoxP3^{lo}

Treg is seen in the positive control (anti-GITR [DTA-1] 10ng/mL) as well as in the anti-IL-23R treated cells.

FIG. 14 provides a schematic illustration of the IL-23 receptor complex. The IL-23 cytokine consisting of p19 and p40 chains, binds to the heterodimeric receptor IL-23R and IL-12Rβ1.

FIG. 15 shows *in vivo* intratumoral Treg conversion and IL-23R expression. Mice were inoculated with B16/F10 cancer cells and assessed for IL-23R expression in splenic (left) and tumor infiltrating (right) Treg populations.

FIG. 16 shows IL-23R expression in tumor dLN and tumor infiltrating Treg cells. IL-23R expression is significantly increased in Treg found in the tumor and tumor draining lymph node (TdLN) compared to the spleen. MC38 cells were subcutaneously inoculated in C57BL/6 mice. Measurements were made (no treatment) on day 25 after inoculation. The representative FACs dot plots gated on $TCR^+CD4^+CD25^+FoxP3^+$ cells with graphical analysis are shown [Mean ± SEM ANOVA ***p>0.001 ****p.0.0001].

FIG. 17 shows that effector Treg (eTr) express high levels of IL-23R. Intratumoral effector Treg (eTr) have significantly increased IL-23R expression compared to TI central Treg (cTr) and splenic Treg. MC38 cells were subcutaneously inoculated in C57BL/6. Measurements were made on day 25 after inoculation. Representative FACs dot plot gated on $TCR^+CD4^+CD25^+FoxP3^+$ cells with graphical analysis are shown.

FIG. 18 shows that tumor infiltrating $CD69^+Icos^+$ Treg express high levels of IL-23R. Double positive $CD69^+Icos^+$ expression in tumor infiltrating (TI) Treg is significantly increased compared to TdLN and Spleen (SP) in MC38 inoculated mice. Representative FACs dot plots gated on $TCR^+CD4^+CD25^+FoxP3^+$ cells with graphical analysis are shown [Mean ± SEM ANOVA: * p>0.05 **p>0.01].

FIG. 19 shows that $Helios^+$ Treg express high levels of IL-23R. $Helios^+$ TI Treg have significantly increased IL-23R expression compared to $Helios^-$ TI Treg. Representative FACs dot plots gated on $TCR^+CD4^+CD25^+FoxP3^+$ cells with graphical analysis are shown[Mean ± SEM Student's T test: **p>0.01].

FIG. 20 shows that $Helios^{+WT}$ tumor infiltrating Treg have significantly increased IL-23R expression in CD69+ Treg compared to $Helios^{-WT}$ and $Helios^{KO}$ TI Treg. $Helios^{f/f}$ (WT) and $Helios^{f/f}$ $FoxP3^{cre}$ (KO) mice were inoculated subcutaneously with F10/B16 collected on day 21. The graphical analysis gated on $TCR^+CD4^+CD25^+FoxP3^+$ for IL-23R expression (top), Treg subsets including CD69+ eTr and cTr (middle), and IL-23R expression in Treg subsets (bottom) are shown [Mean ± SEM ANOVA or Student's T test: **p>0.01].

FIG. 21 shows that IL-23 regulates expression of FoxP3 and IFN-γ. Sorted Treg (>90% pure $CD3^+CD4^+CD25^+$) were plated with plate bound anti-CD3/anti-CD28 (10μg/mL in 1xPBS) and in the presence of IL2 (20ng/mL) and IL4 (50ng/mL) and cultured for 5 days (120 hours). IL-23 recombinant protein was added at 25ng/mL time 0 (T0) of culture and $p(40)_2$ was added for the last 24 hours of culture (T96). Representative dot plots are shown for decreased expression of FoxP3 (top left) and increased expression of IFN-γ (bottom left). The graphical representation (right) of the fold change from baseline (negative control or no antibody) is shown as mean±SEM. ANOVA: $p<0.05$.

FIG. 22 shows CD25 expression in sorted Treg plated with plate bound anti-CD3/anti-CD28 and cultured for 5 days in the presence of IL2 (20ng/mL) and IL4 (50ng/mL). Antibodies and p40 homodimer were added at 0 and 96 hours and then cultured for 1 or 5 days.

FIG. 23 shows IFN-γ expression and fractions of IFN-γ expressing cells in sorted Treg plated with plate bound anti-CD3/anti-CD28 and cultured for 5 days in the presence of IL2 (20ng/mL) and IL4 (50ng/mL). Antibodies and p40 homodimer were added at 0 and 96 hours and then cultured for 1 or 5 days.

FIG. 24 shows tumor outgrowth in control (1xPBS) and p40 homodimer treated mice. Treatment (50μg/mouse using an intraperitoneal injection) control (n=3) or p40 homodimer (n=4) was given on day 0, 4, and 8 as indicated by the arrows (↓). The tumor volume (mm^3) is represented as an average (left) and as individual values (right).

FIGs. 25A-25E show *in vivo* effects of anti-IL-23R treatment. **FIG. 25A** shows the protocol for the *in vivo* experiment. **FIGs. 25B-25C** show tumor outgrowth curves. **FIG. 25B** shows the individual growth curves. The number in the bottom corner indicates how many mice developed tumors/total sample size (day 21). **FIG. 25C** illustrates the mean tumor outgrowth ± SD. Student's T Test (day 21). **FIG. 25D** shows that anti-IL-23R antibody treatment delays tumor outgrowth. Tumor outgrowth in isotype antibody and anti-IL-23R antibody treated mice is shown. Treatment (100mg/mouse using an intraperitoneal injection) isotype (n=3) anti-IL-23R (n=3) was given on day 8, 11, and 14 as indicated by the arrows (↓). The tumor volume (mm^3) is represented as an average (left) and as individual values (right). Student's T test (day 16 data points). **FIG. 25E** shows the tumor out growth curve treatment given intraperitoneally (100μg/mouse) on day 8, 11 and 14.

FIGs. 26A-26B show that anti-IL-23R induces intratumoral Treg conversion and increased antitumor activity. **FIG. 26A** shows CD8 activity as shown by IFN-γ expression. (Representative FACs plots gated on TCR^+CD8^+ cells are shown. **FIG. 26B** shows

intratumoral Treg conversion as indicated by IFN-γ expression Representative FACs plots gated on $TCR^+CD4^+CD25^+FoxP3^+$ cells are shown. ANOVA or Student's T test: * $p<0.05$ ***$p<0.001$ ****$p<0.0001$.

FIG. 27 shows that IL-23R expression is significantly decreased in anti-IL-23R antibody treated tumor and in tumor infiltrating activated Treg. Representative FACs dot plot gating strategy on single cells lymphocytes to aTreg (top left to right), graphical analysis of $IL\text{-}23R^+$ intratumoral cell proportion (bottom left), and $IL\text{-}23R^+$aTreg numbers (bottom right) collected on day 21 are shown [Mean ± SEM Student's t test: *$p>0.05$ **p.0.01].

FIG. 28 shows that IL-23R expression is significantly decreased in eTr found in the tumor of anti-IL-23r antibody treated mice. Representative FACs dot plots gated on $TCR^+CD4^+CD25^+FoxP3^+$ cells with graphical analysis of $IL\text{-}23r^+$ eTr and cTr collected on day 21 are shown [Mean ± SEM Student's t test *$p>0.05$].

FIG. 29 shows that anti-IL-23R tumor infiltrating Treg have decreased Helios expression. Representative dot plots with graphical analysis gated on $IL\text{-}23R^+TCR^+CD4^+FoxP3^+$ for Helios expression (left) and graphical analysis (right) are shown [Mean ± SEM Student's T test *$p>0.05$].

FIG. 30 shows that anti-IL-23R treated mice have significantly reduced intratumoral suppressive Treg compared to isotype. The representative dot plots with graphical analysis gated on TCR^+CD4^+ $CD25^+FoxP3$ and IFN-γ expression with graphical analysis on number of tumor infiltrating (TI) Treg, $FoxP3^{hi}$, and IFN-γ expression are shown [Mean ± SEM Student's T test *$p>0.05$ **$p>0.01$ ****$p>0.0001$].

FIG. 31 shows that anti-IL-23R antibody treatment increases intratumoral CD8/Treg ratio and CD8 IFN-γ expression. Representative FACs dot plots gated on single cells lymphocytes (left) and $TCR^+CD4^+CD25^+FoxP3^+$ (right) cells with graphical analysis are shown [Mean ± SEM Student's t test *$p>0.05$].

FIG. 32 provides a schema for a Treg conversion screening protocol using a $FoxP3^{RFP}$ $IFN\text{-}\gamma^{YFP}$ reporter system.

FIG. 33 shows tumor volume in mice inoculated with MC38 cells and treated with isotype or 12B2B64 or 258018 IL-23R antibodies.

FIGs. 34A-34B show conversion of Tregs upon administration of IL-23R antibodies. Sorted Treg (WT) were plated for 5 days with plate bound anti-CD3/anti-CD28 and in the presence of IL-2 (20ng/mL) and IL-4 (50ng/mL) with 10ng/mL 12B2B64 or 258018 IL-23R antibodies or DTA-1. All samples were compared to Negative Control (no antibody). **FIG. 34A** shows percent change of FOXP3 expression. **FIG. 34B** shows percent change of IFN-

gamma expression. Graphs show combined data from three experiments (mean±SEM ANOVA $p<0.05$ * $p<0.01$ ** $p<0.001$ *** $p<0.0001$ ****.

FIG. 35 shows the eTr phenotype in intratumoral Treg in mice treated with IL-23R antibodies. All mice were inoculated with MC38 and treated with isotype or 12B2B64 or 258018 IL-23r Ab. Representative dot plots are gated on Treg ($TCR^+CD4^+CD25^+FoxP3^+$) and eTre ($TCR^+CD4^+CD25^+FoxP3^+CD44^+CD62L^-$). Graphic analysis is based on IL-$23r^+$ eTr. Mean±SEM ** $p<0.01$.

FIG. 36 shows the pSTAT5:pSTAT3 ratio in mice treated with IL-23R antibodies. All mice were inoculated with MC38 and treated with isotype or 12B2B64 or 258018 IL-23r Ab. The STAT5 activation and STAT3 activation was measured in tumor and spleen. Representative histograms are shown as are the pSTAT5:pSTAT3 ratios.

FIG. 37 shows an alignment of murine and human IL-23R and a schematic illustration of the domains of IL-23R.

FIG. 38 shows the regions in the cytokine binding domain of IL-23R where the peptides identified in the epitope mapping study are located.

FIGs. 39A-39B show that loss of IL-23R enhances conversion of Treg→Teff generally in a Treg conversion assay. **FIG. 39A** shows FoxP3 expression levels and **FIG. 39B** shows IFN-γ expression levels in $IL\text{-}23R^{KO}$ and C57BL/6 Tregs.

FIGs. 40A-40C shows that loss of IL-23R enhances conversion of Treg→Teff in tumor cells. Genetic deletion of IL-23R in Tregs injected into $Rag2^{-/-}$ mice leads to a decrease in tumor volume in mice inoculated with the MC38 cancer cell line **(FIG. 40A)**, a decrease in FoxP3 expression **(FIG. 40B)** and an increase in IFN-γ expression **(FIG. 40C)** compared to $IL\text{-}23R^{WT}$ Treg controls. Expression levels from TIL and spleen cells were independently tested.

FIGs. 41A-41B show that anti-IL-23R antibody induces conversion of Treg→Teff in EL4 lymphoma tumor cells. Treatment of mice inoculated with EL4 cancer cells with anti-IL-23R antibody leads to a decrease in FoxP3 expression **(FIG. 41A)** and an increase in IFN-γ expression **(FIG. 41B)** compared to treatment with isotype. Expression levels from TIL and spleen cells were independently tested.

FIGs. 42A-42C shows that show that treatment with anti-IL-23R antibody in vitro alters STAT5 and STAT3 expression. Treatment of $CD25^+FoxP3^+$ Tregs with anti-IL-23R antibody leads to a decrease in pSTAT5 expression and an increase in pSTAT3 expression compared to treatment with isotype **(FIG. 42A)**. These expression changes result in a decrease in the pSTAT5:pSTAT3 ratio for Tregs treated with anti-IL-23R antibody compared

to isotype (**FIG. 42B**). Treatment with anti-IL-23R antibody also leads to increased expression of IFN-γ (**FIG. 42C**)

FIGs. 43A-43B shows that treatment with anti-IL-23R antibody reduces STAT5 expression in Treg at multiple timepoints. The reduction in pSTAT5 expression resulting in treatment of $CD25^{+}FoxP3^{+}$ Tregs with anti-IL-23R antibody compared to isotype occurs at one hour and persists at five days as shown by flow cytometry (**FIG. 43A**) and as a percentage of initial expression (**FIG. 43B**).

FIGs. 44A-44F shows the effect of an anti-IL-23R antibody treatment in mice that have been inoculated with MC38 cancer cells. **FIGs. 44A-44C** show that treatment with anti-IL-23R antibody provides a reduction in the pSTAT5:pSTAT3 ratio compared to treatment with isotype. STAT5 expression is shown in **FIG. 44A** and STAT3 expression is shown in **FIG. 44B**. **FIG 44D s**hows that treatment with anti-IL-23R antibody decreases the numbers of $CD25^{+}FoxP3^{+}$ Tregs, compared to treatment with isotype. **FIGs 44E-44F** show that treatment with anti-IL-23R antibody increases IFN-γ expression and decreases IL-23R expression levels.

FIGs. 45A-45B show the effect of an anti-IL-23R antibody treatment in mice that have been inoculated with EL4 cancer cells on STAT5 and STAT3 expression. Expression of STAT5 in mice inoculated with EL4 cancer cells and treated with anti-IL-23R antibody is shown in **FIG. 45A**. The pSTAT5:pSTAT3 ratio, compared to treatment with isotype, is shown in **FIG. 45B**.

FIGs. 46A-46C show the effect of STAT3 expression by Treg on tumor growth CD69 and IFN-γ expression. **FIG. 46A** shows that tumor growth, as assessed by tumor volume, progresses with delayed kinetic in $STAT3^{KO}$Treg mice than it does in $STAT3^{WT}$Treg mice. **FIGs 46B-46C** show that $STAT3^{KO}$Treg mice have increased expression levels of CD69 and IFN-γ compared to $STAT3^{WT}$Treg mice in CD8 TIL.

FIGs 47A-47B show that anti-IL23-R antibody interacts with Tregs. **FIG. 47A** shows that treatment of C57BL/6 mice with anti-IL-23R antibody reduces tumor volumes compared to C57BL/6 mice treated with isotype. **FIG. 47B** shows that treatment of $Rag2^{-/-}$ mice that have been injected with Tregs alone or in conjunction with CD8+ T cells with anti-IL-23R antibody reduces tumor volume compared to treatment with isotype.

DETAILED DESCRIPTION OF INVENTION

The present disclosure, in one aspect, relates to the surprising discovery that IL-23R modulates Treg differentiation, and in particular, that inhibition of IL-23R induces Treg

differentiation. The IL-23R cytokine receptor subunit dimerizes with IL-12 β1 to form the IL-23 receptor, which binds the IL-23 cytokine. It has surprisingly been found that IL-23R is expressed on Treg cells the and inhibition of IL-23R induces differentiation of these Treg cells (e.g., to Teff cells).

The present disclosure, in another aspect, relates to the surprising discovery that IL-23R modulates cancer. Regulatory T cells are critically important for maintenance of a balanced immune system, in part because they help to dampen excessive immune responses and prevent autoimmunity. However, immune suppression exerted by Treg can impede anti-tumor immune responses and promote cancer growth. Inducing differentiation of Treg via decreasing IL-23R activity has the capacity to slow cancer progression and agents that decrease IL-23R activity represent novel therapeutics for treating cancer.

Thus, in one aspect, the present disclosure provides agents for treating cancer by decreasing IL-23R activity. In another aspect, the present disclosure provides agents for inducing differentiation of a regulatory T (Treg) cell to an effector T cell by decreasing IL-23R activity. Such agents can include antibodies, polypeptides, nucleic acids, or small molecules.

Interleukin-23 Receptor (IL-23R)

IL-23R mediates signaling by IL-23. Interleukin-23 (IL-23) is a heterodimeric cytokine comprised of two subunits, p19 which is unique to IL-23, and p40, which is shared with IL-12. IL-23 mediates signaling by binding to the heterodimeric IL-23 receptor, comprised of an IL-23R subunit and an IL-12Rβ1, which is shared by the IL-12 receptor. The p19 subunit of IL-23 interacts with the IL-23R subunit of the IL-23 receptor and the p40 subunit of IL-23 interacts with the IL-12Rβ1 subunit of the IL-23 receptor. IL-23 interacts with the IL-23 receptor at the extracellular region.

IL-23-R is a protein with isoforms ranging in size from 20 kDa to 43 kDa. The sequence of IL-23-R can be found at NCBI Ref. No. NM_144701.2.

IL-23R associates with Janus kinase 2 (Jak2) and in a ligand-dependent manner with Signal transducer and activator of transcription STAT3. IL-12Rβ1 interacts directly with Tyrosine kinase 2 (Tyk2).

IL-23 induced activation of STAT3 leads to direct binding of phosphorylated STAT3 to Interleukin-17 (IL-17) and Interleukin 17F (IL-17F) promoters. STAT3 up-regulates the expression of Retinoic Acid Receptor-Related Orphan Receptor Gamma-T (ROR-gamma), a Th17 specific transcriptional regulator that is critical for the expression of two members of

Interleukin-17 family, IL-17A (IL-17) and IL-17F. IL-23 induced JAK2 activation triggers Phosphoinositide-3-kinase (PI3K)/RAC-alpha serine/threonine kinase (AKT) and Nuclear factor kappaB (NF-kB) pathways which are required for IL-17 production. PI3K/AKT pathway is involved in STAT3 phosphorylation through an undetermined mechanism.

In embodiments, an agent described herein decreases IL-23R activity. In general, a decrease means any statistically significant decrease in activity by any one or more measures. The measures of activity may be direct or indirect, using one or more of the measures described herein or otherwise known to those of ordinary skill in the art. The measure may be of a cell in a resting state or a cell contacted with exogenous ligand to IL-23R. The measure may be as illustrated in the examples. The measure typically will be carried out *in vitro*, to establish that the agent has the desired properties of decreasing IL-23R activity but not IL-12R activity. Such agents then can be administered in therapeutically effective amounts. Alternatively, the measure may be an improvement in the subject being treated, such as the slowing of progression of, the halting of the growth of, or the shrinkage of the cancer.

In some embodiments, IL-23R activity is decreased relative to a control at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, and even more than 75%. In some embodiments, IL-23R activity is decreased 1%-5%, 1%-10%, 5%-10%, 5%-25%, 10%-50%, 20%-40%, 40%-60%, 50%-90%, 50%-70%, 60%-80%, 80%-90%, 90%-95%, 90%-99%, or 95%-99%.

In some embodiments, an agent described herein is administered to a subject and IL-23R activity is decreased in the subject. In some embodiments, the agent is administered to a subject and IL-23R activity is decreased relative to the level of IL-23R activity prior to administration of the agent. In some embodiments, the IL-23R activity is decreased relative to the level of IL-23R activity in the subject 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours 12 hours 24 hours, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks or more prior to administration of the agent. In some embodiments, multiple doses of the agent are administered to a subject and IL-23R activity is decreased relative to the level of IL-23R activity prior to the first administration of the agent, e.g., the beginning of treatment. In some embodiments, the IL-23R activity is decreased relative to the level of IL-23R activity in the subject 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours 12 hours 24 hours, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks or more prior to the first administration of the agent. In some embodiments, multiple doses of the agent are administered to a subject and IL-23R activity is decreased relative to the level of IL-23R activity prior to the current administration of the

agent. In some embodiments, the IL-23R activity is decreased relative to the level of IL-23R activity in the subject 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours 12 hours 24 hours, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks or more prior to the current administration of the agent.

In some embodiments, the agent is administered to a subject and IL-23R activity is decreased relative to the level of IL-23R activity in a control subject who is not treated with the agent. In some embodiments, the control subject has the same or a similar type of cancer as the subject. In some embodiments, the control subject has the same or a similar severity of cancer as the subject. In some embodiments, the control subject does not have cancer. In some embodiments, the control subject is the same or a similar age as the subject. In some embodiments, the control subject is the same sex as the subject. In some embodiments, the control subject is related to the subject. In some embodiments, the control subject is a twin of the subject.

In some embodiments, the agent is administered to a subject in amounts effective to treat cancer in the subject. Effective amounts are described in greater detail below.

In some embodiments, Treg cells are contacted *in vitro* with an agent described herein and IL-23R activity is decreased. In some embodiments, IL-23R activity is decreased relative a control cell, e.g., a cell treated with a compound known to not decrease IL-23R activity and IL-12Rβ activity. In some embodiments, IL-23R activity is decreased relative a cell of the same clonal population, e.g., of the same culture, that are not administered the agent. In some embodiments, the IL-23R activity of the cell of the same clonal population is measured simultaneously or near simultaneously with the IL-23R activity of the Treg cell. In some embodiments, the IL-23R activity of the cell of the same clonal population is measured before or after the IL-23R activity of the Treg cell. In some embodiments, the IL-23R activity is decreased relative to cells of the same cell type, e.g., are derived from the same source, that are not administered the agent.

In some embodiments, a decrease in IL-23R activity comprises a decrease in expression of FoxP3, Helios, IL-17A, IL-17F, ROR-gamma, and/or IL-23. In some embodiments, expression of FoxP3, Helios, IL-17A, IL-17F, ROR-gamma, and/or IL-23 is decreased 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, expression of FoxP3, Helios, IL-17A, IL-17F, ROR-gamma, and/or IL-23 is decreased more than 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%. In some embodiments, expression of FoxP3, Helios, IL-17A, IL-17F,

ROR-gamma, and/or IL-23 is decreased 1%-5%, 1%-10%, 5%-10%, 5%-25%, 10%-50%, 20%-40%, 40%-60%, 50%-90%, 50%-70%, 60%-80%, 80%-90%, 90%-95%, 90%-99%, 90%-99%, or 95%-99%.

Gene expression can be measured at either the RNA or protein level. Assays for detecting RNA include, but are not limited to, Northern blot analysis, RT-PCR, sequencing technology, RNA in situ hybridization (using e.g., DNA or RNA probes to hybridize RNA molecules present in the sample), in situ RT-PCR (e.g., as described in Nuovo GJ, et al. Am J Surg Pathol. 1993, 17: 683-90; Komminoth P, et al. Pathol Res Pract. 1994, 190: 1017-25), and oligonucleotide microarray (e.g., by hybridization of polynucleotide sequences derived from a sample to oligonucleotides attached to a solid surface (e.g., a glass wafer with addressable location, such as Affymetrix microarray (Affymetrix®, Santa Clara, CA)).

Assays for detecting protein levels include, but are not limited to, immunoassays (also referred to herein as immune-based or immuno-based assays, e.g., Western blot, ELISA, proximity extension assays, and ELISpot assays), Mass spectrometry, and multiplex bead-based assays. Other examples of protein detection and quantitation methods include multiplexed immunoassays as described for example in U.S. Patent Nos. 6939720 and 8148171, and published U.S. Patent Application No. 2008/0255766, and protein microarrays as described for example in published U.S. Patent Application No. 2009/0088329.

In some embodiments, IL-23 activity can be measured by measuring IL-17 and/or interferon-gamma secretion following treatment of activated preipheral blood mononuclear cells (Dallas et al., Drug Metabolism and Disposition, 2013, 41:689–693). In some embodiments, IL-23 activity can be measured by measuring differentiation of CD4+ T cells to IL-17-producing pathogenic Th17 cells. (Reddy et al., Cell Immunol, 2007, 47:1–11).

In some embodiments, a decrease in IL-23R activity stimulates Teff development (i.e., Treg differentiation) as is described in further detail below.

Interleuken 12

Interleukin-12 (IL-12) is a heterodimeric molecule composed of p35 and p40 subunits. IL-12 plays a role in the differentiation of naïve T cells into T-helper type 1 CD4 lymphocytes that secrete IFNγ. IL-12 is also important for T cell dependent immune and inflammatory responses in vivo. The IL-12 receptor (IL-12Rβ) is composed of IL-12Rβ1 and IL-12Rβ2 subunits.

The main role of IL-12 is activation of interferon gamma production. Upon binding to its receptor, IL-12 activates Janus family kinases Tyrosine kinase 2 (Tyk2) and Janus kinase

2 (Jak2). Interleukin 12 receptor, beta 1 (IL-12Rβl) binds Tyk2, whereas Interleukin 12 receptor, beta 2 (IL-12Rβ2) associates with Jak2. Jak2 phosphorylates the tyrosine residues of STAT3 and STAT4. They translocate to the nucleus and bind to the promoter site of IFN-gamma. STAT4 also induces transcription of IL-12Rβ2, IL18-Rβ1, Interferon regulatory factor 1 (IRF1), Interleukin 2 receptor, alpha (IL-2R), and GCNT1 glucosaminyl (N-acetyl) transferase 1 core 2 (G6NT).

In embodiments, agents described herein do not appreciably decrease IL-12Rβ activity. It will be understood by one of ordinary skill in the art, that according to the invention, agents are employed that decrease IL-23R activity and do not decrease IL-12Rβ activity. In general, it is expected that such agents will not appreciably decrease IL-12Rβ activity because the agents do not interact directly with IL-12Rβ. The IL-12Rβ activity, therefore, will not vary more than would be expected in control cells.

In embodiments, the absence of a decrease in activity may be established by any one or more measures. The measures of activity may be direct or indirect, using one or more of the measures described herein or otherwise known to those of ordinary skill in the art. The measure may be of a cell in a resting state or a cell contacted with exogenous ligand to IL-12R. The measure may be as illustrated in the examples. The measure typically will be carried out *in vitro*, to establish that the agent has the desired properties of decreasing IL-23R activity but not IL-12Rβ activity. The measure typically will be carried out *in vitro*, to establish that the agent has the desired property of not decreasing IL-12Rβ activity. As used herein "does not decrease" means IL-12Rβ activity will decrease significantly less than the decrease observed in IL-23 activity. that the IL-12Rβ activity levels do not decrease in a statistically significant manner, relative to a control. Typically, the IL-12Rβ activity does not decrease in a statistically significant manner, relative to a control. In general, the decrease in IL-12Rβ activity, relative to a control, would be less than 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%. Typically, there would be no statistically significant decrease in IL-12Rβ activity.

In some embodiments, the agent is administered to a subject and IL-12Rβ activity is not decreased relative to the level of IL-12Rβ activity prior to administration of the agent. In some embodiments, the IL-12Rβ activity is not decreased relative to the level of IL-12Rβ activity in the subject 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours 12 hours 24 hours, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks or more prior to administration of the agent. In some embodiments, multiple doses of the agent

are administered to a subject and IL-12Rβ activity is not decreased relative to the level of IL-12Rβ activity prior to the first administration of the agent, e.g., the beginning of treatment. In some embodiments, the IL-12Rβ activity is not decreased relative to the level of IL-12Rβ activity in the subject 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours 12 hours 24 hours, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks or more prior to the first administration of the agent. In some embodiments, multiple doses of the agent are administered to a subject and IL-12Rβ activity is not decreased relative to the level of IL-12Rβ activity prior to the current administration of the agent. In some embodiments, the IL-12Rβ activity is not decreased relative to the level of IL-12Rβ activity in the subject 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks or more prior to the current administration of the agent.

In some embodiments, the agent is administered to a subject and IL-12Rβ activity is not decreased relative to the level of IL-12Rβ activity in a control subject who is not treated with the agent. In some embodiments, the control subject has the same or a similar type of cancer as the subject. In some embodiments, the control subject has the same or a similar severity of cancer as the subject. In some embodiments, the control subject does not have cancer. In some embodiments, the control subject is the same or a similar age as the subject. In some embodiments, the control subject is the same sex as the subject. In some embodiments, the control subject is related to the subject. In some embodiments, the control subject is a twin of the subject.

In some embodiments, the agent is administered to a Treg cell in vitro and IL-12Rβ activity is not decreased relative a cell of the same clonal population, e.g., of the same culture, that are not administered the agent. In some embodiments, the IL-12Rβ activity of the cell of the same clonal population is measured simultaneously or near simultaneously with the IL-12Rβ activity of the Treg cell. In some embodiments, the IL-12Rβ activity of the cell of the same clonal population is measured before or after the IL-12Rβ activity of the Treg cell. In some embodiments, the agent is administered to a Treg in vitro and IL-12Rβ activity is not decreased relative to cells of the same cell type, e.g., are derived from the same source, that are not administered the agent.

In some embodiments, IL-12Rβ activity comprises STAT4 phosphorylation. In some embodiments, STAT4 phosphorylation levels do not decrease by more than 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%.

In some embodiments, IL-12Rβ activity comprises a change in expression of IL-12Rβ2, IL18-Rβ1, IRF1, IL-2R, and/or G6NT. In some embodiments, expression of IL-12Rβ2, IL18-Rβ1, IRF1, IL-2R, and/or G6NT do not decrease by more than 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%.

In some embodiments, IL12 activity can be measured by measuring IL-17 and/or interferon-gamma secretion following treatment of activated peripheral blood mononuclear cells (Dallas et al., Drug Metabolism and Disposition, 2013, 41:689–693). In some embodiments, IL-23 activity can be measured by measuring differentiation of CD4+ T cells to interferon-gamma (IFN-gamma)-producing T helper 1 (Th1) cells. (Reddy et al., Cell Immunol, 2007, 47:1–11.)

Induction of Treg Differentiation

In some embodiments, the agents described herein are useful in a method of inducing differentiation of cells. In some embodiments, an agent described herein induces differentiation of a T cell. In some embodiments, an agent described herein induces differentiation of a regulatory T cell (i.e., a Treg). In some embodiments, an agent described herein induces differentiation of a Treg cell to a Teff cell.

In some embodiments, Treg cells are Helios+ Treg cells. In some embodiments, Treg cells are TIGIT+ Treg cells. In some embodiments, Treg cells are $CD4^+$ Treg cells. In some embodiments, the CD4+ Treg cells are CD4+CD25+ Treg cells. CD4+CD25+ regulatory T cells are thought to function as a regulator of autoimmunity by suppressing the proliferation and/or cytokine production of CD4+CD25− T cell responder cells at the site of inflammation. In some embodiments, the CD4+ Treg cells are CD4+FoxP3+ Treg cells. In some embodiments, the CD4+ Treg cells are CD4+FoxP3+CD25+ Treg cells.

In some embodiments, Treg cells are $CD8^+$ Treg cells. In some embodiments, the $CD8^+$ Treg cells are CD8+CD28- Treg cells. In some embodiments, the CD8+ Treg is positive for Killer cell immunoglobulin like receptor (Kir+).

In some embodiments, Treg cells are Tr1 Treg cells. In some embodiments, Treg cells are Th3 Treg cells. In some embodiments, Treg cells are Qa-1 restricted Treg cells.

In some embodiments, Teff cells are marked by the expression of certain cytokines. Examples of effector cytokines expressed by Teff include tumor necrosis factor alpha (TNF-α), interferon-γ (IFN-γ), interleukin-17 (IL-17), interleukin-2 (IL-2), and Granzyme B.

However, the skilled artisan appreciates that other effector cytokines may also be expressed by differentiated T effector cells.

In some embodiments, effector cells are $CD4^+$ effector T cells. As used herein the term "CD4 effector T cells" refers to a subset of T cells which are associated with cell-mediated immune response. They are characterized by the secretion of one or more effector cytokines such as, but not limited to, IFN-γ, TNF-α, IL-17, IL-2 and granzyme B.

In some embodiments, the effector cells are $CD8^+$ effector T cells. In some embodiments, $CD8^+$ effector T cells are cytotoxic T cells.

In some embodiments, when differentiation is induced, 0.01%-100% of the Treg, e.g., intratumoral Treg, are converted to Teff. In some embodiments, when differentiation is induced, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the Treg, e.g., intratumoral Treg, are converted to Teff. In some embodiments, when differentiation is induced, more than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, or 20% of the Treg, e.g., intratumoral Treg, are converted to Teff. In some embodiments, when differentiation is induced, 0.01%-1%, 0.1%-1%, 0.5%-2%, 1%-10%, 2%-15%, 5%-20%, 10%-30%, 20%-50%, 20%-70%, 40%-50%, 40%-60%, 50%-70%, 50%-80%, 60%-70%, 60%-90%, 70%-90%, 70%-99%, or 80%-99% of the Treg, e.g., intratumoral Treg, are converted to Teff.

In some embodiments, when differentiation is induced, 0.01%-100% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to CD8+ cells. In some embodiments, when differentiation is induced, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to CD8+ cells. In some embodiments, when differentiation is induced, more than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, or 20% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to CD8+ cells. In some embodiments, when differentiation is induced, 0.01%-1%, 0.1%-1%, 0.5%-2%, 1%-10%, 2%-15%, 5%-20%, 10%-30%, 20%-50%, 20%-70%, 40%-50%, 40%-60%, 50%-70%, 50%-80%, 60%-70%, 60%-90%, 70%-90%, 70%-99%, or 80%-99% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to CD8+ cells.

In some embodiments, when differentiation is induced, 0.01%-100% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to cells that secrete one or more of IFN-γ, TNF-α, IL-17, IL-2 and granzyme B. In some embodiments, when differentiation is induced, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to cells that secrete one or more of IFN-γ, TNF-α, IL-17, IL-2 and granzyme B. In some embodiments, when differentiation is induced, more than 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, 10%, or 20% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to cells that secrete one or more of IFN-γ, TNF-α, IL-17, IL-2 and granzyme B. In some embodiments, when differentiation is induced, 0.01%-1%, 0.1%-1%, 0.5%-2%, 1%-10%, 2%-15%, 5%-20%, 10%-30%, 20%-50%, 20%-70%, 40%-50%, 40%-60%, 50%-70%, 50%-80%, 60%-70%, 60%-90%, 70%-90%, 70%-99%, or 80%-99% of CD4+CD25+ cells, e.g., intratumoral CD4+CD25+ cells, are converted to cells that secrete one or more of IFN-γ, TNF-α, IL-17, IL-2 and granzyme B.

In some embodiments, the phenotype of the cells is determined using any method known in the art, for example, flow cytometry.

In some embodiments, the cells are differentiated in vitro. In some embodiments, In some embodiments, one or more parameters identified herein, e.g., IL-23R or IL-12Rβ activity, stat3 phosphorylation, expression of one or more targets of IL-23R and/or IL-12Rβ, and/or conversion of Treg to Teff, are measured in the in vitro cells.

Agents

The methods described herein contemplate treating cancer with agents that decrease IL-23R activity and do not decrease IL-12Rβ activity.

Examples of inhibitor agents that may be used in accordance with the present disclosure include, without limitation, nucleic acids (e.g., DNA and/or RNA) and nucleic acid analogues; antibodies, including full-length antibodies and antigen-binding antibody fragments, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, and humanized antibodies; proteins, including peptides, peptide-mimetics and aptamers; and small molecules.

In some embodiments, an agent described herein binds the extracellular domain of IL-23R.

Antibodies

An antibody (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term "antibody" encompasses not only intact (*i.e.*, full-length) polyclonal or monoclonal antibodies, but also antigen-binding fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, linear antibodies, single chain antibodies, multispecific antibodies (e.g., bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. An antibody includes an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The antibodies to be used in the methods described herein can be murine, rat, human, or any other origin (including chimeric or humanized antibodies). In some examples, the antibody comprises a modified constant region, such as a constant region that is immunologically inert, e.g., does not trigger complement mediated lysis, or does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC). ADCC activity can be assessed using methods disclosed in U.S. Pat. No. 5,500,362. In other embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or UK Patent Application No. 9809951.8.

Any of the antibodies described herein can be either monoclonal or polyclonal. A "monoclonal antibody" refers to a homogenous antibody population and a "polyclonal antibody" refers to a heterogenous antibody population. These two terms do not limit the source of an antibody or the manner in which it is made.

In one example, the antibody used in the methods described herein is a humanized antibody. Humanized antibodies refer to forms of non-human (*e.g.*, murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or antigen-binding fragments thereof that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody. Humanized antibodies may also involve affinity maturation.

In another example, the antibody described herein is a chimeric antibody, which can include a heavy constant region and a light constant region from a human antibody. Chimeric antibodies refer to antibodies having a variable region or part of variable region from a first species and a constant region from a second species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals (*e.g.*, a non-human mammal such as mouse, rabbit, and rat), while the constant portions are homologous to the sequences in antibodies derived from another mammal such as human. In some embodiments, amino acid modifications can be made in the variable region and/or the constant region.

In some examples, the antibody disclosed herein specifically binds a target antigen, such as IL-23R. In some embodiments, the antibody disclosed herein specifically binds the extracellular domain of IL-23R. An antibody that "specifically binds" (used interchangeably

herein) to a target or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit "specific binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody "specifically binds" to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically (or preferentially) binds to an IL-23R epitope is an antibody that binds this IL-23R epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other IL-23R epitopes or non-IL-23R epitopes. It is also understood by reading this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

Antibodies capable of interfering with the IL-23R signaling pathway can be an antibody that binds an IL-23R (*e.g.*, the extracellular domain of IL-23R) and inhibits IL-23R biological activity and/or downstream pathways mediated by IL-23R. Exemplary antibodies that bind IL-23R are shown in Table 1. In some embodiments, the antibodies shown in Table 1 bind the extracellular domain of IL-23R.

Table 1: Anti-IL23-R Antibodies

Source	Catalog No.	Host Species	Species Reactivity	Clonality	Immunogen	Conjugated?
Invitrogen	MA-24804	Mouse	Human, mouse, non-human primate	Monoclonal	Amino acids 288-315 of human IL-23R	No
Invitrogen	MA5-23906	Rat	Mouse	Monoclonal	Recombinant mouse IL-23 R Gly24-Asp372	No
LifeSpan Biosciences	LS-C83986	Goat	Human	Polyclonal	Amino acids 59-87 of human IL-23R	No
LifeSpan Biosciences	LS-C37068-100	Mouse	Human	Monoclonal	NP_653302	No
LifeSpan Biosciences	LS-C37069-100	Mouse	Human	Monoclonal	Unknown	PE
LifeSpan Biosciences	LS-C125821-100	Mouse	Human	Monoclonal	Unknown	PerCP

LifeSpan Biosciences	ABIN393707	Mouse	Human	Monoclonal	Unknown	No
Aviva Systems Biology	OABF00713	Rabbit	Human, mouse, rat	Polyclonal	KLH-conjugated synthetic peptide of human IL-23R	No
United States Biological	223595	Rabbit	Human	Polyclonal	Full-length human IL-23R	No
Abeomics	10-4030	Mouse	Human	Monoclonal	250-450 of IL-23R	No
St. John's Laboratory	STJ24181	Rabbit	Human, Mouse, Rat	Polyclonal	Recombinant peptide from human IL-23R	No
Biorbyt	Orb140302	Rabbit	Human	Polyclonal	Amino acids 428-457 of human IL-23R	No
Biorbyt	Orb47095	Rabbit	Human	Polyclonal	Full length human IL-23R	No
ProSci, Inc.	58-876	Rabbit	Human	Polyclonal	Amino acids 428-457 of human IL-23R	No
Atlas Antibodies	HPA056427	Rabbit	Human	Polyclonal	Full length human IL-23R	No
MyBioSource	MBS854115	Rabbit	Human	Polyclonal	Full length human IL-23R	No
MyBioSource	MBS9404638	Rabbit	Human, Mouse, Rat	Polyclonal	Full length human IL-23R	No
ABclonal	A1613	Rabbit	Human, Mouse, Rat	Polyclonal	Recombinant Human Protein	No
Invitrogen	MA5-23935	Mouse	Human	Monoclonal	Mouse-myeloma cell line NSO-derived recombinant IL-23R amino acids 24-354	No
Invitrogen	MA5-23906	Rat	Mouse	Monoclonal	Mouse-myeloma cell line NSO-derived	No

					recombinant IL-23R amino acids 24-372	
BioMatik	CAC07795	Rabbit	Human, Mouse	Polyclonal	Human IL-23R amino acids 15-125	No
BioMatik	CAU22706	Rabbit	Rat	Polyclonal	IL-23R amino acids 25-355	No
Origene Technologies	TA327101	Rabbit	Human, Mouse, Rat	Polyclonal	Recombinant human IL-23R	No
Origene Technologies	TA321935	Rabbit	Human, Mouse, Rat	Polyclonal	Human IL-23R amino acids 21-227	No
LifeSpan Biosciences	LS-C403797	Rabbit	Human	Polyclonal	Human IL-23R	No
LifeSpan Biosciences	LS-C192978	Rabbit	Human, Mouse, Rat	Polyclonal	Human IL-23R	No
Abnova	PAB0224	Rabbit	Human, Mouse	Polyclonal	Human IL-23R amino acids 400-470	No
Abnova	H00149233	Mouse	Human	Monoclonal	Human IL-23R amino acids 553-628	No
NSJ Bioreagents	R32848	Rabbit	Mouse, Rat	Polyclonal	Amino acids 25-233	No
Antibodies Online	ABIN342781	Goat	Human	Polyclonal	Human IL-23R amino acids 59-87	No
Antibodies Online	ABIN221348	Mouse	Human	Monoclonal	Human IL-23R amino acids 24-354	PE (Phycoerythrin)
Antibodies Online	ABIN530970	Mouse	Human	Monoclonal	LNQGECSSPD IQNSVEEETT MLLENDSPSETIPEQ TLLPDEFVSCLGIVNE ELPSINTYF PQNILESH FN RISLLE	No
Antibodies Online	ABIN360919	Mouse	Human	Monoclonal	Recombinant protein of IL-23R extracellular domain	No
Signalway	31023	Rabbit	Human	Polyclonal	Human IL-	No

Antibody					23R amino acids 31-227	
Signalway Antibody	32341	Rabbit	Human, Mouse, Rat	Polyclonal	Human IL-23R	No
United States Biological	036998	Rabbit	Human	Polyclonal	Human IL-23R amino acids 427-457	No
United States Biological	128452	Mouse	Human	Monoclonal	553-629 from human IL23R	No
United States Biological	247572	Mouse	Unknown	Monoclonal	NP_653302, 553aa-628aa	No
United States Biological	247573	Mouse	Unknown	Monoclonal	IL23R	No
Bioworld Technology	BS6809	Rabbit	Human, Mouse, Rat	Polyclonal	Human IL-23R full-length protein	No
Abbexa	Abx130103	Rabbit	Rat	Polyclonal	IL-23R	No
Cloud Clone	MAE765Hu21	Mouse	Human	Monoclonal	Human IL-23R	No
Cloud Clone	PAE765Hu21	Rabbit	Human	Polyclonal	Human IL-23R	No
Enzo Life Sciences	ALX-210-636-C200	Goat	Human, Mouse	Polyclonal	Human IL-23R amino acids 59-87	No
Bio-Rad	VMA00297KT	Mouse	Human	Monoclonal	Human IL-23R	No
R&D Systems	FAB14001P	Mouse	Human	Monoclonal	Human IL-23R amino acids 24-354	PE (Phycoerythrin)
R&D Systems	FAB14001A	Mouse	Human	Monoclonal	Human IL-23R amino acids 24-354	Allophycocyanin
Bio-Rad	AHP1821	Goat	Mouse, Human	Polyclonal	Human IL-23R N-terminus	No
Santa Cruz Biotechnology, Inc.	sc-293485	Mouse	Human	Monoclonal	IL-23R	No
Bio-Rad	VMA00297K	Mouse	Human	Monoclonal	IL-23 R	No
Novus Biologicals	NB600-1147	Rabbit	Human, Mouse	Polyclonal	Human IL-23R amino acids 439-455	No
Novus Biologicals	NB2-47419	Rabbit	Human	Polyclonal	Human IL-23R amino acids 258-346	No
Novus	NBP2-	Mouse	Human,	Monoclonal	Amino acids	No

Biologicals	27091SS		Primate		288-315 of hIL-23 receptor	
Bioss	Bs-1460R	Rabbit	Human, Mouse, Rat	Polyclonal	Human IL-23R	No
BosterBio	A00607	Rabbit	Mouse, Rat	Polyclonal	Mouse IL-23R amino acids 25-233	No
Millipore Sigma	06-1331	Rabbit	Human, Mouse, Rat, Ox, Horse	Polyclonal	Human IL-23R	No
Sigma-Aldrich	WH0142923 3M1	Mouse	Human	Monoclonal	Human IL-23R	No
Creative Biolabs	TAB-421CL	Unknown	Human	Monoclonal	Human IL-23R	Unknown
Biolegend	Unknown: Clone 12B2B64	Rat	Mouse	Monoclonal	Mouse IL-23R amino acids 24-374	No
R&D	Unknown: Clone 258018	Unknown	Mouse	Monoclonal	Unknown	Unknown

In some embodiments, the antibody has one, two or all of a light chain CDR1, CDR2, and/or CDR3 and/or one, two or all of a heavy chain CDR1, CDR2 and/or CDR3. In some embodiments, the antibody has an amino acid sequence having one, two or all of a light chain CDR1, CDR2, and/or CDR3 and/or one, two or all of a heavy chain CDR1, CDR2 and/or CDR3 of an antibody in Table 1. In some embodiments, the antibody has an amino acid sequence having at least one, two or three modifications (e.g., substitutions, e.g., conservative substitutions) but not more than 30, 20 or 10 modifications (e.g., substitutions, e.g., conservative substitutions) to one, two or all of a light chain CDR1, CDR2, and/or CDR3 and/or one, two or all of a heavy chain CDR1, CDR2 and/or CDR3 of an antibody in Table 1.

In some embodiments, the antibody has one or both of a VH and VL. In some embodiments, the antibody has an amino acid sequence having one or both of a VH and VL of an antibody in Table 1, or a sequence with 95-99% identity thereof, e.g., 95%, 96%, 97%, 98% or 99% identity, e.g., 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity.

In some embodiments, the antibody binds IL-23R and does not bind IL-12Rβ. In some embodiments, the IL-12Rβ binding affinity of an anti-IL23R antibody measured. In some embodiments, the anti-IL23-R antibodies shown in Table 1 are screened to identify anti-IL23-R antibodies with low or no binding affinity for IL-12Rβ, e.g., has greater binding affinity for IL-23R than for IL-12Rβ. Binding affinity can be expressed KD or dissociation constant, and an increased binding affinity corresponds to a decreased KD. One way of

determining binding affinity of antibodies to IL-23R is by measuring binding affinity of monofunctional Fab fragments of the antibody. To obtain monofunctional Fab fragments, an antibody (for example, IgG) can be cleaved with papain or expressed recombinantly. The affinity of an anti- IL-23R Fab fragment of an antibody can be determined by surface plasmon resonance (BIAcore3000TM surface plasmon resonance (SPR) system, BIAcore, INC, Piscaway N.J.). Kinetic association rates (kon) and dissociation rates (koff) (generally measured at 25 ºC.) are obtained; and equilibrium dissociation constant (KD) values are calculated as koff/kon. In some embodiments, an antibody described herein has 1, 2, 5, 10, 20, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 2,000, 5,000, 10,000, 50,000, 100,000-fold or more greater binding affinity for IL-23R than for IL-12Rβ. In some embodiments, an antibody described herein has greater than 1, 2, 5, 10, 20, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 2,000, 5,000, 10,000, 50,000, 100,000-fold more binding affinity for IL-23R than for IL-12Rβ.

Methods of producing antibodies (e.g., monoclonal antibodies or polyclonal antibodies) are known in the art. For example, a polyclonal antibody may be prepared by immunizing an animal, preferably a mammal, with an allergen of choice followed by the isolation of antibody-producing B-lymphocytes from blood, bone marrow, lymph nodes, or spleen. Alternatively, antibody-producing cells may be isolated from an animal and exposed to an allergen in vitro against which antibodies are to be raised. The antibody-producing cells may then be cultured to obtain a population of antibody-producing cells, optionally after fusion to an immortalized cell line such as a myeloma. In some embodiments, as a starting material B-lymphocytes may be isolated from the tissue of an allergic patient, in order to generate fully human polyclonal antibodies. Antibodies may be produced in mice, rats, pigs (swine), sheep, bovine material, or other animals transgenic for the human immunoglobulin genes, as starting material in order to generate fully human polyclonal antibodies. In some embodiments, mice or other animals transgenic for the human immunoglobulin genes (e.g. as disclosed in U.S. Pat. No. 5,939,598), the animals may be immunized to stimulate the in vivo generation of specific antibodies and antibody producing cells before preparation of the polyclonal antibodies from the animal by extraction of B lymphocytes or purification of polyclonal serum.

Monoclonal antibodies are typically made by cell culture that involves fusing myeloma cells with mouse spleen cells immunized with the desired antigen (i.e., hyrbidoma technology). The mixture of cells is diluted and clones are grown from single parent cells on microtitre wells. The antibodies secreted by the different clones are then assayed for their

ability to bind to the antigen (with a test such as ELISA or Antigen Microarray Assay) or immuno-dot blot. The most productive and stable clone is then selected for future use.

In some embodiments, the antibodies described herein are "humanized" for use in human (e.g., as therapeutics). In some embodiments, an antibody shown in Table 1 is a murine antibody and the antibody is humanized. "Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. Humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992).

Nucleic Acids

In some embodiments, the agent is a nucleic acid. In some embodiments, the agent binds a nucleic acid expressing IL-23R. e.g., having a sequence shown at NCBI Ref. No. NM_144701.2. In some embodiments, the agent binds a sequence encoding the extracellular domain of IL-23R.

As used herein, "gene silencing," refers to post-transcriptional gene silencing, which may be the result of mRNA of a particular gene being degraded or blocked. The RNAi inhibitor agents provide herein may, in some embodiments, decrease the expression level of IL-23R mRNA by at least about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 55%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99% or about 100% of the mRNA level found in the cell without the presence of an RNAi inhibitor agent.

As used herein, "RNA interference" is a biological process in which RNA molecules inhibit gene expression, typically by causing the degradation of specific mRNA molecules.

In some embodiments, the agent is an siRNA. As used herein, "siRNA" is a class of double-stranded RNA molecules, which interferes with the expression of specific genes having a nucleotide sequence complementary to the siRNA. siRNAs typically have a well-defined structure: a short (e.g., 21 base pair) double-stranded RNA (dsRNA) with phosphorylated 5' ends and hydroxylated 3' ends with two overhanging nucleotides. The Dicer enzyme catalyzes production of siRNAs from long dsRNAs and small hairpin RNAs (shRNAs). An siRNA for use in accordance with the present disclosure may be about 15 to about 35 base pairs, or about 20 to about 25 base pairs, in length. In some embodiments, the siRNA may be about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 base pairs in length. In some embodiments, the siRNA is complementary to IL-23R, e.g., having a sequence shown at NCBI Ref. No. NM_144701.2. In some embodiments, the siRNA is complementary to a sequence encoding the extracellular domain of IL-23R.

Exemplary siRNAs against IL23-R are shown in Table 2. In some embodiments, the siRNA comprises a nucleic acid sequence of any one of SEQ ID NOs; 19-31. In some embodiments, the siRNA comprises a nucleic acid sequence having 70%, 71%. 72%. 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% to any one of SEQ ID NOs; 19-31. In some embodiments, the siRNA comprises a nucleic acid sequence having at least 5, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 consecutive nucleotides of a nucleic acid sequence of any one of SEQ ID NOs; 19-31, or a sequence having 70%, 71%. 72%. 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a nucleic acid sequence having at least 5, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 consecutive nucleotides of a nucleic acid sequence of any one of SEQ ID NOs; 19-31.

Table 2: siRNAs against IL23-R generated using siRNA Wizard v3.1 at http://www.invivogen.com/sirnawizard/design.php

Number	Sequence	Position	Length (bp)	SEQ ID NO:
1	GACCTGGAACGTGAAAGAATT	27 – 48	21	19
2	GCAAAGCGTTTCAGCATGATA	209 – 230	21	20
3	GATTCCGAAATGGCTGTATGA	411 – 432	21	21

4	GAAATGGCTGTATGAAGATAT	417 – 438	21	22
5	GCGAACTGATGAACAACAACA	485 – 506	21	23
6	GGTGTATATTCCGGATCTGAA	660 – 681	21	24
7	GGATCTGAACACCGGCTATAA	672 – 693	21	25
8	GCAGCCATCTGAGCAACAACA	673 – 694	21	26
9	GCAGCCATCTGAGCAACAACA	722 – 743	21	27
10	GCAACAACAACGAAATTACCA	734 – 755	21	28
11	GCGAACTGAGCCTGATTCTGA	878 – 899	21	29
12	GGGCATTGTGAACGAAGAACT	1032 – 1053	21	30
13	GCCGAGCATTAACACCTATTT	1053 – 1074	21	31

In some embodiments, the agent is an shRNA. As used herein, "shRNA" refers to a sequence of RNA that makes a tight hairpin turn that can be used to silence target gene expression through RNA interference (RNAi). Expression of shRNA in cells may be accomplished by delivery of plasmids or through viral or bacterial vectors. For example, in some embodiments, shRNA targeting IL-23R may be delivered to Treg cells by transfecting the cells with a plasmid that contains a nucleic acid encoding the shRNA. In some embodiments, bacterial vectors may be used to obtain shRNA expression in cells. In some embodiments, viral vectors (e.g., adeno-associated viruses (AAVs), adenoviruses, and lentiviruses) may be used to obtain shRNA expression in cells. Due to the ability of shRNA to provide specific, long-lasting, gene silencing, shRNA may be used for gene therapy applications. In some embodiments, the shRNA is complementary to IL-23R, e.g., having a sequence shown at NCBI Ref. No. NM_144701.2. In some embodiments, the shRNA is complementary to a sequence encoding the extracellular domain of IL-23R.

As used herein, a "stem-loop structure" refers to a nucleic acid having a secondary structure that includes a region of nucleotides which are known or predicted to form a double strand (stem portion) that is linked on one side by a region of predominantly single-stranded nucleotides (loop portion). The term "hairpin" may be used herein to refer to a stem-loop structure.

Peptides

The terms "protein," "peptide," and "polypeptide" are used interchangeably herein, and refer to a polymer of amino acid residues linked together by peptide (amide) bonds. The terms refer to a protein, peptide, or polypeptide of any size, structure, or function. Typically, a protein, peptide, or polypeptide will be at least three amino acids long. A protein, peptide, or polypeptide may refer to an individual protein or a collection of proteins. One or more of the amino acids in a protein, peptide, or polypeptide may be modified, for example, by the

addition of a chemical entity such as a carbohydrate group, a hydroxyl group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc. A protein, peptide, or polypeptide may also be a single molecule or may be a multi-molecular complex. A protein, peptide, or polypeptide may be just a fragment of a naturally occurring protein or peptide. A protein, peptide, or polypeptide may be naturally occurring, recombinant, or synthetic, or any combination thereof.

A peptide that is "derived from" a protein (e.g., a peptide derived from IL-23R) means the peptide is obtained from the protein and has an amino acid sequence that shares homology with the fragment of the protein it corresponds to. The amino acid sequence of the peptide may be at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or 100% identical to the amino acid sequence of the fragment of the protein it corresponds to. A peptide that is derived from a protein may also contain chemical modifications, amino acid substitutions, and/or unnatural amino acids.

Useful peptides ing to the invention include antibodies, zinc finger proteins which can target and cleave DNA, and protein antagonists which mimic the structure of the native ligand for a receptor and block binding of the native ligand without agonizing the receptor.

Small molecules

In other embodiments, the IL-23R inhibitory compounds described herein are small molecules, which can have a molecular weight of about any of 100 to 20,000 daltons, 500 to 15,000 daltons, or 1000 to 10,000 daltons. Libraries of small molecules are commercially available. The small molecules can be administered using any means known in the art, including inhalation, intraperitoneally, intravenously, intramuscularly, subcutaneously, intrathecally, intraventricularly, orally, enterally, parenterally, intranasally, or dermally. In general, when the agent according to the invention is a small molecule, it will be administered at the rate of 0.1 to 300 mg/kg of the weight of the patient divided into one to three or more doses. For an adult patient of normal weight, doses ranging from 1 mg to 5 g per dose can be administered.

The above-mentioned small molecules can be obtained from compound libraries. The libraries can be spatially addressable parallel solid phase or solution phase libraries. See, *e.g.*, Zuckermann et al. J. Med .Chem. 37, 2678-2685, 1994; and Lam Anticancer Drug Des. 12:145, 1997. Methods for the synthesis of compound libraries are well known in the art, e.g., DeWitt et al. PNAS USA 90:6909, 1993; Erb et al. PNAS USA 91:11422, 1994;

Zuckermann et al. J. Med. Chem. 37:2678, 1994; Cho et al. Science 261:1303, 1993; Carrell et al. Angew Chem. Int. Ed. Engl. 33:2059, 1994; Carell et al. Angew Chem. Int. Ed. Engl. 33:2061, 1994; and Gallop et al. J. Med. Chem. 37:1233, 1994. Libraries of compounds may be presented in solution (e.g., Houghten Biotechniques 13:412-421, 1992), or on beads (Lam Nature 354:82-84, 1991), chips (Fodor Nature 364:555-556, 1993), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent No. 5,223,409), plasmids (Cull et al. PNAS USA 89:1865-1869, 1992), or phages (Scott and Smith Science 249:386-390, 1990; Devlin Science 249:404-406, 1990; Cwirla et al. PNAS USA 87:6378-6382, 1990; Felici J. Mol. Biol. 222:301-310, 1991; and U.S. Patent No. 5,223,409).

In vitro differentiation

In some embodiments, cells, e.g., Treg cells, are contacted in vitro with an agent described herein and IL-23R activity is decreased. In some embodiments, differentiation is induced.

In some embodiments, the cells, e.g., Treg cells, are isolated. In some embodiments, the cells are isolated from mice. In some embodiments, the cells are isolated from murine spleens. In some embodiments, the cells are isolated from murine lymph nodes. In some embodiments, the cells, e.g., Treg cells, are isolated from a human subject. In some embodiments, the cells are isolated from peripheral blood mononuclear cells.

In some embodiments, the cells, e.g., Treg cells, are contacted in a buffer, e.g., PBS. In some embodiments, the Treg cells are contacted in a cell culture media, e.g., a hematopietic cell culture medium (e.g., X-VIVO®15) or a T cell expansion media (e.g., CTS OpTmizer T Cell Expansion SFM®).

In some embodiments, the cells, , e.g., Treg cells, are contacted in the presence of anti-CD3 antibodies. In some embodiments, the cells, e.g., Treg cells are contacted in the presence of anti-CD28 antibodies. In some embodiments, the cells, e.g., Treg cells are contacted in the presence of anti-CD3 antibodies and anti-CD28 antibodies. In some embodiments, the anti-CD3 antibodies and/or anti-CD28 antibodies are attached to a surface. In some embodiments, the anti-CD3 antibodies and/or anti-CD28 antibodies are attached to a plate. In some embodiments, the anti-CD3 antibodies and/or anti-CD28 antibodies are attached to a bead.

In some embodiments, the cells, e.g., Treg cells, are contacted in the presence of one or more inflammatory cytokines. Inflammatory cytokines include, for example, interleukin-1 (IL-1), tumor necrosis factor (TNF), gamma-interferon (IFN-gamma), IL-12, IL-18,

granulocyte-macrophage colony stimulating factor, IL-2, IL-4, IL-10, IL-13, IFN-alpha and TGF-β.

In some embodiments, the cells, e.g., Treg cells, are contacted in the presence of IL-2. In some embodiments, T reg cells are contacted in 0-1000 ng, 0-500 ng, 0-200 ng, 0-100 ng, or 0-50 ng IL-2. In some embodiments, the cells, e.g., Treg cells, are contacted in the presence of IL-4. In some embodiments, the T reg cells are contacted in 0-1000 ng, 0-500 ng, 0-200 ng, 0-100 ng, 0-50 ng, or 0-20 ng IL-4.

Therapeutic Uses

In some embodiments, provided herein are methods for treating cancer by administering an agent described herein.

The subject to be treated by the methods described herein are human.

A human subject who needs the treatment may be a human patient having, at risk for, or suspected of having cancer. A subject having cancer can be identified by routine medical examination, *e.g.*, laboratory tests, functional tests, biopsy, CT scans, or ultrasounds. A subject suspected of having cancer might show one or more symptoms of the disorder. A subject at risk for cancer can be a subject having one or more of the risk factors for that disorder. For example, risk factors associated with cancer include (a) hereditary cancer, (b) age, and (c) family history of cancer.

Cancers include but are not limited to: Oral: buccal cavity, lip, tongue, mouth, pharynx; Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: non-small cell lung cancer (NSCLC), small cell lung cancer, bronchogenic carcinoma (squamous cell or epidermoid, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, larynx, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel or small intestines (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel or large intestines (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma), rectal, colon, colon-rectum, colorectal; Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma),

prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma, biliary passages; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), head and neck cancer, meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-thecal cell tumors, SertolI-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes (carcinoma), breast; Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma] hairy cell; lymphoid disorders; Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, keratoacanthoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis, Thyroid gland: papillary thyroid carcinoma, follicular thyroid carcinoma; medullary thyroid carcinoma, multiple endocrine neoplasia type 2A, multiple endocrine neoplasia type 2B, familial medullary thyroid cancer, pheochromocytoma, paraganglioma; and Adrenal glands: neuroblastoma.

"An effective amount" as used herein refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the

duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of cancer. Alternatively, sustained continuous release formulations of agent that decreases IL-23R activity may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

In one example, dosages for an agent that decreases IL-23R activity as described herein may be determined empirically in individuals who have been given one or more administration(s) of the agent. Individuals are given incremental dosages of the agent. To assess efficacy of the agent, an indicator of IL-23R activity can be followed.

For the purpose of the present disclosure, the appropriate dosage of an agent that decreases IL-23R activity will depend on the specific agents (or compositions thereof) employed, the type and severity of cancer, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician.

As used herein, the term “treating” refers to the application or administration of a composition including one or more active agents to a subject, who has cancer, a symptom of cancer, or a predisposition toward the disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward the disease.

Alleviating cancer includes delaying the development or progression of the disease, or reducing disease severity. Alleviating the disease does not necessarily require curative results. As used therein, "delaying" the development of a disease (such as cancer) means to

defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

"Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of cancer includes initial onset and/or recurrence.

Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the agent to the subject, depending upon the type of disease to be treated or the site of the disease. This composition can also be administered via other conventional routes, *e.g.*, administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term “parenteral” as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, and intracranial injection or infusion techniques. In addition, it can be administered to the subject via injectable depot routes of administration such as using 1-, 3-, or 6-month depot injectable or biodegradable materials and methods.

Combination Therapies

In some embodiments, the methods described herein comprise administering an agent described herein in in conjunction with an additional therapy. In some embodiments, the additional therapy is an immunomodulatory agent. In some embodiments, the additional therapy is an agent that inhibits cancer cell growth.

Immunomodulators

In any of the foregoing aspects and following embodiments, the one or more immune checkpoint inhibitors are, for example, each an antagonist of programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), 15 V-domain Ig suppressor of T cell activation (VISTA), programmed death ligand 2 (PD-L2), indoleamine 2,3-dioxygenase (IDO), arginase, B7 family inhibitory ligand B7-H3, B7 family inhibitory ligand B7-H4, lymphocyte activation gene 3 (LAG3), 2B4, B and T lymphocyte attenuator (BTLA), T cell membrane protein 3 (TIM3; also known as HAVcr2), adenosine A2a receptor (A2aR), a killer inhibitory receptor, and/or signal transducer and activator of 20 transcription (STAT)3. In particular embodiments, the one or more immune checkpoint inhibitors are each an antagonist of programmed death 1 (PD-1), an antagonist of programmed death ligand 1 (PD-L1), an antagonist of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), and/or an antagonist of V-domain Ig suppressor of T cell activation (VISTA).

In any of the foregoing aspects and embodiments, the PD-1 antagonist is, for example, an 25 agent that binds to and antagonizes PD-1. Such agents can be, for example, a peptide that binds PD-1. Such agents can be a humanized antibody that selectively binds PD-1. In some embodiments, the humanized antibody that selectively binds PD-1 is nivolumab, pembrolizumab, pidilizumab, MEDI-0680, REGN2810, or AMP-224. In some embodiments, the humanized antibody that selectively binds PD-1 is nivolumab, pembrolizumab, or 30 pidilizumab. In some embodiments, the antagonist is (i) an antisense molecule directed against PD-1 nucleic acid, (ii) an adnectin directed against PD-1 nucleic acid, (iii) a single stranded or double stranded RNAi inhibitor of PD-1, and/or (iv) a small molecule inhibitor of PD-1.

In any of the foregoing aspects and embodiments, the PD-L1 antagonist is, for example, an agent that binds to and antagonizes PD-L1. Such agents can be, for example, a peptide that binds PD-L1. Such agents can be a humanized antibody that selectively binds PD-L1. In some embodiments, the humanized antibody that selectively binds PD-L1 is BMS-936559/ MDX-1105, MPDL3280A/ RG7446/ atezolizumab, MSB0010718C/ avelumab, or MEDI4736/ durvalumab. In some embodiments, the antagonist is (i) an antisense molecule directed against PD-L1, (ii) an adnectin directed against PD-L1, (iii) a single stranded or double stranded RNAi inhibitor of PD-L1, or (iv) a small molecule inhibitor of PD-L1. 10
In any of the foregoing aspects and embodiments, the CTLA-4 antagonist is, for example, an agent that binds to and antagonizes CTLA-4. Such agents can be, for example, a peptide that binds CTLA-4. Such agents can be a humanized antibody that selectively binds CTLA-4. In

some embodiments, the humanized antibody that selectively binds CTLA-4 is ipilimumab or tremelimumab. In some embodiments, the CTLA-4 antagonist is (i) an antisense molecule directed against CD80, CD86, and/or CTLA-4 nucleic acid, (ii) an adnectin directed against CD80, CD86, and/or CTLA-4 nucleic acid, (iii) a single stranded or double stranded RNAi inhibitor of CD80, CD86, and/or CTLA-4, or (iv) a small molecule inhibitor of CD80, CD86, or CTLA-4.

In any of the foregoing aspects and embodiments, the VISTA antagonist is, for example, an agent that binds to and antagonizes VISTA. Such agents can be, for example, a peptide. Such agents can be an inhibitory antibody directed to VISTA. In some embodiments, the agent that binds to and antagonizes VISTA is a humanized antibody. In some embodiments, the agent that binds to and antagonizes VISTA is (i) an antisense molecule directed against VISTA nucleic acid, (ii) an adnectin directed against VISTA nucleic acid, (iii) a single stranded or double stranded RNAi inhibitor of VISTA, or (iv) a small molecule inhibitor of VISTA.

Chemotherapeutic Agents

In some embodiments, the methods described herein comprise administering an agent described herein in in conjunction with an agent that inhibits cancer cell growth.

In some embodiments, an agent that inhibits cancer cell growth comprises a chemotherapeutic agent. Chemotherapeutic agents include, for example, including alkylating agents, anthracyclines, cytoskeletal disruptors (Taxanes), epothilones, histone deacetylase inhibitors, inhibitors of topoisomerase I, inhibitors of topoisomerase II, kinase inhibitors, nucleotide analogs and precursor analogs, peptide antibiotics, platinum-based agents, retinoids, vinca alkaloids and derivatives thereof. Non-limiting examples include: (i) anti-angiogenic agents (e.g., TNP-470, platelet factor 4, thrombospondin-1, tissue inhibitors of metalloproteases (TIMP1 and TIMP2), prolactin (16-Kd fragment), angiostatin (38-Kd fragment of plasminogen), endostatin, bFGF soluble receptor, transforming growth factor beta, interferon alpha, soluble KDR and FLT-1 receptors, placental proliferin-related protein, as well as those listed by Carmeliet and Jain (2000)); (ii) a VEGF antagonist or a VEGF receptor antagonist such as anti-VEGF antibodies, VEGF variants, soluble VEGF receptor fragments, aptamers capable of blocking VEGF or VEGFR, neutralizing anti-VEGFR antibodies, inhibitors of VEGFR tyrosine kinases and any combinations thereof; and (iii) chemotherapeutic compounds such as, e.g., pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine), purine analogs, folate antagonists and related

inhibitors (mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine (cladribine)); antiproliferative/antimitotic agents including natural products such as vinca alkaloids (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristine, vinblastine, nocodazole, epothilones, and navelbine, epidipodophyllotoxins (etoposide and teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethyhnelamineoxaliplatin, iphosphamide, melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, teniposide, triethylenethiophosphoramide and etoposide (VP16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycin, plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates-busulfan, nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes-dacarbazinine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole, anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel, abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds (e.g., TNP-470, genistein, bevacizumab) and growth factor inhibitors (e.g., fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab); cell cycle inhibitors and differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase inhibitors (doxorubicin (adriamycin), amsacrine, camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin, mitoxantrone, topotecan, and irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylprednisolone,

prednisone, and prednisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers and caspase activators; and chromatin disruptors.

In some embodiments, an agent that inhibits cancer cell growth comprises external radiation therapy and internal radiation therapy (also called brachytherapy). Energy sources for external radiation therapy include x-rays, gamma rays and particle beams, energy sources used in internal radiation include radioactive iodine (iodine125 or iodine131), strontium89, or radioisotopes of phosphorous, palladium, cesium, indium, phosphate, or cobalt. Methods of administering radiation therapy are well known to those of skill in the art.

In some embodiments, an agent that inhibits cancer cell growth comprises a CD-44 antagonist. In some embodiments, the CD-44 antagonist is hyaluronic acid. In some embodiments, the CD-44 antagonist is an anti-CD-44 antibody.

In some embodiments, an agent that inhibits cancer cell growth comprises a CD133 antagonist.

In some embodiments, the methods described herein comprise administering an agent described herein in in conjunction with an agent that is an antagonist of inhibitory macrophages and/or dendritic cells.

Screening methods

In some embodiments, the methods described herein provide for identifying a test compound that will convert a Treg into a Teff. The method comprises:

contacting a regulatory T cell (Treg) with a test compound;

measuring IL-23R activity in the cell;

measuring IL-12Rβ activity in the cell;

wherein the test compound is a compound for converting a regulatory T cell (Treg cell) into an effector cell if

(i) the IL-23R activity is decreased relative to a control cell that has been treated with a compound known to not decrease IL-23R activity and IL-12Rβ activity; and

(ii) the IL-12Rβ activity is not decreased relative to the control cell.

In some embodiments, contacting a regulatory T cell (Treg) with a test compound comprises contacting a regulatory T cell (Treg) with a test compound in vitro using the methods for in vitro differentiation described herein.

In some embodiments, IL-23R activity in the cell is measured. In some embodiments, measuring IL-23R activity comprises measuring expression of FoxP3, Helios, IL-17A, IL-17F, ROR-gamma, and/or IL-23. In some embodiments, measuring IL-23R activity

comprises measuring expression of interferon-gamma. In some embodiments, measuring IL-23R activity comprises measuring STAT3 phosphorylation.

In some embodiments, a decrease in IL-23R activity comprises a decreased in IL-23R activity relative to a control as described supra. In some embodiments, a decrease in IL-23R activity comprises a decrease in expression of FoxP3, Helios, IL-17A, IL-17F, ROR-gamma, and/or IL-23, as is described supra.

In some embodiments, IL-12Rβ activity in the cell is measured. In some embodiments, measuring IL-12Rβ activity comprises measuring expression of interferon-gamma.

In some embodiments, IL-12Rβ activity does not decrease if IL-12Rβ activity does not decrease relative to a control as described supra. In some embodiments, IL-12Rβ activity does not decrease if IL-12Rβ2, IL18-Rβ1, IRF1, IL-2R, and/or G6NT expression does not decrease as described supra.

In some embodiments, the test compound is a polypeptide, a nucleic acid, or a small molecule, as is described herein.

In some embodiments, effector cytokines produced by the cell are measured. Effector cytokines include, but are not limited to TNF-α, IFN-γ, IL-17, IL-10, or IL-2.

EXAMPLES

Example 1: IL-23R as a target in T regulatory (Treg) conversion and anti-tumor therapy

Immunological approaches to the treatment of cancer have shown great promise for increased long-term survival for a number of cancers and complete remission in others. The number of clinical trials using immunotherapy alone or in combination with conventional cancer treatments has grown remarkably, based on increased understanding of the critical contribution of T cell subsets to the control of cancer growth.

Regulatory T cells are critically important for maintenance of a balanced immune system, in part because they help to dampen excessive immune responses and prevent autoimmunity. However, immune suppression exerted by Treg can impede anti-tumor immune responses and promote cancer growth. The efficacy of several successful immunotherapies has been associated with depletion or blockade of CD4 regulatory T-cells (Treg) (Mahoney et al., Nat Rev Drug Discov 14:561-584). Described in this example is a

more advanced strategy that depends on selective induction of Treg phenotypic instability and effector cell conversion of intratumoral but not systemic Treg.

Impact of Helios regulated STAT5 activation on nTreg stability

It was previously determined that the Helios transcription factor (TF) maintains stable expression of FoxP3 under inflammatory conditions via regulation of STAT5b (Mahoney et al., Nat Rev Drug Discov 14:561-584). However the precise mechanism of Helios-dependent Treg stability and function has not yet been fully clarified. The data provided below defines the molecular basis of Helios-mediated stability and the biological activity of conventional Treg in the tumor microenvironment (TME).

Helios was recently defined as a critical transcriptional factor that ensures stable expression of the Treg phenotype, which shows that inhibition of Helios expression in intratumoral CD4+ Treg induces reprogramming of Treg into T-effector cells that can destroy tumor cells (Nakagawa et al., Proc Natl Acad Sci U S A 113:6248-6253, Kim et al., Science 350:334-339). The response is marked by decreased numbers of intratumoral CD4 Treg, increased conversion of intratumoral Treg to T effector cells and increased production of effector cytokines by CD8 T cells. Since Treg conversion is restricted to intratumoral CD4 Treg, potential systemic toxicity and IRAEs may be avoided.

It has recently been shown that Helios$^{-/-}$ nTreg exhibit reduced expression of T cell anergy markers CD73 and FR4 which correlated with increased proinflammatory cytokine expression under inflammatory conditions (Kim et al., Science 350:334-339). Using Helios$^{-/-}$ mice with solid tumors (B16/F10 melanoma and MC38 colorectal cancer) as a source of inflammation, nTreg anergic phenotype was characterized (FIGs. 1A and 1B). nTreg loss of function was highlighted by reduced tumor size (FIG. 1A) and increased cytotoxic T cell activity (FIG. 1B).

Chromatin immunoprecipitation DNA sequencing (ChIP-seq) analysis of Treg cells indicated that the STAT5b locus contains multiple Helios binding regions including a prominent Helios binding region located within the 5' enhancer intron of STAT5b (illustrated in FIGs. 2A-B). It is possible that Helios-dependent STAT5b interaction enhances Stat5b expression and stabilizes the nTreg phenotype via binding to the FoxP3 conserved noncoding sequence 2 (CNS2) region. CRISPR/ Cas9 genome editing can be used to delete the putative Helios binding sites on STAT5b and generate genetically modified mice in which the interaction between Helios and Stat5b is interrupted.

Immunological activity of converted Th1-like cells

Selective deletion of Helios in FoxP3$^+$ Treg was found to result in decreased Treg-dependent inhibition of an anti-tumor response in B16/F10 inoculated *Rag2*$^{-/-}$ reconstituted mice (2×10^6 CD4: 1×10^6 CD8: 0.5×10^6 Helios^{WT} or Helios^{KO} nTreg) (FIG. 3A) which correlated with reduced FoxP3 expression (Fig. 3B) and increased production of the pro-inflammatory cytokine IFN-γ (FIG. 3C). Interestingly, the reduced FoxP3 expression and increased IFN-γ production by *Rag2*$^{-/-}$Helios$^{fl/fl}$FoxP3^{Cre} mice was only noted in the tumor infiltrate (Figs. 3B-3C). No significant changes were observed in spleen.

Helios deletion was also found to specifically destabilize Tregs in a second tumor model (MC38 colorectal cancer cell line) within the tumor microenvironment (FIGs. 4A and 4B). Loss of the Treg-mediated suppressive function (FIG. 4) resulted in decreased FoxP3 expression (FIG. 4A) as well as anergy markers CD73 and FR4 (FIG. 4B) suggesting that nTreg stability in solid tumors is Helios dependent. Furthermore, TCR activation of nTreg under inflammatory conditions (anti-CD3, anti-CD28, IL-2, IL-4) was found to reduce the proportion of nTreg cells positive for Helios and the surface receptor TIGIT (FIGs. 5A and 5B). Helios$^+$ nTreg cells were decreased by nearly 50% (FIG. 5A) when co-cultured with IL-4 and Helios$^+$TIGIT$^+$ cells by ~50% (FIG. 5B) with increased IL-4 levels.

Antibodies that induce Treg to Teff conversion.

Two major signaling pathways maintain Treg stability within the tumor microenvironment (TME): 1) a Helios-dependent pathway that enhances IL-2R responsiveness and STAT5 activation and/or 2) an IL-23R signaling pathway that activates STAT5 within intratumoral Treg (FIG. 6). Blockade of either one of these signaling pathways induces Treg conversion as described below.

FIGs. 7A-7D shows antibodies found to induce conversion of Treg→Teff. Anti-IL-23R antibody treated Tregs showed decreased FoxP3 and increased IFN-γ expression compared to controls (FIGs. 7A-7D).

Converted Th1-like nTregs mediate stronger antitumor activity

Isolated pure populations of homogeneous CD4 Treg cells were tested for differentiation into CD4 Teff cells after antibody engagement of Treg target receptors that included TNFRSF members (anti-GITR, anti-OX-40, anti-4-1BB) and others (e.g., anti-CD47 and anti-Nrp-1). This initial analysis of isolated CD4 Treg indicated that engagement of GITR by antibodies in the presence of IL-4 leads to a Treg→Teff conversion associated with downregulation of FoxP3 and Helios and upregulation of IFNγ production (FIG. 8).

The GITR agonist, DTA-1, has been suggested to alter the stability of Treg cells. An *in vitro* assay was used to test if loss of stability resulted in Treg conversion to Th1-like effector cells. FIGs. 9A and 9B shows data from an experiment in which sorted C57BL/6 (WT) Treg (>90% pure CD4+CD25+) were cultured with plate bound anti-CD3/anti-CD28 in the presence of IL-2 and IL-4 for 24 hours or 5 days, and then assessed for conversion defined as loss of FoxP3 and gain of IFN-γ expression. In this system, GITR agonist DTA-1 showed loss of FoxP3 and increase in IFN-γ expression (FIGs. 9 and 9B). It can be seen that when the Ab was used either prophylactically or therapeutically, engagement of GITR on Tregs by anti-GITR Ab (DTA-1) led to reduction in tumor size compared to the negative control (FIG. 9A). Anti-GITR also led to an effector phenotype in tumors, but not in the spleen, as shown in FIGs. 10B and 10C.

Treg conversion by anti-IL-23R antibody

Blocking of IL-23R signaling in Treg under inflammatory conditions ($IL\text{-}2^{+}IL\text{-}4^{+}$) was found to induce Treg conversion as judged from reduced expression of FoxP3 and production of IFN-γ (FIGs. 11A and 11B). As can be seen in FIG. 12, like DTA-1, anti-IL-23R antibody lowers the expression of FoxP3 and increases the expression of IFN-γ in Tregs plated with plate bound anti-CD3/anti-CD28 and cultured in the presence of IL2 and IL4. This effect was found to be dependent on the dose of anti-IL-23R antibody (FIG. 13).

Moreover, unlike other anti-IL-23R antibodies which fail to induce conversion (not shown), the anti-IL-23R antibody with conversion activity recognizes the extracellular domain of the IL-23R chain rather than the IL-12Rβ common chain that also binds to IL-12p40 (FIG. 14).

IL-23R expression

In terms of being able to target tumors specifically, it is relevant and important to note that Treg from tumors express high levels of IL-23R, while splenic Treg were found not to do so (FIG. 15). IL-23R expression is significantly increased in Treg found in the tumor and tumor draining lymph node (TdLN) compared to the spleen (FIG. 16). It was also found that effector Treg (eTr) express high levels of IL-23R (FIG. 17). Tumor infiltrating $CD69^{+}Icos^{+}$ also express high IL-23R (FIG. 18). FIG. 20 shows that $Helios^{+}$ Treg express high levels of IL-23R. Indeed, it was found that IL-23R expression is reduced in cells in which Helios is knocked out (FIG. 20), demonstrating the dependence of IL-23R expression on Helios.

IL-23R regulates FoxP3 and IFN-γ expression

In an *in vitro* experiment in which sorted Treg were plated with plate bound anti-CD3/anti-CD28 and cultured in IL-2 and IL-4 for 5 days, it was found that FoxP3 expression is increased after treatment with recombinant IL-23 protein and then somewhat decreased in cells treated with recombinant IL-23 protein and $p(40)_2$ (FIG. 21). Conversely, the IFN-γ levels increased after treatment with recombinant IL-23 protein and $p(40)_2$ (FIG. 21).

Anti- IL-23R antibody effects on FoxP3 and IFN-γ expression

Data in FIG. 22 shows that blocking IL-23R ligation by either anti-IL-23R antibody or $P(40)_2$ must occur after activation of the receptor to reduce FoxP3 expression. FIG. 23 also shows that blocking IL-23R ligation likely occurs after activation to induce IFN-γ expression in $FoxP3^{lo}$ cells.

Anti-tumor activity of anti-IL-23R antibody in vivo

Tumor outgrowth was observed to be delayed in MC38 inoculated C57BL/6 mice treated with $(p40)_2$ homodimer (FIG. 24).

FIGs. 25A-25E show results from an experiment in which C57BL/6 mice were inoculated with MC38 cancer cells subcutaneously and thereafter administered anti-IL-23R antibody (or isotype control) before assessment of Treg subsets and IL-23R expression in various immune cell subsets (FIG. 25A). Growth curves for individual mice are shown in FIG. 25B. The graph in FIG. 25C shows that the third mouse that was administered anti-IL-23R antibody remained tumor-free. The data plotted as the average per treatment condition can be seen in FIGs. 25D and 25E.

Treatment of mice inoculated with MC38 cancer cells with anti-IL-23R resulted in increased CD8 activity as indicated by IFN-γ expression (FIG. 26A) and induced intratumoral Treg conversion as indicated by increased IFN-γ expression (FIG. 26B).

IL-23R expression was found to be significantly decreased in anti-IL-23R treated tumor and tumor infiltrating activated Treg (FIG. 27). It was also decreased in eTr found in the tumor (FIG. 28). These anti-Il-23R tumor infiltrating Treg have decreased Helios expression (FIG. 29), and reduced intratumoral suppressive Treg compared to isotype-control treated mice (FIG. 30). Anti-Il-23R antibody treatment of cancer cells inoculated mice also increases CD8/Treg ratio and CD8 IFN-γ expression (FIG. 31).

Summary

The data discussed in this example show that anti-IL-23R antibody that recognizes the extracellular domain of the IL-23R chain rather than the IL-12Rβ common chain that also binds to IL-12p40, converts Tregs to effector type cells in the tumor microenvironment specifically.

Example 2: Identifying and validating antibodies that induce T regulatory (Treg) conversion and anti-tumor activity

An in vitro assay to measure Treg conversion

Two major signaling pathways maintain Treg stability within the tumor microenvironment (TME): 1) a Helios-dependent pathway that enhances IL-2R responsiveness and STAT5 activation and/or 2) an IL-23R signaling pathway that activates STAT5 within intratumoral Treg (FIG. 6). Blockade of either one of these signaling pathways induces Treg conversion as described below. Based on this knowledge, an *in vitro* screen was developed to identify target surface molecules expressed by Treg that induce Treg→Teff conversion after engagement by an antibody. Verification of the efficacy of antibodies that induce Treg reprogramming as judged by conversion of intratumoral Treg and enhanced anti-tumor immunity is performed *in vivo* thereafter.

The *in vitro* screening system allows identification of Treg surface molecules that, after engagement by antibodies, induce conversion of Treg to T effector cells (FIG. 32). A reporter system that allows detection of a converted Treg phenotype by levels of fluorescence intensity is used: red fluorescence protein (RFP) for FoxP3 and yellow fluorescence protein (YFP) for IFNγ is utilized. Isolated CD4 Treg (RFP^+YFP^+) are stimulated with anti-

CD3/CD28 antibody in the presence of IL-2 and IL-4 to mimic an inflammatory environment. The effect of surface molecule engagement by antibodies is tested in 96 well plates that contain triplicates of test- and control antibody treated wells. Candidate surface molecules and Treg converting antibodies are identified based on reduced RFP and increased YFP signals, which indicate conversion of Treg to T effector cells. Candidate antibodies that display Treg reprogramming activity are validated individually *in vitro* before testing them *in vivo*.

Use of epitope mapping to identify anti-IL-25R Treg converting antibodies

The anti-IL-25R antibody that is discussed in Example 1 is a rat anti-mouse antibody. Epitope mapping can be used to develop more antibodies, including anti-human antibodies, that block IL-25R.

Binding sites of the rat anti- mouse IL-25R antibody on IL-25R are mapped using standard epitope mapping techniques. Identification and characterization of the binding sites of anti-IL-25R antibody, which has the ability to block IL-25R and induce Treg conversion (see Example 1) is then used to design and develop new antibodies against IL-25R. This data is also used to develop anti-human IL-23R antibodies. The sequence of human IL-25R (SEQ ID NO. 1) is 67% identical to the sequence of mouse IL-23R (SEQ ID NO. 2). Since antibody binding sites are expected to be on the extracellular domain of IL-23R, sequence identity in only that region between species is used for further fine-tuning. The sequence of extracellular domain of human IL-25R (SEQ ID NO. 1, underlined) is 70.5% identical to the sequence of the extracellular domain of mouse IL-23R (SEQ ID NO. 2, underlined).

Amino acid sequence of human IL-23R (extracellular domain is underlined):

MNQVTIQWDAVIALYILFSWCHGGITNINCSGHIWVEPATIFKMGMNISIYCQAAIKNCQPRKLHFYK
NGIKERFQITRINKTTARLWYKNFLEPHASMYCTAECPKHFQETLICGKDISSGYPPDIPDEVTCVIY
EYSGNMTCTWNAGKLTYIDTKYVVHVKSLETEEEQQYLTSSYINISTDSLQGGKKYLVWVQAANALGM
EESKQLQIHLDDIVIPSAAVISRAETINATVPKTIIYWDSQTTIEKVSCEMRYKATTNQTWNVKEFDT
NFTYVQQSEFYLEPNIKYVFQVRCQETGKRYWQPWSSLFFHKTPETVPQVTSKAFQHDTWNSGLTVAS
ISTGHLTSDNRGDIGLLLGMIVFAVMLSILSLIGIFNRSFRTGIKRRILLLIPKWLYEDIPNMKNSNV
VKMLQENSELMNNNSSEQVLYVDPMITEIKEIFIPEHKPTDYKKENTGPLETRDYPQNSLFDNTTVVY
IPDLNTGYKPQISNFLPEGSHLSNNNEITSLTLKPPVDSLDSGNNPRLQKHPNFAFSVSSVNSLSNTI
FLGELSLILNQGECSSPDIQNSVEEETTMLLENDSPSETIPEQTLLPDEFVSCLGIVNEELPSINTYF
PQNILESHFNRISLLEK (SEQ ID NO. 1)

Amino acid sequence of mouse IL-23R (extracellular domain is underlined):

MKREREMRGFYYIWDMSHLTLQLHVVIALYVLFRWCHGGITSINCSGDMWVEPGEIFQMGMNVSIYCQ
EALKHCRPRNLYFYKNGFKEEFDITRINRTTARIWYKGFSEPHAYMHCTAECPGHFQETLICGKDISS
GHPPDAPSNLTCVIYEYSGNMTCTWNTGKPTYIDTKYIVHVKSLETEEEQQYLASSYVKISTDSLQGS
RKYLVWVQAVNSLGMENSQQLHVHLDDIVIPSASIISRAETTNDTVPKTIVYWKSKTMIEKVFCfhkt
petvpqv
VHQTSQETGKRNWQPWSSPFVHQTSQTVSQVTAKSSHEPQKMEMLSATIFRGHPASGNHQDIGLLSGM
VFLAIMLPIFSLIGIFNRSLRIGIKRKVLLMIPKWLYEDIPNMENSNVAKLLQEKSVFENDNASEQAL
YVDPVLTEISEISPLEHKPTDYKEERLTGLLETRDCPLGMLSTSSSVVYIPDLNTGYKPQVSNVPPGG
NLFINRDERDPTSLETTDDHFARLKTYPNFQFSASSMALLNKTLILDELCLVLNQGEFNSLDIKNSRQ
EETSIVLQSDSPSETIPAQTLLSDEFVSCLAIGNEDLPSINSYFPQNVLESHFSRISLFQK (SEQ ID NO. 2)

Example 3: IL-23R antibodies in tumor immunity

Tumor volume was measured in mice treated with IL-23R antibodies. All mice were inoculated with MC38 cells ($0.2x10^6$) and treated with isotype or IL-23R antibodies (100ug/mouse) given 3 times (i.p 100 uL every third day after tumor formation). Both 12B2B6 (Biolegend clone) and 258018 (R&D clone) antibodies were used. As is shown in FIG. 33, significant delay in tumor growth can be seen in the IL-23R (12B2B64) antibody treated group tumor volume (mm^3). Mean±SEM * $p<0.05$.

Treg conversion was measured in mice treated with IL-23R antibodies. Sorted Treg (WT) were plated for 5 days with plate bound anti-CD3/anti-CD28 and in the presence of IL-2 (20ng/mL) and IL-4 (50ng/mL). 12B2B64 and 258018 antibodies were added at 10 ng/mL on Day 0 of culture. All samples were compared to Negative Control (no antibody). DTA-1 was used as a positive control for Treg conversion. FoxP3 expression is shown in FIG. 34A and IFN-gamma expression is shown in FIG. 34B. Graphs show combined data from three experiments (mean±SEM ANOVA $p<0.05$ * $p<0.01$ ** $p<0.001$ *** $p<0.0001$ ****). IL-23R antibody (12B2B64- Biolegend) induces Treg conversion in vitro under inflammatory conditions. Additional IL-23 receptor antibody (258018- R&D Systems) did not show evidence of Treg converting (↓FoxP3 and ↑IFN-g) ability.

The eTr phenotype was also measured in intratumoral Treg in mice treated with IL-23R antibodies. All mice were inoculated with MC38 ($0.2x10^6$) and treated with isotype or 12B2B64 or 258018 IL-23R antibodies (100ug/mouse) given 3 times (i.p 100uL every third

day after tumor formation). Tumors were collected and digested on day 21 to extract infiltrating immune cells. Cells were then assessed for activation markers without stimulation. Significantly reduced expression of IL-23R$^+$ effector Treg (eTr) (TCR$^+$CD4$^+$CD25$^+$FoxP3$^+$CD44$^+$CD62L$^-$IL-23r$^+$) was observed in the Biolegend (12B2B64) antibody treated group. Representative dot plots shown in FIG. 35 were gated on Treg (TCR$^+$CD4$^+$CD25$^+$FoxP3$^+$) and eTre (TCR$^+$CD4$^+$CD25$^+$FoxP3$^+$CD44$^+$CD62L$^-$). Graphic analysis is based on IL-23R$^+$ eTr. Mean±SEM ** $p<0.01$.

The pSTAT5:pSTAT3 ratio was also assessed in mice treated with IL-23R antibodies. All mice were inoculated with MC38 ($0.2x10^6$) and treated with isotype or 12B2B64 or 258018 IL-23R antibodies (100 ug/mouse) given 3 times (i.p 100uL every third day after tumor formation). Tumors and spleen were immediately fixed and assessed for STAT activation (pSTAT5 and pSTAT3) without stimulation. Reduced expression of pSTAT5 was observed in the IL-23R (12B2B64) antibody treated group resulting in a reduced overall pSTAT5/pSTAT3 ratio. Representative histograms are depicted in FIG. 36 and ratios are shown.

Epitope mapping was done for the 12B2B64 and 258018 IL-23R antibodies. FIG. 37 shows an alignment of murine and human IL-23R and a schematic illustration of the domains of IL-23R. Using the proposed cytokine binding domain, a peptide library constructed of 26 peptides. The peptides were 15 amino acids long with an additional 5 amino acids of overhang. Binding of the antibodies to the peptides was assessed via ELISA. The peptides were bound to a plate, the antibodies were incubated with the individual peptides at various concentrations, and the analysis was completed in a plate reader. Of the 26 peptides in the library 23 were not bound by either antibody. Antibody 12B2B64 bound WNAGKLTYIDTKYVV (SEQ ID NO. 3) and LTYIDTKYVVHVKSL (SEQ ID NO. 4). Antibody 258018 bound WNAGKLTYIDTKYVV (SEQ ID NO. 3), LTYIDTKYVVHVKSL (SEQ ID NO. 4), and KYLVWVQAANALGME (SEQ ID NO. 5). FIG. 38 shows the regions in the cytokine binding domain where the peptides are located.

Example 4: IL-23R modulates Treg conversion via STAT5 and STAT3

Loss of IL-23R enhances Treg conversion generally and in tumor cells specifically. FIGs. 39A-39B show that loss of IL-23R enhances conversion of Treg→Teff generally in a Treg conversion assay. IL-23R^{KO} Tregs showed decreased FoxP3 and increased IFN-γ expression compared to C57BL/6 controls (FIGs. 39A-39B). FIGs. 40A-40C show that loss of IL-23R enhances conversion of Treg→Teff in tumor cells and decreases tumor volume.

Treg isolated from IL-23R^{KO} or C57BL/6 mice were FACS sorted and transferred into Rag2$^{-/-}$ mice via injection into the tail vein along with CD8^{+} T cells. The mice were then inoculated with MC38 cancer cell line and tumor volume, and FoxP3 and IFN-γ expression in Treg from TIL and the spleen were measured. The results presented herein show that loss of IL-23R decreases tumor volume, decreased FoxP3 expression and increased IFN-γ expression compared to C57BL/6 controls. FIGs. 40A-40C show knockout of IL-23R enhances conversion of Treg→Teff in tumor cells.

Treatment of mice having tumors from inoculation with the EL4 cancer cell line with anti-IL-23R antibody shows that treatment with anti-IL-23R antibody also enhances conversion of Treg→Teff in lymphoma tumor cells. Briefly, mice were inoculated with EL4 cancer cells ($0.4x10^6$) for 21 days before being treated with isotype or anti-IL-23R antibody (100μg/mouse) three times (i.p 100 μL every third day after tumor formation). As shown in FIGs. 41A-41B, treatment of mice with EL4 tumors with anti-IL-23R antibody decreased FoxP3 expression and increased IFN-γ expression compared to controls. These results further demonstrate that treatment with anti-IL-23R antibody enhances conversion of Treg→Teff in tumor cells.

To demonstrate that knockdown of IL-23R, via neutralizing antibody, impacts STAT5 and STAT3 expression levels, CD25^{+}FoxP3^{+} Tregs were treated with either isotype or anti-IL-23R antibody *in vitro*. As shown in FIGs. 42A-C, CD25^{+}FoxP3^{+} Tregs treated with anti-IL-23R antibody had significant reduction in the pSTAT5:pSTAT3 ratio compared to isotype-treated. This resulted from a decrease in pSTAT5 expression and an increase in pSTAT3 expression in anti-IL-23R antibody-treated cells compared to isotype-treated cells as determined by flow cytometry (FIGs. 42A-42B). Furthermore, as shown in FIGs 43A-43B, this significant reduction in pSTAT5 expression occurs shortly after treatment (54% pSTAT5 expression in anti-IL-23R antibody-treated compared to 63.8% pSTAT5 expression in isotype-treated at one hour) and persists on longer timescales (5.97% pSTAT5 expression in anti-IL-23R antibody-treated compared to 22.8% pSTAT5 expression in isotype-treated at five days).

To corroborate the effectiveness of anti-IL-23R antibodies to enhance conversion of Treg→Teff in tumor cells via STAT5 and STAT3 expression, a *in vivo* model was tested with anti-IL-23R antibody. Similar to previous experiments, mice were inoculated with MC38 cancer cells ($0.2x10^6$), treated with isotype or anti-IL-23R antibody (100μg/mouse) three times (i.p 100 μL every third day after tumor formation), and FACS analysis was performed on Day 25 after inoculation. FIGs. 44A-44C show that treatment of mice with MC38 tumors

with anti-IL-23R antibody provided a significant reduction in the pSTAT5:pSTAT3 ratio compared to mice treated with isotype, as anti-IL-23R antibody-treated mice had a reduction in pSTAT5 expression and an increase in pSTAT3 expression compared to isotype-treated mice. Mice treated with anti-IL-23R antibody also had increased IFN- γ expression and decreased IL-23R expression levels compared to isotype-treated mice, as shown in FIGs. 44E-44F. FIG. 44D demonstrates that anti-IL-23R antibody treatment decreases the numbers of $CD25^{+}FoxP3^{+}$ Tregs, compared to isotype treatment. In a similar experiment, mice were inoculated with EL4 cancer cells ($0.4x10^{6}$), treated with isotype or anti-IL-23R antibody (100μg/mouse) three times (i.p 100 μL every third day after tumor formation), and FACs analysis was completed 21 days after inoculation. As shown in FIGs. 45A-45B, treatment of mice with anti-IL-23R antibody decreased pSTAT5 expression and provided a reduction in the pSTAT5:pSTAT3 ratio, compared to isotype-treated mice.

The role of STAT3 in tumor growth was further investigated. $STAT3^{WT}$Treg mice and $STAT3^{KO}$Treg mice were inoculated with MC38 cancer cells ($0.2x10^{6}$). $STAT3^{KO}$Treg mice have been genetically engineered for knockout of STAT3 in Tregs ($STAT3^{fl/fl}FoxP3^{cre}$). FIG. 46A shows that tumor growth, as assessed by tumor volume, progresses more slowly in $STAT3^{KO}$Treg mice then it does in $STAT3^{WT}$Treg mice. Additionally, $STAT3^{KO}$Treg mice have increased expression levels of CD69 and IFN-γ compared to $STAT3^{WT}$Treg mice in CD8 TIL (FIGs. 46B-46C).

It was next investigated whether the anti-IL23R antibody directly interacts with IL23-R Treg. This was first investigated by looking at tumor volume in C57BL/6 and $Rag2^{-/-}$ mice inoculated with MC38 cancer cells ($0.2x10^{6}$) for nine days before being treated with isotype or anti-IL-23R antibody (100μg/mouse) three times (i.p 100 μL every third day after tumor formation). FIG. 47A shows that C57BL/6 mice treated with anti-IL-23R antibody had a reduction in tumor volume at the end of the treatment period compared to C57BL/6 mice treated with isotype. There was no difference observed for $Rag2^{-/-}$ mice.

Whether the anti-IL23R antibody directly interacts with IL23-R Treg was next investigated by looking at tumor volume in mice in which isolated Tregs alone or in conjunction with $CD8^{+}$ T cells were injected into the tail veins of $Rag2^{-/-}$ mice. The mice were then inoculated with MC38 cancer cells ($0.2x10^{6}$) for nine days before being treated with isotype or anti-IL-23R antibody (100μg/mouse) three times (i.p 100 μL every third day after tumor formation). FIG. 47B demonstrates that anti-IL-23R antibody decreases tumor volume, compared to isotype, in $Rag2^{-/-}$ mice that have been injected with Tregs alone or in conjunction with $CD8^{+}$ T cells.

_EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements

specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts

of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

CLAIMS

What is claimed is:

1. A method for treating cancer in a human subject, the method comprising:

administering to a human subject in need thereof an agent that decreases IL-23R activity and does not decrease IL-12Rβ activity, in an amount effective to treat the cancer.

2. The method of claim 1, wherein the agent is a polypeptide, nucleic acid, or small molecule.

3. The method of claim 1, wherein the agent binds the extracellular domain of IL-23R.

4. The method of claim 3, wherein the agent is a human or humanized monoclonal antibody.

5. The method of claim 4, wherein the antibody binds IL-23R and does not bind IL-12Rβ.

6. The method of claim 4, wherein the antibody has 100-fold, 1,000-fold, or 10,000-fold greater binding affinity for IL-23R than for IL-12Rβ.

7. The method of any one of claims 4-6, wherein the antibody is free of antibody-dependent cellular cytotoxicity (ADCC) activity.

8. The method of any one of claims 4-6, wherein the antibody has an IgG4 heavy chain immunoglobulin constant domain.

9. The method of claim 1, wherein the agent binds a nucleic acid expressing IL-23R.

10. The method of any one of claims 1-9, further comprising administering to the human subject an immunomodulatory agent.

11. The method of claim 10, wherein the immunomodulatory agent is an immune checkpoint inhibitor.

12. The method of claim 11, wherein the immune checkpoint inhibitor is an antagonist of a molecule selected from the group consisting of PD-1, TIM-3, TIGIT, VISTA, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR and LAG3.

13. The method of claim 10, wherein the immunomodulatory agent in an antagonist of inhibitory macrophages and/or dendritic cells.

14. The method of any one of claims 1-13, further comprising administering an agent that inhibits cancer cell growth.

15. A method for inducing differentiation of a regulatory T (Treg) cell to an effector T cell, the method comprising contacting the Treg cell in vitro with an agent that decreases IL-23R activity and does not decrease IL-12Rβ activity.

16. The method of claim 15, wherein the agent is a polypeptide, nucleic acid, or small molecule.

17. The method of claim 15, wherein the agent binds the extracellular domain of IL-23R.

18. The method of claim 17, wherein the agent is a human or humanized monoclonal antibody.

19. The method of claim 18, wherein the antibody binds IL-23R and does not bind IL-12Rβ.

20. The method of claim 18, wherein the antibody has 100-fold, 1,000-fold, or 10,000-fold greater binding affinity for IL-23R than for IL-12Rβ.

21. The method of any one of claims 18-20, wherein the antibody is free of antibody-dependent cellular cytotoxicity (ADCC) activity.

22. The method of any one of claims 18-20, wherein the antibody has an IgG4 heavy chain immunoglobulin constant domain.

23. The method of claim 15, wherein the agent binds a nucleic acid expressing IL-23R.

24. The method of any one of claims 15-23, wherein the Treg cell is a CD4+ Treg cell, a CD8+ Treg cell, a Helios+ Treg cell, or a CD25+ and FoxP3+ Treg cell.

25. The method of any one of claims 15-24, further comprising contacting the Treg cell in the presence of one or more inflammatory cytokines.

26. The method of claim 25, wherein the one or more inflammatory cytokines is selected from the group consisting of IL-2, IL-4, and combination thereof.

27. A method for identifying a compound that will convert a regulatory T cell (Treg cell) into an effector cell, comprising:

contacting a regulatory T cell (Treg) with a test compound;

measuring IL-23R activity in the cell; and

measuring IL-12Rβ activity in the cell;

wherein the test compound is a compound for converting a regulatory T cell (Treg cell) into an effector cell if:

(i) the IL-23R activity is decreased relative to a control cell that has been treated with a compound known to not decrease IL-23R activity and IL-12Rβ activity; and

(ii) the IL-12Rβ activity is not decreased relative to the control cell.

28. The method of claim 27, further comprising measuring FoxP3 activity in the cell.

29. The method of claim 27, further comprising measuring an effector cytokine produced by the cell.

30. The method of claim 29, wherein the effector cytokine is TNF-α, IFN-γ, IL-17, IL-10, or IL-2.

31. The method of any one of claims 27-30, wherein the test compound is selected from the group consisting of a polypeptide, a nucleic acid and a small molecule.

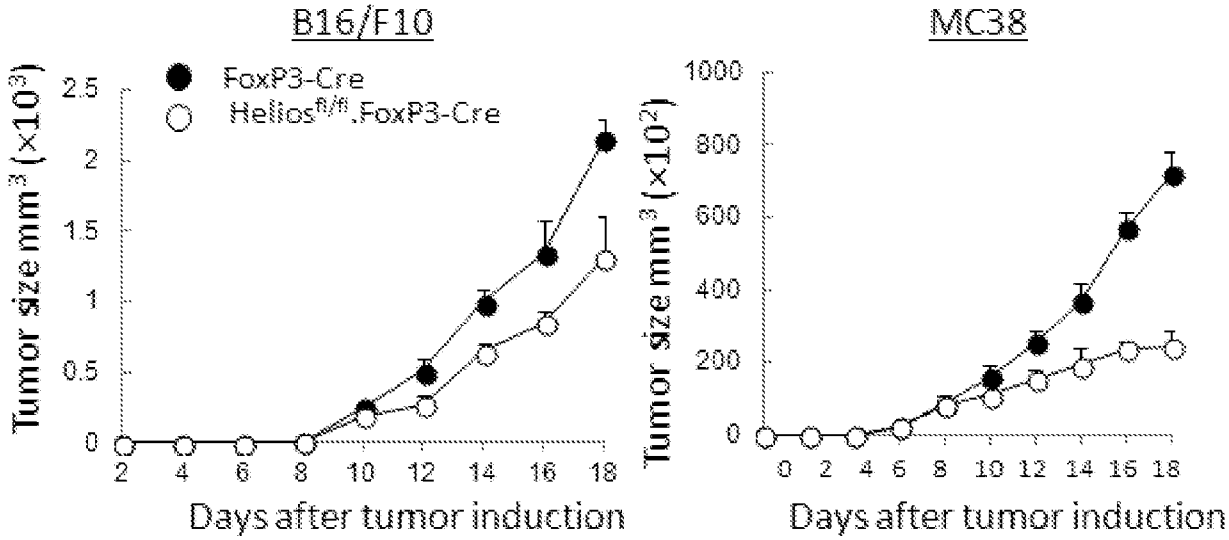

B16/F10
MC38
FoxP3-Cre
Helios^fl/fl.FoxP3-Cre
Tumor size mm^3 (×10^3)
Tumor size mm^3 (×10^2)
Days after tumor induction
Days after tumor induction

FIG. 1A

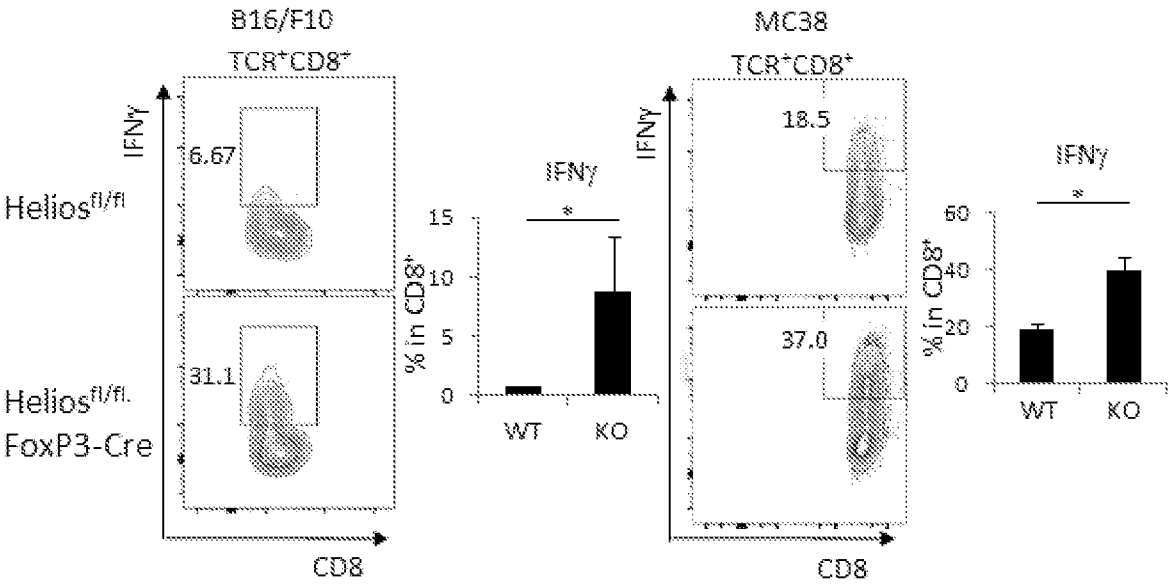
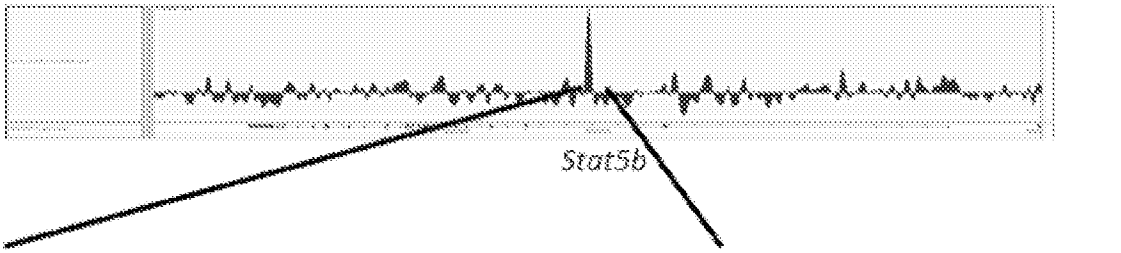

B16/F10
TCR+CD8+
MC38
TCR+CD8+
IFNγ
CD8
Helios^fl/fl
Helios^fl/fl.
FoxP3-Cre
6.67
31.1
18.5
37.0
% in CD8+
WT
KO

FIG. 1B



GTCCTAAAAATATGATGGTGAATCCAGTTCCCAGGGT
TTAGAGCGGCCCCTGTGATGATTTACCAGCGATACAA
ACCACTTCCCTTGTCAGAATGTCACGAACTGAAAACTG
AGCTGCCGGGTCAATAAGCAGAGAAGAGCAT GCGCA
TGCGC GGGGTAGGCTGTCTCCATGCGGGT GGAGG AG
CCGCCCGGTGGCTGGATTGACCATGGGTACAGGGCC
GGTGGGGGCCCCCCCACTTACACCTGTGTGCACAATG
TTTCCTGGTGAGTGT GGAGAGTTCTGACAGGCATG GG
AGAAAAGCACTAACCAGACCACAGGTCTTGAG

SEQ ID NO: 6

FIG. 2A

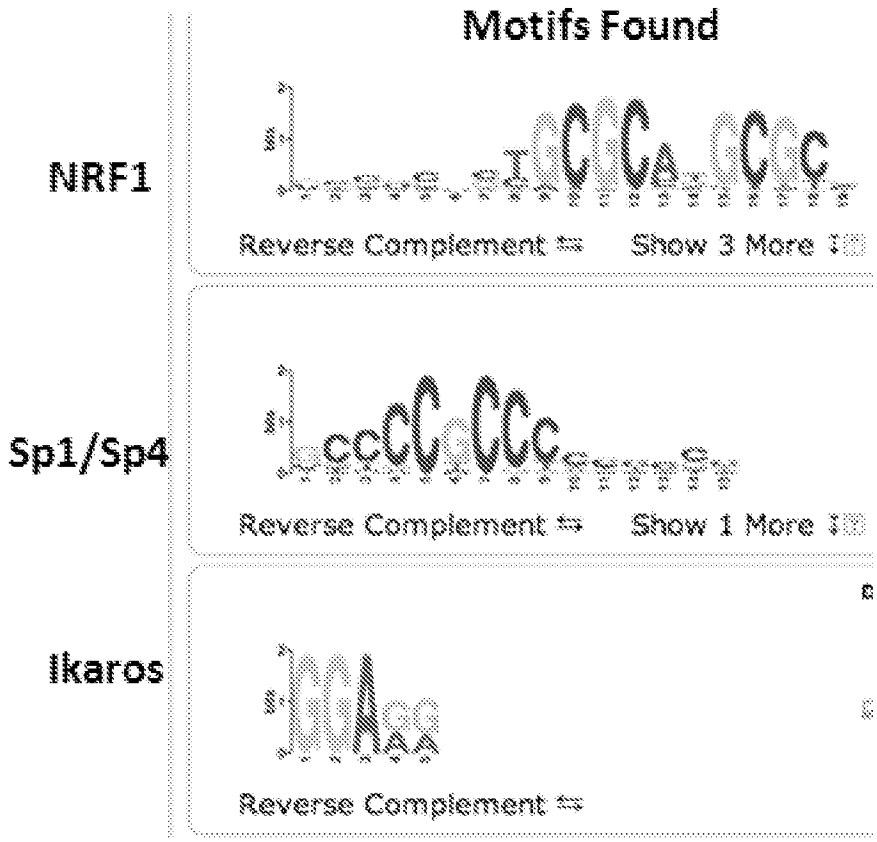


FIG. 2B

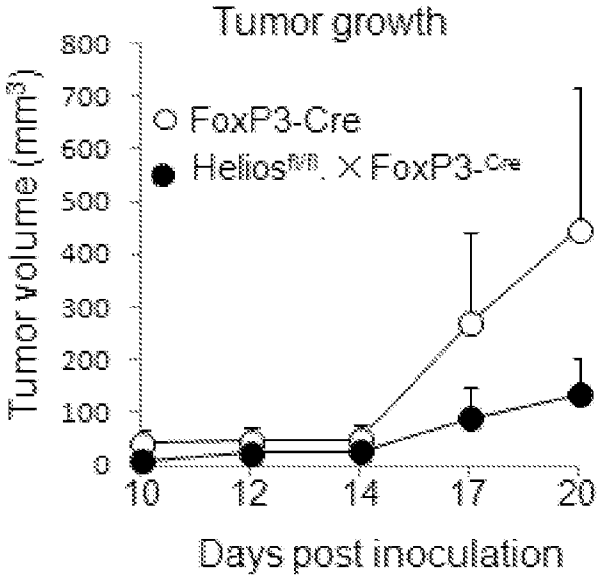


FIG. 3A

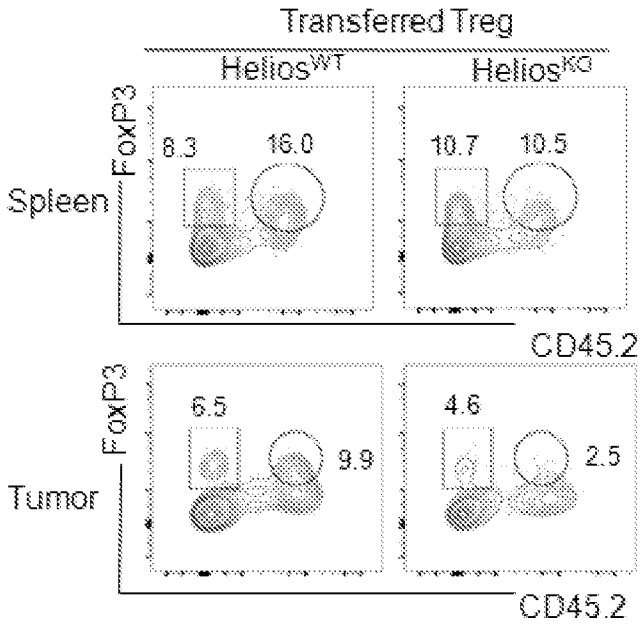


FIG. 3B

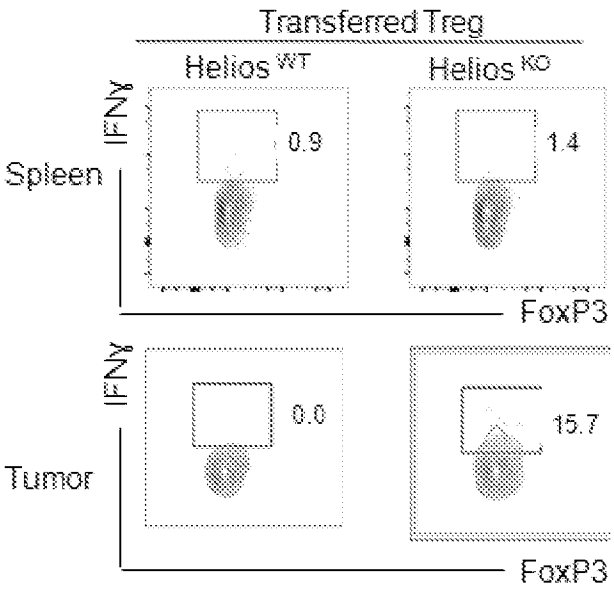


FIG. 3C

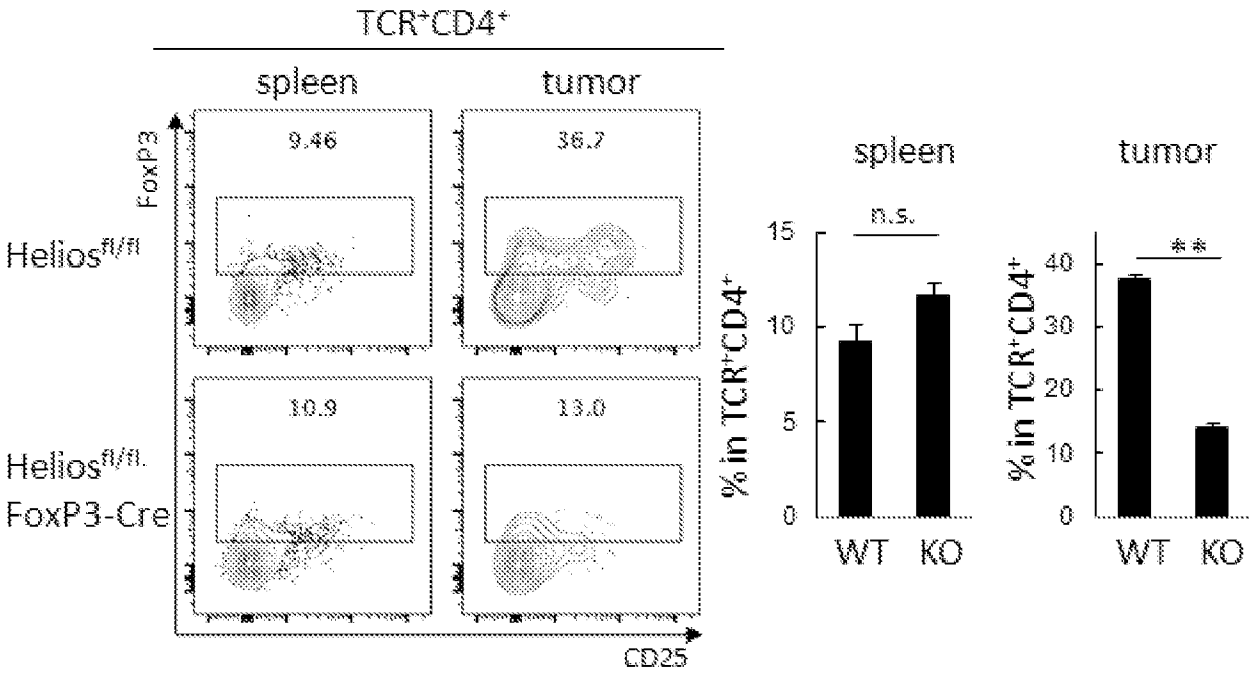

TCR+CD4+
spleen
tumor
FoxP3
9.46
36.7
Helios fl/fl
10.9
13.0
Helios fl/fl.
FoxP3-Cre
CD25
spleen
tumor
n.s.
**
% in TCR+CD4+
15
10
5
0
40
30
20
10
0
WT
KO

FIG. 4A

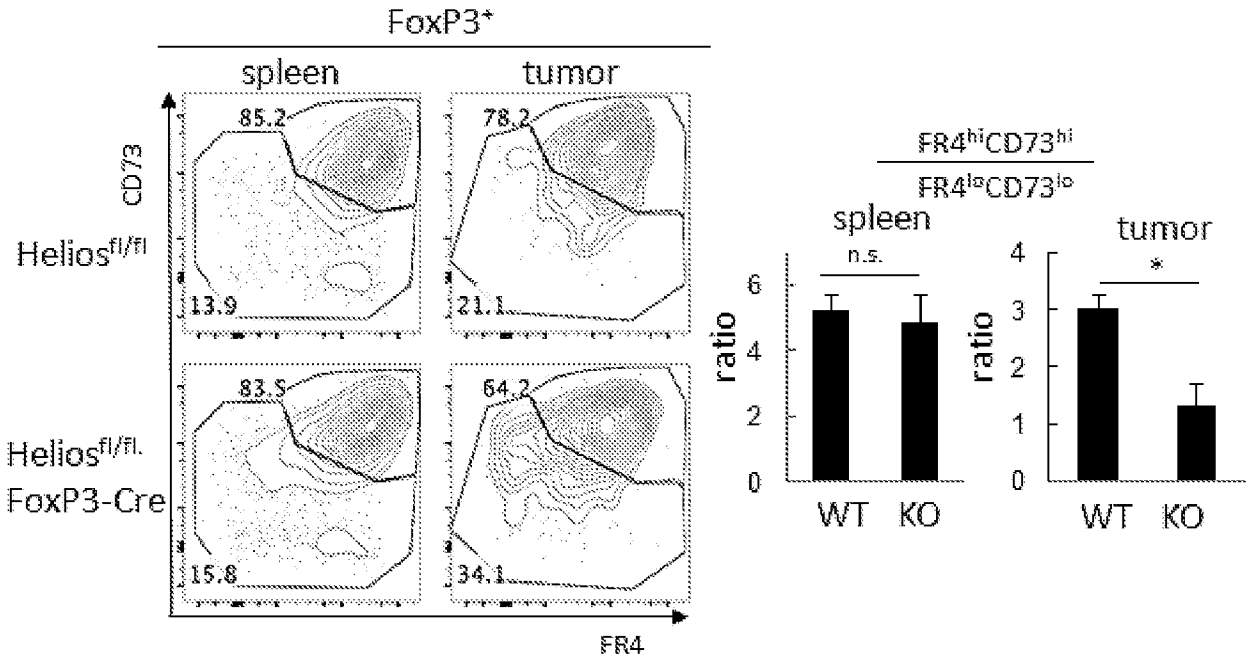

FoxP3+
spleen
tumor
CD73
85.2
78.2
13.9
21.1
Helios fl/fl
83.9
64.2
15.8
34.1
Helios fl/fl.
FoxP3-Cre
FR4
FR4hiCD73hi
FR4loCD73lo
spleen
tumor
n.s.
*
ratio
6
4
2
0
3
1
WT
KO

FIG. 4B

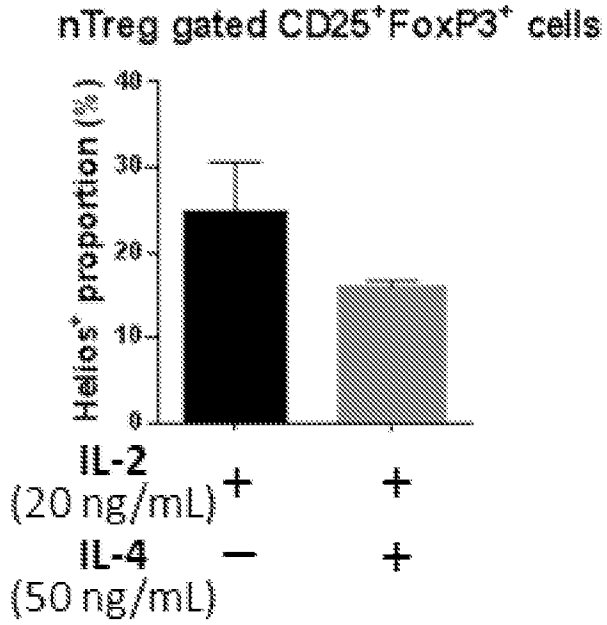

nTreg gated CD25+FoxP3+ cells
Helios+ proportion (%)
40
30
20
10
0
IL-2
(20 ng/mL)
+
+
IL-4
(50 ng/mL)
−
+

FIG. 5A

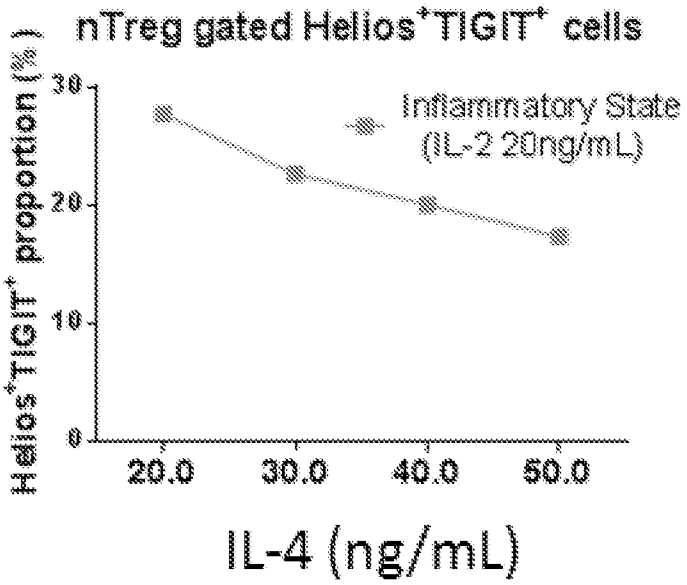

nTreg gated Helios+TIGIT+ cells
Helios+TIGIT+ proportion (%)
30
20
10
0
Inflammatory State
(IL-2 20ng/mL)
20.0
30.0
40.0
50.0
IL-4 (ng/mL)

FIG. 5B

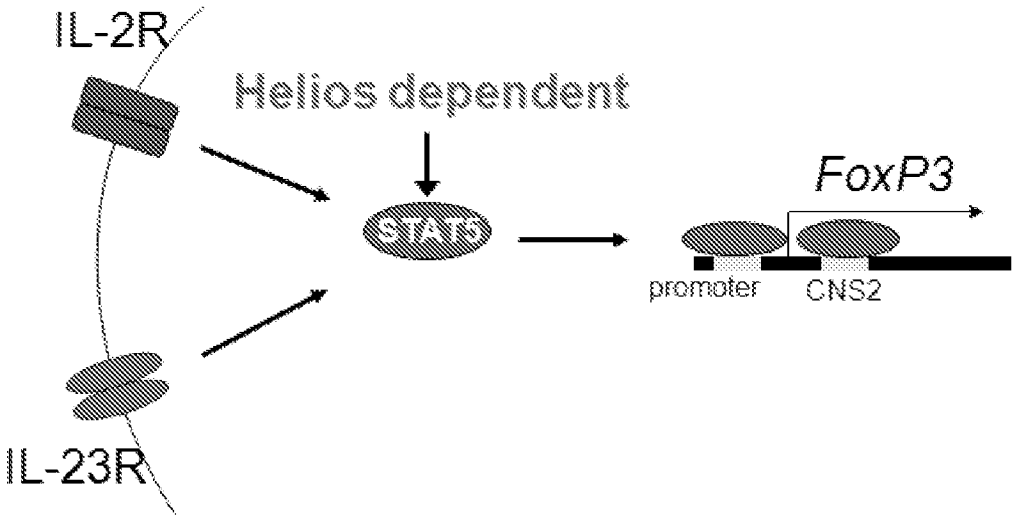


FIG. 6

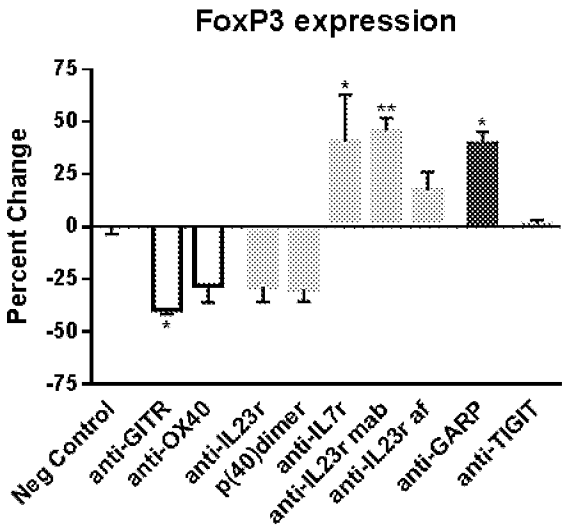


FIG. 7A

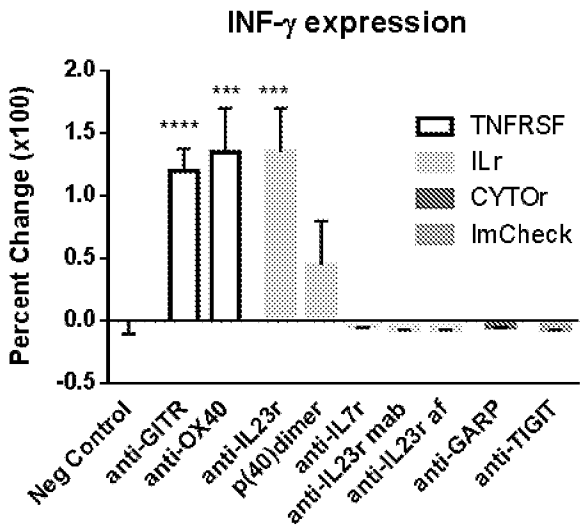


FIG. 7B

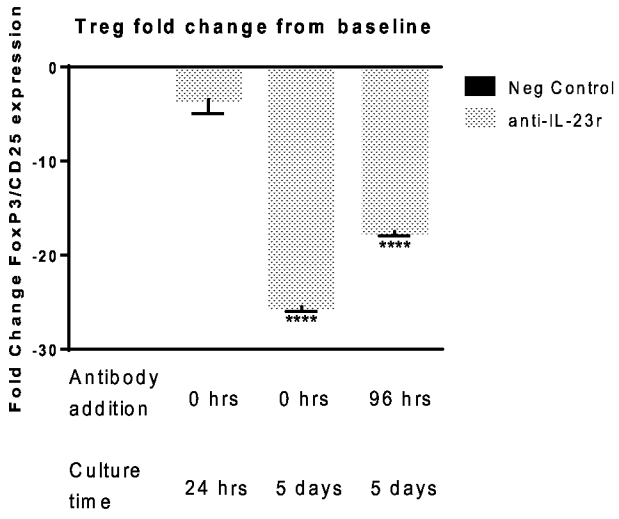


FIG. 7C

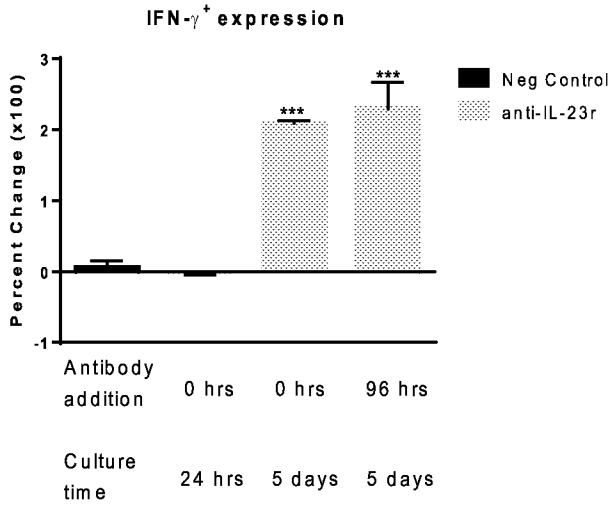


FIG. 7D

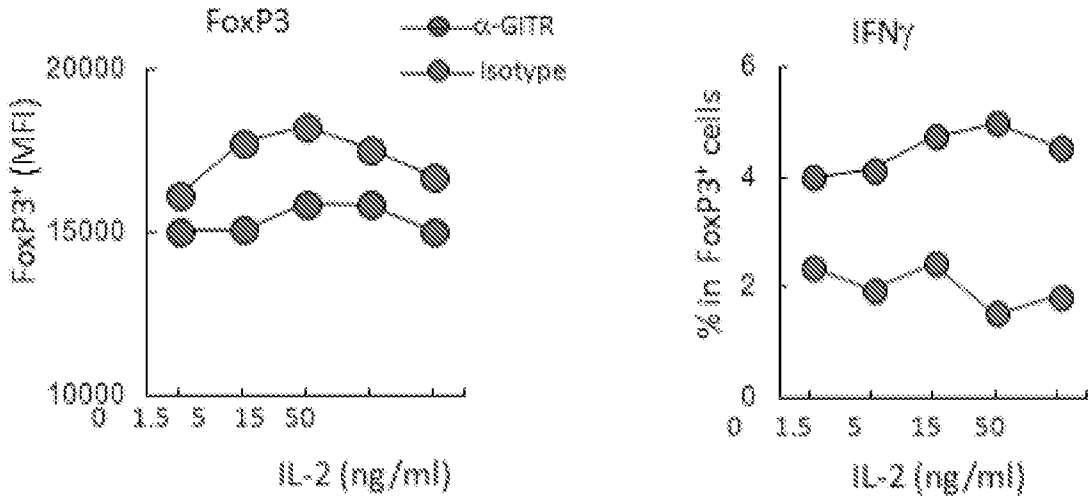


FIG. 8

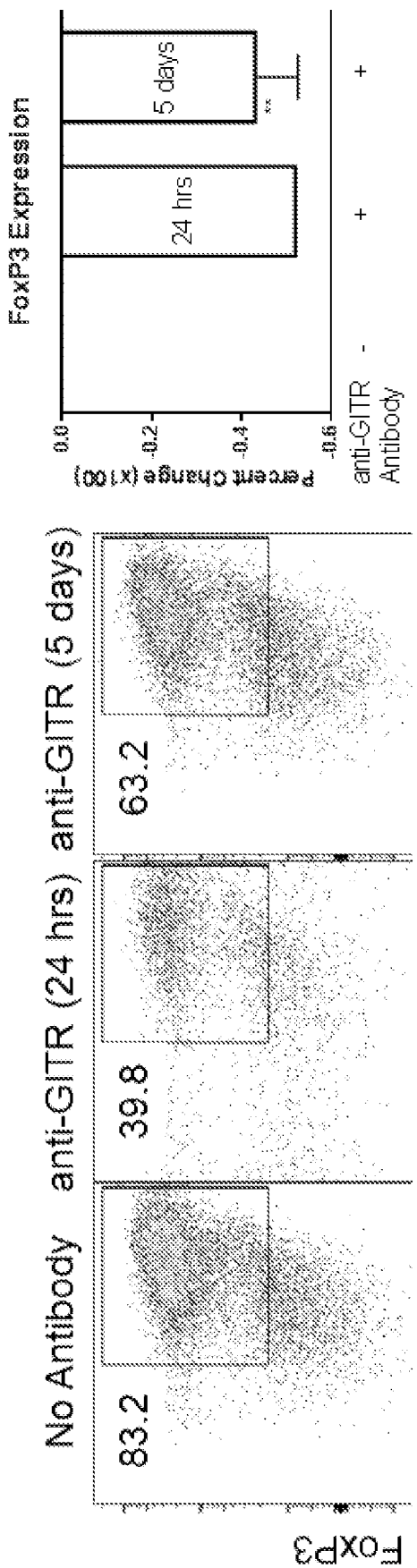

No Antibody
anti-GITR (24 hrs)
anti-GITR (5 days)
83.2
39.8
63.2
FoxP3
CD25
FoxP3 Expression
Percent change (x10)
0.0
-0.2
-0.4
-0.6
24 hrs
5 days
anti-GITR Antibody
-
+
+

FIG. 9A

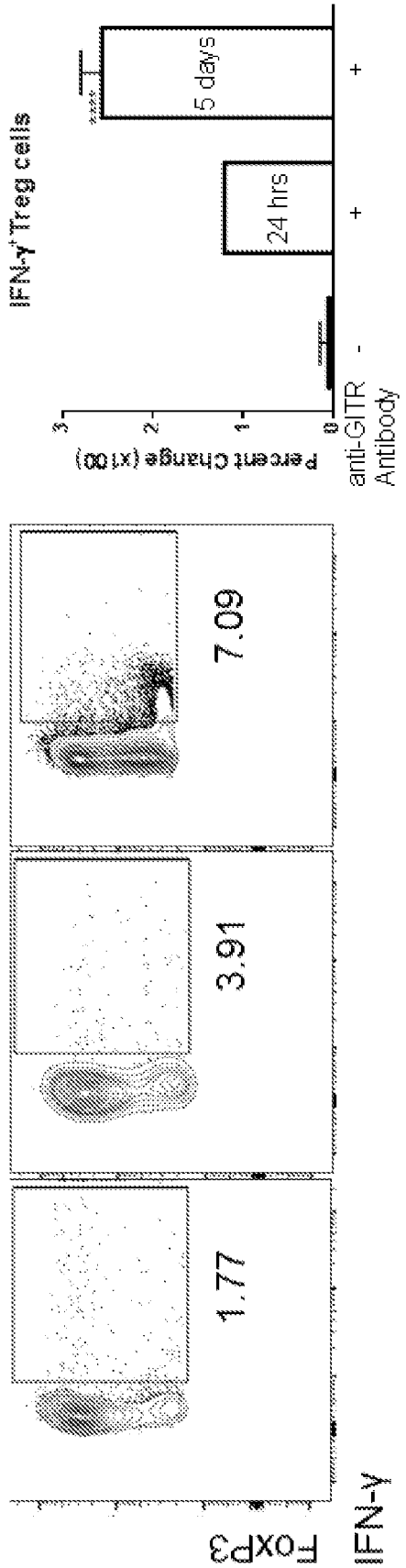

IFN-γ+ Treg cells
Percent change (x10)
3
2
1
0
24 hrs
5 days
anti-GITR Antibody
-
+
+
1.77
3.91
7.09
FoxP3
IFN-γ

FIG. 9B

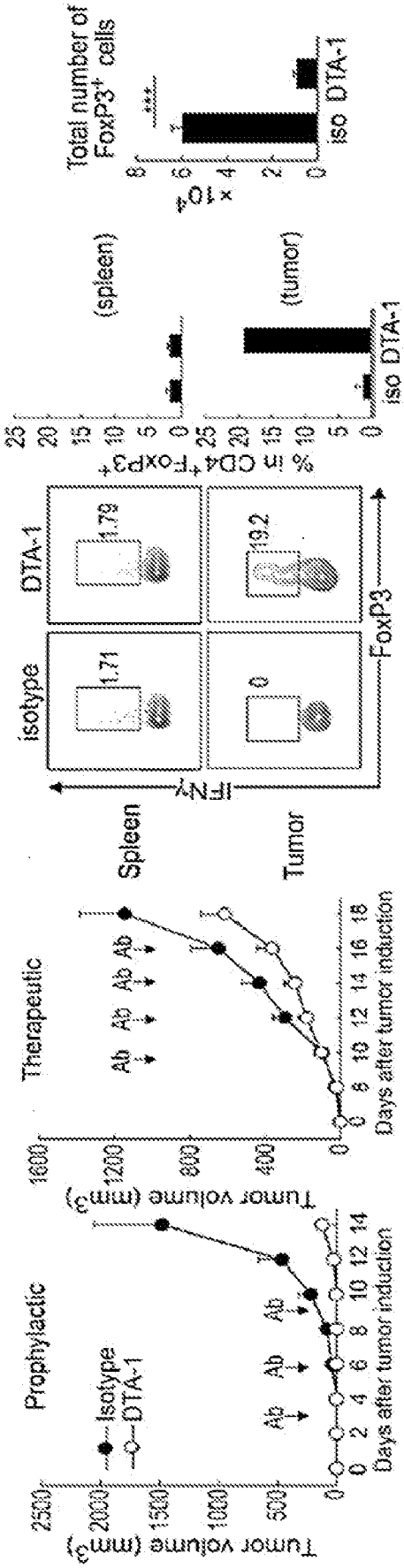


FIG. 10A

FIG. 10B

FIG. 10C

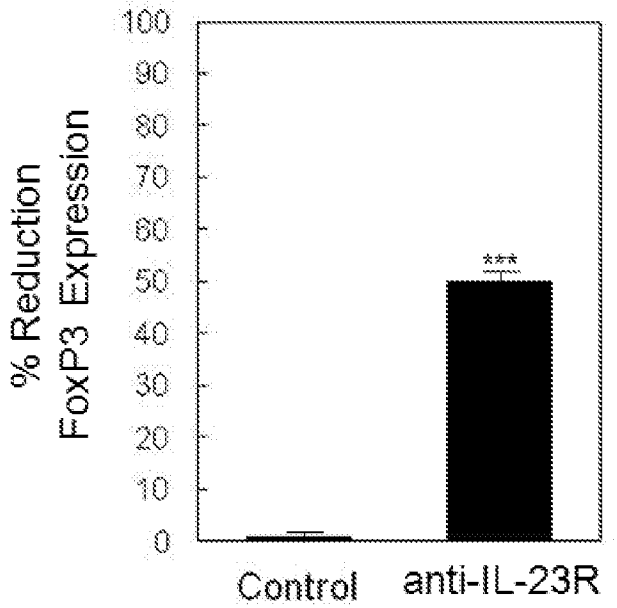


FIG. 11A

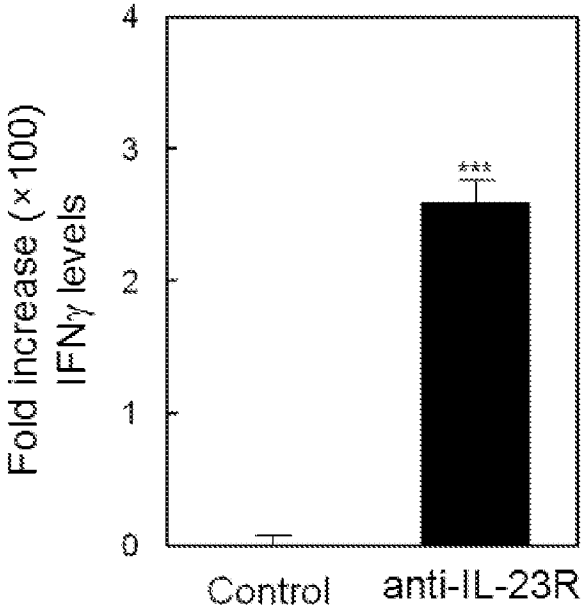
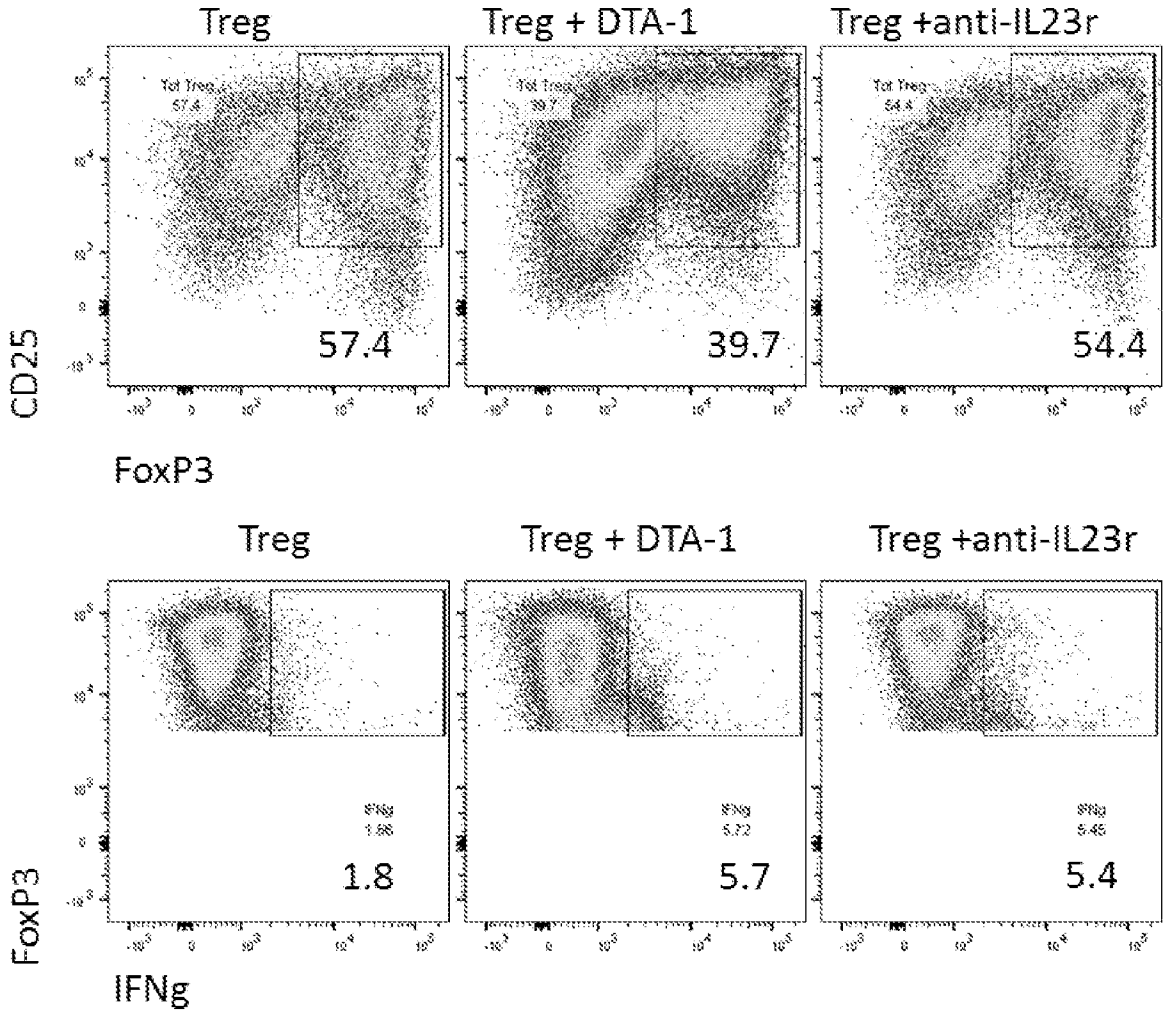


FIG. 11B


Treg
Treg + DTA-1
Treg +anti-IL23r
CD25
FoxP3
57.4
39.7
54.4
Treg
Treg + DTA-1
Treg +anti-IL23r
FoxP3
IFNg
1.8
5.7
5.4

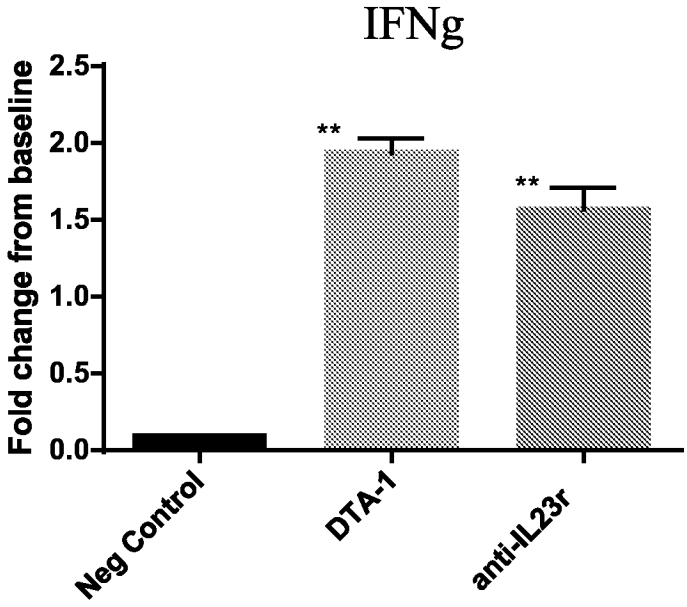

IFNg
Fold change from baseline
2.5
2.0
1.5
1.0
0.5
0.0
**
**
Neg Control
DTA-1
anti-IL23r

FIG. 12

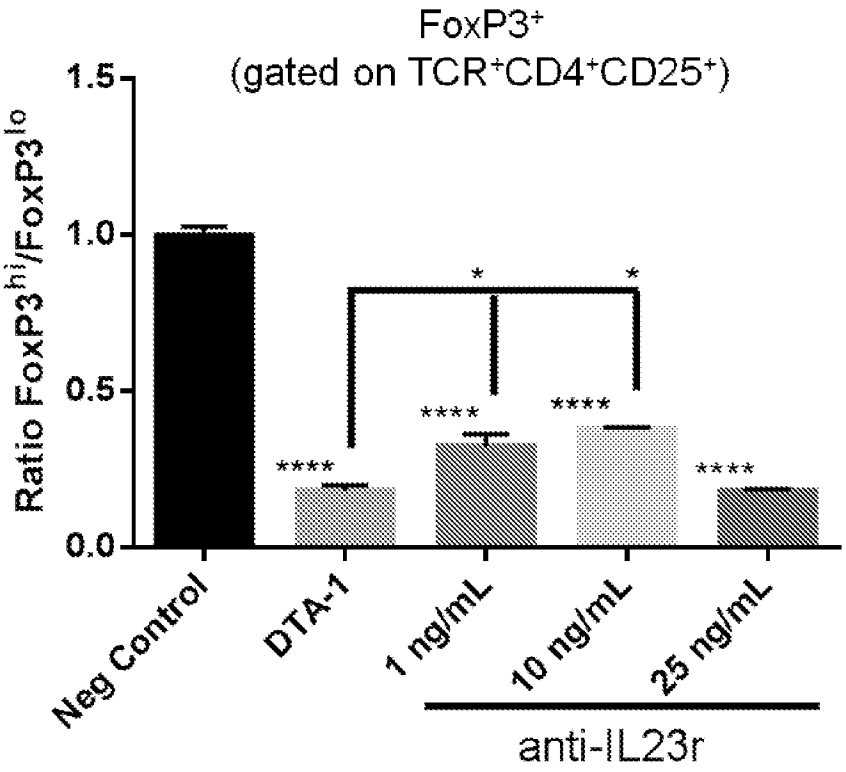

FoxP3+
(gated on TCR+CD4+CD25+)
Ratio FoxP3hi/FoxP3lo
1.5
1.0
0.5
0.0
*
*
****
****
****
Neg Control
DTA-1
1 ng/mL
10 ng/mL
25 ng/mL
anti-IL23r

FIG. 13

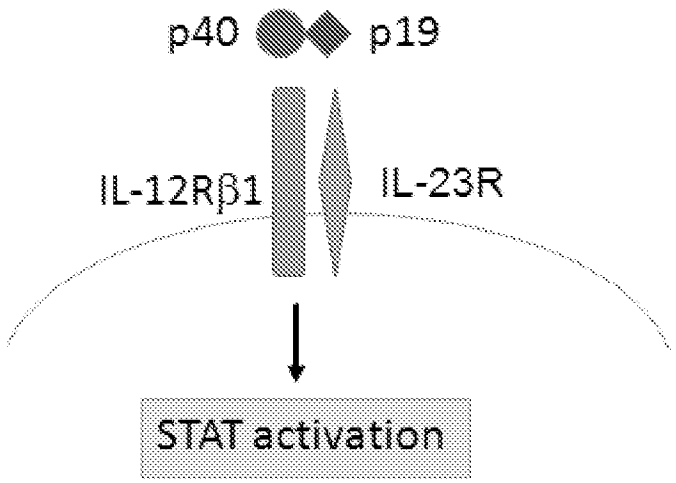

p40
p19
IL-12Rβ1
IL-23R
STAT activation

FIG. 14

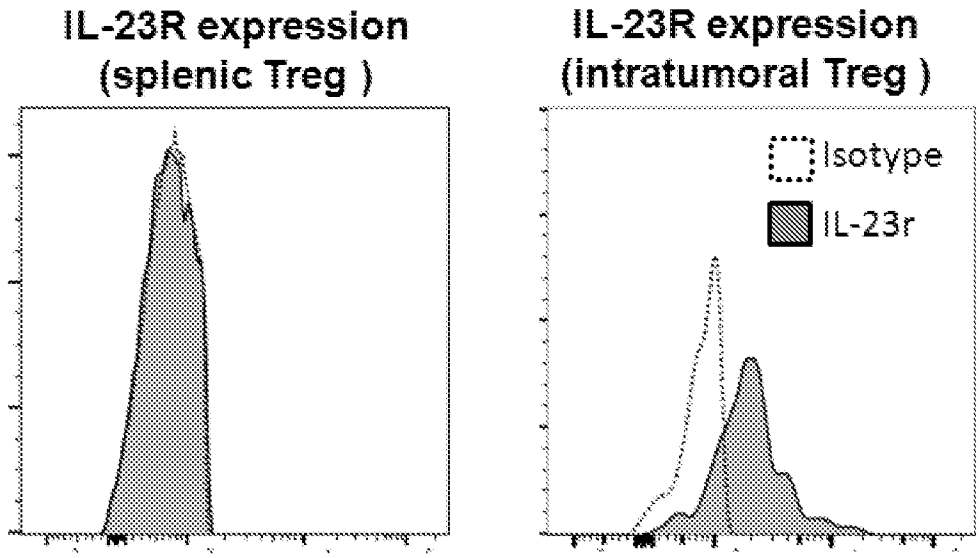

IL-23R expression
(splenic Treg)
IL-23R expression
(intratumoral Treg)
Isotype
IL-23r

FIG. 15

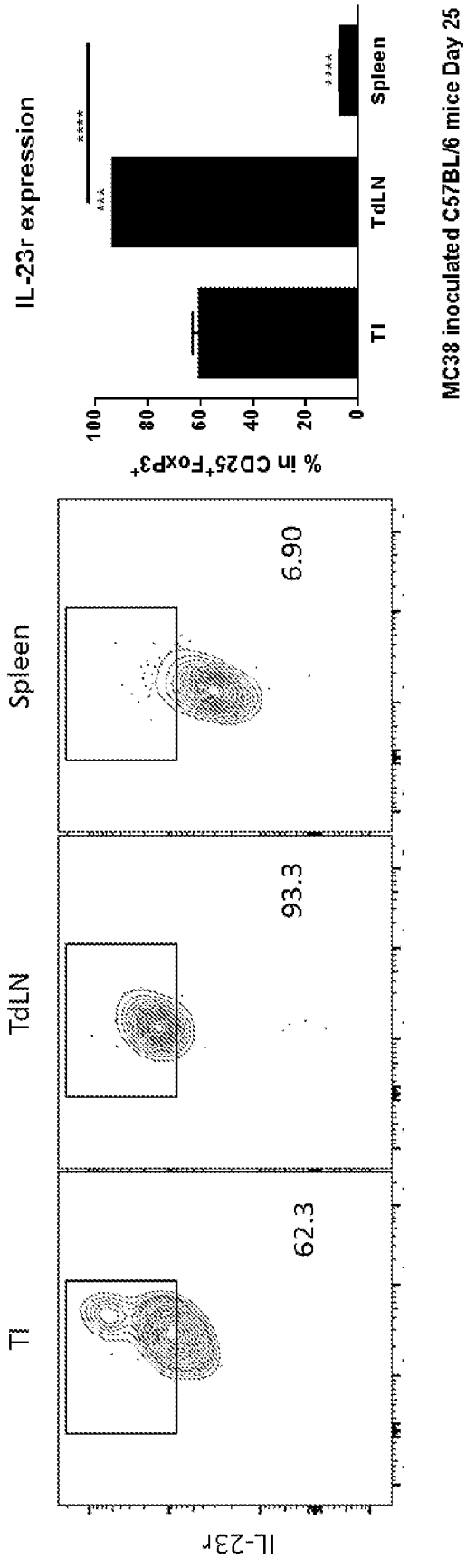

IL-23r expression
% in CD25+FoxP3+
100
80
60
40
20
0
TI
TdLN
Spleen
***
****
****
MC38 inoculated C57BL/6 mice Day 25
TI
TdLN
Spleen
62.3
93.3
6.90
IL-23r

FIG. 16

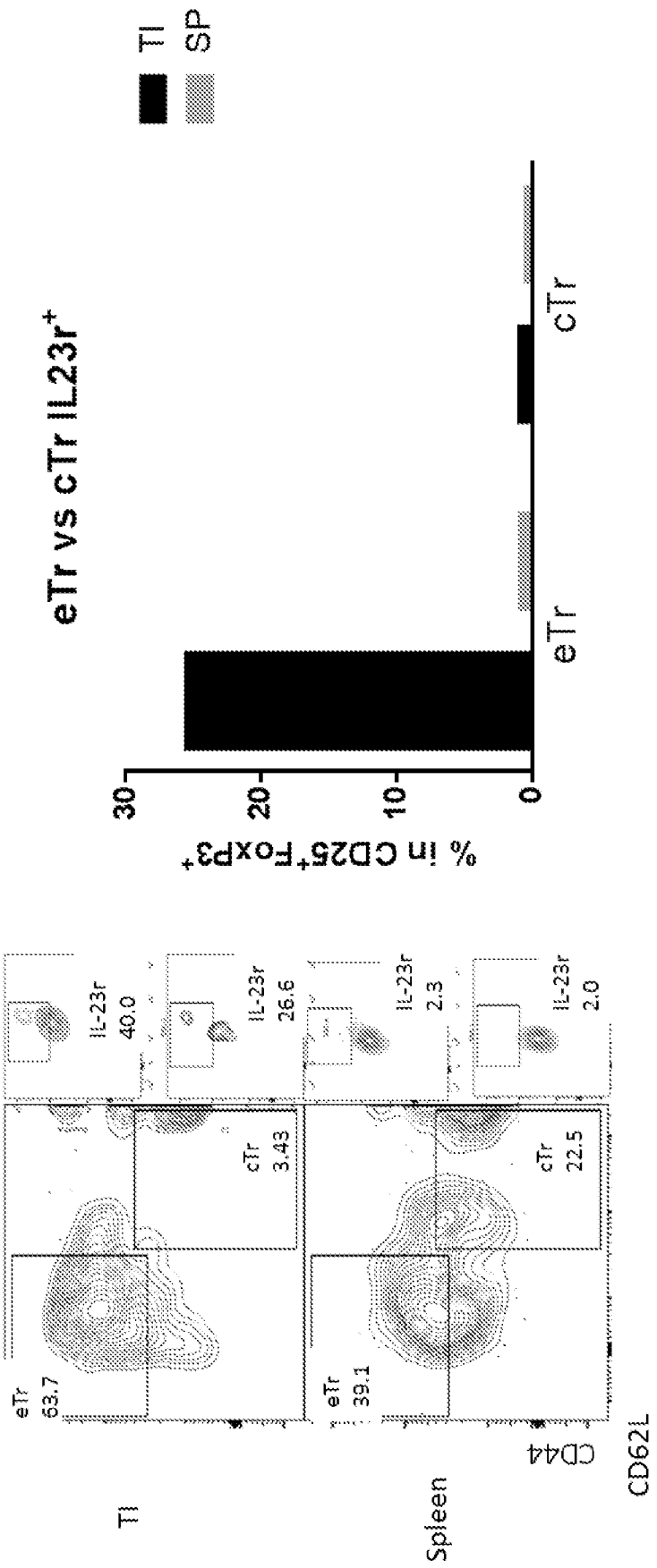

TI
SP
eTr vs cTr IL23r+
% in CD25+FoxP3+
30
20
10
0
eTr
cTr
TI
Spleen
eTr
63.7
IL-23r
40.0
cTr
3.43
IL-23r
26.6
eTr
39.1
IL-23r
2.3
cTr
22.5
IL-23r
2.0
CD44
CD62L

FIG. 17

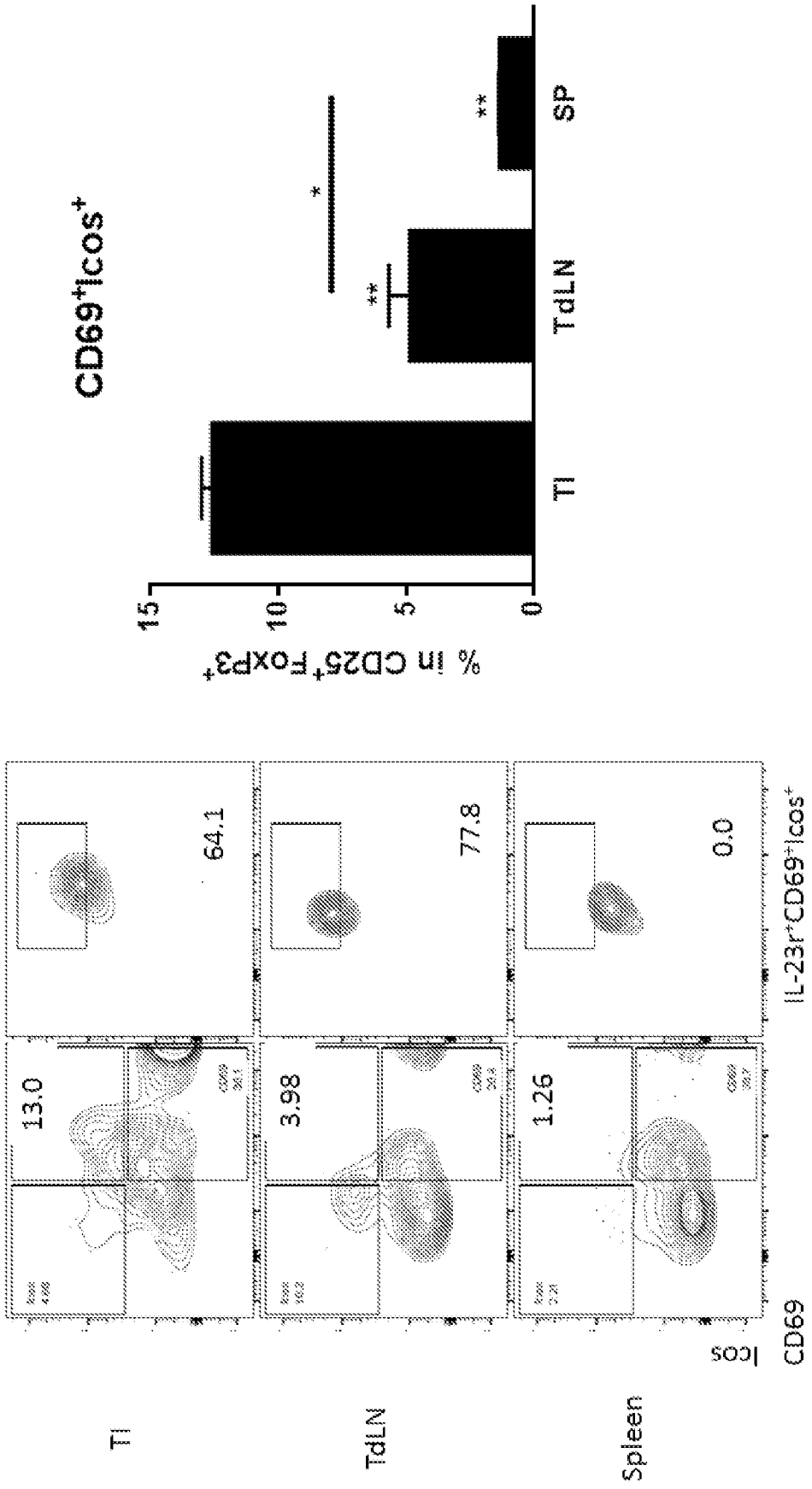

% in CD25+FoxP3+
15
10
5
0
TI
TdLN
SP
*
**
**
CD69+Icos+
TI
TdLN
Spleen
13.0
64.1
3.98
77.8
1.26
0.0
Icos
CD69
IL-23r+CD69+Icos+

FIG. 18

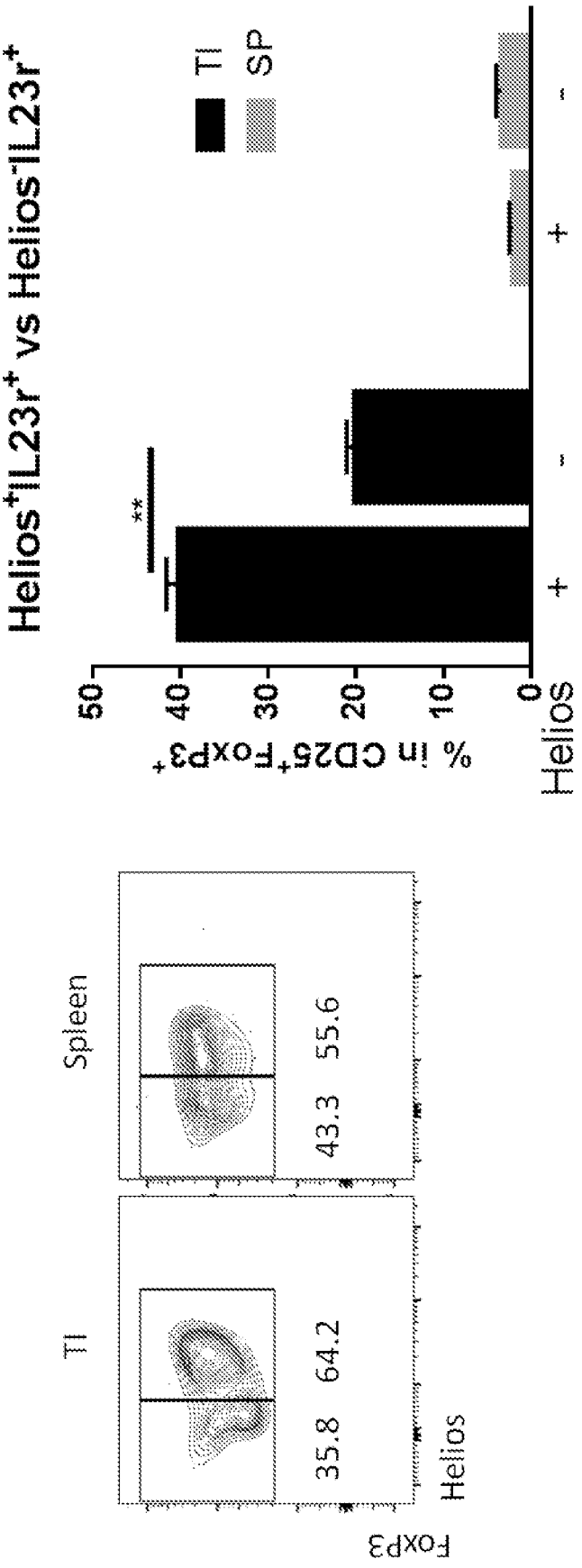

Helios+IL23r+ vs Helios-IL23r+
TI
SP
**
% in CD25+FoxP3+
50
40
30
20
10
0
Helios
+
-
+
-
Spleen
TI
35.8
64.2
43.3
55.6
FoxP3
Helios

FIG. 19

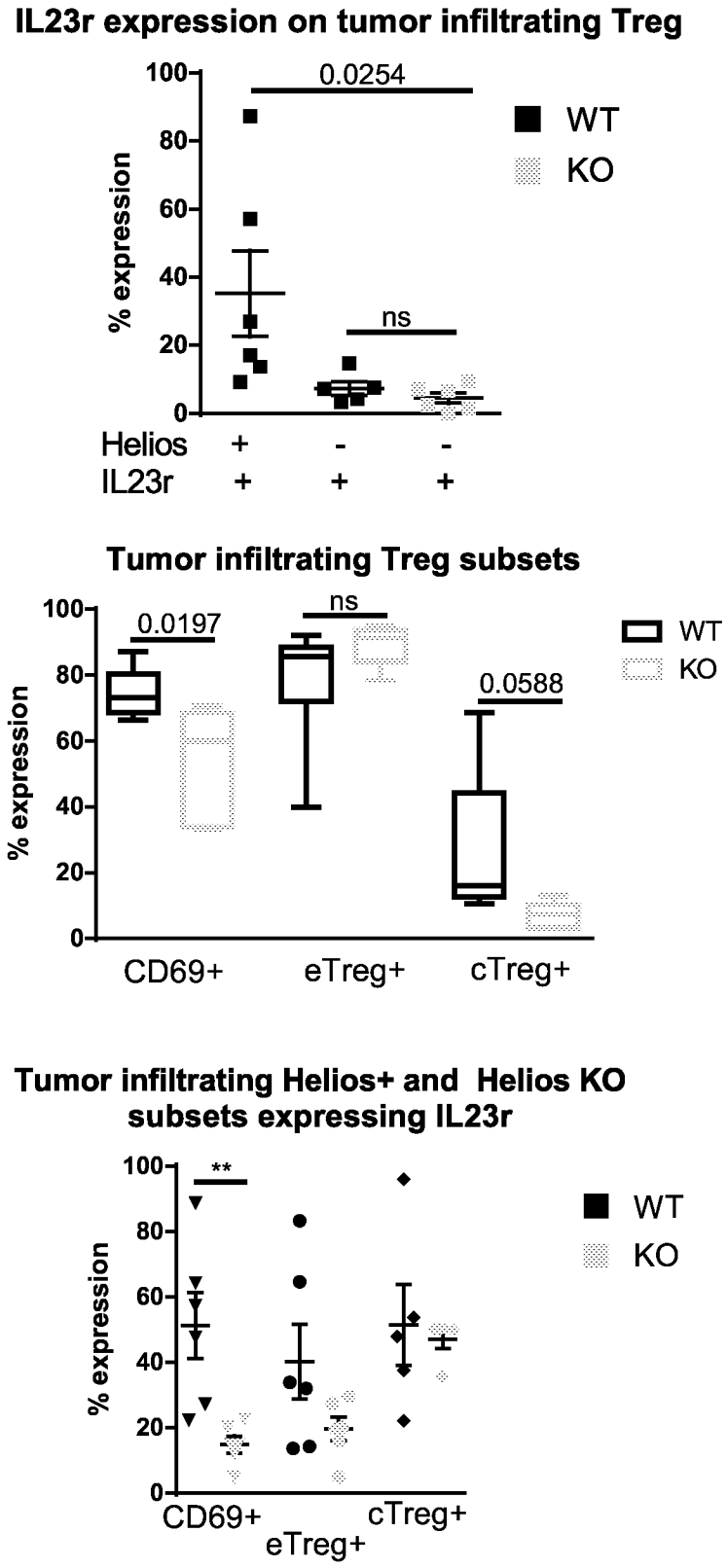

IL23r expression on tumor infiltrating Treg
0.0254
ns
WT
KO
% expression
100
80
60
40
20
0
Helios + - -
IL23r + + +
Tumor infiltrating Treg subsets
0.0197
ns
0.0588
WT
KO
% expression
CD69+
eTreg+
cTreg+
Tumor infiltrating Helios+ and Helios KO subsets expressing IL23r
**
WT
KO
% expression
CD69+
eTreg+
cTreg+

FIG. 20

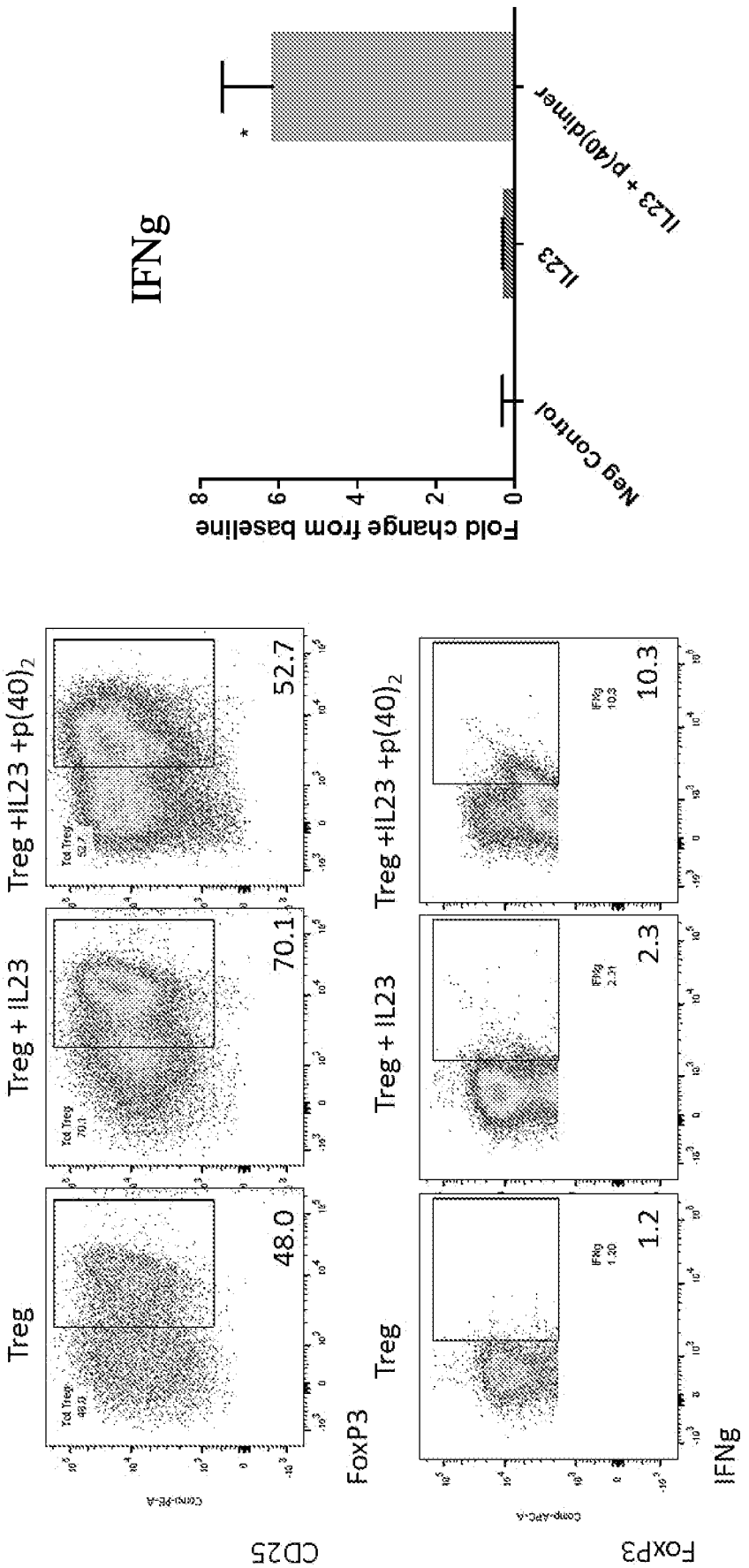

IFNg
Fold change from baseline
0
2
4
6
8
Neg Control
IL23
IL23 + p(40)dimer
*
CD25
FoxP3
Treg
Treg + IL23
Treg +IL23 +p(40)2
48.0
70.1
52.7
FoxP3
IFNg
1.2
2.3
10.3

FIG. 21

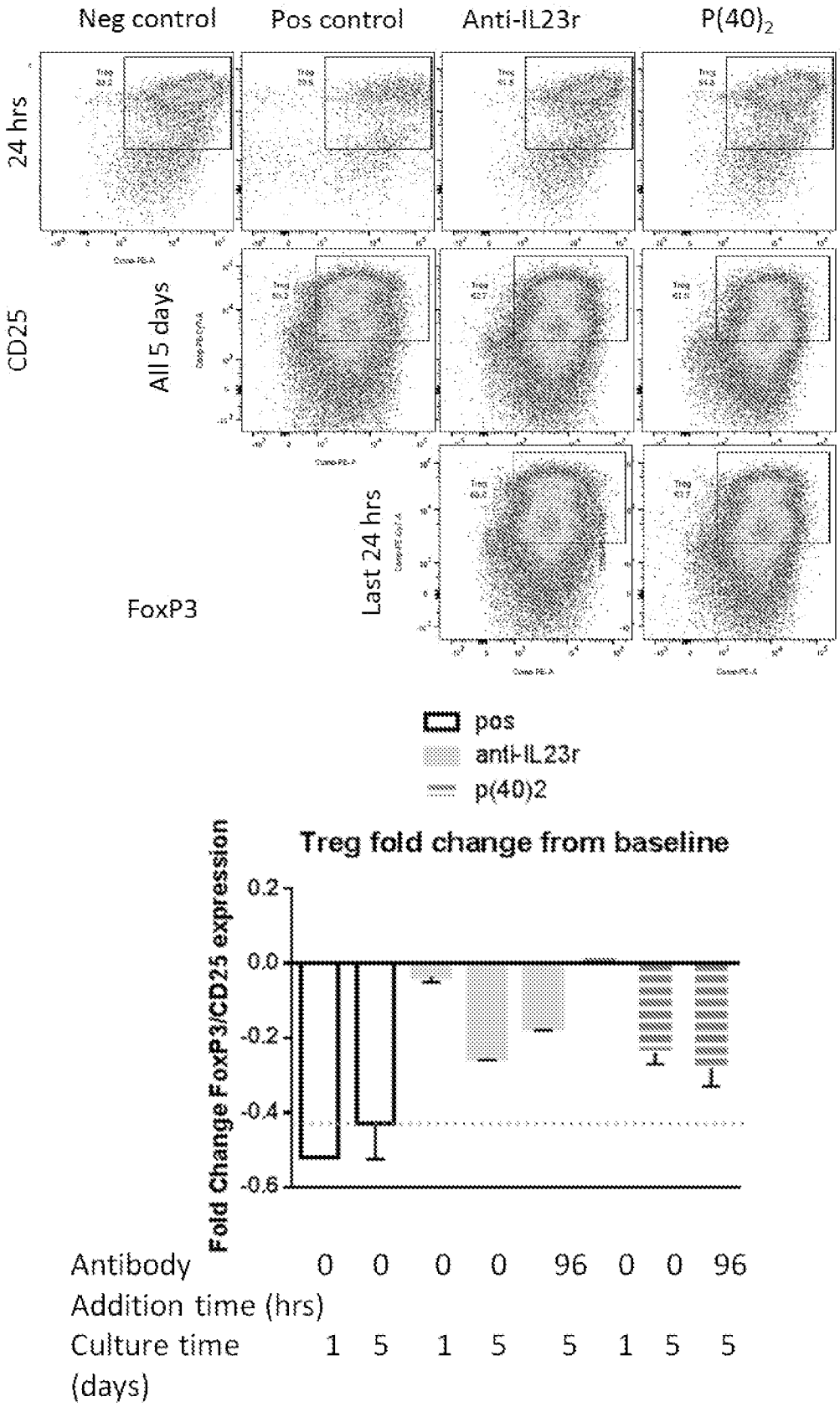
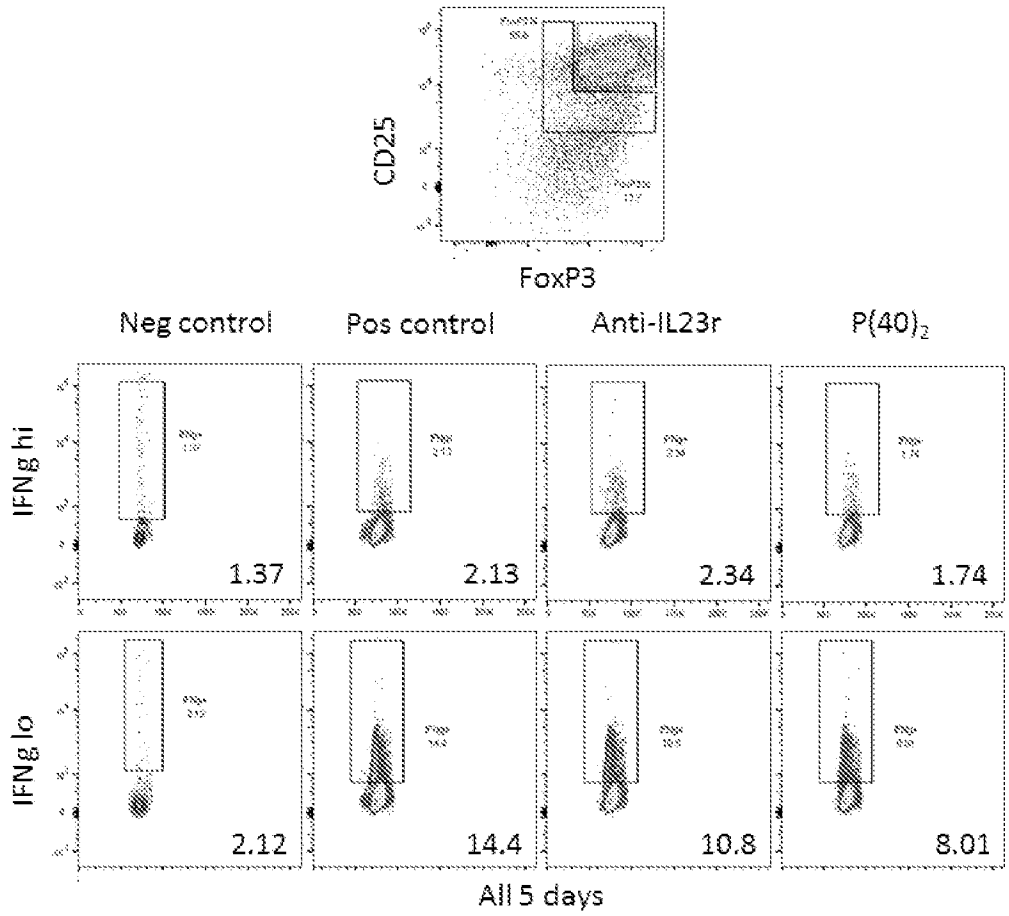

Neg control
Pos control
Anti-IL23r
P(40)2
24 hrs
CD25
All 5 days
Last 24 hrs
FoxP3
pos
anti-IL23r
p(40)2
Treg fold change from baseline
Fold Change FoxP3/CD25 expression
0.2
0.0
-0.2
-0.4
-0.6
Antibody 0 0 0 0 96 0 0 96
Addition time (hrs)
Culture time 1 5 1 5 5 1 5 5
(days)

FIG. 22


CD25
FoxP3
Neg control
Pos control
Anti-IL23r
P(40)2
IFNg hi
IFNg lo
1.37
2.13
2.34
1.74
2.12
14.4
10.8
8.01
All 5 days

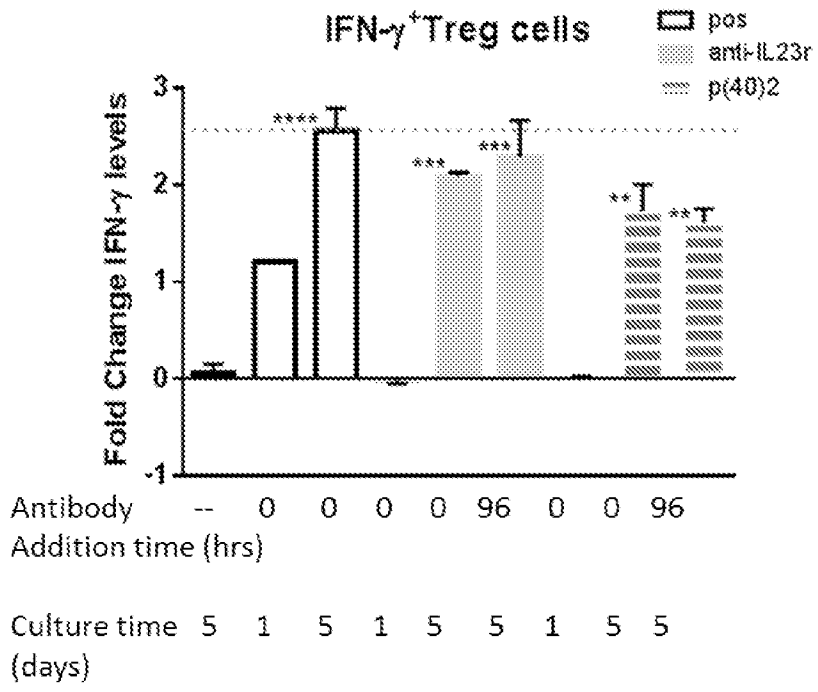

IFN-γ+Treg cells
pos
anti-IL23r
p(40)2
Fold Change IFN-γ levels
3
2
1
0
-1
****
***
***
**
**
Antibody -- 0 0 0 0 96 0 0 96
Addition time (hrs)
Culture time 5 1 5 1 5 5 1 5 5
(days)

FIG. 23

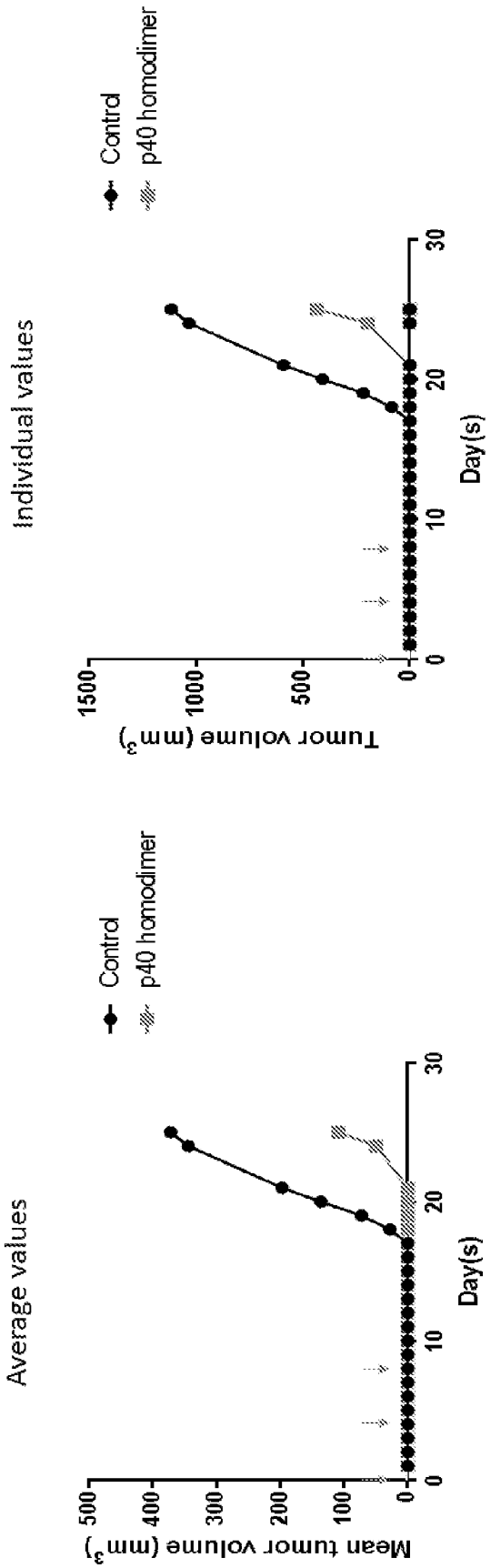

Individual values
Tumor volume (mm³)
1500
1000
500
0
0
10
20
30
Day(s)
Control
p40 homodimer
Average values
Mean tumor volume (mm³)
500
400
300
200
100
0
0
10
20
30
Day(s)
Control
p40 homodimer

FIG. 24

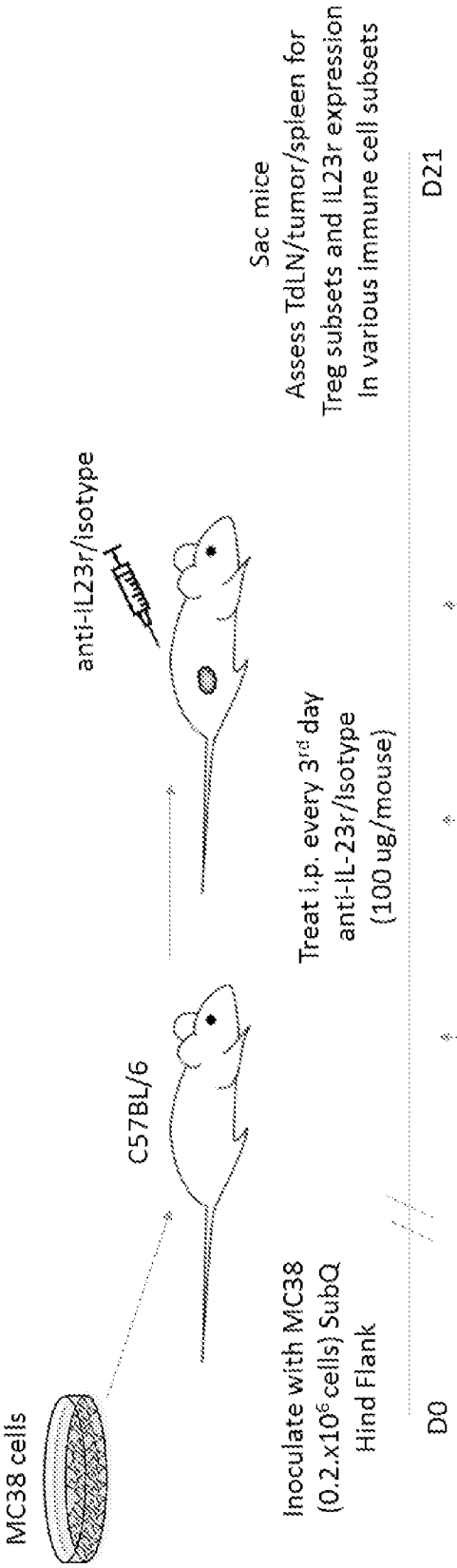

MC38 cells
C57BL/6
anti-IL23r/isotype
Inoculate with MC38
(0.2.x10^6 cells) SubQ
Hind Flank
Treat i.p. every 3rd day
anti-IL-23r/isotype
(100 ug/mouse)
Sac mice
Assess TdLN/tumor/spleen for
Treg subsets and IL23r expression
In various immune cell subsets
D0
D8
D11
D14
D21

FIG. 25A

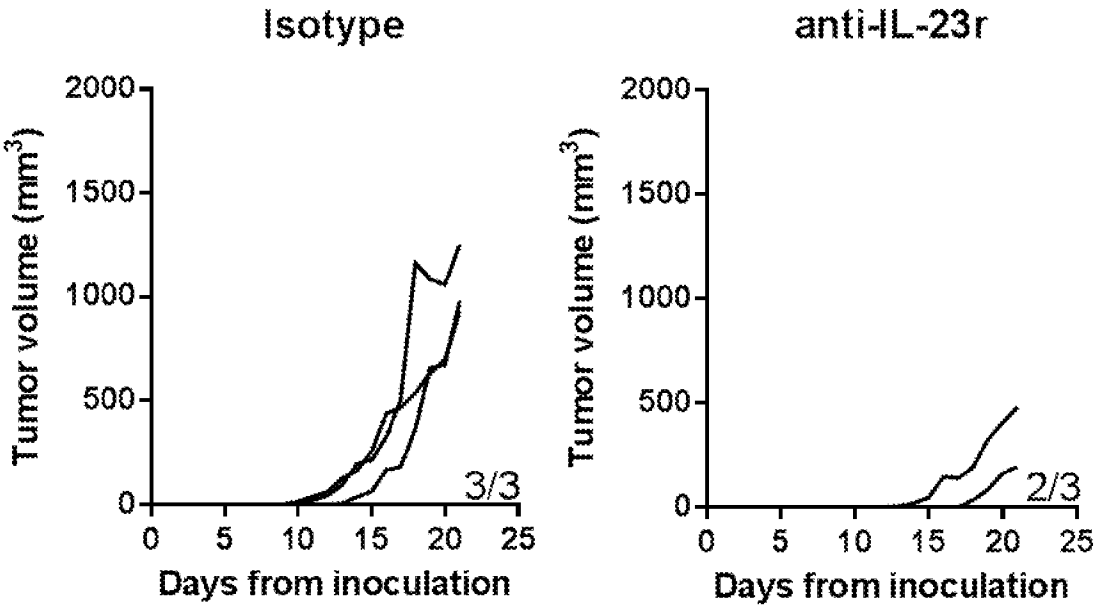


FIG. 25B

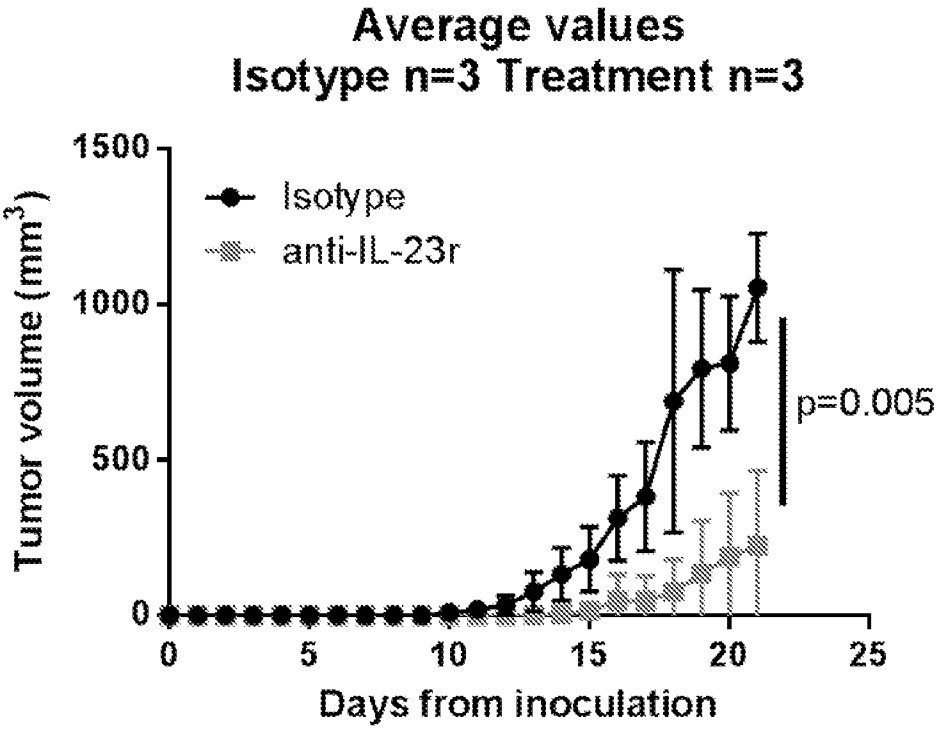


FIG. 25C

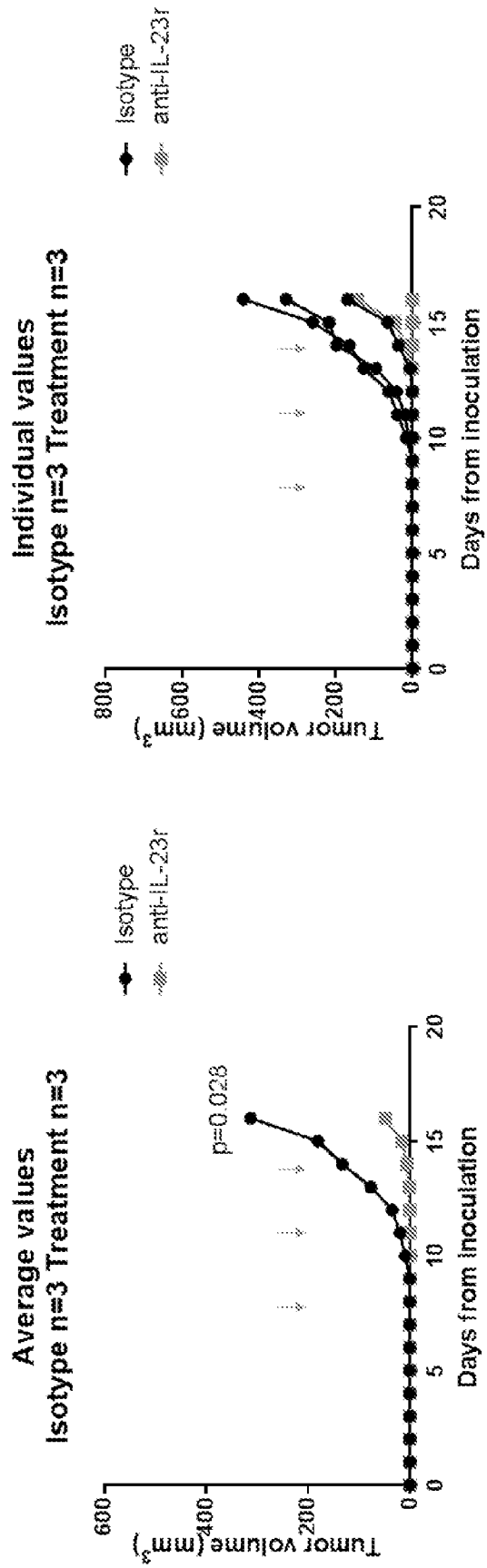

Individual values
Isotype n=3 Treatment n=3
Isotype
anti-IL-23r
Tumor volume (mm³)
800
600
400
200
0
0
5
10
15
20
Days from inoculation
Average values
Isotype n=3 Treatment n=3
p=0.028
Isotype
anti-IL-23r
Tumor volume (mm³)
600
400
200
0
0
5
10
15
20
Days from inoculation

FIG. 25D

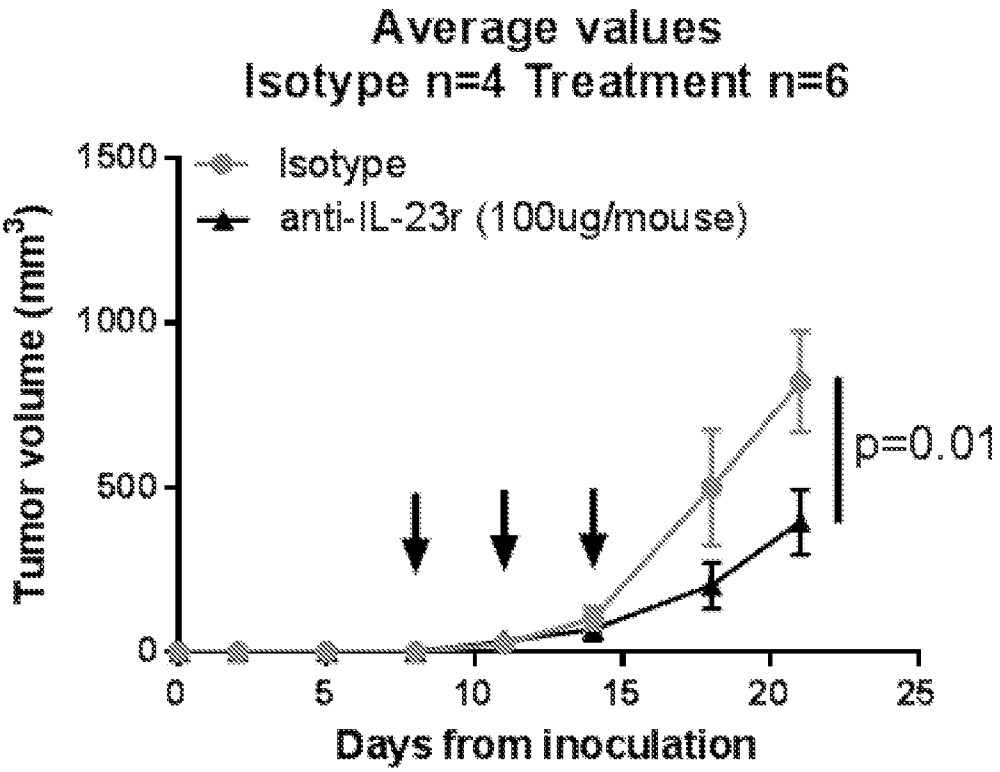

Average values
Isotype n=4 Treatment n=6
Isotype
anti-IL-23r (100ug/mouse)
Tumor volume (mm3)
1500
1000
500
0
0
5
10
15
20
25
Days from inoculation
p=0.01

FIG. 25E

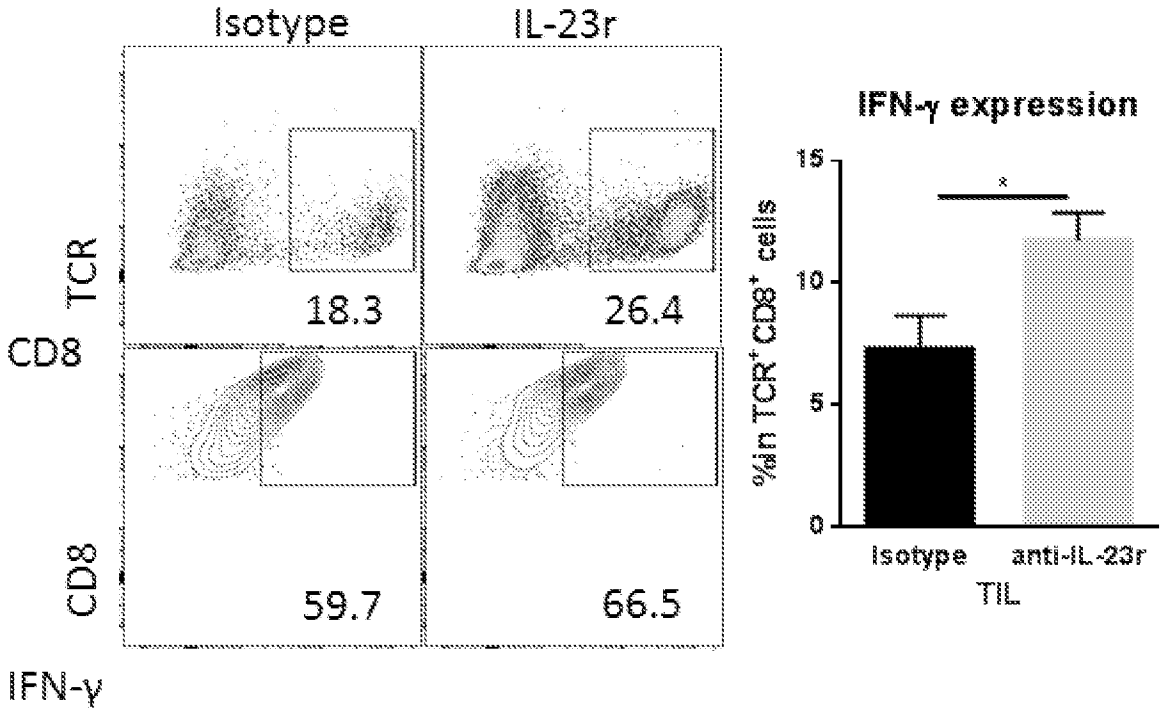


FIG. 26A

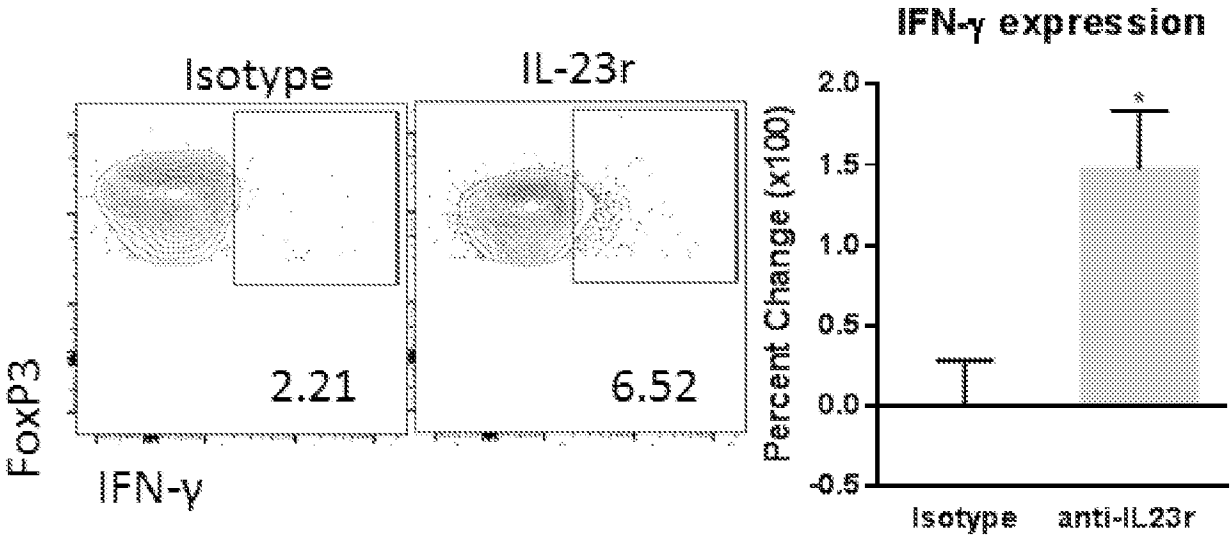


FIG. 26B

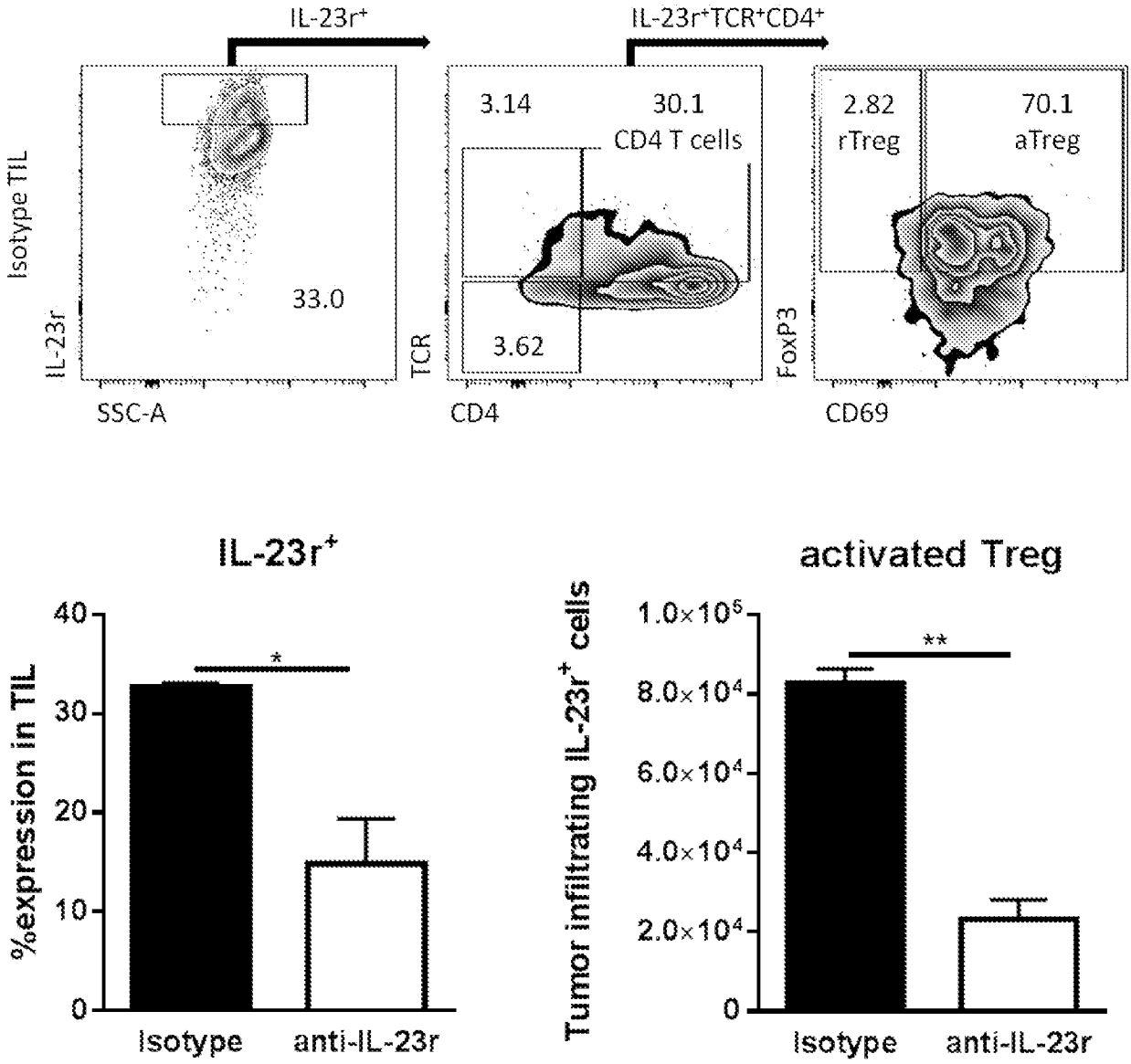
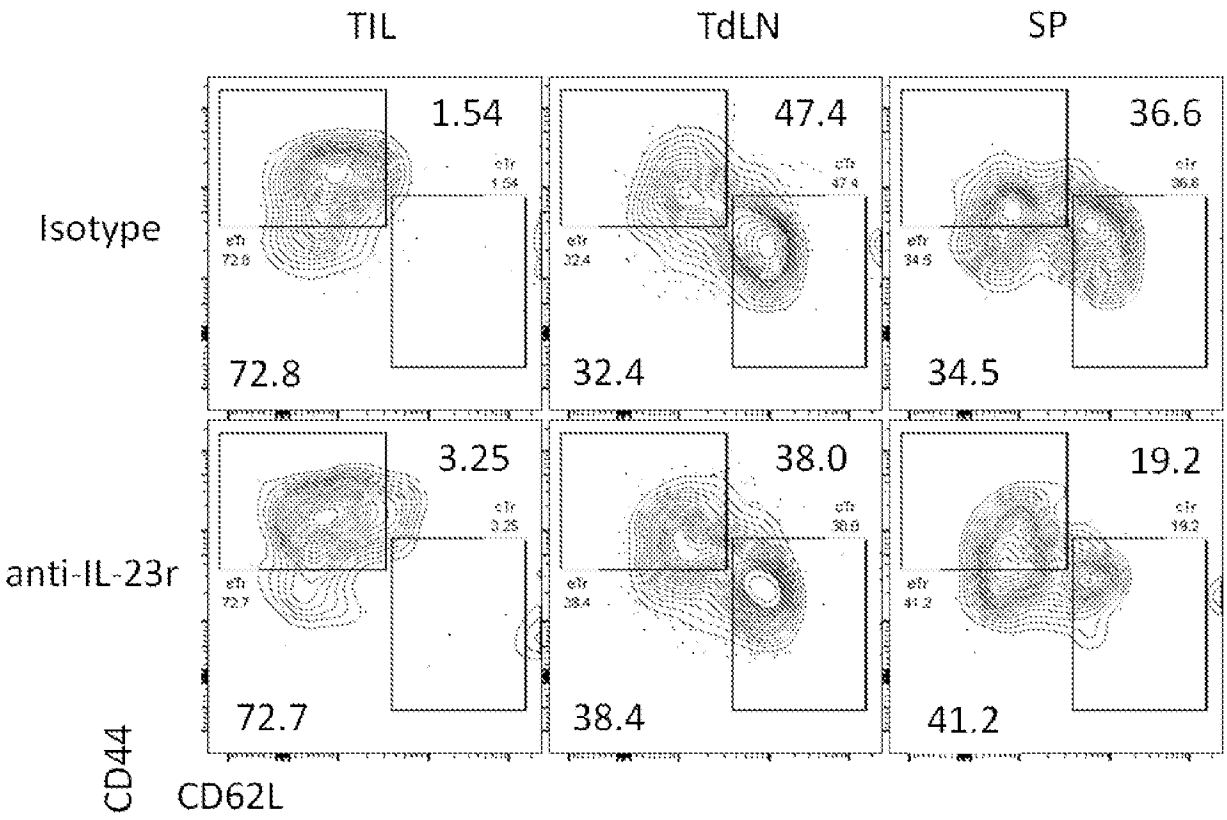

IL-23r+
IL-23r+TCR+CD4+
Isotype TIL
IL-23r
SSC-A
33.0
TCR
CD4
3.14
30.1
CD4 T cells
3.62
FoxP3
CD69
2.82
rTreg
70.1
aTreg
IL-23r+
%expression in TIL
40
30
20
10
0
*
Isotype
anti-IL-23r
activated Treg
Tumor infiltrating IL-23r+ cells
1.0×10^5
8.0×10^4
6.0×10^4
4.0×10^4
2.0×10^4
0
**
Isotype
anti-IL-23r

FIG. 27


TIL
TdLN
SP
Isotype
anti-IL-23r
1.54
47.4
36.6
72.8
32.4
34.5
3.25
38.0
19.2
72.7
38.4
41.2
CD44
CD62L

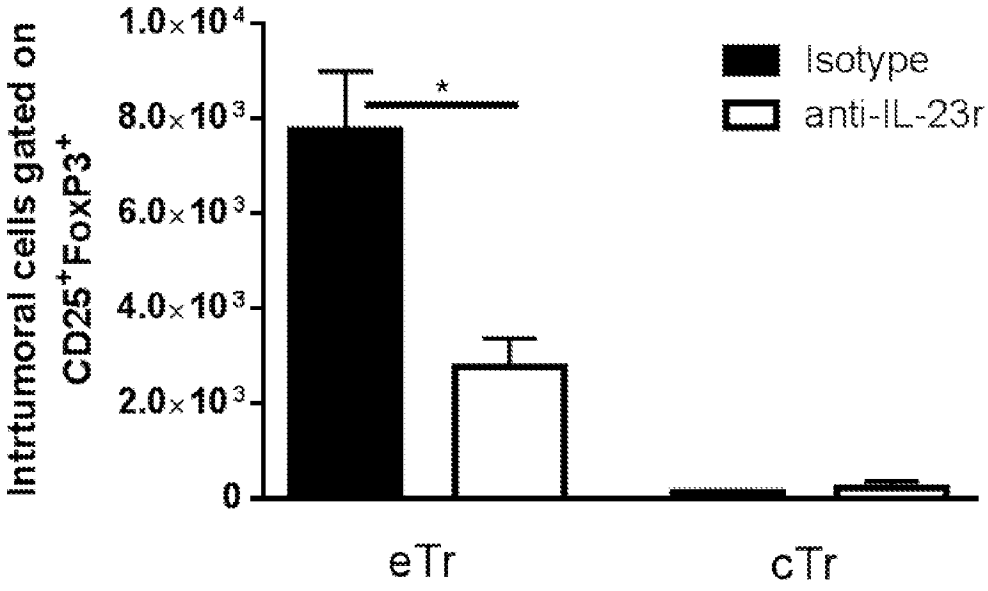

Intrtumoral cells gated on CD25+FoxP3+
1.0×10^4
8.0×10^3
6.0×10^3
4.0×10^3
2.0×10^3
0
*
Isotype
anti-IL-23r
eTr
cTr

FIG. 28

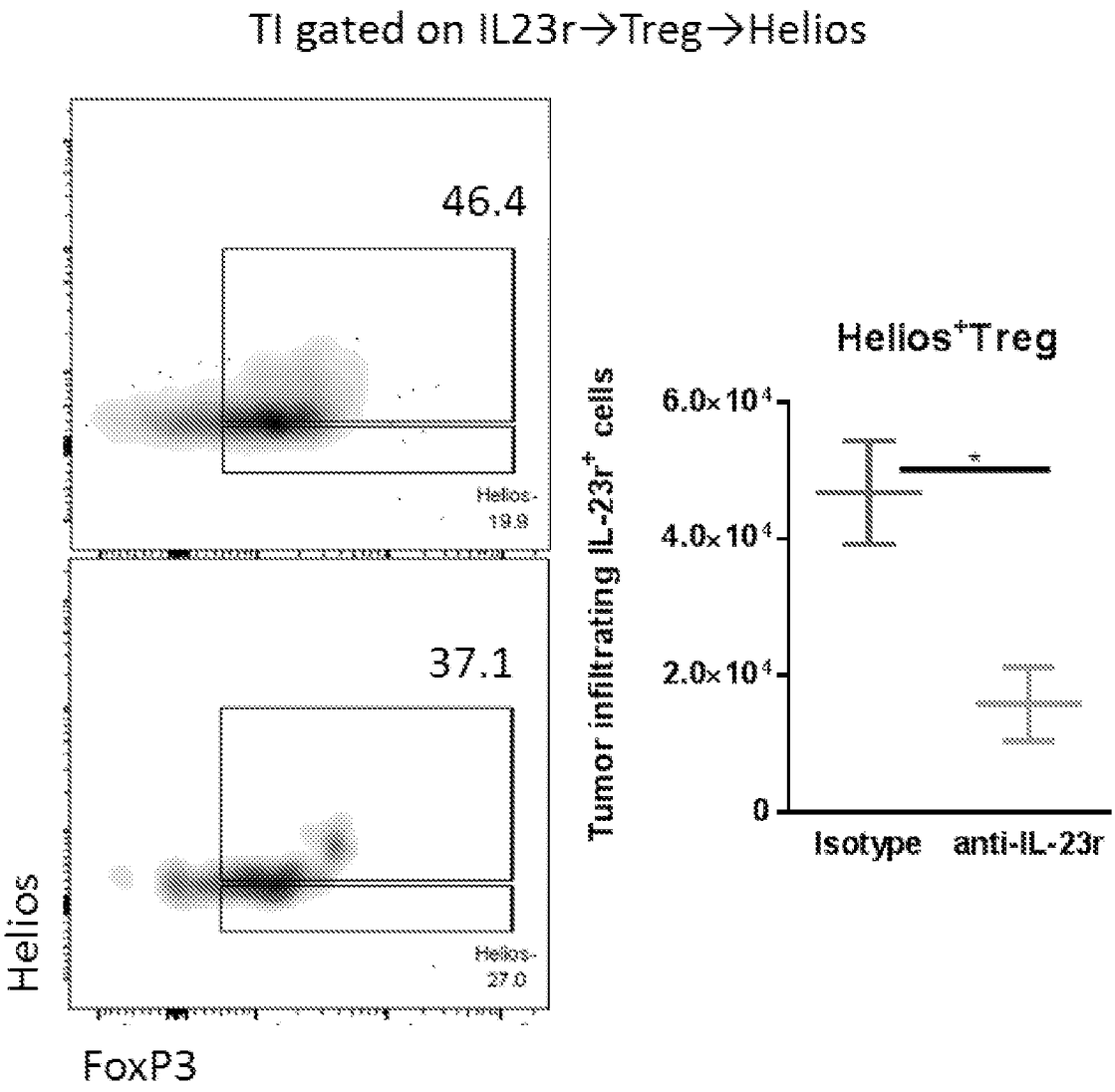
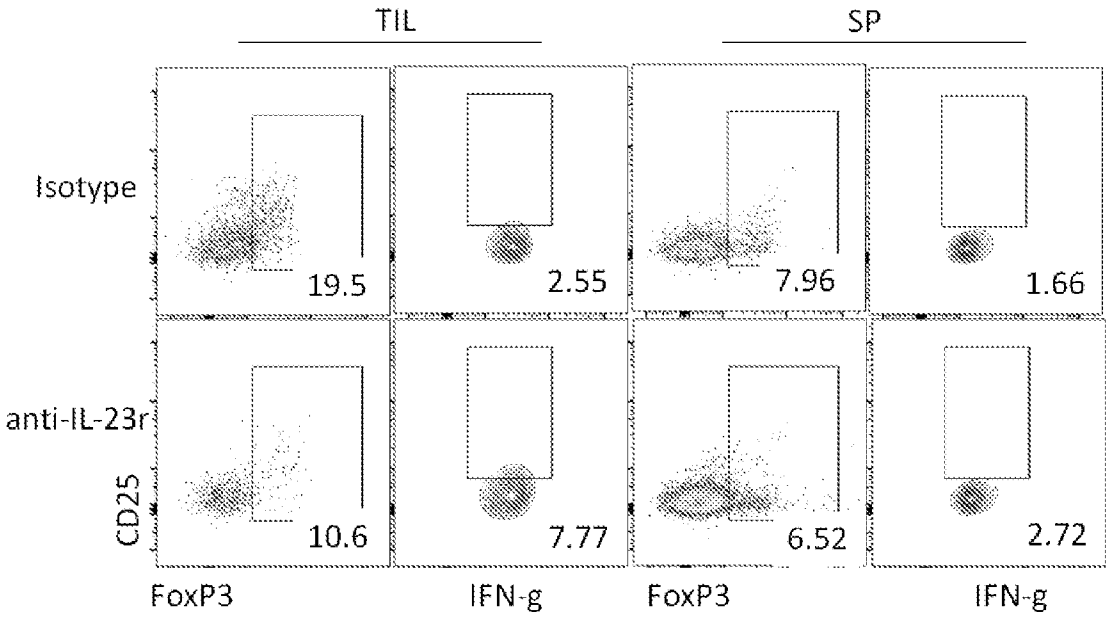

TI gated on IL23r→Treg→Helios
46.4
Helios-
19.9
37.1
Helios-
27.0
Helios
FoxP3
Helios+Treg
6.0x10^4
4.0x10^4
2.0x10^4
0
Tumor infiltrating IL-23r+ cells
Isotype
anti-IL-23r

FIG. 29


TIL
SP
Isotype
anti-IL-23r
CD25
19.5
2.55
7.96
1.66
10.6
7.77
6.52
2.72
FoxP3
IFN-g
FoxP3
IFN-g

TL

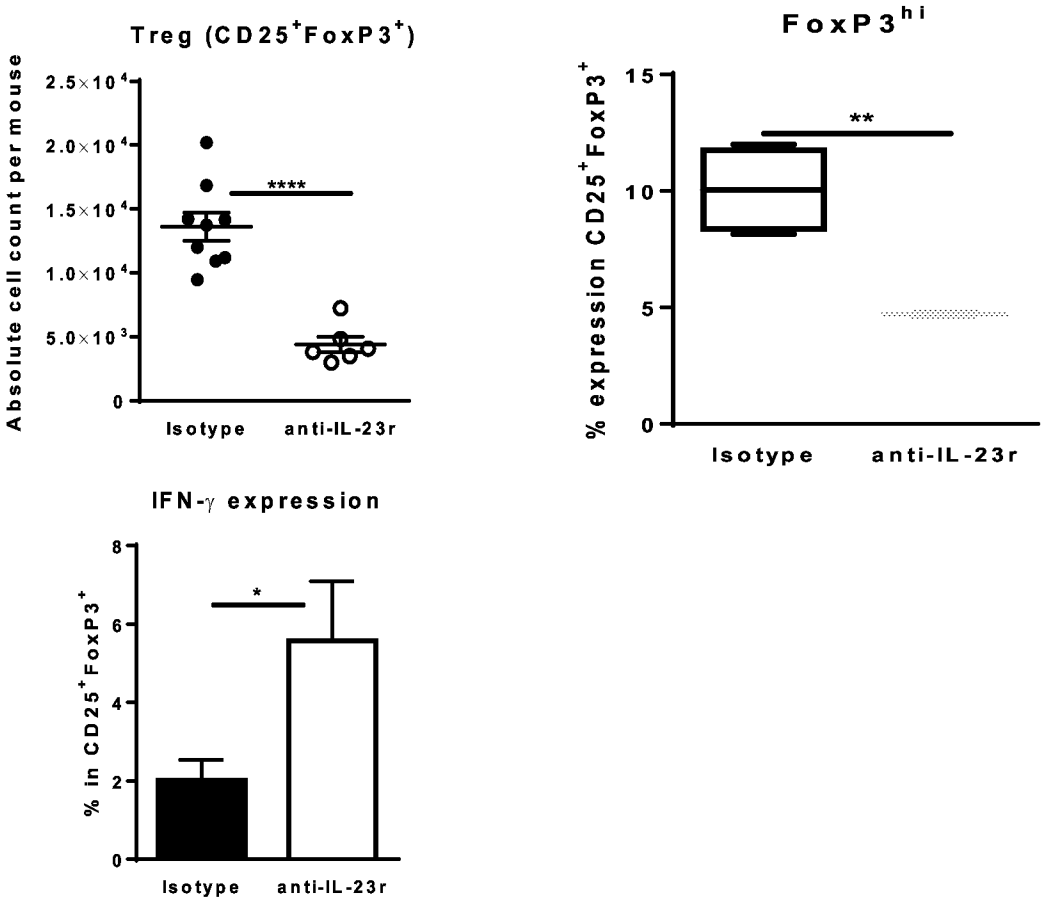

Treg (CD25+FoxP3+)
Absolute cell count per mouse
2.5×10^4
2.0×10^4
1.5×10^4
1.0×10^4
5.0×10^3
0
****
Isotype
anti-IL-23r
FoxP3hi
% expression CD25+FoxP3+
15
10
5
0
**
Isotype
anti-IL-23r
IFN-γ expression
% in CD25+FoxP3+
8
6
4
2
0
*
Isotype
anti-IL-23r

FIG. 30

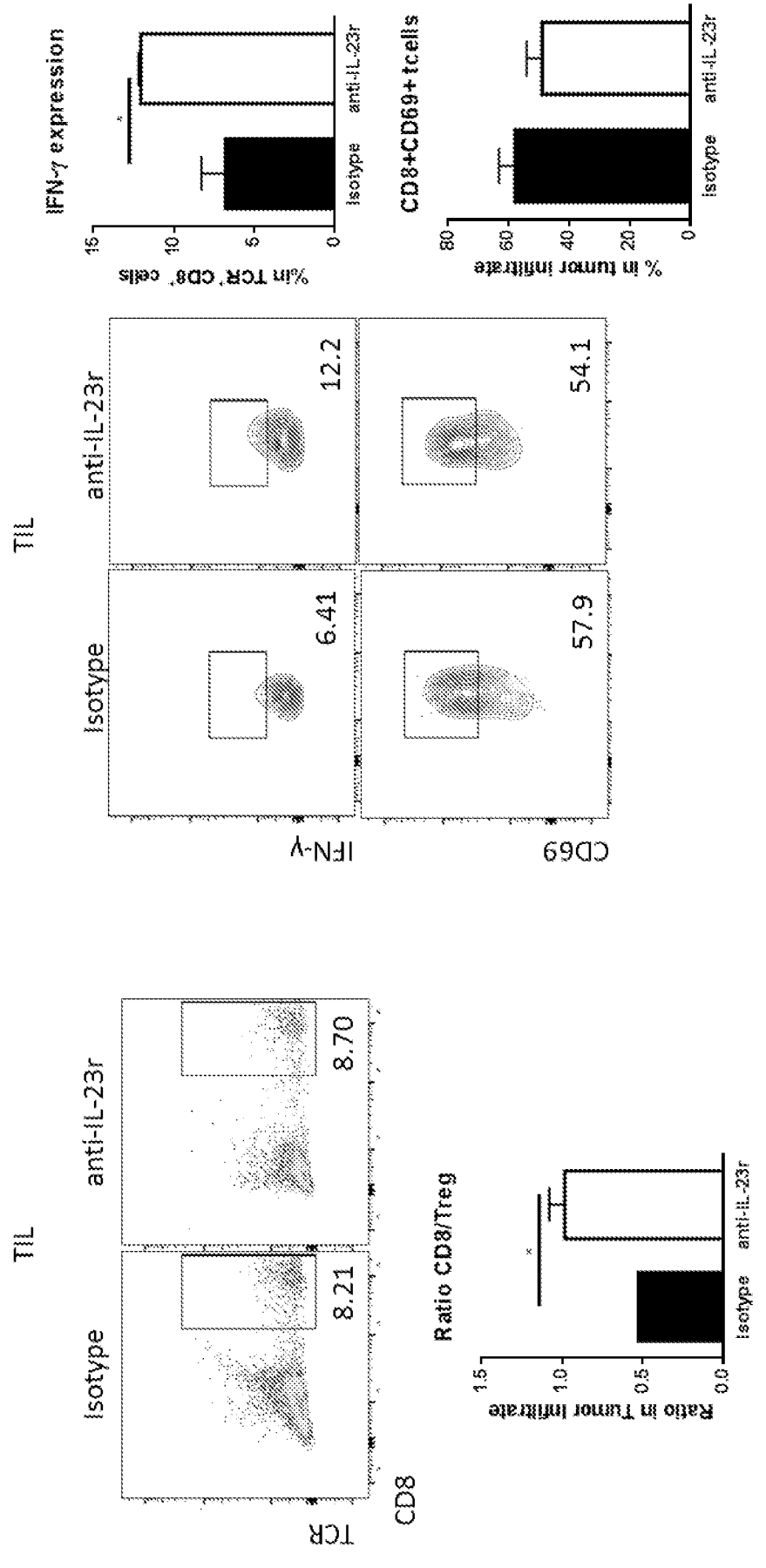

TIL
Isotype
anti-IL-23r
TCR
CD8
8.21
8.70
Ratio CD8/Treg
Ratio in Tumor Infiltrate
1.5
1.0
0.5
0.0
Isotype
anti-IL-23r
TIL
Isotype
anti-IL-23r
IFN-γ
CD69
6.41
12.2
57.9
54.1
IFN-γ expression
%in TCR+CD8+ cells
15
10
5
0
Isotype
anti-IL-23r
CD8+CD69+ tcells
% in tumor infiltrate
80
60
40
20
0
Isotype
anti-IL-23r

FIG. 31

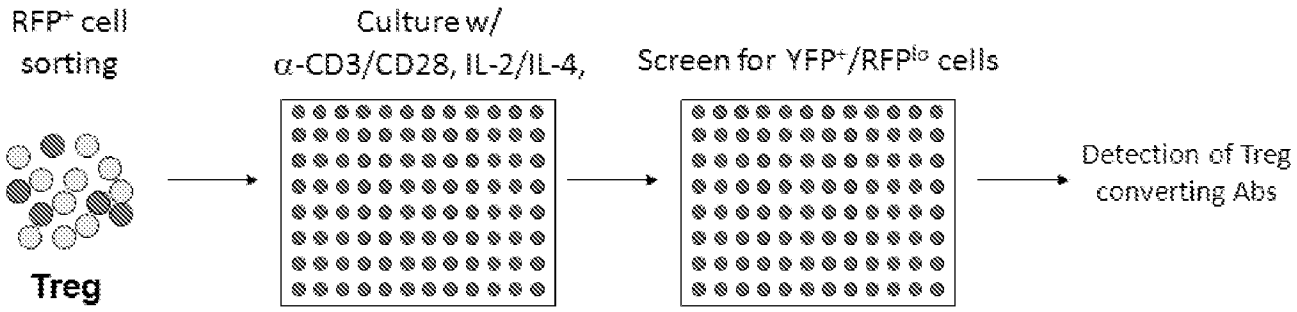

RFP+ cell sorting
Treg
Culture w/ α-CD3/CD28, IL-2/IL-4,
Screen for YFP+/RFPlo cells
Detection of Treg converting Abs

FIG. 32

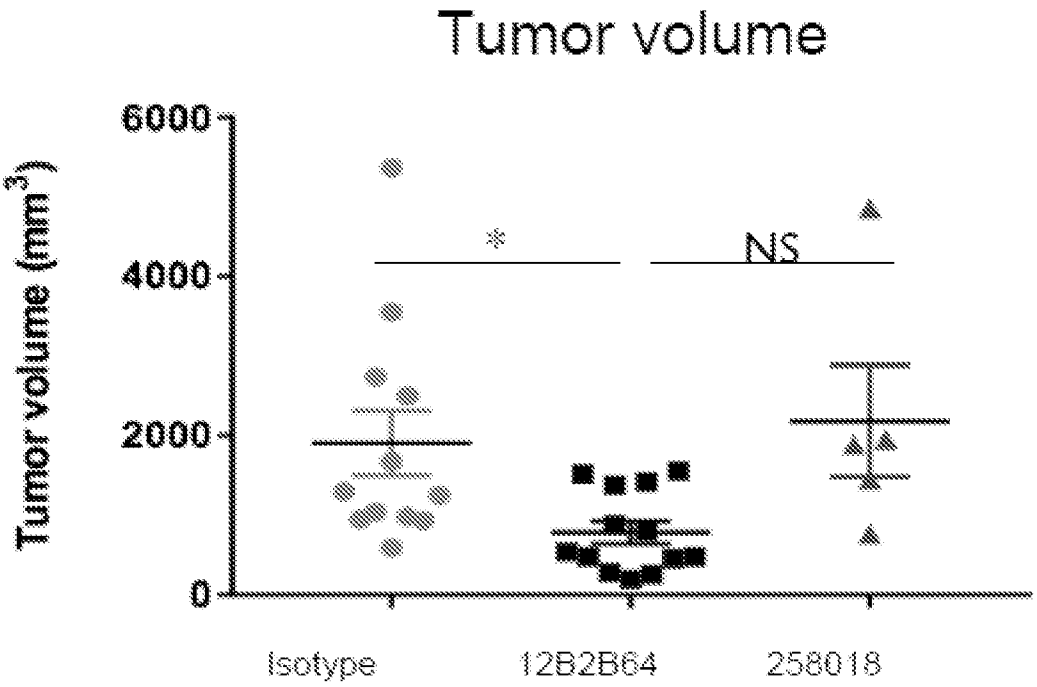

Tumor volume
Tumor volume (mm3)
6000
4000
2000
0
*
NS
Isotype
12B2B64
258018

FIG. 33

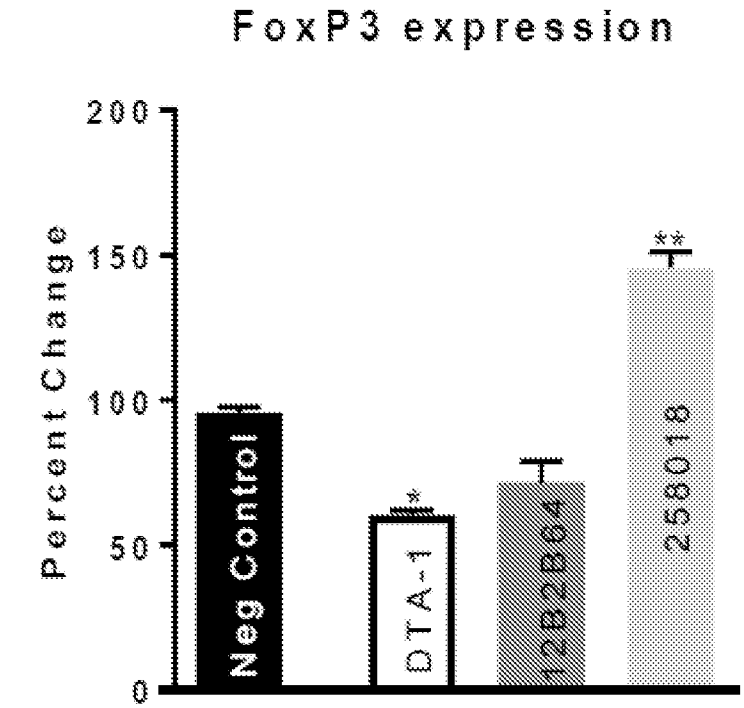


FIG. 34A

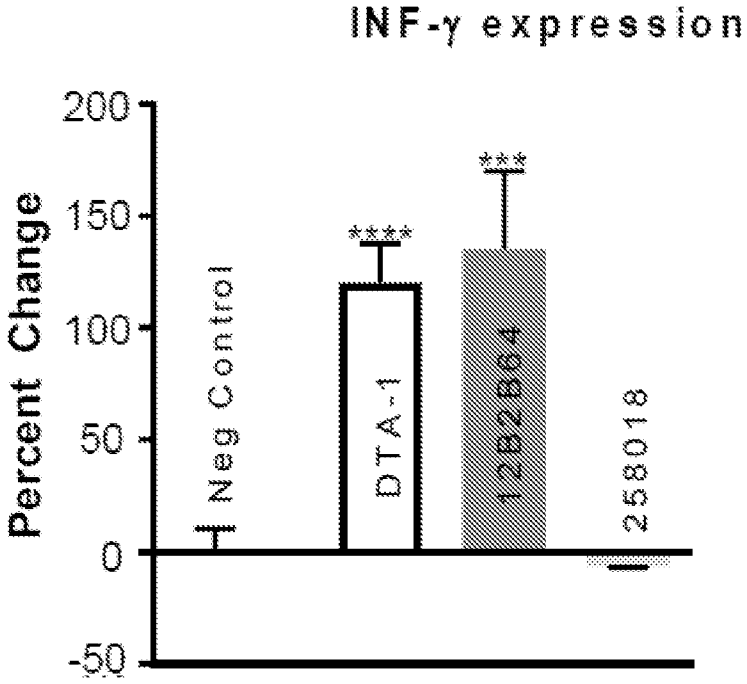


FIG. 34B

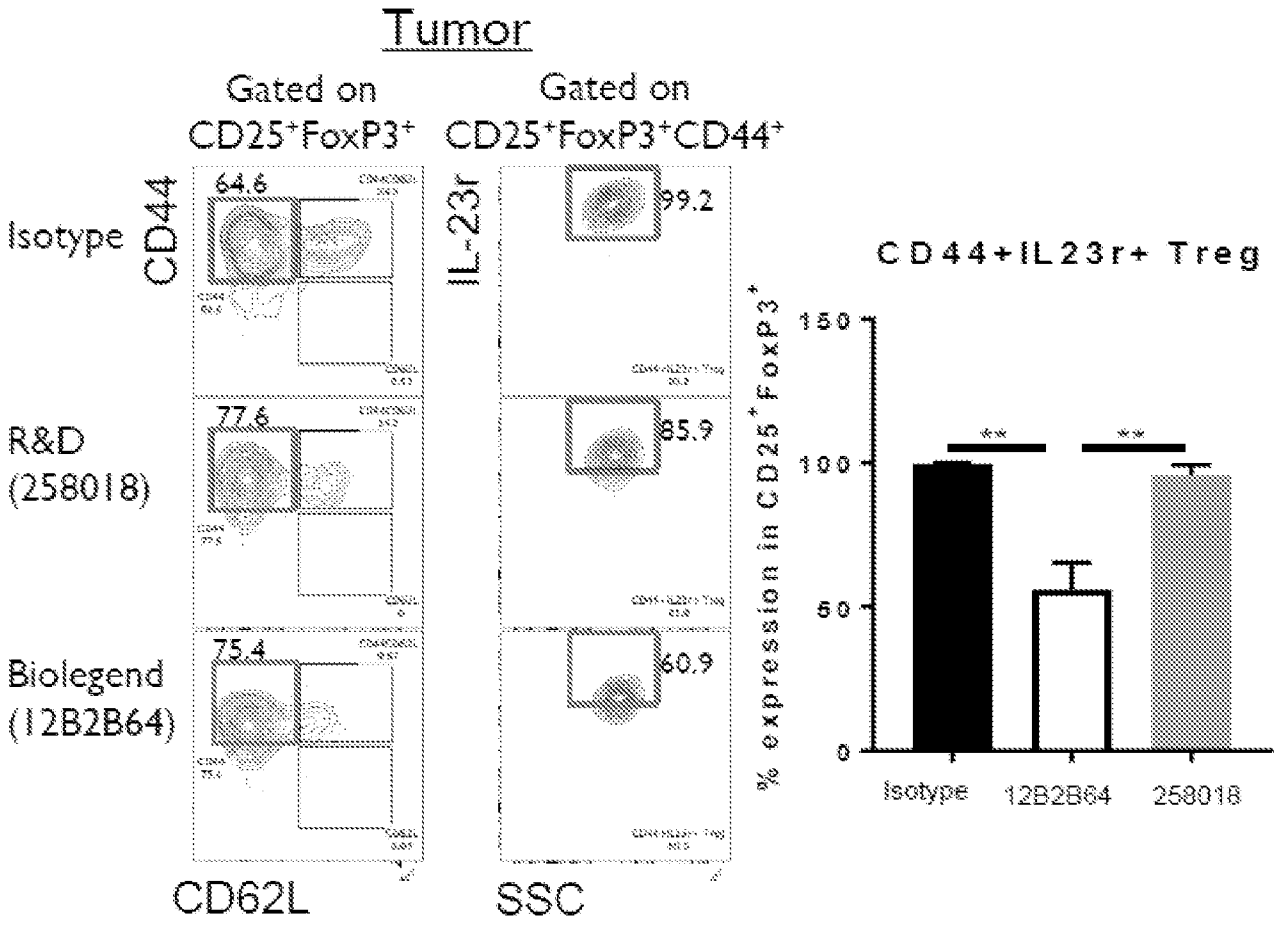
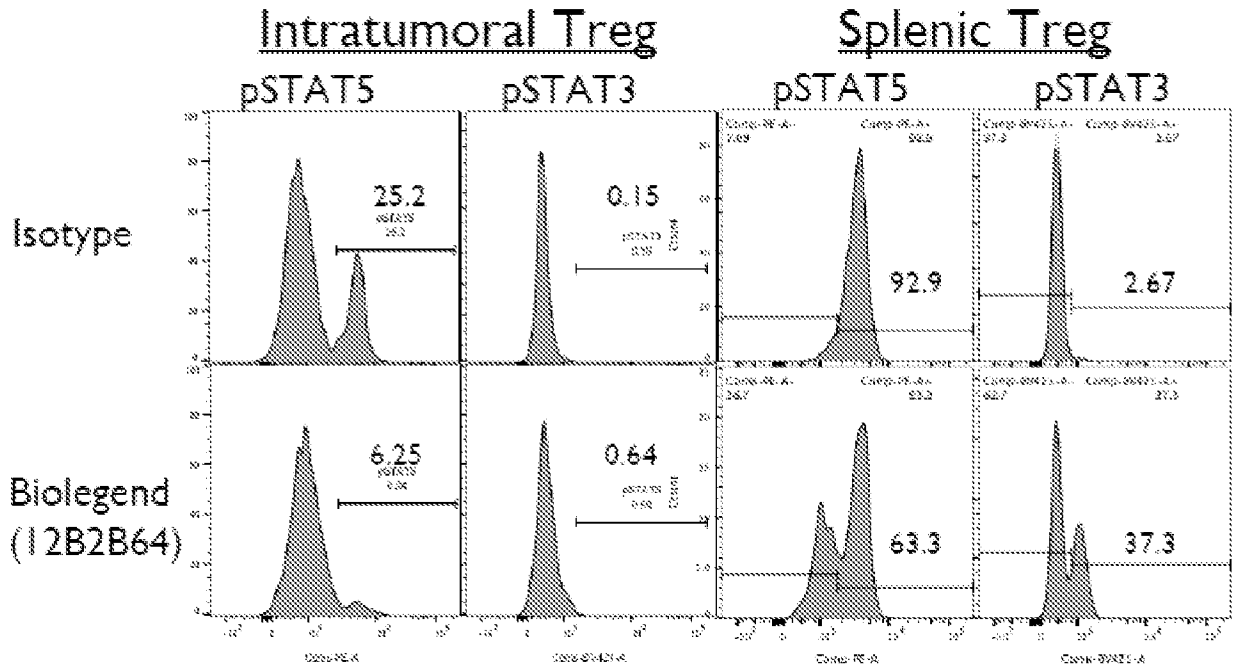

Tumor
Gated on CD25+FoxP3+
Gated on CD25+FoxP3+CD44+
Isotype
R&D (258018)
Biolegend (12B2B64)
CD44
CD62L
IL-23r
SSC
64.6
77.6
75.4
99.2
85.9
60.9
CD44+IL23r+ Treg
% expression in CD25+FoxP3+
150
100
50
0
**
**
Isotype
12B2B64
258018

FIG. 35



pSTAT5/pSTAT3 Ratio

	Tumor	Spleen
Isotype	168	34.8
Clone 12B2B64	9.8	1.7

FIG. 36

m IL-23r vs h IL-23r homology schematic

m IL-23r GITNINCSGHI WVEPATIFK MGMNI SIYCGAAIKNCQPRKLHFYKNGI SEQ ID NO: 7
h IL-23r GITSINCSGDMWVEPGEIFQMGMNVSIYCGEALKHCRPRNLYFYKNGE SEQ ID NO: 8

m IL-23r KERFQITRINKTTARLWYKNFLEPHASMYCTAECPKHFQETLICGKDISSGYPP SEQ ID NO: 9
h IL-23r KEEFDITRINETTARIWYKGFSEPHAYMHCTAECPGHFQETLICGKDISSGHPP SEQ ID NO: 10

m IL-23r DIPDEVTCVIYEYSGNMTCTWNAGKLTYIDTKYVVHVKSLETEEEQQYLTSSYINISTDSLQGGK
h IL-23r DAPSMLTCVIYEYSGNMTCTWNTGKETYIDTKYIVHVKSLETEEEQQYLASSYVKISTDSLQGGR
m IL-23r KYLVWVQAANALGMEESKQLQI HLDDIVIPSAAVISRAETINATVPKTI I YWDSQTTIEKVSC
h IL-23r KYLVWVQAVNSLGMENSQQLHVHLDDIVIPSASIISRAETTNDTVPKTIVYWKSKTMIEKVEC

Cytokine Binding Domain

SEQ ID NOS: 11-14

m IL-23r EMRYKATTNQTWNVKEFDTNFTYVQQSEFYLEPNI KYVFQVRCQETGKRYWQPWSSLF SEQ ID NO: 15
h IL-23r EMRYKTTTNQTWSVKEFDANFTYVQQSEFYLEPDSKYVFQVRCQETGKRNWQPWSSPF SEQ ID NO: 16

m IL-23r FHKTPETVPQVTSKAFQHDTWNSGLTVASISTGHLTSDNRGDKG SEQ ID NO: 17
h IL-23r VHQTSQETGKRNWQPWSSPFVHQTSQTVSQVTAKSSHEPQKMEMLSATIFRGHPASGNHQDKG SEQ ID NO: 18

Amino acids highlighted in yellow represent homology between human and mouse IL-23r

Representation of IL-23r cytokine binding domain

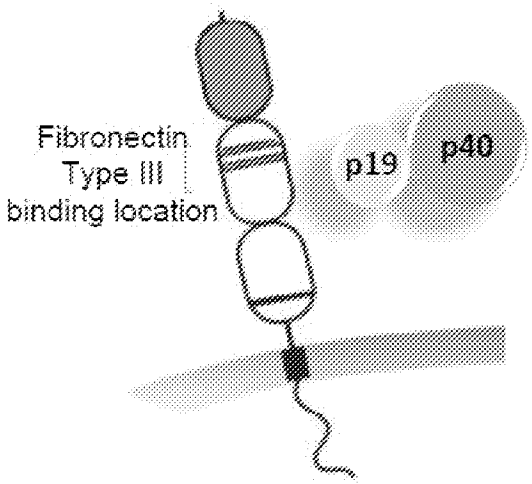
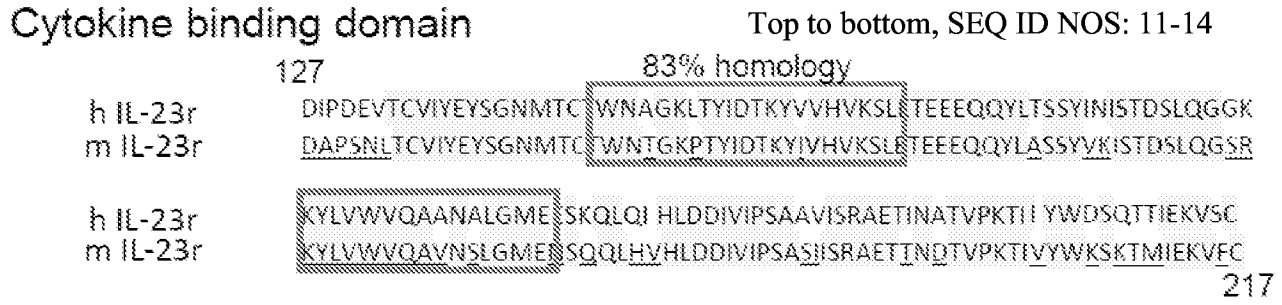


FIG. 37



IL-23r antibody clones	Clone 12B2B64	Clone 25R13
Peptide library matches	WNAGKLTYIDTKYVV	WNAGKLTYIDTKYVV
	LTYIDTKYVVHVKSL	LTYIDTKYVVHVKSL
	SEQ ID NOS: 3-4	KYLVWVQAANALGME *
		SEQ ID NOS: 3-5

Amino acids highlighted in yellow represent homology between human and mouse IL-23r

Peptide binding of IL-23r antibody

FIG. 38

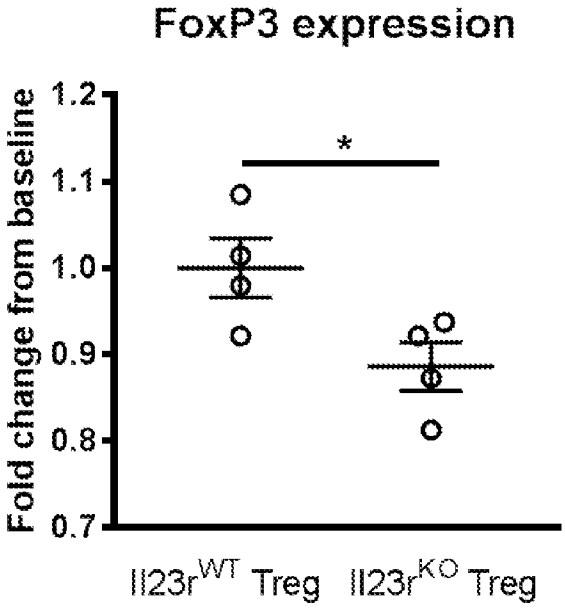


FIG. 39A

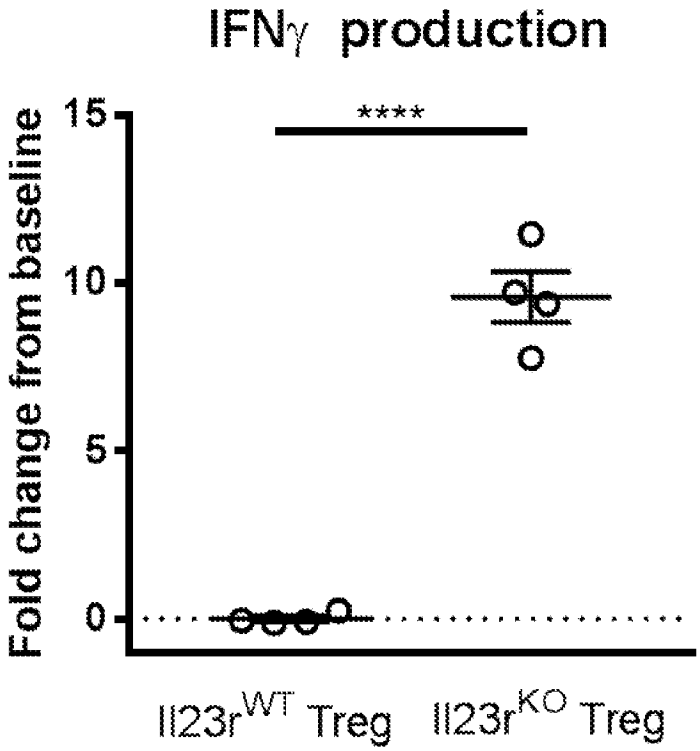


FIG. 39B

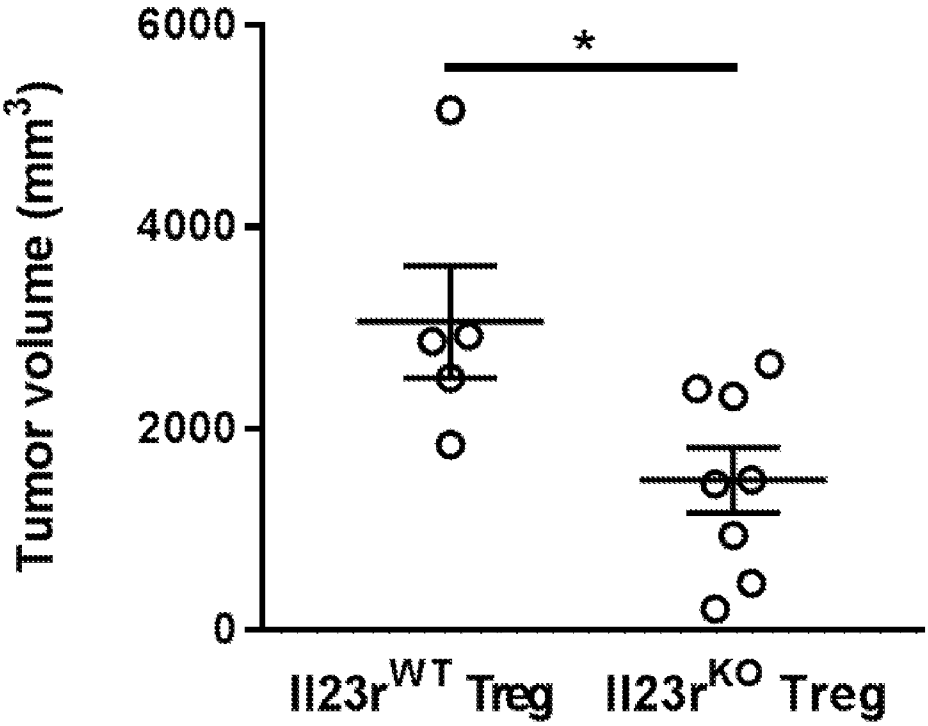


FIG. 40A

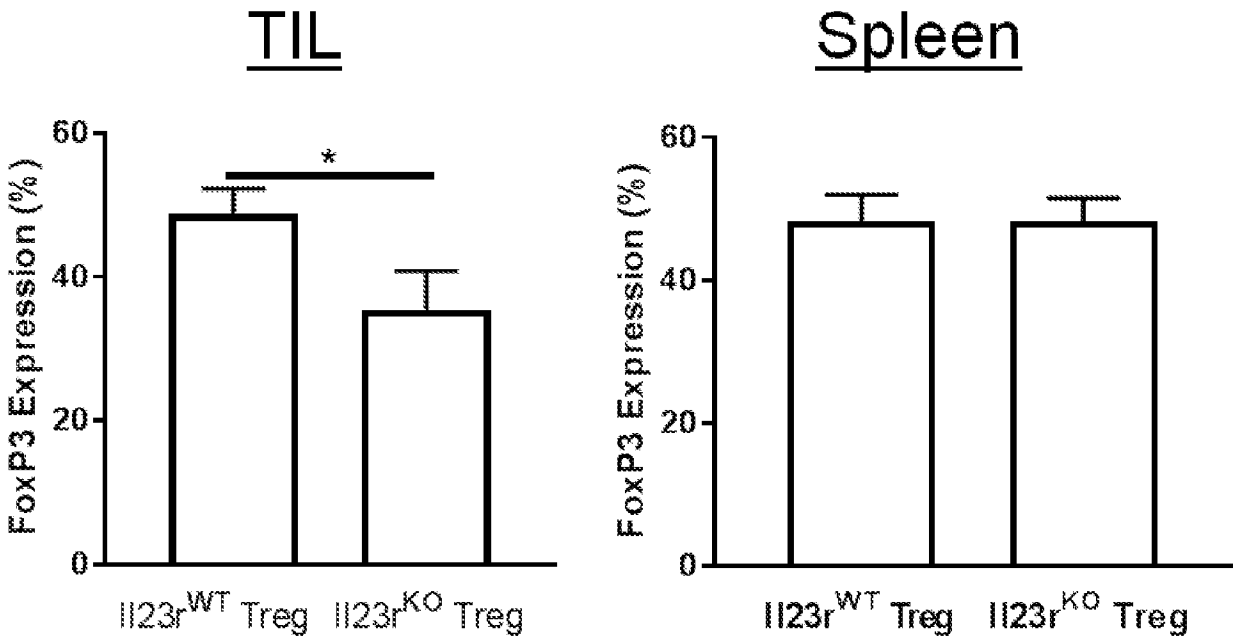


FIG. 40B

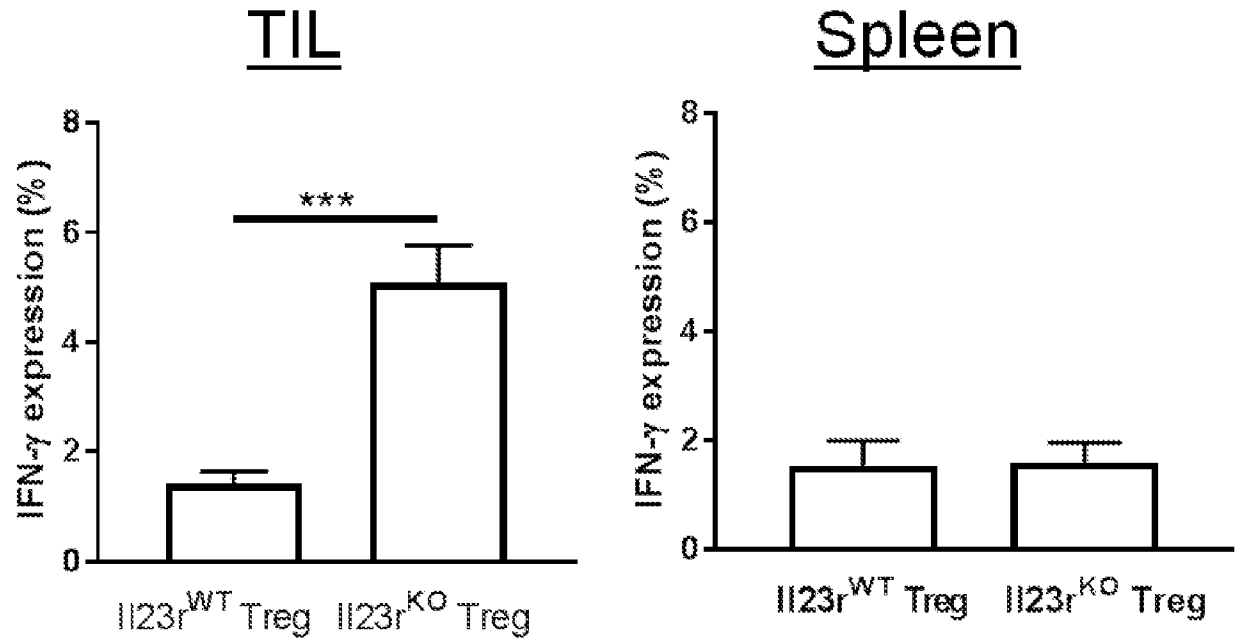


FIG. 40C

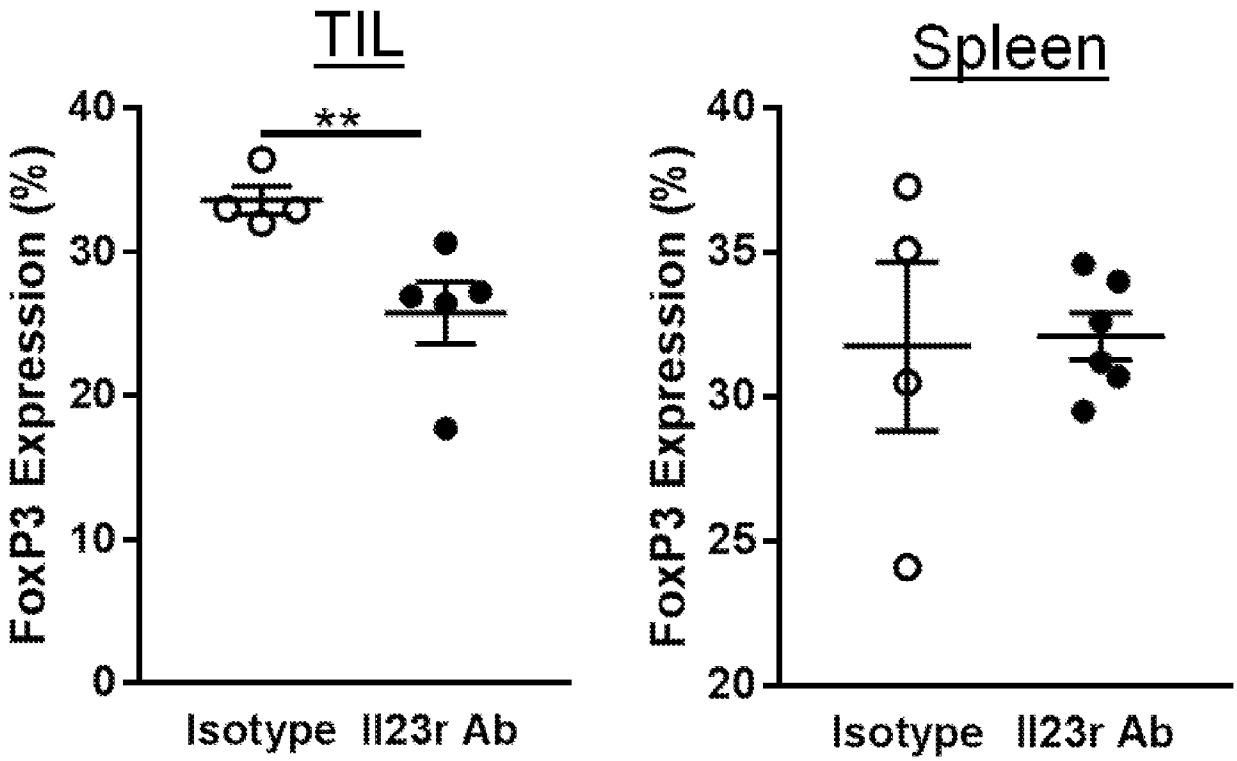


FIG. 41A

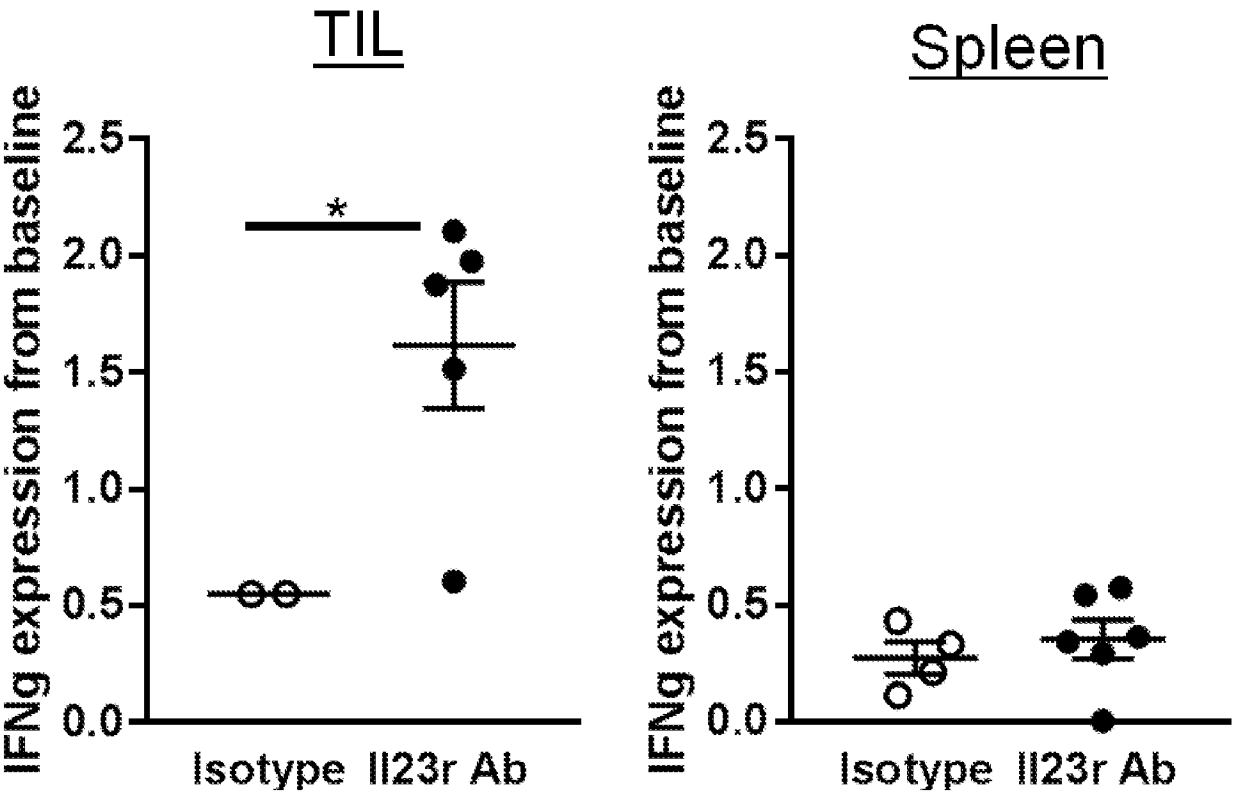


FIG. 41B

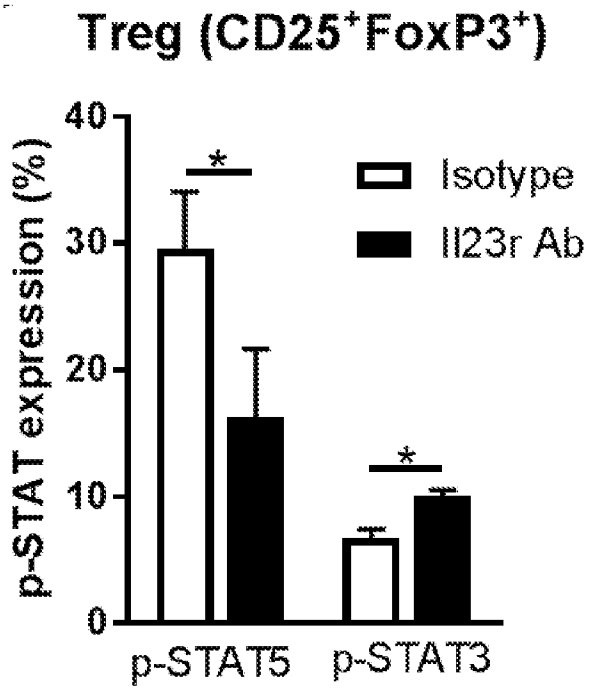


FIG. 42A

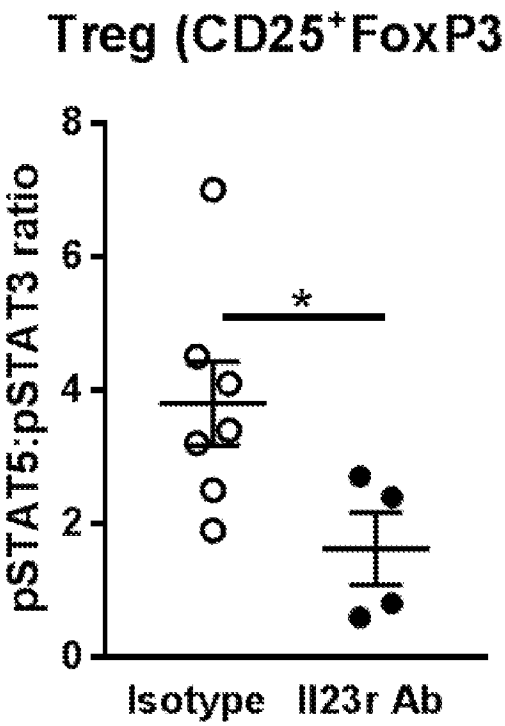


FIG. 42B

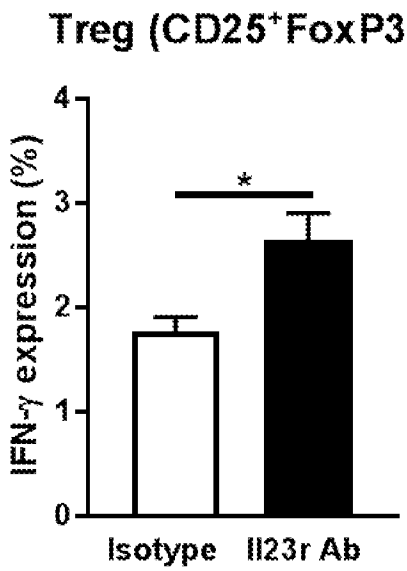


FIG. 42C

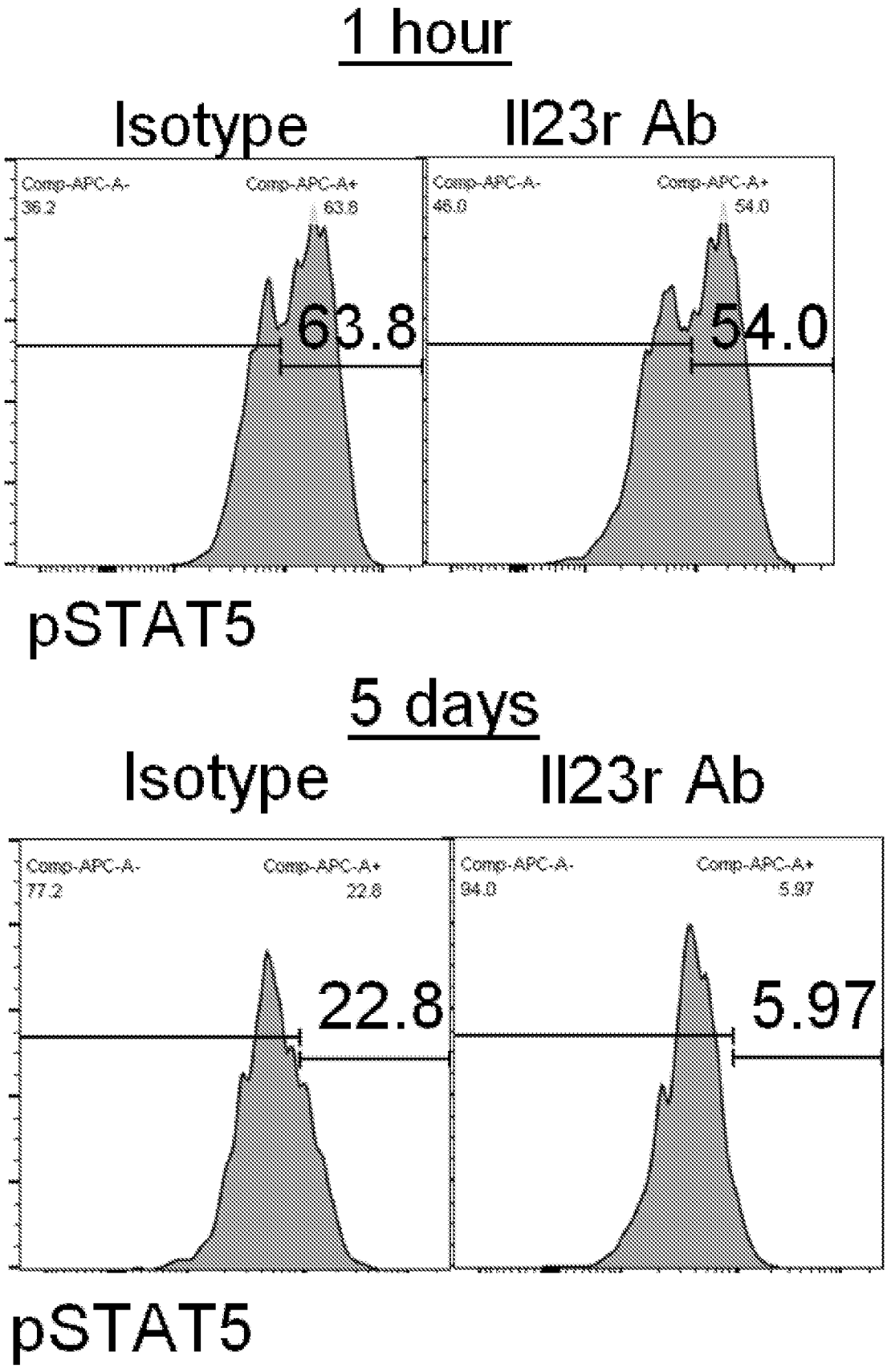

1 hour
Isotype
Il23r Ab
Comp-APC-A-
36.2
Comp-APC-A+
63.8
63.8
Comp-APC-A-
46.0
Comp-APC-A+
54.0
54.0
pSTAT5
5 days
Isotype
Il23r Ab
Comp-APC-A-
77.2
Comp-APC-A+
22.8
22.8
Comp-APC-A-
94.0
Comp-APC-A+
5.97
5.97
pSTAT5

FIG. 43A

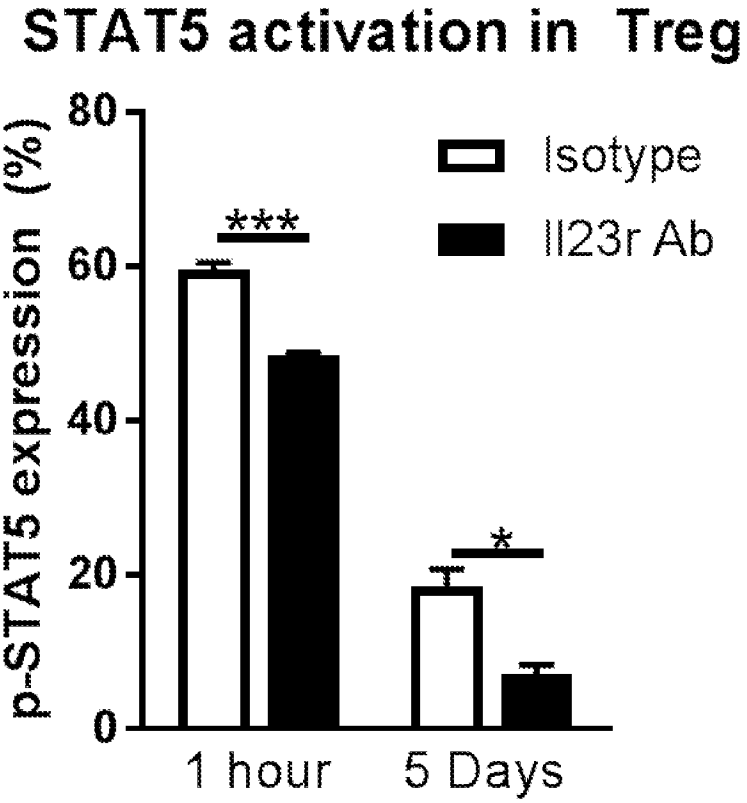


FIG. 43B

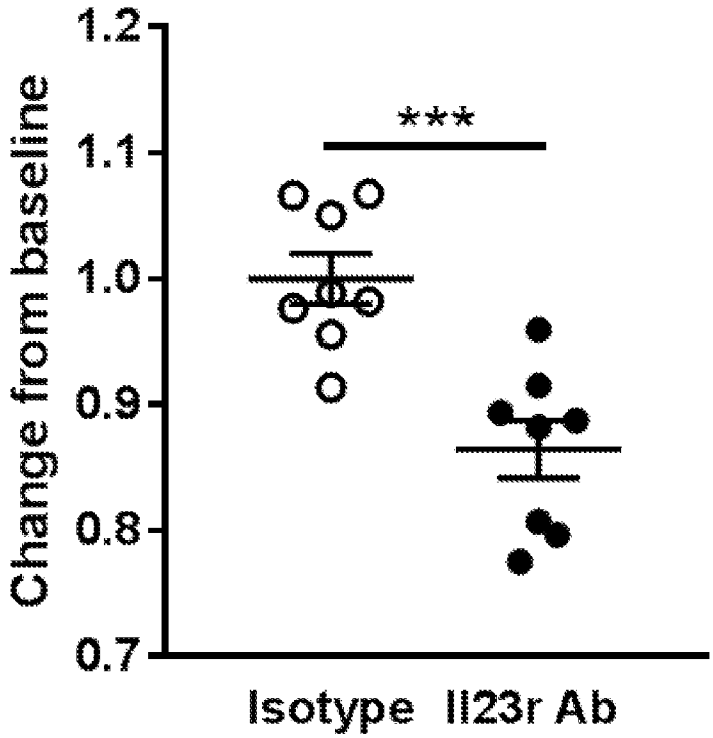


FIG. 44A

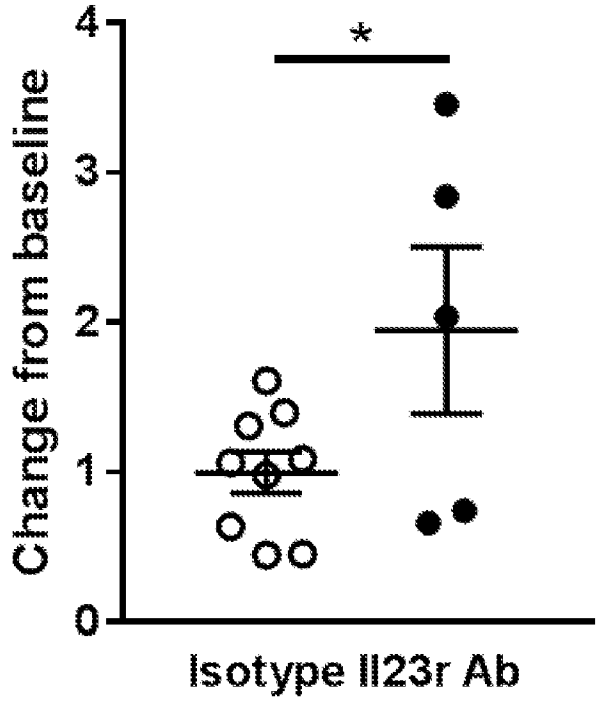


FIG. 44B

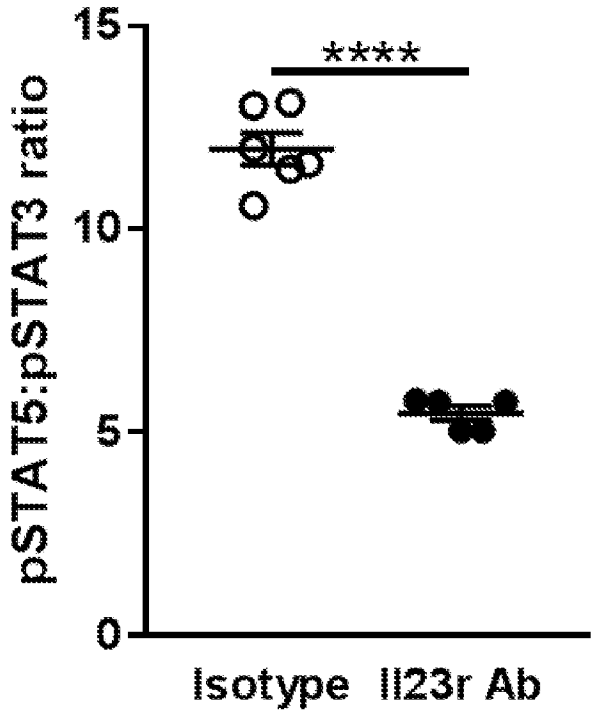


FIG. 44C

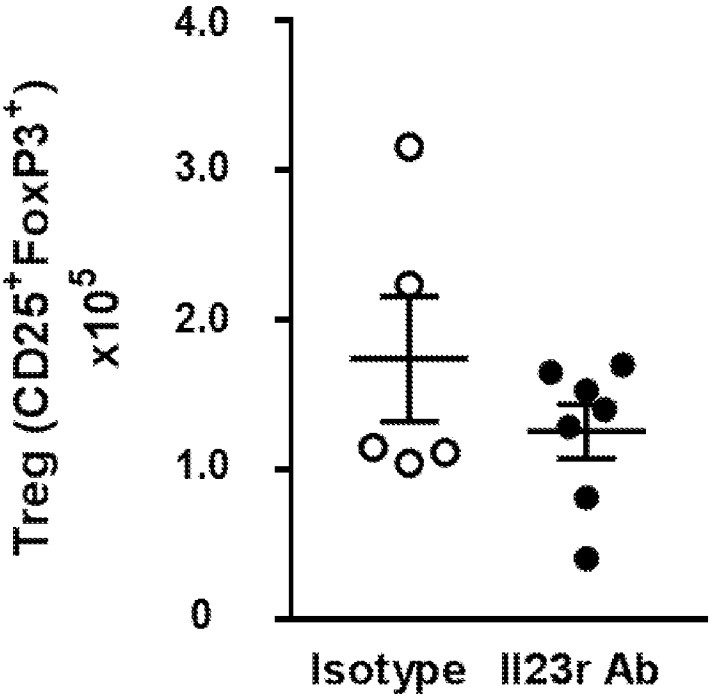


FIG. 44D

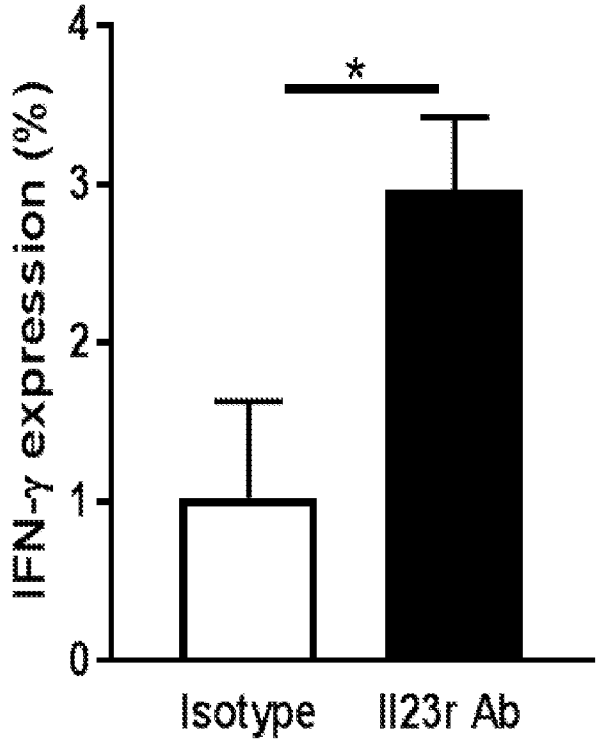


FIG. 44E

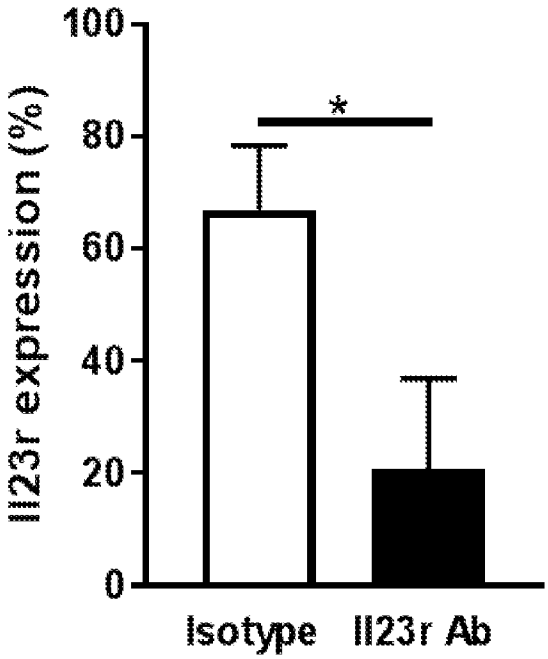


FIG. 44F

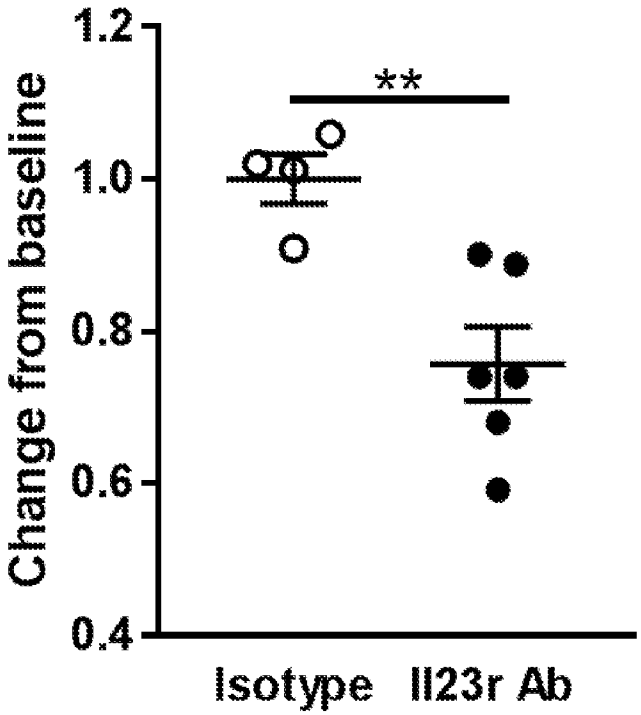


FIG. 45A

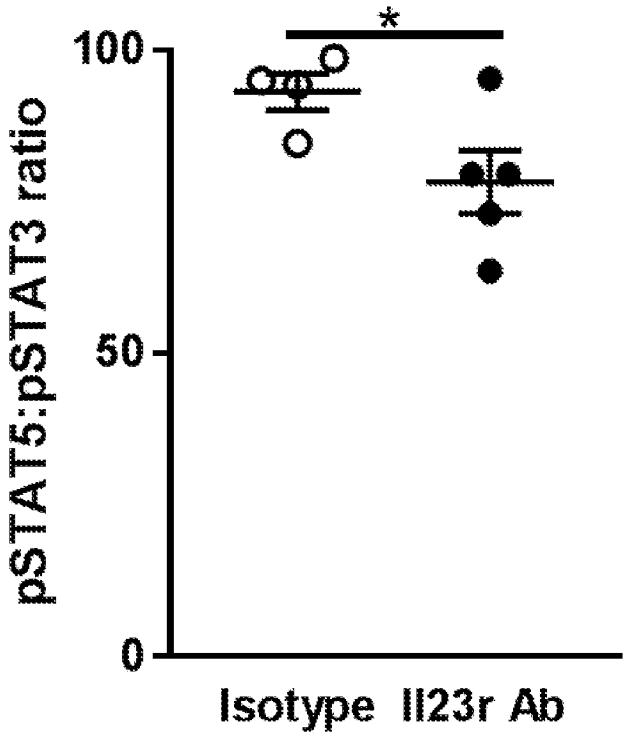


FIG. 45B

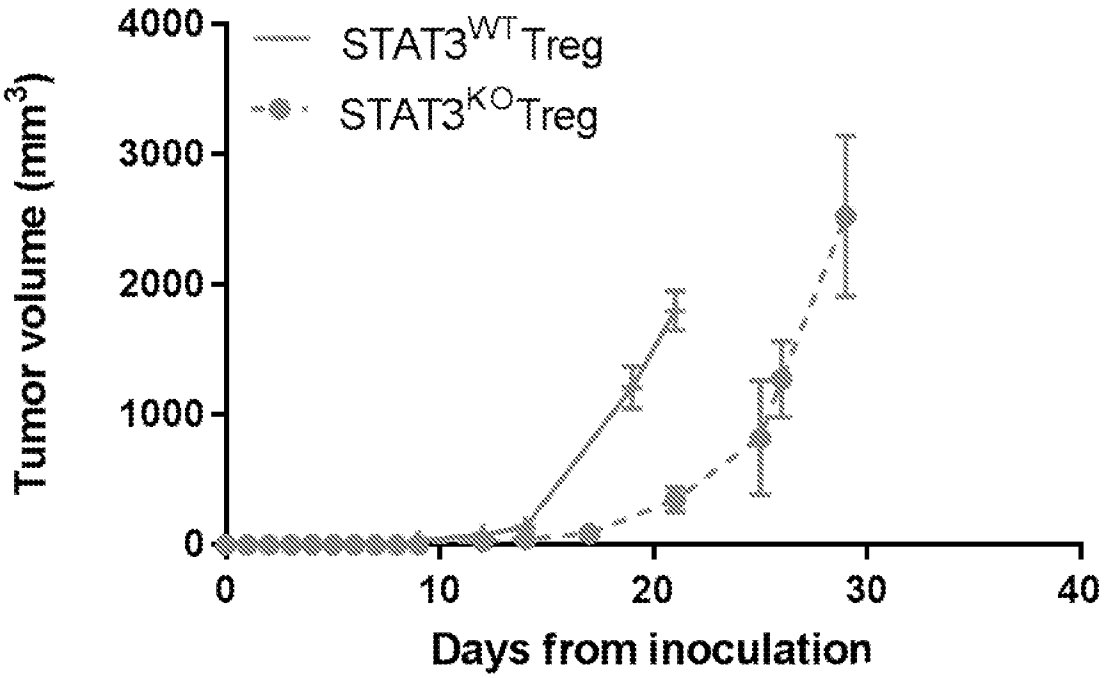


FIG. 46A

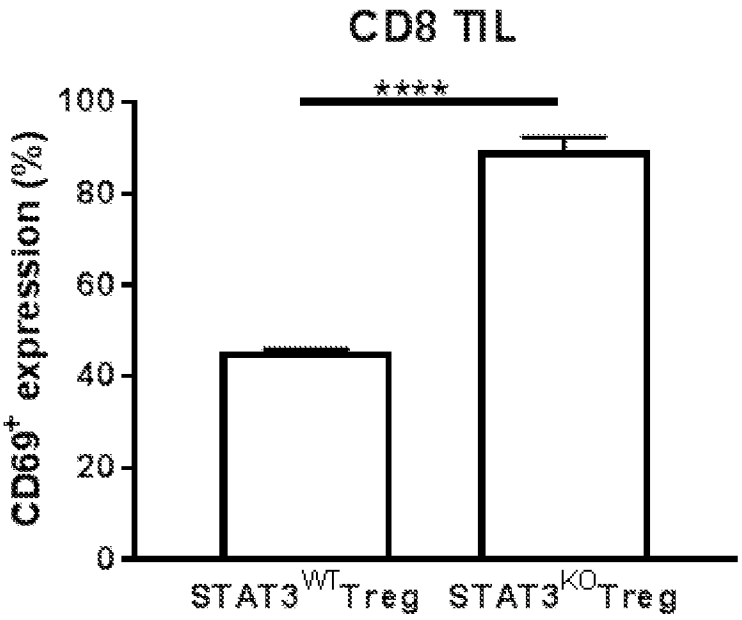


FIG. 46B

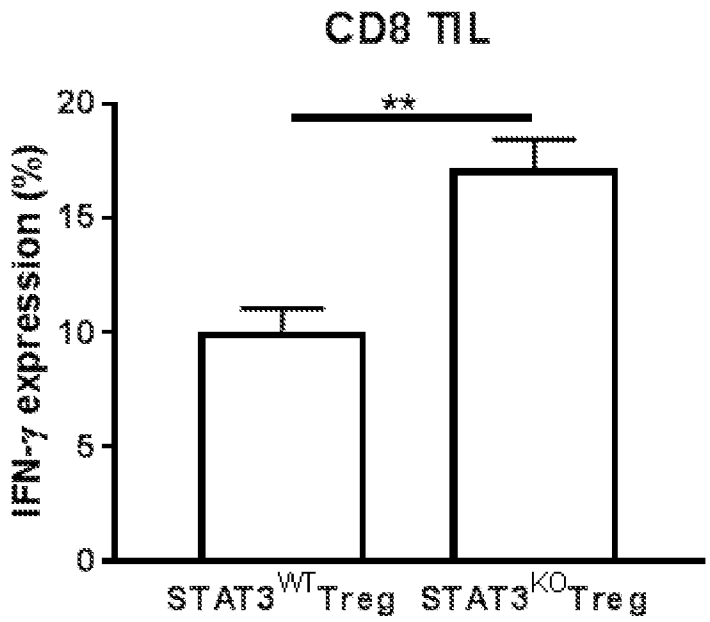
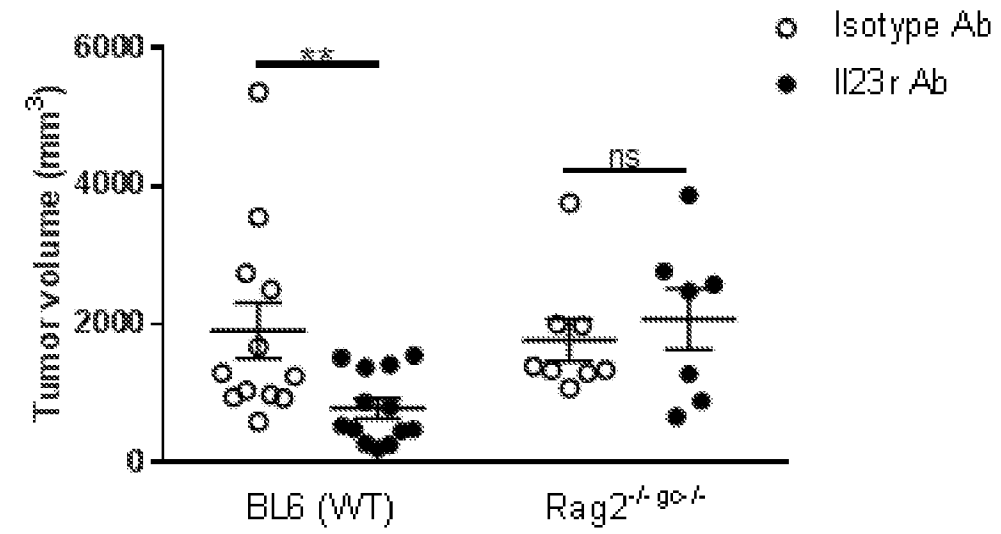


FIG. 46C


Isotype Ab
Il23r Ab
Tumor volume (mm3)
6000
4000
2000
0
**
ns
BL6 (WT)
Rag2-/- gc-/-

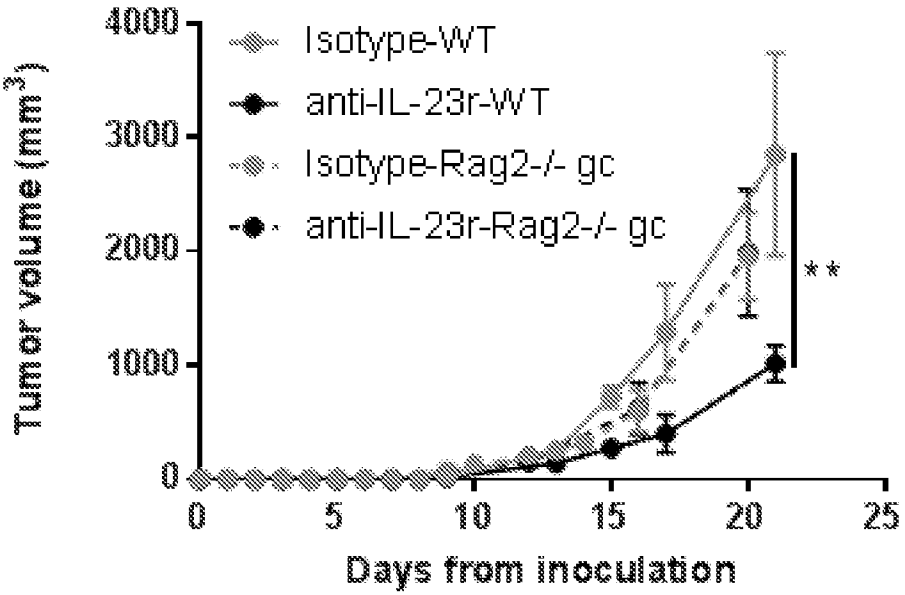

Isotype-WT
anti-IL-23r-WT
Isotype-Rag2-/- gc
anti-IL-23r-Rag2-/- gc
Tumor volume (mm3)
4000
3000
2000
1000
0
**
0
5
10
15
20
25
Days from inoculation

FIG. 47A

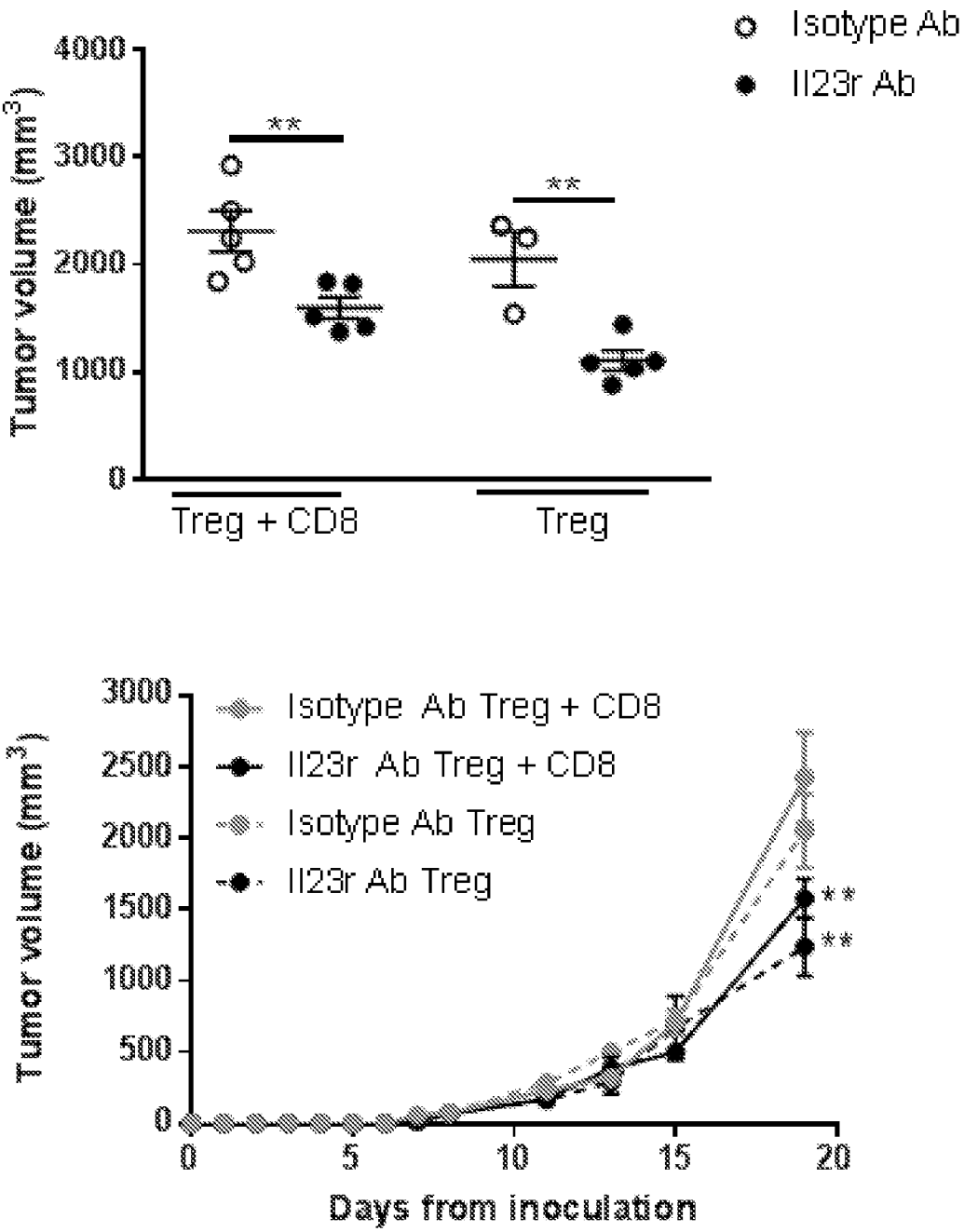

Isotype Ab
Il23r Ab
Tumor volume (mm3)
4000
3000
2000
1000
0
**
**
Treg + CD8
Treg
Isotype Ab Treg + CD8
Il23r Ab Treg + CD8
Isotype Ab Treg
Il23r Ab Treg
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
Days from inoculation
**
**

FIG. 47B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/30636

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/54, 14/705, 14/715, 16/26, 16/28; C12N 5/0783 (2018.01)
CPC - C07K 14/5434, 14/7155, 16/2866, 2317/32, 14/57, 14/705; C12N 5/0637, 5/0636, 2501/2323, 2501/2312

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2010/0166767 A1 (PRESTA) 1 July 2010 (01.07.2010). Especially para [0011], [0080], [0084], [0107], [0115], [0156]	1-8 ------ 9 ------ 15-23, 27-31
Y	US 2013/0035365 A1 (GALLAGHER et al.) 7 February 2013 (07.02.2013). Especially abstract, para [0093].	9
A	BOEVENSCHEN et al. Foxp3+ regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin. J Invest Dermatol September 2011 Vol 131 No 9 Pages 1853-1860. Especially abstract, pg 1856 col 2 para 2.	15-23, 27-31
A	KANNAN et al. IL-23 induces regulatory T cell plasticity with implications for inflammatory skin diseases. J. Immunol 1 May 2017 Vol 198 Suppl 1 Abstract 220.13. Especially abstract.	15-23, 27-31
A	ZHOU et al. Plasticity of CD4+ FoxP3+ T cells. Curr Immunol June 2009 Vol 21 No 3 Pages 281 -285. Especially abstract, pg 3 para 1.	15-23, 27-31
A	WO/2016/196912 (DANA FARBER CANCER INSTITUTE, INC.) 8 December 2016 (08.12.2016). Especially claims 1-50.	15-23, 27-31

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 2018	19 JUL 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/30636

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

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

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

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

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

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

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

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

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

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/30636

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

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

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

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

3. ☒ Claims Nos.: 10-14, 24-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.