Generation and Characterization of Mirikizumab, a Humanized Monoclonal Antibody Targeting the p19 Subunit of IL-23

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ABSTRACT

Interleukin (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 CD4+ 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 (miri), a humanized IgG4 monoclonal antibody directed against the p19 subunit of IL-23. Miri 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. Miri 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, miri blocked IL-23-induced keratin mRNA or IL-17 production, respectively. These data provide a comprehensive preclinical characterization of miri, for which efficacy and safety have been demonstrated in human clinical trials for psoriasis, ulcerative colitis, and Crohn’s disease.

SIGNIFICANCE STATEMENT

This article describes the generation and characterization of mirikizumab, a high affinity, neutralizing IgG4 variant monoclonal antibody that is under development for the treatment of ulcerative colitis and Crohn’s disease. Neutralization of interleukin (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.

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 et al., 2012). By itself, p19 has no determined biologic 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, whereas 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 CD4+ 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 et al., 2014; Gheita et al., 2014; Globig et al., 2014; El-Bassat et al., 2016). Simply blocking IL-17, however, has been shown to either be ineffective or to worsen disease in patients with CD (Hueber et al., 2012; Targan 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 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 et al., 2015). Because Th17 cells also produce several other cytokines in addition to IL-17, such as IL-21 and IL-22 (Croxford et al., 2012), preventing differentiation and activation of Th17 cells would have a different effect than neutralizing IL-17 alone.

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 (miri).

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) 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 fluorescence-activated cell sorted for either IL-23 antigen or CD80/CD35 binding activity and cocultured with EL4-B cells. After 2 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 reverse-transcription polymerase chain reaction from those cells having the desired activity and cloned into the murine antibody expression vectors pEMO1 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 (Miri). 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 and completely inhibited IL-23 binding to its receptor but did not bind human IL-12. Unique beneficial mutations were sequence analyzed and confirmed 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 divergent sequence were converted into full-length human IgG4PA containing the hinge region mutation L334A and L335A to minimize effector functions (Liu 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 Chinese hamster ovary 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 (K(D) = K(D))/K(U) 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 (Calibiochem) was coupled via free amine groups to carboxyl groups on flow cells 1 and 2 of a CM4 biosensor chip using a mixture of N-ethyl-N-(dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide. Flow cells were monitored with a flow rate of 80 μl/min using a buffer composed of 0.01 M HEPES, pH 7.4, 150 mM NaCl, and 0.005% surfactant P20.

LY3074828 was captured on flow cell 2 to yield a total of 40 to 60 response units, and flow cell 1 was used as a control to monitor nonspecific 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 two 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 Biologic) at 833 nM.

In Vitro Inhibition of IL-23 Binding to IL-23 Receptor by LY3074828: SPR Analysis. Recombinant human IL-23/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 nonspecific binding. Mouse anti-4X HIS antibody was coupled to flow cell 4 of the same chip to pre-activate HIS-tagged human IL-12/1/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 afterward using glycine-HCl (pH 1.5) between each test. Overlays of individual 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), nonessential amino acids (HyClone), 55 μM 2-mercaptoethanol, (Gibco), and Pen/Strep (HyClone) at 37°C, 5% CO2. T-75 flasks were seeded with 107 cells per flask in 10 ml of RPMI-1640 plus 0.1% bovine albumin fraction V (Gibco), and then incubated for 4 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% bovine albumin fraction V 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 β-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-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 700 nm and 800 nm. 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 produced IL-17 (Aggarwal et al., 2003). Splenocytes were resuspended at 5 × 10^6 cells/ml in assay media (RPMI-1640 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, and 50 ng/ml human IL-2) and dispensed in volumes of 100 µl per well into a 96-well culture plate. LY3074828 (ranging from 0.01 to 100 nM) and ethanol, and 50 ng/ml human IL-2) and dispensed in volumes of 100 µl per well into a 96-well culture plate. LY3074828 (ranging from 0.01 to 100 nM) and ethanol, and 50 ng/ml human IL-23 (Lilly) yielding 160 ng/ml, 500 ng/ml, and 5 ng/ml 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 o-phenylenediamine dihydrochloride 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 = Ec50*(Top-Bottom)/(A394-Bottom) - 1/(Hill). IC50 values were calculated with the converted values using a standard four-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 3 mg IL-2 20 hours before being injected intraperitoneally with 1.5 mg of either LY3074828 or isotype-matched control antibody. Mice received three additional intraperitoneal injections of mL-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 hamster anti-mouse CD3 and anti-mouse CD28 (BD Biosciences; each at 2 µg/ml). The plate was incubated for 48 hours at 37°C, 5% CO2. A sandwich ELISA kit for mouse IL-17A (R&D Systems) was used to measure IL-17A concentrations in each culture supernatant. Neutralization activity was assessed by comparing the absorbance of treated cultures to untreated controls, and neutralization was defined as an IC50 < 5%.

Intradermal Neutralization of Human IL-23. Hair was removed from the back of C57BL/6 mice (8-week-old females; Jackson Laboratories) using electric clippers. Three days later, mice (n = 10

<table>
<thead>
<tr>
<th>Name</th>
<th>Light Chain CDRs</th>
<th>Heavy Chain CDRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>hu9F2.25</td>
<td>GATSLAT</td>
<td>QMYWSTPFT</td>
</tr>
<tr>
<td>hu9F2.25.16.4</td>
<td>YINPYNGTNYNEKFG</td>
<td>NWDVGA</td>
</tr>
<tr>
<td>hu9F2.25.23</td>
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<td>hu9F2.25.23.5</td>
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<td>NWDVGA</td>
</tr>
<tr>
<td>hu9F2.25.38</td>
<td>GATSLAT</td>
<td>QMYWSTPFT</td>
</tr>
</tbody>
</table>

Table 1 CDR sequences of hu9F2 and optimized antibodies

Amino acid sequences of humanized anti-IL-23 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.

Table 2 Miri Binding Kinetics and Affinity to Human, Cynomolgus Monkey, and Rabbit IL-23 and Human IL-23 Family Members

<table>
<thead>
<tr>
<th>Antigen</th>
<th>K_a (avg ± S.D.)</th>
<th>K_d (avg ± S.D.)</th>
<th>K_d (avg ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu IL-12</td>
<td>2.43 ± 0.16</td>
<td>0.52 ± 0.21</td>
<td>No binding</td>
</tr>
<tr>
<td>Hu IL-23</td>
<td></td>
<td>0.09 ± 0.001</td>
<td>53,000 ± 1131</td>
</tr>
<tr>
<td>Hu IL-27</td>
<td></td>
<td>47.9 ± 0.4</td>
<td>No binding</td>
</tr>
<tr>
<td>Hu IL-35</td>
<td></td>
<td>55 ± 6.4</td>
<td>No binding</td>
</tr>
<tr>
<td>Monkey IL-23</td>
<td>1.28 ± 0.05</td>
<td>0.7 ± 0.11</td>
<td>No binding</td>
</tr>
<tr>
<td>Rabbit IL-23</td>
<td>0.09 ± 0.001</td>
<td>47.9 ± 0.4</td>
<td>No binding</td>
</tr>
<tr>
<td>Mouse IL-23</td>
<td></td>
<td>55 ± 6.4</td>
<td>No binding</td>
</tr>
<tr>
<td>Rat IL-23</td>
<td></td>
<td>53,000 ± 1131</td>
<td>No binding</td>
</tr>
</tbody>
</table>

avg. average.

N = 2 for each antigen. IL-12 was tested at 400× concentration of what is detectable for IL-23. IL-27 and IL-35 were tested at 800× what is detectable for IL-23. Mouse and rat IL-23 were tested at 500× and 300× concentrations of what is detectable for human IL-23, respectively. Dashes indicate no binding signals were detected.
per group) received a subcutaneous injection of LY3074828 or an isotype control antibody (0.54 mg per mouse) and an intradermal injection with human IL-23 in one location on one side of the back (1 mg 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/Bio101 Systems) followed by RNeasy Mini kit cleanup (Qiagen). 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 \( \Delta \Delta C_t \) 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**

**Miri 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 was further characterized for affinity by SPR and for the neutralization of biologic 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 four 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 miri.

**Miri Is Specific for IL-23.** SPR was used to determine both the family and species specificity of miri. Miri 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 miri captured on the chip surface. Under the conditions tested, the binding affinity ($K_D$) of human, monkey, and rabbit IL-23 was 21, 55 and 53,000 pM respectively (Table 2). Miri did not bind to mouse IL-23, rat IL-23, human IL-12, human IL-27, or human IL-35 under these conditions.
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 two 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 et al., 2002). SPR was used to monitor the interactions between a complex of miri/human IL-23 and the extracellular domain of IL-23R or IL-12Rβ1. Miri 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. Miri was able to completely neutralize human or cynomolgus monkey IL-23-induced IL-17A production in this assay (Fig. 1, A and B). The calculated IC50 is 82 pM for human and 120 pM for cynomolgus monkey IL-23, n = 2 for each. These results demonstrate that miri 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. Miri was able to completely inhibit the IL-23–stimulated IL-17 secretion from two different pools of human PBMCs costimulated with anti-CD3/anti-CD28 (Fig. 1C). The calculated IC50 values are 22 μg/ml for PBMC Prep 1 and 29 μg/ml for PBMC Prep 2. These results demonstrate that miri is able to inhibit IL-23–induced IL-17 secretion in vitro in primary human PBMCs.

Two human T cell lines that respond to IL-12, Kit225 and TALL-104, were used to confirm that miri did not affect IL-12 signaling by determining IL-12–induced phosphorylation of STAT4 in the presence of the miri/IL-23 complex. In both Kit225 cells (Fig. 2A) and TALL-104 cells (Fig. 2B), the ratio of pSTAT4:STAT4 demonstrated that pSTAT4 was only found in the samples that were stimulated with IL-12. Preincubation with the miri/IL-23 complex before IL-12 stimulation did not block phosphorylation of STAT4 in Kit225 and TALL-104 cell lines, indicating that the miri/IL-23 complex did not interfere with IL-12 signaling. The same results were obtained when these experiments were repeated with miri alone instead of the miri/IL-23 complex.

Assessment of Fc Receptor Activation and Complement Binding. In vitro binding assays were performed to determine whether miri 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). Miri binding to all four was equal to the negative control antibody (Supplemental Fig. 1), suggesting that would be highly unlikely for miri to induce Fc- or complement-mediated biologic effects in vivo.

Miri Neutralization of IL-23 In Vivo. Intraperitoneal injection of a combination of human IL-23 and IL-2 is able to prime rodent splenocytes for ex vivo production of IL-17 on restimulation with anti-CD3 and anti-CD28. Concurrent administration of miri in this system resulted in significant inhibition of the resulting mouse IL-17 production (Fig. 3A).

Human IL-23 protein was then injected intradermally into mice to assess whether systemic administration of miri 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 mouse IL-17F mRNA (Table 4). Treatment with miri, 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 (Fig. 3B). However, keratin-16 induction was significantly inhibited by administration of miri. Overall, these results showed that miri effectively inhibits human IL-23–induced mouse IL-17A, IL-17F, and keratin16 mRNA production in an acute local in vivo assay.

### Discussion

This report describes the generation and characterization of miri, a humanized IL-23 mAb. The humanization process (Vasserot et al., 2003; Liu et al., 2016) used to engineer miri included steps to optimize its affinity for IL-23, resulting in an antibody with high binding affinity. Miri 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, miri 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, miri does not bind to Fc receptors or complement, and the lack of binding was confirmed by ELISA (Supplemental Fig. 1). As illustrated in the visual abstract, miri blocks IL-23 binding to its receptor, IL-23R, and thus prevents activation of the cell’s effector function through the JAK/STAT pathway.

Although IL-23 plays an 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 UC and CD. Miri has completed phase III trials in moderate-to-severe psoriasis and UC (Sandborn et al., 2020, 2022; D’Haens et al., 2022), and phase II trials in CD (Sands 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 miri downregulated several transcripts correlated with increased UC disease activity as well as antitumor necrosis factor resistance, and these changes were maintained through 52 weeks of treatment, suggesting a distinct molecular healing pathway associated with IL-23 inhibition (Steere et al., 2020; Johnson 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 et al., 2008; Papp et al., 2008; Sandborn 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 et al., 2017; Gordon et al., 2018). Risankizumab, guselkumab, and brazikumab, other monoclonal IL-23p19 antibodies, are in different stages of development for UC and CD (Almradi et al., 2020). Miri 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.

### Data Availability

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 United States and Europe 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:** Beidler, Martin, Bright, Kikly.
- **Performed data analysis/interpretation:** Beidler, Martin, Kikly, Benschop.
- **Wrote or contributed to the writing of the manuscript:** Steere, Beidler, Martin, Bright, Kikly, Benschop.

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