

In one example, the disclosure provides an antibody or antigen binding fragment which binds specifically to IL-1RAcP wherein the antibody or antigen binding fragment cross-competes with an antibody or antigen binding fragment comprising:

- (a) the HCDR1 region of SEQ ID No. 4, the HCDR2 region of SEQ ID No.5, the HCDR3 region of SEQ ID No.6, the LCDR1 region of SEQ ID No.1 , the LCDR2 region of SEQ ID No.2 and the LCDR3 region of SEQ ID No. 3 ; or
- (b) the HCDR1 region of SEQ ID No.12, the HCDR2 region of SEQ ID No. 13, the HCDR3 region of SEQ ID No.14, the LCDR1 region of SEQ ID No.9 , the LCDR2 region of SEQ ID No.10 and the LCDR3 region of SEQ ID No.11.

In one example, the disclosure provides an antibody or antigen binding fragment which binds specifically to IL-1RAcP according to the above example, wherein said antibody or antigen binding fragment comprises

- (a) a HCDR1 region comprising the amino acid sequence of SEQ ID No. 4, a HCDR2 region comprising the amino acid sequence of SEQ ID No.5 , a HCDR3 region comprising the amino acid sequence of SEQ ID No.6 , a LCDR1 comprising the amino acid sequence of SEQ ID No.1 , the LCDR2 comprising the amino acid sequence of SEQ ID No.2 and the LCDR3 comprising the amino acid sequence of SEQ ID No. 3 ; or
- (b) the HCDR1 comprising the amino acid sequence of SEQ ID No.12, the HCDR2 comprising the amino acid sequence of SEQ ID No. 13, the HCDR3 comprising the amino acid sequence of SEQ ID No.14 , the LCDR1 comprising the amino acid sequence of SEQ ID No.9 , the LCDR2 comprising the amino acid sequence of SEQ ID No.10 and the LCDR3 comprising the amino acid sequence of SEQ ID No.11.

In one example, the disclosure provides an antibody or antigen binding fragment according to any of the examples above, wherein said antibody or antigen binding fragment

comprises:

- (a) the HCDR1 region of SEQ ID No. 4, the HCDR2 region of SEQ ID No.5 , the HCDR3 region of SEQ ID No.6 , the LCDR1 region of SEQ ID No.1 , the LCDR2 region of SEQ ID No.2 and the LCDR3 region of SEQ ID No. 3 ; or
- (b) the HCDR1 region of SEQ ID No.12, the HCDR2 region of SEQ ID No. 13, the HCDR3 region of SEQ ID No.14 , the LCDR1 region of SEQ ID No.9 , the LCDR2 region of SEQ ID No.10 and the LCDR3 region of SEQ ID No.11.

In one aspect, the disclosure provides an antibody or antigen-binding fragment thereof that binds specifically to IL-1RAcP, wherein the antibody or antigen-binding fragment:

- (a) comprises an HCDR1 region comprising the amino acid sequence of SEQ ID No. 4, an HCDR2 region comprising the amino acid sequence of SEQ ID No. 5, an HCDR3 region comprising the amino acid sequence of SEQ ID No. 6, an LCDR1 comprising the amino acid sequence of SEQ ID No. 1, an LCDR2 comprising the amino acid sequence of SEQ ID No. 2, and an LCDR3 comprising the amino acid sequence of SEQ ID No. 3;
 - (b) comprises an HCDR1 comprising the amino acid sequence of SEQ ID No. 12, an HCDR2 comprising the amino acid sequence of SEQ ID No. 13, an HCDR3 comprising the amino acid sequence of SEQ ID No. 14, an LCDR1 comprising the amino acid sequence of SEQ ID No. 9, an LCDR2 comprising the amino acid sequence of SEQ ID No. 10, and an LCDR3 comprising the amino acid sequence of SEQ ID No. 11; or
 - (c) is an antibody or antigen-binding fragment that cross-competes with the an antibody or antigen-binding fragment thereof of (a) or (b).
- In one example, the disclosure provides an antibody or antigen binding fragment according to any of the preceding examples, wherein the antibody or antigen binding fragment binds specifically to human IL-1RAcP.
- In one example, the disclosure provides an antibody or antigen binding fragment according to the example above, wherein the antibody or antigen binding fragment binds specifically to human IL-1RAcP and Cynomolgus IL-1RAcP.
- In one example, the disclosure provides an antibody or antigen binding fragment according to any of the preceding examples wherein said antibody or antigen binding fragment comprises at least one heavy chain variable domain (VH) of seq. id. No 7 and at least one light chain variable domain (VL) selected from the following: the VH comprising the amino acid sequence of Seq. id. No. 7 or an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical thereto, and at least one light chain variable domain (VL) of Seq. id. No. 8 , or an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical thereto.
- In one example, the disclosure provides an antibody or antigen binding fragment according to any of the preceding examples wherein said antibody or antigen binding fragment comprises at least one heavy chain variable domain (VH) of seq. id. No 7 and at least one light chain variable domain (VL) of Seq. id. No. 8
- In one example, the disclosure provides an antibody or antigen binding fragment according to any of the preceding examples wherein said antibody or antigen binding fragment comprises at least one heavy chain variable domain (VH) of seq. id. No 15 and at least one light chain variable domain (VL) selected from the following: the VH comprising the amino acid sequence of Seq. id. No. 15 or an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical thereto, and at least one light chain variable domain (VL) of Seq. id. No. 16, or an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical thereto.
- In one example, the disclosure provides an antibody or antigen binding fragment according to any of the preceding examples, wherein said antibody or antigen binding

fragment comprises at least one heavy chain variable domain (VH) of seq. id. No 15 and at least one light chain variable domain (VL) of Seq. id. No. 16.

In one example, the disclosure provides an antibody or antigen binding fragment according to one of the preceding examples, wherein said antibody or antigen binding fragment is an isolated antibody or antigen binding fragment.

In one example, the disclosure provides an antibody or antigen binding fragment according to one of the preceding examples, wherein said antibody or antigen binding fragment is a recombinant antibody or antigen binding fragment.

In one example, the disclosure provides an antibody or antigen binding fragment according to one of the preceding examples for use in the treatment of a subject in need thereof.

In one aspect, the disclosure provides use of an IL-1RAcP antibody as disclosed herein in the manufacture of a medicament for the treatment of an IL1RAcP-associated disease or condition.

In one example, the disclosure provides a nucleic acid composition comprising a nucleic acid sequence or a plurality of nucleic acid sequences encoding the antibody or antigen binding fragment according to any one of examples above.

In one aspect, the disclosure provides an isolated polynucleotide encoding the antibody or antigen-binding fragment as disclosed herein.

In one example, the disclosure provides a vector composition comprising a vector or a plurality of vectors comprising the nucleic acid sequence or plurality of nucleic acid sequences of the example above.

In one example, the disclosure provides a cell comprising the vector composition of the example above.

In one aspect, the disclosure provides a host cell comprising the polynucleotide as disclosed herein.

In one example, the disclosure provides a pharmaceutical composition comprising the antibody or antigen binding fragment according to one of the examples above and a pharmaceutically acceptable carrier or excipient.

In one aspect, the disclosure provides a pharmaceutical composition comprising the antibody or antigen-binding fragment as disclosed herein and a pharmaceutically acceptable carrier or excipient.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1-1 :Various concentrations of the antibodies in the IgG1f_AEASS format were tested for binding to human (AG-12210) IL-1RAcP (IL-1RAP).

Figure 1-2: Various concentrations of the antibodies in the IgG1f_AEASS format were tested for binding to cynomolgus (AG-12211) IL-1RAcP (IL-1RAP), by ELISA

Figure 1-3; Various concentrations of the antibodies in the IgG1f_AEASS format were tested for binding to mouse (AG-12212) IL-1RAcP (IL-1RAP) were assessed in Fc-capture mode of the IgGs.

Figure 2-1: Various concentrations of the antibodies in the IgG1f_AEASS format were tested

for binding to human IL1RAcP(IL-1RAP) domain 1 (AG-12335).

Figure 2-2: Various concentrations of the antibodies in the IgG1f_AEASS format were tested for binding to human IL1RAcP (IL-1RAP)domain 1/2 (AG-12337)

Figure 2-3: Various concentrations of the antibodies in the IgG1f_AEASS format were tested for binding to human IL1RAcP (IL-1RAP) domain 3 (AG-12127)

Figure 3-1: FACS analysis of binding of antibodies in the IgG1f_AEASS format to human IL-1RAcP over-expressing CHO cells.

Figure 3-2: FACS analysis of binding of antibodies in the IgG1f_AEASS format to cynomolgus IL-1RAcP over-expressing CHO cells.

Figure 3-3: As control, antibodies were also tested in titration on parental CHO cells not expressing human or cynomolgus IL-1RAcP.

Figure 4-1: Y024149 was tested for binding to primary human dermal fibroblast expressing endogenous IL-1RAcP, by FACS. EC50 value of binding reported in table.

Figure 5-1: Antibody Y024084 in FabCys-AviHis format coated on the surface, and subsequent binding of reference antibodies.

Figure 5-2: Antibody Y024148 in FabCys-AviHis format coated on the surface, and subsequent binding of reference antibodies.

Figure 5-3: Antibody Y024149 in FabCys-AviHis format coated on the surface, and subsequent binding of reference antibodies.

Figure 5-4: Antibody of prior art CAN04 in FabCys-AviHis format coated on the surface, and subsequent binding of reference antibodies.

Figure 5-5: Antibody of prior art 1A05 in FabCys-AviHis format coated on the surface, and subsequent binding of reference antibodies.

Figure 6-1: Inhibition of signalling by anti-IL1RAcP mAbs in primary human dermal fibroblasts stimulated with 10 nM IL-36 α .

Figure 6-2: Inhibition of signalling by anti-IL1RAcP mAbs in primary human dermal fibroblasts stimulated with 3 nM IL-36 β .

Figure 6-3: Inhibition of signalling by anti-IL1RAcP mAbs in primary human dermal fibroblasts stimulated with 1.5 nM IL-36 γ .

Figure 6-4: Inhibition of signalling by anti-IL1RAcP mAbs in primary human dermal fibroblast stimulated with 2pM IL-1 α .

Figure 6-5: Inhibition of signalling by anti-IL1RAcP mAbs in primary cynomolgus dermal fibroblast stimulated with 1 pM IL-1 β .

Figure 7-1: Inhibition of signalling by anti-IL1RAcP mAbs in primary human keratinocytes stimulated with 60 pM IL-1 β .

- 5 Figure 7-2: Inhibition of signalling by anti-IL1RAcP mAbs in primary human keratinocytes stimulated with 250pM IL-36 β .

Figure 8-1: Inhibition of signalling by anti-IL1RAcP mAbs in human PBMCs stimulated with 50 pM IL-33.

- 10 Figure 8-2: Inhibition of signalling by anti-IL1RAcP mAbs in human PBMCs stimulated with 60 pM IL-1 β .

Figure 9-1: Inhibition of signalling by anti-IL1RAcP mAb Y024148, in human PBMC stimulated with 50 pM IL-33, in presence or absence of increasing concentration of exogenously added sIL-1RAcP.

- 15 Figure 9-2: Inhibition of signalling by anti-IL1RAcP mAb Y024149, in human PBMC stimulated with 50 pM IL-33, in presence or absence of increasing concentration of exogenously added sIL-1RAcP.

Figure 10-1: Inhibition of signal by anti-IL1RAcP mAb Y024149 in human skin explants stimulated with 50 ng/mL IL-1 β . Inhibition of IL36A gene expression is expressed as relative gene expression level and percentage of inhibition.

- 20 Figure 10-2: Inhibition of signal by anti-IL1RAcP mAb Y024149 in human skin explants stimulated with 50 ng/mL IL-1 β . Inhibition of IL23A gene expression is expressed as relative gene expression level and percentage of inhibition.

- 25 Figure 10-3: Inhibition of signal by anti-IL1RAcP mAb Y024149 in human skin explants stimulated with 50 ng/mL IL-1 β . Inhibition of DEFB4 gene expression is expressed as relative gene expression level and percentage of inhibition

Figure 11-1: Inhibition of signal IL-1 β induced IL-6 release by anti-IL1RAcP mAb Y024149 in cynomolgus whole blood stimulated with 50 ng/mL IL-1 β . Donor 1.

Figure 11-2: Inhibition of signal by anti-IL1RAcP mAb Y024149 in cynomolgus whole blood stimulated with 50 ng/mL IL-1 β . Donor 2.

- 30 Figure 12-1: Inhibition of signal by anti-IL1RAcP mAb Y024149 in human whole blood stimulated with 1 ng/mL IL-1 β .

Figure 12-2: Inhibition of signal by anti-IL1RAcP mAb Y024149 in human whole blood stimulated with 5 ng/mL IL-1 β

Figure 12-3: Inhibition of signal by anti-IL1RAcP mAb Y024149 in human whole blood stimulated with 10 ng/mL IL-1 β

5 Figure 13-1: Inhibition of signal by anti-IL1RAcP mAb Y024149 in cynomolgus whole blood stimulated with 300 ng/mL IL-33. Donor 1

Figure 13-2: Inhibition of signal by anti-IL1RAcP mAb Y024149 in cynomolgus whole blood stimulated with 300 ng/mL IL-33. Donor 2

10 Figure 14-1: Plasma concentrations of Y024149 versus time profiles for each of the 9 monkeys during the first dose. Plasma concentration (nM) shown are total soluble mAb Y024149

Figure 14-2: Plasma concentrations of Y024149 versus time profiles for each of the 9 monkeys during the first dose. Data shown are free/unbound soluble Y024149

15 Figure 14-3: Plasma concentrations of Y024149 versus time profiles for each of the 9 monkeys during the first dose. Data shown are total soluble IL-1RAcP

Figure 14-4: Plasma concentrations of Y024149 versus time profiles for each of the 9 monkeys during the first dose. Data shown are free/unbound soluble IL-1RAcP.

Figure 14-5: Data from in vivo i.v. dosing of 6 mg/kg of antibodies to cynomolgus monkeys. Top: Cartoons describing the assay set ups for the different assays used in the cynomolgus
20 PK/PD study. Bottom: Curves showing the data on the amount of free antibody, total antibody and the free soluble IL-1RAcP and total sIL-1RAcP for Y024149, Y024084 and reference R001031 (CAN04 in IgG1f_AEASS format) mAbs.

Definitions

25 The terms "antibody" or "antibodies" refer to immunoglobulin molecules comprising four polypeptide chains: two heavy chains and two light chains. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2, and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of
30 one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with

to the wells and incubated for 30 min with gentle agitation. Subsequently, plates were washed 3x using PBS-T.

For detection 20 µl of a prepared streptavidin-alkaline phosphatase conjugate solution was added to the wells in order to detect bound biotinylated human IL-1RAcP. Incubation

5 happened for 30 min with gentle agitation. Following the incubation with streptavidin-alkaline phosphatase conjugate, assay plates were washed 5x with PBS-T. Then 20 µl of AttoPhos substrate (diluted 1:5 in H₂O) was added to the wells. Plates were incubated for 10 min at RT and readout happened using a Tecan M1000 Pro Reader.

10 The different epitope bins are as follows:

- Bin B: CAN04 with a unique competition profile, potentially sharing parts of the epitope (overlapping epitope) with 1A05 (Bin AB), and Y024084 (Bin F) Bin AB: 1A05 with a unique competition profile, potentially sharing parts of the epitope (overlapping epitope) with CAN04 (Bin B), Y024084 (F), Y024148 and
- 15 Y024149 . Thus 1A05 is potentially sharing parts of the epitope with all tested antibodies.
- Bin A: Y024148 and Y024149, with a unique competition profile, potentially sharing parts of the epitope (overlapping) with 1A05 (Bin AB), Y024148, Y024149,
- Bin C: h11C5 with a unique competition profile. Bin C is clearly discriminated
- 20 from bin B, A and AB.
- Bin F: Y024084 potentially overlapping with parts of Bin AB. No apparent overlap with Bin A.

Figure 5-1 - 5-5 shows ELISA based epitope binning. Every graph represents an individual

25 first antibody in the FabCys-AviHis format coated on the surface. A fixed concentration of biotinylated IL-1RAcP (1.5 nM; AG12210) was pre-incubated with increasing concentrations of the second antibody as FabCys-AviHis for 1h. Subsequently, preformed antigen antibody complex was added to the respective wells with the surface coated first antibody. Detection happened *via* the biotin of IL-1RAcP using a streptavidin-alkaline phosphatase

30 conjugate. The dotted line indicates the signal of an IL-1RAcP unspecific isotype control

anti-IL8 cryptate conjugate were thawed and reconstitution buffer was added to dilute 20-fold. After reconstitution, the two reagents were mixed in a 50 ml centrifuge tube and an equal amount of media was added. 2 µl of supernatant per well was transferred to a detection plate and 4 µl of the freshly prepared mix was added. Plates were
 5 sealed, centrifuged for 30 seconds and incubated at room temperature for 2 hours. Readout was done in an Envision plate reader.

Cynomolgus: Cynomolgus Dermal Fibroblasts (cyDF) (Pelo Biotech cat# PB-CY-423-0811) were suspended in cynomolgus fibroblast growth medium (Pelo Biotech cat# PB-MY-400-900) and seeded (3500 cells/well) in collagen coated 384-well plates. 10 µl of the test
 10 antibodies were added to the cells and incubated for 30 min. Subsequently, 10 µl of cyno IL-1β was added to the cells. The final concentration of cytokine was 1 pM of recombinant cyno IL-1 β (Sino Biological cat# 90010-CNAE). The plate was incubated for 24 hours at 37°C in a humidified incubator. 1 µl supernatant was used to quantify IL-8 by proximity Homogenous Time-resolved Fluorescence
 15 (HTRF, CisBio cat#62HIL08PEH) measuring FRET at 665 nm and normalized to the fluorescence of europium cryptate at 620 nm. The assay was modified from the manufacture instructions and run as follows: equal amounts of anti-IL-8-XL conjugate and anti-IL-8-Cryptate conjugate were thawed and reconstitution buffer was added to dilute 20-fold. After reconstitution, the two reagents were mixed in a 50 ml centrifuge tube and
 20 added in an equal amount of media. 1 µl of supernatant per well was transferred to a detection plate and 4 µl of freshly prepared mix was added. Plates were sealed, centrifuged for 30 seconds and incubated at room temperature for 2 hours. Readout in Envision plate reader.

Figure 6-1- 6-5 showing exemplary results of cell based functional assays utilizing primary
 25 dermal fibroblasts, either human dermal fibroblasts stimulated with 10 nM, 3 nM or 1.5 nM of IL-36α, IL-36β, or IL-36γ respectively and with 2 pM IL-1α or Cynomolgus dermal fibroblasts stimulated with 1 pM IL-1β.

Example 9 signalling measured in Keratinocytes

The Human Epidermal Keratinocytes from adult donor (HEKa) (ATCC®-RCS-200-011™) cells
 30 were suspended in EpiLife growth medium (EpiLife-Cascade Biologics, cat no: MEPI500CA) supplemented with HKGS without hydrocortisone (HKGS-kit, Gibco S-001-K, 1819066) and

seeded (6000 cells/well) in 384-well plates in a volume of 30 μ L. Two hours post plating 10 μ L of the test antibodies were added to the cells and incubated for 30 min. Subsequently 10 μ L of IL-1 β (R&D Systems cat# 201-LB-010) was added to the cells to the final concentration of 0.06 nM. Or 10 μ L of IL-36 β (R&D Systems cat# 6834-ILB) was added to the cells to the

5 final concentration of 0.25 nM . The plate was incubated for 24 hours at 37°C in a humidified incubator. 10 μ L supernatant was used to quantify IL-8 levels by using a cytokine-specific detection antibody labelled with MSD SULFO-TAG™ reagent (Mesoscale cat# K211ANB-2). The assay was run according to the manufacturer instructions.

Results in Figure 7-1-7-2 of cell based functional assays utilizing primary human

10 keratinocytes.

Example 10 signalling in PBMCs

Human IL-1 β induced IFN- γ release in human PBMCs :

The human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats by density centrifugation using Lymphoprep™ (Stemcell cat# 07851). On the assay day,

15 cells were thawed and suspended in medium RPMI 1640 with FCS 10%, P/S 1% (both Life Technologies) and recombinant human IL-12 with a concentration of 0.6 nM (R&D Systems Cat# 219-IL-025). Cells were seeded in 384-well plates (60000 cells/well) in a volume of 30 μ L on the assay day. 10 μ L of the test antibodies were added to the cells and incubated for 30 min. 10 μ L of IL-1 β was added to the cells to a final concentration of 0.05 nM (human IL-1 β ,

20 R&D Systems cat# 201-LB-010). The plate was incubated for 24 hours at 37°C in a humidified incubator. 10 μ L supernatant was used to quantify IFN γ levels by using a cytokine-specific detection antibody labelled with MSD SULFO-TAG™ reagent (Mesoscale cat# K211AEB-2). The assay was run according to the manufacturer instructions.

Human IL-33 induced IFN- γ release in human PBMCs :

25 The cells were suspended in Medium RPMI 1640 (Life Technologies cat# M-106-500) with FCS 10%, P/S 1% (both Life Technologies) and recombinant human IL-12 to a final concentration of 0.6 nM (R&D Systems, Cat# 219-IL-025) and seeded in 384-well plates (50000 cells/well) in a volume of 30 μ L on the assay day. 10 μ L of the test antibodies were added to the cells and incubated for 30 min. 10 μ L of IL-33 was added to the cells in a final

30 concentration of 0.05 nM (human IL-33, R&D Systems cat# 3625-IL-010).

The plate was incubated for 24 hours at 37°C in a humidified incubator. 10 µl supernatant was used to quantify IFN γ levels by using a cytokine-specific detection antibody labelled with MSD SULFO-TAG™ reagent (MesoScale cat# K211AEB-2). The assay was run according to the manufacturer instructions.

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Figure 8-1 and 8-2 shows exemplary results of cell based functional assays utilizing human PBMCs, either stimulated with 50 pM IL-33 or 50pM IL-1 β .

Example 11 sIL-1RAcP

10 In vivo the extracellular soluble form of IL-1RAcP (sIL-1RAcP) also exists due to alternative splicing. It is abundantly present in circulation at levels around 8-15 nM. The anti-IL-1RAcP antibody will also bind the soluble form of IL-1RAcP. Therefore, the assessment of the functional inhibition of anti-IL-1RAcP antibody in PBMCs in the presence of low and high level of sIL1RAcP protein was performed and demonstrated that there was still full
15 inhibition of the IL-33-mediated IFN γ release, and that the full inhibition happend at roughly the same concentration, even though the IC₅₀ values were higher with the presence of sIL1RAcP.

Exemplary results in Figure 9-1 and 9-2 of cell based functional assays utilizing human PBMCs, stimulated with 50 pM IL-33 in the presence of 0, 2 or 20 nM soluble IL-1RAcP (sIL-
20 1RAcP).

The antibodies were compared in various assays side by side with the three reference antibodies CAN04, 1A05 and h11C5. Exemplary curves are displayed in Figures 6-1 to 6-5 (assays using human or cynomolgus primary dermal fibroblasts stimulated with human IL-1 α , human IL36 γ , and cynomolgus IL-1 β) and figure 7-1 and 7-2 (assays utilizing primary
25 human keratinocytes stimulated with IL-1 β and IL-36 β). Additionally, the inhibitory effect in human PBMCs stimulated with IL-33 and IL-1 β shown in Figures 8-1 and 8-2. The overview of all assays with an average IC₅₀ value (nM) is displayed in table. All present disclosure antibodies do inhibit signalling with an E_{max} of 100%. In most assays all present disclosure antibodies are superior or similar to the reference antibodies. The direct comparison of the
30 IC₅₀ values of the reference 1A05 and the project antibodies is displayed in Table 2.

the level of IL-6 by using the specific U-plex NHP IL-6 MSD assay (Meso Scale Discovery, Cat# K156TXK-2) accordingly to the manufacturer instructions.

Results:

The functional blockade of IL-1 β induced IL-6 release in cynomolgus whole blood was demonstrated for Y024149. Using whole blood from eight different donors (n=8), Y024149 showed to fully inhibit the release of IL-6 induced by stimulation with 50 ng/ml of rIL-1 β with an IC50 of ~50 nM, with maximal inhibition at ~100 nM. Figure 11-1 and 11-2)

Example 14 Human IL-1 β induced IL-6 release in human whole blood

Human whole blood samples (100 μ l) were seeded in a 96 well culture plate (100 μ l/well) containing 50 μ l/well of RPMI 1640 medium (Gibco, Cat# 42401018) conditioned with 1% P/S and with increasing concentration of test antibody Y024149 or vehicle control. The plate was incubated for 30 minutes at 37°C in a humidified incubator. Subsequently, the samples were stimulated by the addition of 50 μ l/well of RPMI 1640 medium conditioned with 1% P/S and rhIL-1 β at the final concentration of 1 ng/ml, 5 ng/ml and 10 ng/ml (rhIL-1 β from R&D systems, Cat.#201-LB). Unstimulated vehicle control samples were also included. The samples were finally incubated for 22 hours at 37°C in a humidified incubator. At the termination time point, 100 μ l of diluted plasma samples were harvested for each sample by the means of centrifugation at 500 x g for 10 minutes at room temperature and assayed for the level of IL-6 by using the specific U-plex Human IL-6 MSD assay (Meso Scale Discovery, Cat# K151TXK) accordingly to the manufacturer instructions.

Results:

The functional blockade of IL-1 β induced IL-6 release in human whole blood was demonstrated for Y024149. Using whole blood from three different donors (n=3), Y024149 showed to fully inhibit the release of IL-6 induced by stimulation with 1 ng/ml, 5 ng/ml and 10 ng/ml of rhIL-1 β with an IC50 of ~10 nM, ~8 nM and ~20 nM, respectively, with maximal inhibition at 50-100 nM. (Figure 12-1, 12-2 and 12-3)

Example 15: Cynomolgus IL-33 induced IFN- γ release in cynomolgus whole blood.

Cynomolgus whole blood samples (100 μ l) were seeded in a 96 well culture plate (100

5 $\mu\text{l/well}$) containing 50 $\mu\text{l/well}$ of RPMI 1640 medium (Gibco, Cat# 42401018) conditioned with 1% P/S and with increasing concentration of test antibody Y024149 or vehicle control. The plate was incubated for 30 minutes at 37°C in a humidified incubator. Subsequently, the samples were stimulated by the addition of 50 $\mu\text{l/well}$ of RPMI 1640 medium conditioned with 1% P/S, ascorbic acid, rhIL-12 (R&D Systems, Cat# 219-IL-005) and rIL-33 (Sino Biological Cat# 90912-CNAE) at the final concentration of 9 $\mu\text{g/ml}$, 150 ng/ml and 300 ng/ml , respectively. Unstimulated vehicle control samples were also included. The samples were finally incubated for 22 hours at 37°C in a humidified incubator. At the termination time point, 100 μl of diluted plasma samples were harvested for each sample by the means of
 10 centrifugation at 500 x g for 10 minutes at room temperature and assayed for the level of IFN- γ by using the specific customized U-plex HNP IFN- γ MSD assay (Meso Scale Discovery, Cat# K15068L) accordingly to the manufacturer instructions.

Results:

15 The functional blockade of IL-33 induced IFN- γ release in cynomolgus whole blood was demonstrated for Y024149. Using whole blood from two different donors (n=2), Y024149 showed to fully inhibit the release of IFN- γ induced by stimulation with 300 ng/ml of rIL-33 with an IC₅₀ of ~15 nM, with maximal inhibition at 30 nM. (Figure 13-1 and 13-2 Functional concentration dependent blockade of cynomolgus IL-33 induced IFN- γ release in
 20 cynomolgus whole blood from two donors.)

Example 16 Cynomolgus PK included on CAN04, 084 and antibodies of the disclosure

25 The monkeys were dosed IV with vehicle (n=2), 0.6, 2.0, 6.0, 20 and 60 mg/kg respectively (all n=1) as well as SC with 20 mg/kg (n=2). The formulations were made in PBS pH 7.1 and given as a slow bolus over 5 minutes. Four different ligand binding assays were used for PK analysis of the soluble target and soluble drug candidate.

Target mediated drug disposition (TMDD) dominated the clearance/distribution of drug especially at plasma concentrations of Y024149 below 100 nM in the cynomolgus monkeys,
 30 whereas a long half-life was seen for the linear phase when the target was saturated.

Plasma concentrations of Y024149 versus time profiles for each of the 9 monkeys during the first dose. Plasma concentration (nM) shown are total soluble mAb Y024149 (Figure 14-1), free/unbound soluble Y024149 (Figure 14-2), total soluble IL-1RAcP (Figure 14-3) and free/unbound soluble IL-1RAcP (Figure 14-4).

5 Comparison in vivo

The monkeys were dosed IV with vehicle (n=2), 6.0 mg/kg of different antibodies, Y024149, or Morphosys 24084 or CAN04 on a Morphosys IgG backbone (all n=1). The formulations were made in PBS pH 7.1 and given as a slow bolus over 5 minutes. Four different ligand binding assays were used for PK analysis of the soluble target and soluble drug

10 candidate. This clearly demonstrates a better exposure and occupancy of the soluble target by Y024149 compared to both CAN04 (R001031) and Mor Y024084. Additionally when compared to the data published for the antibody h11C5 the exposure and occupancy of Y024149 is similar to the data for the half life extended version of the h11C5 antibody (YTE).

Results in Figure 14-5.

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Incorporation by Reference

Various publications are cited in the current description and throughout the examples. The content of each publication is incorporated by reference herein in its entirety.

20 The present disclosure is not to be limited in scope by the specific embodiments or examples described herein. Indeed, various modifications of the disclosure in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Moreover, all embodiments described herein are considered
25 to be broadly applicable and combinable with any and all other consistent embodiments, as appropriate.

References: