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Title Page

Generation and characterization of mirikizumab, a humanized monoclonal antibody targeting the p19 subunit of IL-23

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Running Title Page

Running title: Generation and characterization of mirikizumab

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Text pages: 16

Tables: 4

Figures: 3

References: 25

Word counts:

Abstract: 179

Introduction: 427

Discussion: 500

Abbreviations:

CD = Crohn's disease

CHO = Chinese hamster ovary

FACS = fluorescence-activated cell sorting

ELISA = enzyme-linked immunosorbent assay

IL-12 = interleukin 12

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IL-23 = interleukin 23

IP= intraperitoneal

miri = mirikizumab

SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis

UC = ulcerative colitis

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Abstract

IL-23 exists as a heterodimer consisting of p19 and p40 and is a key cytokine for promoting inflammatory responses in a variety of target organs. IL-23 plays a key role in the differentiation and maintenance of T helper 17 cells, and deregulation of IL-23 can result in autoimmune pathologies of the skin, lungs, and gut. This study describes the generation and characterization of mirikizumab, a humanized IgG4 monoclonal antibody directed against the p19 subunit of IL-23. Mirikizumab binds human and cynomolgus monkey IL-23 with high affinity and binds rabbit IL-23 weakly but does not bind to rodent IL-23 or the other IL-23 family members IL-12, IL-27, or IL-35. Mirikizumab effectively inhibits the interaction of IL-23 with its receptor, and potently blocks IL-23-induced IL-17 production in cell-based assays while preserving the function of IL-12. In both local and systemic *in vivo* mouse models, mirikizumab blocked IL-23-induced keratin mRNA or IL-17 production, respectively. These data provide a comprehensive preclinical characterization of mirikizumab, for which efficacy and safety have been demonstrated in human clinical trials for psoriasis, ulcerative colitis, and Crohn's disease.

Significance Statement

This manuscript describes the generation and characterization of mirikizumab, a high affinity, neutralizing IgG4 variant monoclonal antibody which is under development for the treatment of ulcerative colitis and Crohn's disease. Neutralization of IL-23 is achieved by preventing the binding of IL-23 p19 subunit to the IL-23 receptor and does not affect the IL-12 pathway.

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Introduction

Interleukin (IL)-23 is a member of the IL-12 family of heterodimeric cytokines, consisting of IL-12, IL-23, IL-27, and IL-35. IL-23 has two components: the p40 subunit, which is shared by IL-12, and the p19 subunit, which is unique to IL-23 (Croxford, Mair et al. 2012). By itself, p19 has no determined biological activity, but when combined with p40 forms biologically active IL-23. The receptor responsible for IL-23 signaling is composed of two subunits: the IL-23R and IL-12Rβ1. IL-23R is responsible for signaling through STAT3, while IL-12Rβ1 stabilizes the binding of IL-23 through its interactions with the p40 subunit of IL-23.

IL-23 functions in innate and adaptive immunity and is a key cytokine for promoting inflammatory responses in a variety of target organs. In its normal physiologic role, signaling of IL-23 through IL-23R stimulates memory CD4+ T cells to produce IL-17 as part of a proinflammatory axis critical for host defense. However, deregulation of IL-23 affects barrier function, resulting in pathologies of the skin, lungs, and gut. Indeed, IL-23 plays a key role in the differentiation and maintenance of T helper 17 (Th17) cells, a subset of human T cells critically important to the pathogenesis of many autoimmune diseases, including ulcerative colitis (UC) and Crohn's disease (CD) (Croxford, Kulig et al. 2014, Gheita, El et al. 2014, Globig, Hennecke et al. 2014, El-Bassat, AboAli et al. 2016). Simply blocking IL-17, however, has been shown to either be ineffective or to worsen disease in patients with CD (Hueber, Sands et al. 2012, Targan, Feagan et al. 2016). The Th17 pathway clearly has a role in the pathology of UC and CD; however, IL-17 itself may have a more protective role supporting intestinal barrier function (Maxwell, Zhang et al. 2015), and IL-23-independent IL-17-producing γδ T cells have been observed to be important for the maintenance barrier function in the intestinal mucosa (Lee, Tato et al. 2015). Because Th17 cells also produce several other cytokines in addition to IL-17, such as IL-21 and IL-22 (Croxford, Mair et al. 2012), preventing differentiation and activation of Th17 cells would have a different effect than neutralizing IL-17 alone.

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A therapeutic antibody specific for IL-23 has the potential to block inflammation and modify the underlying disease pathology of autoimmune diseases involving dysfunction of Th17 cells, while permitting a normal immune response to most pathogens. Such an antibody must have high specificity, high affinity, low immunogenicity, and good chemical stability. We describe here the generation of a neutralizing antibody recognizing human IL-23, LY3074828, which began a first-in-man study on October 2013 and was subsequently named mirikizumab.

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Materials and Methods

Immunizations and Screening Mouse Antibodies

All animal studies were conducted in accordance with, and approved by, the research guidelines of Eli Lilly and Company (Indianapolis, IN, USA) Animal Care and Use Committee. An immune response in BALB/c mice that were immunized with human IL-23 was confirmed with serum titers as follows: spleen cells from the immunized mice were harvested and FACS sorted for either IL-23 antigen or $CD80^{br}/CD35^{-}$ binding activity and co-cultured with EL4-B cells. After two weeks, cell supernatants were assayed for positive binding to human IL-23 and lack of binding to human IL-12. Heavy and light chain variable region genes were isolated by RT-PCR from those cells having the desired activity and cloned into the murine antibody expression vectors pEMG1 and pEMK-NF2. The resulting antibodies were characterized for relative antigen binding affinity by ELISA and their ability to block IL-23 receptor binding by signal neutralization in human Kit225 cells or murine spleen cell assay. Antibody 9F2 and the closely related (by sequence) 8B9 bound human IL-23 and completely inhibited IL-23 binding to its receptor but did not bind human IL-12. In addition, analysis showed 8B9 and 9F2 mutually blocked binding to IL-23, indicating they bind in a similar region on IL-23.

Generation of LY3074828 (mirikizumab)

Starting with the human IL-23 specific mouse antibody clone 9F2, human heavy and light chain frameworks for the synthesis of humanization libraries were chosen based on V-region analysis and canonical structure homology. Libraries were created in a bacteriophage M13 human Fab expression vector containing human frameworks VH1-69, JH6, VK-02, and JK4 fused with the 9F2 complementarity determining regions (CDRs). CDRH3, CDRL3, and heavy chain position S31 were completely randomized by position. Screening of the library was completed by capture lift using biotinylated human IL-23 to identify humanized Fab variants retaining binding

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activity. Variants of interest were further characterized by titration in an antibody capture ELISA format.

An initial humanized Fab clone (9F2.25) was selected for further affinity optimization based on relative binding affinity and chemical nature of the selected mutations. Single amino acid mutations of each CDR residue were screened by capture lift using biotinylated human IL-23. Unique beneficial mutations were sequence confirmed and analyzed by capture ELISA titrations. Selected single mutations were combined into a combinatorial library and further characterized by sequence analysis and ELISA using reduced antigen concentration and prolonged washes, yielding a panel of high affinity humanized antibodies. Four Fabs with diverse sequence were converted into full-length human IgG4PAA containing the hinge region mutation S328P to minimize antibody heterogeneity and CH2 mutations L334A and L335A to minimize effector functions (Liu, Lu et al. 2016) and were expressed and purified for further characterization. The antibody with the most desirable characteristics was designated LY3074828.

Expression and Purification

Heavy and light chain variable regions of LY3074828 were cloned into expression plasmids pEE6.4 and pEE12.4, respectively, which are used in the GS Gene Expression System™ (Lonza Biologics). LY3074828 was expressed in CHO cells. Antibodies were purified by Protein A affinity chromatography and size exclusion chromatography.

Specificity and Affinity Determination

Antibody affinity to human, cynomolgus monkey or rabbit IL-23 ($KD = K_d/K_a$) was established by surface plasmon resonance (SPR) using a BIAcore biosensor 2000 and BIAevaluation software with a 1:1 binding with mass transfer model. Briefly, protein A (Calbiochem) was coupled via free amine groups to carboxyl groups on flow cells 1 and 2 of a CM4 biosensor chip

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using a mixture of N-ethyl-N- (dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS). Flow cells were monitored with a flow rate of 80 µL/minute using a buffer composed of 0.01 M HEPES, pH 7.4, 150 mM NaCl, 0.005% surfactant P20. LY3074828 was captured on flow cell 2 to yield a total of 40 to 60 response units (RU), and flow cell 1 was used as a control to monitor non-specific binding of IL-23. Multiple cycles of increasing concentrations of IL-23 were injected over flow cells 1 and 2 (0.62 nM to 30 nM for human and monkey IL-23 and 30 nM to 240 nM for rabbit IL-23). Each cycle includes an antibody capture step, followed by injection of IL-23 at one concentration with a 30-minute dissociation period, and then a regeneration step between each cycle with glycine-HCl (pH 1.5). Buffer was injected in place of IL-23 for two cycles to serve as a control for baseline subtraction and correct for drift associated with the dissociation of LY3074828 from the protein A surface. Affinity was measured at 37°C using a 30-minute dissociation period. The assay was performed twice using human, monkey, or rabbit IL-23. LY3074828 was tested 2 times each with mouse IL-23 (Lilly) at 333 nM, rat IL-23 at 200 nM (at 25°C), human IL-12 at 333 nM, human IL-27 at 500 nM (all from R&D Systems), or human IL-35 (Sino Biological) at 833 nM.

In Vitro Inhibition of IL-23 Binding to IL-23 Receptor by LY3074828: Surface Plasmon Resonance Analysis

Recombinant human IL-23R/Fc was coupled to carboxyl groups on flow cell 2 of a CM4 biosensor chip. Recombinant human IgG1 Fc was coupled to flow cell 1, which served as control to monitor non-specific binding. Mouse anti-6X HIS antibody was coupled to flow cell 4 of the same chip to pre-capture HIS-tagged human IL-12β1/Fc. Recombinant human IL-23 was preincubated for 90 minutes in the presence or absence of a 16X molar excess of LY3074828. Each combination was injected over flow cells 1, 2 and 4 in a total volume of 150 µL, with regeneration afterwards using glycine-HCl (pH 1.5) between each test. Overlays of individual

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binding sensorgrams were prepared with BIAevaluation software. The assay was performed twice.

Evaluation of IL-23 and IL-12 Receptor Inhibition

Two human T cell lines reported to respond to IL-12, Kit-225, and TALL-104, were selected for these studies. The presence of cytokine receptors IL-12R-β1, IL-12R-β2, and IL-23R were verified on each cell line by flow cytometry. Both cell lines were cultured in RPMI-1640 (HyClone), 10% FBS (Gibco), 1 μM Na Pyruvate (HyClone), Non-Essential Amino Acids (HyClone), 55 μM 2-Mercaptoethanol, (Gibco), and PenStrep (HyClone) at 37°C, 5% CO_2.

T-75 flasks were seeded with 10^7 cells per flask in 10 mL of RPMI-1640 plus 0.1% Bovine Albumin Fraction V (BAFV; Gibco), then incubated for four hours to allow the cells to serum starve. LY3074828 (50.0 μg/mL) +/- IL-23 (5.0 μg/mL) were added to 1.0 mL of RPMI-1640 plus 0.1% BAFV and incubated at 37°C for 1.5 hours to allow formation of LY3074828/IL-23 complexes. The LY3074828/IL-23 complexes were added to the serum-starved cells and incubated for 1.5 hours, after which IL-12 was added and the flasks were incubated an additional 20 minutes. Cells were harvested, lysed, and analyzed by Western blot for phospho-STAT4 production as described below.

Proteins from each treated sample (50 μg for Kit-225 and 15 μg for TALL-104 cells) were separated by SDS-PAGE (Novex), transferred to nitrocellulose membranes (Novex), and the membranes were blocked with LiCOR Odyssey membrane-blocking buffer . Rabbit anti-human phospho-STAT4), mouse anti-human STAT4), and rabbit anti-human beta-actin monoclonal antibodies (all from Cell Signaling) were used at 1:1000 dilution. The antibodies were incubated for 16 hours with the membranes at 4°C, and the excess and nonspecifically bound antibodies were removed by three 10-minute washes with Tris-Buffered Saline Tween-20 (TBST) (KPL) at room temperature. The specifically bound antibodies were detected with anti-Rabbit-

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IRDye680RD and anti-mouse-IRDye800CW (LiCor) antibodies used at 1:10,000 dilution in TBST for 2 hours at room temperature. The membranes were washed by three 10-minute washes with TBST at room temperature and scanned with a LiCor Odyssey CLx at 700nm and 800nm. The images were analyzed with Image Studio 2.0 Software (LiCor).

Neutralization of IL-23 In Vitro Using Mouse Splenocytes

Splenocytes from C57BL/6 mice stimulated with IL-23 and IL-2 produce IL-17 (Aggarwal, Ghilardi et al. 2003). Splenocytes were resuspended at $5x10^6$ cells/mL in assay media (RPMI1640 with L-glutamine containing 10% FBS, 1% non-essential amino acids, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.00035% 2-mercaptoethanol, 50 ng/mL human IL-2) and dispensed in volumes of 100 μL per well into a 96-well culture plate. LY3074828 (ranging from 800,000 to 4.4 pM) or LSN2835015 (IgG4) control were pre-incubated with human or cynomolgus monkey IL-23 (16 pM) in a separate well for 90 minutes at 37°C.

The pre-incubated mix of LY3074828/IL-23 (100μL per well) was added to the mouse splenocytes and incubated at 37°C in 5% CO_2 for 48 hours. Culture supernatants were tested for mIL-17 using a commercial ELISA kit from R&D Systems based upon manufacturer instructions. IC_{50} was established using a 4-parameter curve fit. The assay was performed twice each for human and cynomolgus monkey IL-23.

Neutralization of Human IL-23 In Vitro Using Human Peripheral Blood Mononuclear Cells

LY3074828 was tested for its ability to inhibit IL-17 secretion from anti-CD3, anti-CD28, and IL-23 stimulated human peripheral blood mononuclear cells (PBMC). PBMC were isolated from whole blood buffy coats from 20 donors (Prep 1) or 29 donors (Prep 2) and stored frozen in liquid nitrogen. On the first day of the assay, PBMC were thawed and resuspended in RPMI

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1640 (HyClone) supplemented with additional HEPES (5 mM) and L-glutamine (1.2 mM) plus Fetal Bovine Serum (10%), Pen/Strep (100 U/mL), and 2-mercaptoethanol (200 nM). After resuspension, 100,000 PBMC (30 µL per well) were plated on 384-well tissue culture plates. LY3074828 (5 µL) was added to the cells in a ten-point concentration response (n=8) and the plates were pre-incubated for 30 minutes at 37°C, 5% CO_2. IL-17 secretion was stimulated by the addition of 30 µL of media containing anti-human CD3 (Lilly), anti-human CD28 (Lilly, IBA083), and human IL-23 (Lilly) yielding 160 ng/mL, 500 ng/mL, and 5 ng/mL final concentrations in the assay, respectively. Following a 48-hour incubation at 37°C, IL-17 concentrations were determined with ELISA. Capture and detection anti-human IL-17 antibodies were purchased from R&D Systems, streptavidin-HRP from Thermo, and colorimetric OPD substrate from Sigma.

Absorbance at 394 nm (A394) was converted to pg/mL IL-17 based on a standard curve using the equation pg/mL IL-17 = EC_{50}*([(Top-Bottom)/(A394-Bottom)]-1)$^{(1/-Hill)}$. IC_{50} values were calculated with the converted values using a standard 4 parameter fit. Maximum inhibition was determined from wells with anti-CD3 and anti-CD28 without IL-23, and minimum inhibition was determined from wells containing all three stimulants.

Neutralization of Human IL-23 In Vivo in an Acute, Systemic Mouse Model

C57BL/6 mice were primed with 3µg mIL-2 20 hours before being injected intraperitoneally (IP) with 1.5 mg of either LY3074828 or isotype matched control antibody. Mice received 3 additional IP injections of mIL-2 (3 µg) and huIL-23 (10 µg) 2, 9, and 25 hours after antibody administration. Mice were sacrificed 7 hours after the final injection, and splenocytes were prepared as a single cell suspension. Splenocytes were washed in complete RPMI (1% non-essential amino acids, 1% sodium pyruvate, 2.5 mM HEPES, 1% L-glutamine, 0.00035% 2-mercaptoethanol, 1% Pen/Strep, 10% heat-inactivated fetal calf serum). Splenocytes were then seeded at 200,000 cells/200 µL/well in a 96-well culture plate and stimulated with soluble

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hamster anti-mouse CD3 and anti-mouse CD28 (BD Biosciences; each at 2 μg/mL). The plate was incubated for 48 hours at 37ºC, 5%CO_2. A sandwich ELISA kit for mouse IL-17A (R&D Systems) was used to measure IL-17A concentrations in each culture supernatant. The assay was performed twice, with 4 mice tested in each antibody group). A t-test was used to assess statistical significance.

Intradermal Neutralization of Human IL-23

Hair was removed from the back of C57BL/6 mice (8-week-old females; Jackson Labs) using electric clippers. Three days later, mice (n=10 per group) received a subcutaneous injection of LY3074828 or an isotype control antibody (0.54 μg per mouse), and an intradermal injection with human IL-23 in one location on one side of the back (1 μg in 50 μL diluted with sterile saline) using a 29-gauge needle for the subsequent 2 days. A vehicle control (saline) was injected on the contralateral side. Mice were sacrificed 24 hours after the last IL-23 injection. Skin samples were removed from both sides, and frozen directly in liquid nitrogen. Total RNA was isolated by homogenization in Lysing Matrix A shaker tubes (Qbiogene Inc./Bio101 Systems) followed by RNeasy Mini kit cleanup (Qiagen, Inc.). RNA concentrations were determined from spectrophotometric absorption at 260 nm. RNA was reverse-transcribed into cDNA using High-Capacity cDNA Reverse Transcription Kit (PE Applied Biosystems). All reactions were performed in triplicate on an ABI Prism 7900HT (PE Applied Biosystems). Primer probe sets for mouse IL17A, mouse IL17F, and mouse keratin-16 were obtained from PE Applied Biosystems. Both 18S and GAPD were measured as endogenous controls to normalize variability in gene expression levels. Expression data were analyzed using Δ-Δ Ct method, and individual Ct values were calculated as means of triplicate measurements. Experiments were performed twice. Unpaired t-test was used with $P< 0.05$ considered to be statistically significant.

Results

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Mirikizumab Generation

Antibodies generated by immunized mice were screened for binding to human IL-23 and human IL-12 by ELISA. A panel of antibodies specific for human IL-23 were further characterized for affinity by SPR and for the neutralization of biological activity in Kit225 cells and the *in vitro* mouse spleen cell assay. The preferred mouse antibody, clone 9F2, was selected for high affinity and complete neutralization of IL-23 (data not shown). 9F2 was successfully humanized and optimized for affinity and biophysical properties. Relative to the murine parent 9F2, the resulting humanized clone, hu9F2.25, had changes of S31R in HCDR1, Y104A in HCDR3 and Q90M in LCDR3 (all linear numbering). For clone hu9F2.25, antigen binding by ELISA was equivalent to the murine 9F2 antibody and BIAcore analysis provided a K_D of 905 pM at 37°C. In analysis for protein stability, hu9F2.25 showed good solubility with concentrations of 125 mg/mL achievable. Chemical stability evaluation at 40°C for 4 weeks showed less than 1% aggregate and minor degradation products in all buffer conditions (data not shown). Further CDR optimization of the humanized antibody resulted in a panel of 4 high affinity variants (K_D<50 pM) (Table 1). Based on protein characteristics, including affinity, aggregation and expression levels, clone 9F2.25.38 (LY3074828) was selected and designated mirikizumab.

Mirikizumab is specific for IL-23

SPR was used to determine both the family and species specificity of mirikizumab. Mirikizumab produced a concentration-dependent binding response with human, cynomolgus monkey and rabbit IL-23 using this method. Saturation of binding of IL-23 was attained at 30 nM (human and monkey) and 240 nM (rabbit) using 80-100 response units of mirikizumab captured on the chip surface. Under the conditions tested, the binding affinity (KD) of human, monkey and rabbit IL-23 was 21, 55 and 53,000 pM respectively (Table 2). Mirikizumab did not bind to mouse IL-23, rat IL-23, human IL-12, human IL-27, or human IL-35 under these conditions.

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IL-23 Receptor Inhibition

The most common mechanism of action for a neutralizing antibody against a cytokine is prevention of binding to the receptor. The receptor responsible for IL-23 signaling is composed of 2 subunits; the IL-23R and IL-12Rβ1, where IL-23R is responsible for signaling through STAT3 and IL-12Rβ1 stabilizes the binding through interactions with the p40 subunit of IL-23.(Parham, Chirica et al. 2002) SPR was used to monitor the interactions between a complex of mirikizumab/human IL-23 and the extracellular domain of IL-23R or IL-12Rβ1. Mirikizumab effectively prevented the interaction between IL-23 and IL-23R, but not the interaction of IL-23 with IL-12Rβ1 (Table 3).

Neutralization of IL-23 but not IL-12 In Vitro

Mouse splenocytes can produce IL-17A in response to human or cynomolgus monkey IL-23. Mirikizumab was able to completely neutralize human or cynomolgus monkey IL-23-induced IL-17A production in this assay (Figure 1A-B). The calculated IC_{50} is 82 pM for human and 120 pM for cynomolgus monkey IL-23, n= 2 for each. These results demonstrate that mirikizumab can neutralize human or cynomolgus monkey IL-23 *in vitro*.

Polyclonal activation of T cells with anti-CD3/anti-CD28 leads to cytokine secretion. Addition of IL-23 to the stimulus mixture will preferentially stimulate IL-23R positive Th17 cells to secrete cytokines such as IL-17. Mirikizumab was able to completely inhibit the IL-23 stimulated IL-17 secretion from two different pools of human PBMC co-stimulated with anti-CD3/anti-CD28 (Figure 1C). The calculated IC50 values are 22 µg/mL for PBMC Prep 1 and 29µg/mL for PBMC Prep 2. These results demonstrate that mirikizumab is able to inhibit IL-23 induced IL-17 secretion *in vitro* in primary human PBMC.

Two human T cell lines that respond to IL-12, Kit225 and TALL-104, were used to confirm that mirikizumab did not affect IL-12 signaling by determining IL-12 induced phosphorylation of

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STAT4 in the presence of the mirikizumab/IL-23 complex. In both Kit225 cells (Figure 2A) and TALL-104 cells (Figure 2B), the ratio of pSTAT4:STAT4 demonstrated that pSTAT4 was only found in the samples that were stimulated with IL-12. Pre-incubation with the mirikizumab/IL-23 complex before IL-12 stimulation did not block phosphorylation of STAT4 in Kit225 and TALL-104 cell lines, indicating that the mirikizumab/IL-23 complex did not interfere with IL-12 signaling. The same results were obtained when these experiments were repeated with mirikizumab alone instead of the mirikizumab/IL-23 complex.

Assessment of Fc Receptor Activation and Complement Binding

In vitro binding assays were performed to determine whether mirikizumab was capable of binding either Fcγ receptors or components of the complement system. Binding was assessed for human Fcγ receptors I (CD64), IIa (CD32a), and IIIa (CD16a) and the complement component C1q (See Supplemental Appendix for Methods). Mirikizumab binding to all four was equal to the negative control antibody (Suppl. Fig. 1), suggesting that would be highly unlikely for mirikizumab to induce Fc- or complement-mediated biological effects *in vivo*.

Mirikizumab neutralization of IL-23 in vivo

IP injection of a combination of human IL-23 and IL-2 is able to prime rodent splenocytes for *ex vivo* production of IL-17 upon restimulation with anti-CD3 and anti-CD28. Concurrent administration of mirikizumab in this system resulted in significant inhibition of the resulting mouse IL-17 production (Figure 3A).

Human IL-23 protein was then injected intradermally into mice to assess whether systemic administration of mirikizumab could neutralize the local response to human IL-23. Skin from wild-type mice injected daily with human IL-23 induced mRNA expression of mouse IL-17A and mouse IL-17F, whereas daily saline solution did not show detectable levels of mouse IL-17A or

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mouse IL-17F mRNA (Table 4). Treatment with mirikizumab, but not isotype control antibody, abrogated the human IL-23-induced IL-17A and IL-17F mRNA expression.

Furthermore, human IL-23 injection also induced keratin-16 mRNA expression, which could not be prevented by treatment with the isotype control antibody (Figure 3B). However, keratin-16 induction was significantly inhibited by administration of mirikizumab. Overall, these results showed that mirikizumab effectively inhibits human IL-23-induced mouse IL-17A, IL-17F, and keratin16 mRNA production in an acute local *in vivo* assay.

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Discussion

This report describes the generation and characterization of mirikizumab, a humanized IL-23 mAb. The humanization process (Vasserot, Dickinson et al. 2003, Liu, Lu et al. 2016) used to engineer mirikizumab included steps to optimize its affinity for IL-23, resulting in an antibody with high binding affinity. Mirikizumab binds to human and cynomolgus monkey IL-23 but not mouse or rat IL-23 and is able to completely inhibit IL-23 activity both *in vitro* and *in vivo*. Importantly, because of its specificity to the p19 subunit of IL-23, mirikizumab does not bind to IL-23 family members IL-12, IL-27, or IL-35. Moreover, as a human IgG4 with the addition of two alanine mutations in the lower hinge region, mirikizumab does not bind to Fc receptors or complement, and the lack of binding was confirmed by ELISA (Suppl Figure 1). As illustrated in the visual abstract, mirikizumab blocks IL-23 binding to its receptor, IL-23R, and thus prevents activation of the cell's effector function through the JAK/STAT pathway.

While IL-23 plays in important role in host defense, dysregulation of the IL-23 pathway has been shown to result in a variety of autoimmune disorders, including psoriasis and inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Mirikizumab has completed Phase III trials in moderate-to-severe psoriasis and ulcerative colitis (Sandborn, Ferrante et al. 2019, Sandborn, Ferrante et al. 2020, D'Haens, Kobayashi et al. 2022), and Phase II trials in Crohn's disease (Sands, Peyrin-Biroulet et al. 2022), showing rapid and significant improvements in clinical endpoints and quality of life. Biopsies taken from the colon in the UC trial and from colon and terminal ileum in the CD trial demonstrated significantly reduced neutrophil infiltration of the mucosa in both cases. Furthermore, transcriptional analysis of the colonic biopsies from the UC trial demonstrated that mirikizumab down-regulated several transcripts correlated with increased UC disease activity as well as anti-TNF resistance, and that these changes were maintained through 52 weeks of treatment, suggesting a distinct

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molecular healing pathway associated with IL-23 inhibition (Steere, Schmitz et al. 2020, Johnson, Steere et al. 2021).

Other antibodies targeting the IL-23 pathway have been approved by regulatory agencies. Ustekinumab, a monoclonal antibody directed to the shared p40 subunit of IL-12 and IL-23 has been approved for use in psoriasis, UC, and CD (Leonardi, Kimball et al. 2008, Papp, Langley et al. 2008, Sandborn, Gasink et al. 2012). However, multiple studies in patients with psoriasis have suggested that more selective targeting of the IL-23 pathway by blocking the p19 subunit of IL-23 is more effective than ustekinumab (Papp, Blauvelt et al. 2017, Gordon, Strober et al. 2018). Risankizumab, guselkumab, and brazikumab, other monoclonal IL-23p19 antibodies, are in different stages of development for ulcerative colitis and Crohn's disease (Almradi, Hanzel et al. 2020).Mirikizumab is a high affinity, neutralizing IgG subclass 4 variant monoclonal antibody with specificity for human IL-23, which targets the p19 subunit of IL-23. Neutralization is achieved by preventing the binding of IL-23 p19 subunit to the IL-23 receptor and does not affect the IL-12 pathway.

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Data Availability Statement: Lilly provides access to all individual participant data collected during the trial, after anonymization, with the exception of pharmacokinetic or genetic data. Data are available to request 6 months after the indication studied has been approved in the US and EU and after primary publication acceptance, whichever is later. No expiration date of data requests is currently set once data are made available. Access is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of a signed data sharing agreement. Data and documents, including the study protocol, statistical analysis plan, clinical study report, blank or annotated case report forms, will be provided in a secure data sharing environment. For details on submitting a request, see the instructions provided at www.vivli.org.

Authorship Contributions:

Participated in research design: Steere, Beidler, Martin, Bright, Kikly, Benschop

Conducted experiments: Martin, Beidler, Bright, Kikly

Performed data analysis/interpretation: Martin, Beidler, Kikly, Benschop

Wrote or contributed to the writing of the manuscript: Steere, Beidler, Martin, Bright, Kikly, Benschop

References

Aggarwal, S., N. Ghilardi, M. H. Xie, F. J. de Sauvage and A. L. Gurney (2003). "Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17." J Biol Chem **278**(3): 1910-1914.

Almradi, A., J. Hanzel, R. Sedano, C. E. Parker, B. G. Feagan, C. Ma and V. Jairath (2020). "Clinical Trials of IL-12/IL-23 Inhibitors in Inflammatory Bowel Disease." BioDrugs **34**(6): 713-721.

Croxford, A. L., P. Kulig and B. Becher (2014). "IL-12-and IL-23 in health and disease." Cytokine Growth Factor Rev **25**(4): 415-421.

Croxford, A. L., F. Mair and B. Becher (2012). "IL-23: one cytokine in control of autoimmunity." Eur J Immunol **42**(9): 2263-2273.

D'Haens, G., T. Kobayashi, N. Morris, T. Lissoos, A. Hoover, X. Li, V. Arora, C. Milch, W. J. Sandborn and B. E. Sands (2022). "OP26 Efficacy and safety of mirikizumab as induction therapy in patients with moderately to severely active Ulcerative Colitis: Results from the Phase 3 LUCENT-1 study." Journal of Crohn's and Colitis **16**(Supplement_1): i028-i029.

El-Bassat, H., L. AboAli, S. El Yamany, H. Al Shenawy, R. A. Al Din and A. Taha (2016). "Interleukin-23p19 expression in patients with ulcerative colitis and its relation to disease severity." Advances in Digestive Medicine **3**(3): 88-94.

Gheita, T. A., G. El, II, H. S. El-Fishawy, M. A. Aboul-Ezz and S. A. Kenawy (2014). "Involvement of IL-23 in enteropathic arthritis patients with inflammatory bowel disease: preliminary results." Clin Rheumatol **33**(5): 713-717.

Globig, A. M., N. Hennecke, B. Martin, M. Seidl, G. Ruf, P. Hasselblatt, R. Thimme and B. Bengsch (2014). "Comprehensive intestinal T helper cell profiling reveals specific accumulation of IFN-gamma+IL-17+coproducing CD4+ T cells in active inflammatory bowel disease." Inflamm Bowel Dis **20**(12): 2321-2329.

Gordon, K. B., B. Strober, M. Lebwohl, M. Augustin, A. Blauvelt, Y. Poulin, K. A. Papp, H. Sofen, L. Puig, P. Foley, M. Ohtsuki, M. Flack, Z. Geng, Y. Gu, J. M. Valdes, E. H. Z. Thompson and H. Bachelez (2018). "Efficacy and safety of risankizumab in moderate-to-severe plaque psoriasis (UltIMMa-1 and UltIMMa-2): results from two double-blind, randomised, placebo-controlled and ustekinumab-controlled phase 3 trials." Lancet **392**(10148): 650-661.

Hueber, W., B. E. Sands, S. Lewitzky, M. Vandemeulebroecke, W. Reinisch, P. D. Higgins, J. Wehkamp, B. G. Feagan, M. D. Yao, M. Karczewski, J. Karczewski, N. Pezous, S. Bek, G. Bruin, B. Mellgard, C. Berger, M. Londei, A. P. Bertolino, G. Tougas, S. P. Travis and G. Secukinumab in Crohn's Disease Study (2012). "Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial." Gut **61**(12): 1693-1700.

Johnson, T., B. Steere, P. Zhang, Y. Zang, R. Higgs, K. Gottlieb, W. Reinisch, J. Panés and G. Krishnan (2021). "DOP09 Mirikizumab-induced transcriptome changes in patient biopsies at Week 12 are maintained through Week 52 in patients with Ulcerative Colitis." Journal of Crohn's and Colitis **15**(Supplement_1): S047-S048.

Lee, J. S., C. M. Tato, B. Joyce-Shaikh, M. F. Gulen, C. Cayatte, Y. Chen, W. M. Blumenschein, M. Judo, G. Ayanoglu, T. K. McClanahan, X. Li and D. J. Cua (2015). "Interleukin-23-Independent IL-17 Production Regulates Intestinal Epithelial Permeability." Immunity **43**(4): 727-738.

Leonardi, C. L., A. B. Kimball, K. A. Papp, N. Yeilding, C. Guzzo, Y. Wang, S. Li, L. T. Dooley, K. B. Gordon and P. s. investigators (2008). "Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1)." Lancet **371**(9625): 1665-1674.

Liu, L., J. Lu, B. W. Allan, Y. Tang, J. Tetreault, C. K. Chow, B. Barmettler, J. Nelson, H. Bina, L. Huang, V. J. Wroblewski and K. Kikly (2016). "Generation and characterization of ixekizumab, a humanized monoclonal antibody that neutralizes interleukin-17A." J Inflamm Res **9**: 39-50.

Maxwell, J. R., Y. Zhang, W. A. Brown, C. L. Smith, F. R. Byrne, M. Fiorino, E. Stevens, J. Bigler, J. A. Davis, J. B. Rottman, A. L. Budelsky, A. Symons and J. E. Towne (2015). "Differential Roles for Interleukin-23 and Interleukin-17 in Intestinal Immunoregulation." Immunity **43**(4): 739-750.

Papp, K. A., A. Blauvelt, M. Bukhalo, M. Gooderham, J. G. Krueger, J. P. Lacour, A. Menter, S. Philipp, H. Sofen, S. Tyring, B. R. Berner, S. Visvanathan, C. Pamulapati, N. Bennett, M. Flack, P. Scholl and S. J. Padula (2017). "Risankizumab versus Ustekinumab for Moderate-to-Severe Plaque Psoriasis." N Engl J Med **376**(16): 1551-1560.

Papp, K. A., R. G. Langley, M. Lebwohl, G. G. Krueger, P. Szapary, N. Yeilding, C. Guzzo, M. C. Hsu, Y. Wang, S. Li, L. T. Dooley, K. Reich and P. s. investigators (2008). "Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2)." Lancet **371**(9625): 1675-1684.

Parham, C., M. Chirica, J. Timans, E. Vaisberg, M. Travis, J. Cheung, S. Pflanz, R. Zhang, K. P. Singh, F. Vega, W. To, J. Wagner, A. M. O'Farrell, T. McClanahan, S. Zurawski, C. Hannum, D. Gorman, D. M. Rennick, R. A. Kastelein, R. de Waal Malefyt and K. W. Moore (2002). "A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R." J Immunol **168**(11): 5699-5708.

Sandborn, W. J., M. Ferrante, B. R. Bhandari, E. Berliba, B. G. Feagan, T. Hibi, J. L. Tuttle, P. Klekotka, S. Friedrich, M. Durante, M. Morgan-Cox, J. Laskowski, J. Schmitz and G. R. D'Haens (2019). "Efficacy and Safety of Mirikizumab in a Randomized Phase 2 Study of Patients With Ulcerative Colitis." Gastroenterology.

Sandborn, W. J., M. Ferrante, B. R. Bhandari, E. Berliba, T. Hibi, G. R. D'Haens, J. L. Tuttle, K. Krueger, S. Friedrich, M. Durante, V. Arora, A. N. Naegeli, J. Schmitz and B. G. Feagan (2020). "Efficacy and Safety of Continued Treatment With Mirikizumab in a Phase 2 Trial of Patients With Ulcerative Colitis." Clin Gastroenterol Hepatol.

Sandborn, W. J., C. Gasink, L. L. Gao, M. A. Blank, J. Johanns, C. Guzzo, B. E. Sands, S. B. Hanauer, S. Targan, P. Rutgeerts, S. Ghosh, W. J. de Villiers, R. Panaccione, G. Greenberg, S. Schreiber, S. Lichtiger, B. G. Feagan and C. S. Group (2012). "Ustekinumab induction and maintenance therapy in refractory Crohn's disease." N Engl J Med **367**(16): 1519-1528.

Sands, B. E., L. Peyrin-Biroulet, J. Kierkus, P. D. R. Higgins, M. Fischer, V. Jairath, F. Hirai, G. D'Haens, R. M. Belin, D. Miller, E. Gomez-Valderas, A. N. Naegeli, J. L. Tuttle, P. F. Pollack and W. J. Sandborn (2022). "Efficacy and Safety of Mirikizumab in a Randomized Phase 2 Study of Patients With Crohn's Disease." Gastroenterology **162**(2): 495-508.

Steere, B., J. Schmitz, N. Powell, R. Higgs, K. Gottlieb, Y. Liu, B. Jia, J. L. Tuttle, W. J. Sandborn, B. E. Sands and V. Krishnan (2020). "DOP65 Mirikizumab regulates genes involved in anti-TNF resistance and ulcerative colitis disease activity." Journal of Crohn's and Colitis **14**(Supplement_1): S103-S104.

Targan, S. R., B. Feagan, S. Vermeire, R. Panaccione, G. Y. Melmed, C. Landers, D. Li, C. Russell, R. Newmark, N. Zhang, Y. Chon, Y. H. Hsu, S. L. Lin and P. Klekotka (2016). "A Randomized, Double-Blind, Placebo-Controlled Phase 2 Study of Brodalumab in Patients With Moderate-to-Severe Crohn's Disease." Am J Gastroenterol **111**(11): 1599-1607.

Vasserot, A. P., C. D. Dickinson, Y. Tang, W. D. Huse, K. S. Manchester and J. D. Watkins (2003). "Optimization of protein therapeutics by directed evolution." Drug Discov Today **8**(3): 118-126.

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Figure Legends

Figure 1: Mirikizumab (LY3074828) neutralizes IL-17 expression *in vitro*. Mirikizumab inhibition of IL-17 production in mouse splenocytes in response to human (A) or cynomolgus monkey (B) IL-23. C) Mirikizumab inhibition of IL-17 production in human PBMCs in response to human IL-23.

Figure 2: Mirikizumab (LY3074828) specifically inhibits IL-23 but not IL-12 signaling *in vitro*. Ratio of pSTAT4:STAT4 in Kit225 (A) or TALL-104 (B) cells following stimulation with IL-12 in the presence of the mirikizumab/IL-23 complex.

Figure 3: Mirikizumab (LY3074828) neutralizes IL-23 in systemic and local *in vivo* models. (A) Mirikizumab inhibition of IL-17 production from mouse splenocytes following priming by intraperitoneal injection of human IL-23 and IL-2. (B) Mirikizumab inhibition of murine keratin-16 mRNA expression in skin following intradermal injection of human IL-23.

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Tables

Table 1: CDR sequences of hu9F2 and optimized antibodies

Name	**pI***	**Light Chain CDRs**		
hu9F2.25	7.5	KASDHI**NNY**L**A**	GATSLET	Q**M**YWSTPFT
hu9F2.25.16.4	7.9	KASDHILK**Y**LL	GATSL**S**T	Q**M**YWSTPFT
hu9F2.25.23	7.7	KASDHILKFLL	GATSLET	Q**M**YWSTPFT
hu9F2.25.23.5	8.0	KAS**RP**I**S**KFLL	GATSL**A**T	Q**M**YWSTPFT
hu9F2.25.38	7.7	KASDHILKFL**T**	GATSLET	Q**M**YWSTPFT

Name	**Heavy Chain CDRs**		
hu9F2.25	GYKFT**R**YV**M**H	YINPYNDGTNYNEKFKG	NWD**V**G**A**
hu9F2.25.16.4	GYKFT**R**YV**M**H	YINPYN**A**GTNYNEKFKG	NWDTGL
hu9F2.25.23	GYKFT**R**YV**T**H	YINPYNDGTNYNEKFKG	NWDTGL
hu9F2.25.23.5	GYKFT**R**YV**T**H	YINPYNDGTNYNEKFKG	NWDTGL
hu9F2.25.38	GYKFT**R**YV**M**H	YINPYNDGTNYNEKFKG	NWDTGL

Amino acid sequences of humanized anti-IL23 antibody 9F2. R (heavy chain region 1), A (heavy chain region 3) and M (light chain region 3) residues are mutations introduced during the humanization step. Additional residues in bold indicate optimization mutations.

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Table 2: Mirikizumab binding kinetics and affinity to human, cynomolgus monkey, and rabbit IL-23 and human IL-23 family members

Antigen	K_{on} (avg±SD) $M^{-1}s^{-1}(10^6)$	K_{off} (avg±SD) $s^{-1}(10^{-4})$	KD (avg±SD) pM
Hu IL-12	-	-	No binding
Hu IL-23	2.43 ± 0.16	0.52 ± 0.21	21 ± 9.9
Hu IL-27	-	-	No binding
Hu IL-35	-	-	No binding
Monkey IL-23	1.28 ± 0.05	0.7 ± 0.11	55 ± 6.4
Rabbit IL-23	0.09 ± 0.001	47.9 ± 0.4	53,000 ± 1131
Mouse IL-23	-	-	No binding
Rat IL-23	-	-	No binding

N=2 for each antigen. IL-12 was tested at 400x concentration of what is detectable for IL-23. IL-27 and IL-35 were tested at 800x what is detectable for IL-23. Mouse and rat IL-23 were tested at 500x and 300x concentrations of what is detectable for human IL-23, respectively.

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Table 3: SPR analysis of IL-23/IL-23R Interactions

Antibody	Binding to IL-23R	Binding to IL-12Rβ1
none	yes	yes
Anti-p40 subunit	yes	no
Mirikizumab	no	yes

N=2 experiments for each antibody; yes/no refers to whether IL-23 binds to the IL-23R/IL-12RB1 complex in the presence of the indicated antibody

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Table 4: Mirikizumab In Vivo Neutralization of Human IL-23 Induced Murine IL-17A and IL-17F mRNA Expression

	Ct values			
	Saline		IL-23	
	IL-17A	IL-17F	IL-17A	IL-17F
Isotype control	≥40	≥40	35.4	31.6
Mirikizumab	≥40	≥40	≥40	≥40

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Figure 1

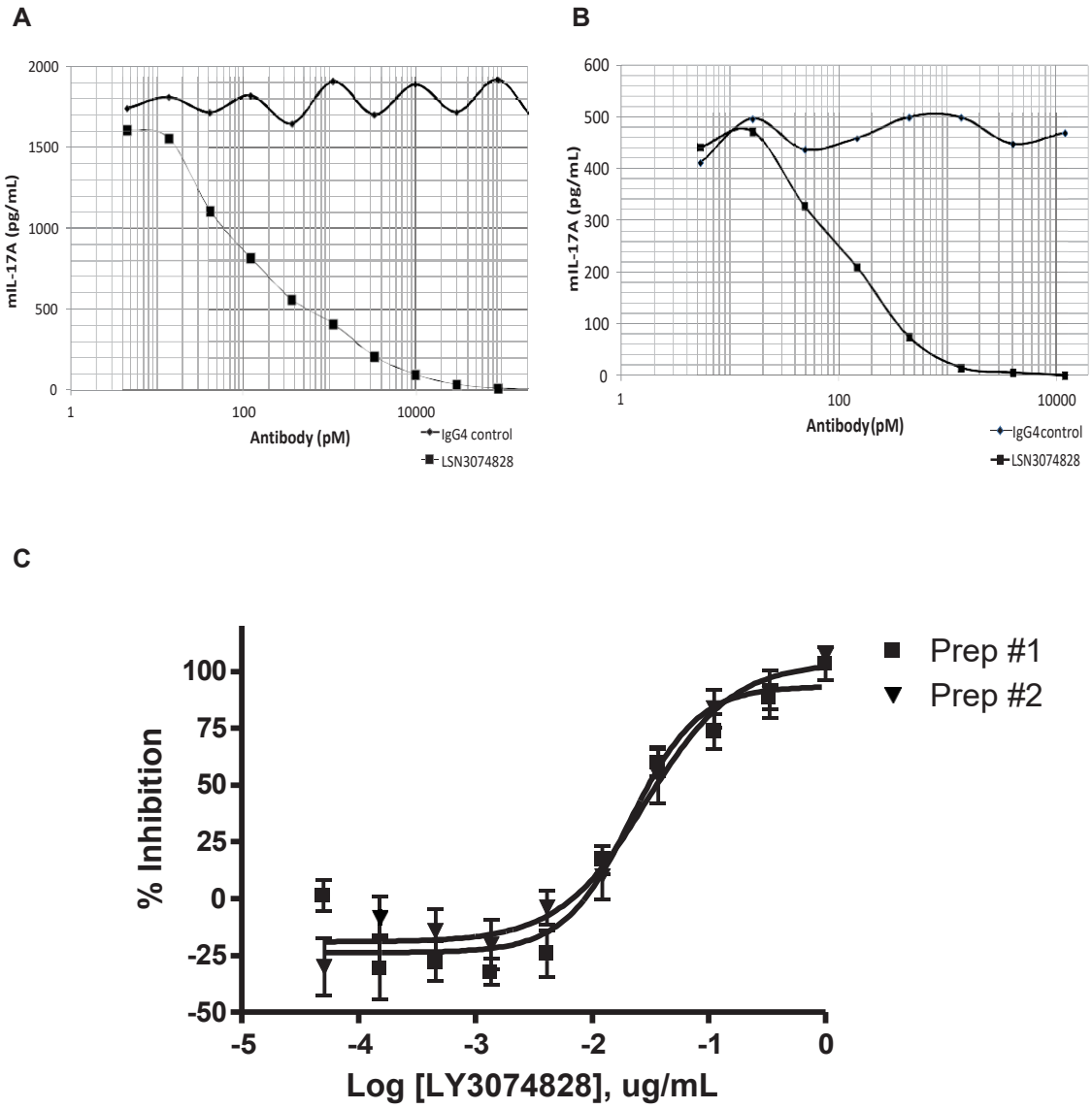

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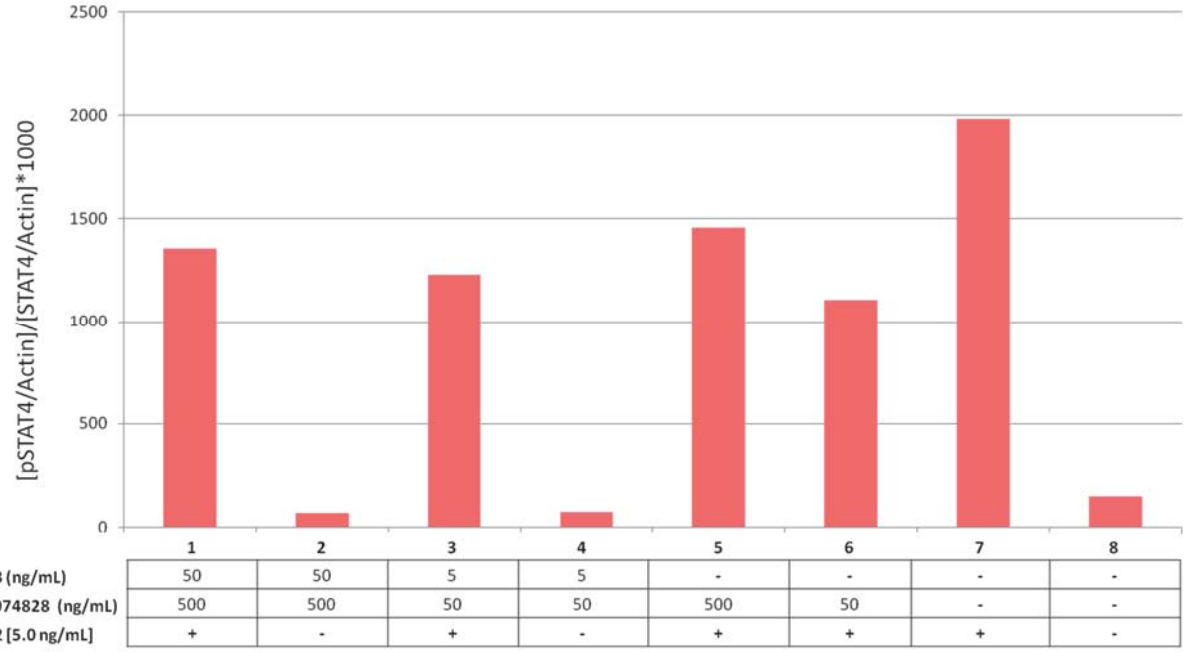
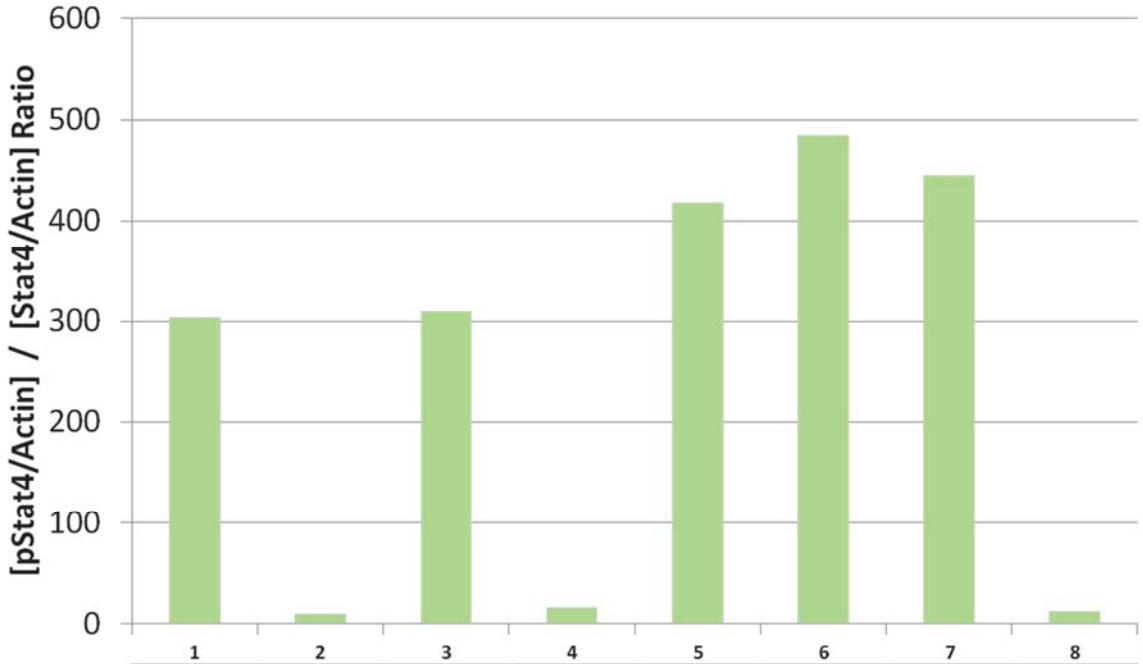
Figure 2

A



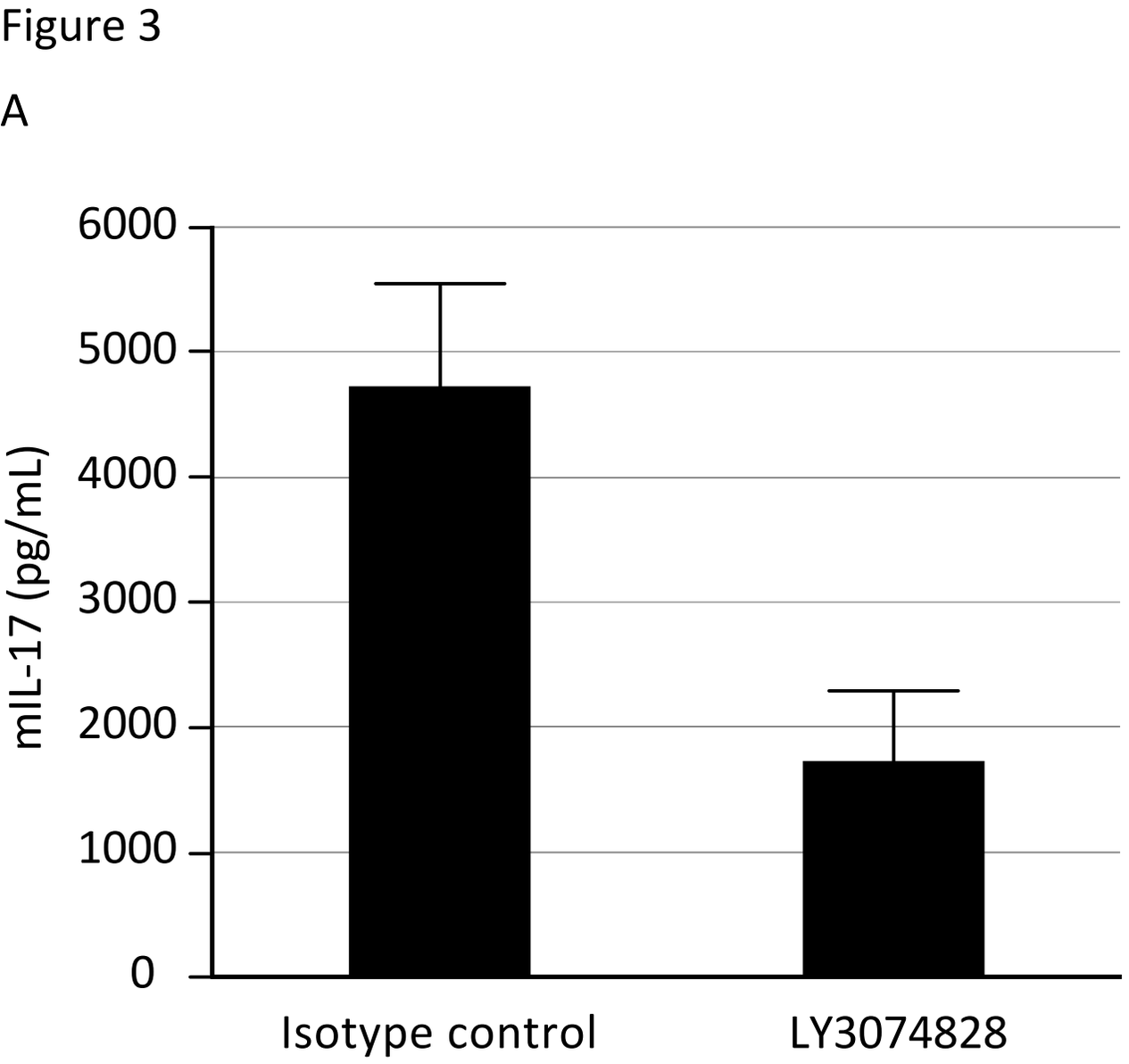
	1	2	3	4	5	6	7	8
IL-23 (ng/mL)	50	50	5	5	-	-	-	-
LY3074828 (ng/mL)	500	500	50	50	500	50	-	-
IL-12 [5.0 ng/mL]	+	-	+	-	+	+	+	-

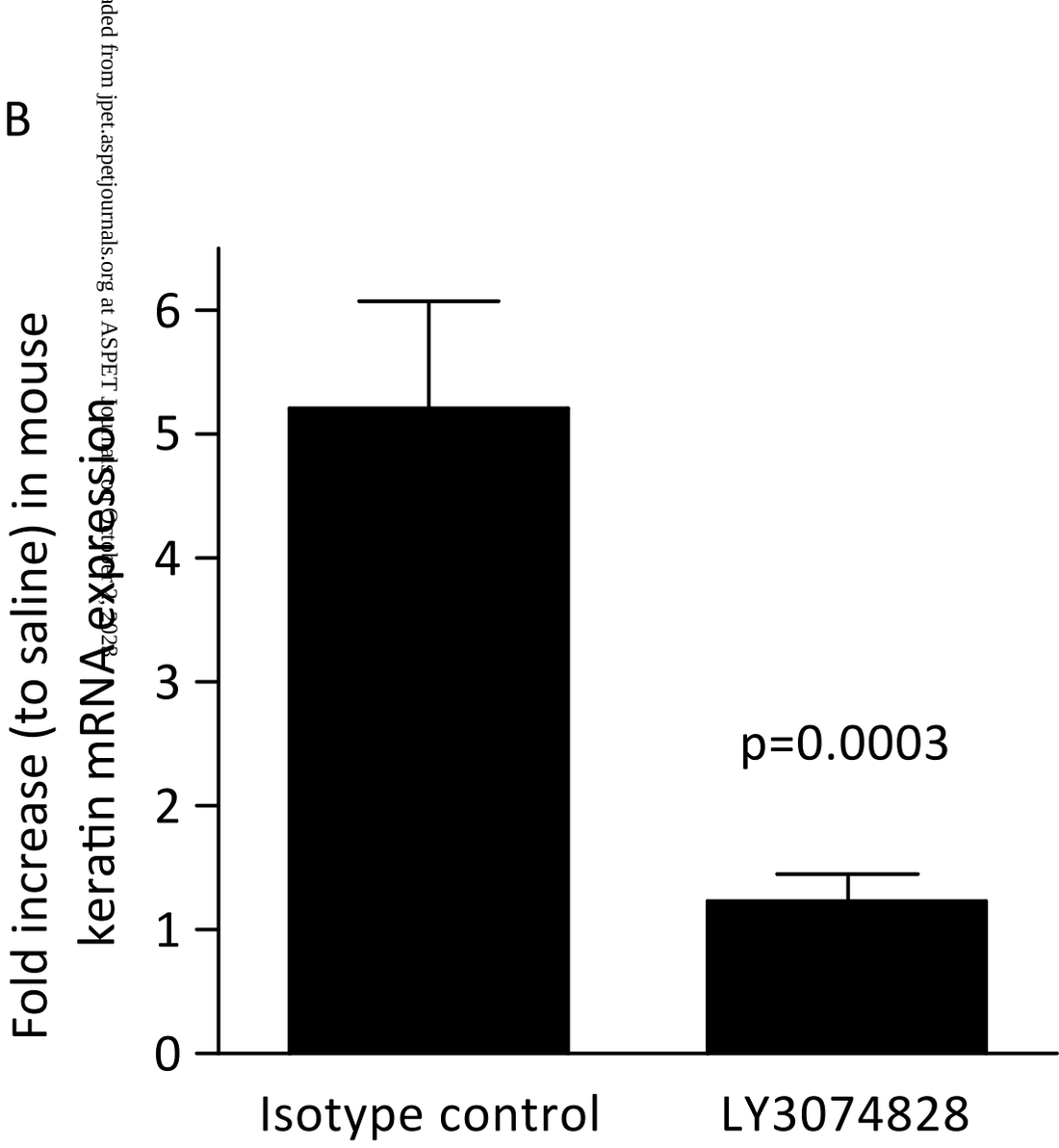
B



	1	2	3	4	5	6	7	8
IL-23 (ng/mL)	50	50	5	5	-	-	-	-
LY3074828 (ng/mL)	500	500	50	50	500	50	-	-
IL-12 [5.0 ng/mL]	+	-	+	-	+	+	+	-

Figure 3





LY3074828 = mirikizumab
Note: One representative study shown (n = 4 mice/group);
Mean+SD; p<0.01, t-test.

Supplemental Appendix

Generation and characterization of mirikizumab, a humanized monoclonal antibody targeting the p19 subunit of IL-23

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METHODS

In Vitro Analysis of Human Fc Receptor and Complement Binding

CD16a, CD32a, and C1q

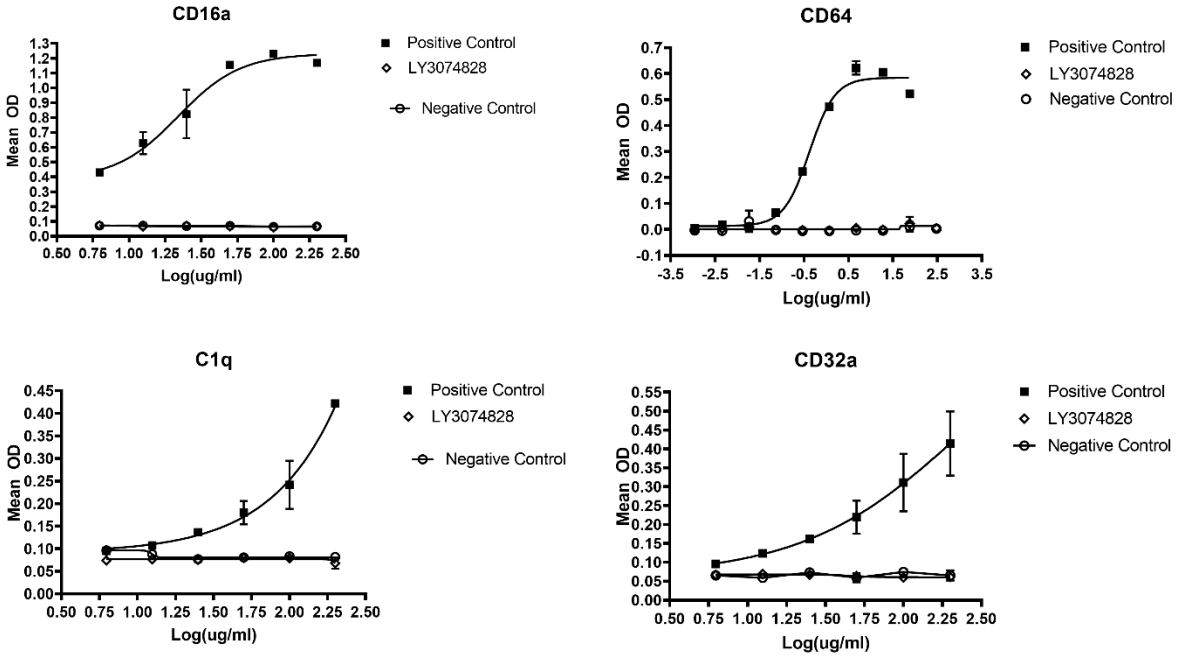
A 96-well microplate was coated with CD-32a with a C-terminal 10-His tag or recombinant human CD-16a with a C-terminal 6-His Tag (R&D Systems for both) at 1 µg/mL in Phosphate Buffered Saline (PBS), or Human C1q (Quidel) at 2 µg/mL in PBS. The plate was incubated overnight at 4°C, then the coating reagent was removed from each well, 200 µL/well of casein blocking reagent (Thermo) was added, and the plate was incubated for 1 hour at room temperature (RT). Each well was washed two times with wash buffer. Serial dilutions of LY3074828, human IgG1 positive control (LSN2436595; Lot No. 17670-16), or human IgG4 negative control (LSN2835015; Lot No. 16396-028) diluted in casein blocking reagent, were added to each well and incubated for 2 hours at RT. Antibodies were tested with a range of 6.25-200 µg/mL in two-fold serial dilutions). Testing was performed in duplicate wells. The plate was then washed three times with wash buffer before a 1:12,500 dilution of HRP-conjugated Goat Anti-Human IgG, F(ab')2 (Jackson ImmunoResearch Catalog No. 109-036-097) in casein blocker was added and incubated for 1 hour at RT. This polyclonal antibody recognizes both human IgG1 and IgG4 (data not shown). The plate was washed four times with wash buffer, and 100 µL/well of TMB Substrate (Pierce) was added, and incubated for 4.5 minutes for CD16a, 9 minutes for CD32a, or 30 minutes for C1q at RT, at which time 100 µL of 1.0 N HCl was added to each well. Optical density was measured using a colorimetric microplate reader set to 450 nm.

CD64

The assay was performed as described above. A 96-well microplate was coated with 100 µL/well of CD64 with a C-terminal 6-His Tag (R&D Systems) at 1 µg/mL in PBS. Antibodies were tested with a concentration range of 0.001 to 300 µg/mL in 4-fold serial dilutions. The plate was incubated with TMB Substrate for 4.5 minutes and developed as described above.

RESULTS

Supplemental Figure 1: Assessment of Fc Receptor Activation and Complement Binding



Abbreviations: IgG1= immunoglobulin G subclass 1; IgG4= immunoglobulin G subclass 4; SD= standard deviation; HRP= horseradish peroxidase

Note: Data are mean±SD of duplicate wells.