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(56) Documents Cited:
US 20050142108 A
Cancer Immunology Immunotherapy (2007); Vol 56, pp 205-215, "Human interleukin 24 (MDA-7/IL-24) protein...", Zheng et al
Molecular Therapy (2005); VOl 11, pp 724-733, "mda-7/IL24 kills pancreatic cancer cells ...", Chada et al
Molecular Human Reproduction (2013); Vol 19, pp 655-664, "Estrogen promotes the growth of decidual...", Shao et al
Immunology (2005); Vol 114, pp 166-170, "Interleukin-24 and its receptors", Wang & Liang

(58) Field of Search:
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Other: **WPI, EPODOC, BIOSIS, MEDLINE, CAS-ONLINE, BLASTP**

(54) Title of the Invention: **Antibodies to IL-24**
Abstract Title: **Antibodies to IL-24**

(57) Monoclonal antibodies or antigen binding fragments thereof which bind human Interleukin-24 (IL-24) protein and block IL-24 signalling through the IL-22R1/IL-20R2 receptor complex may be used to treat dermatological and/or inflammatory conditions. Such dermatological conditions may include atopic dermatitis, contact dermatitis, contact hypersensitivity, plaque psoriasis and pustular psoriasis, while the inflammatory conditions may include IBD, colitis, endotoxemia, arthritis, rheumatoid arthritis, psoriatic arthritis, adult respiratory disease (ARD), septic shock, multiple organ failure, inflammatory lung injury such as asthma or bronchitis, bacterial pneumonia, psoriasis, eczema, ulcerative colitis and Crohn's disease. The invention also relates more generally to the treatment of such dermatological and/or inflammatory conditions with an IL-24 antagonist.

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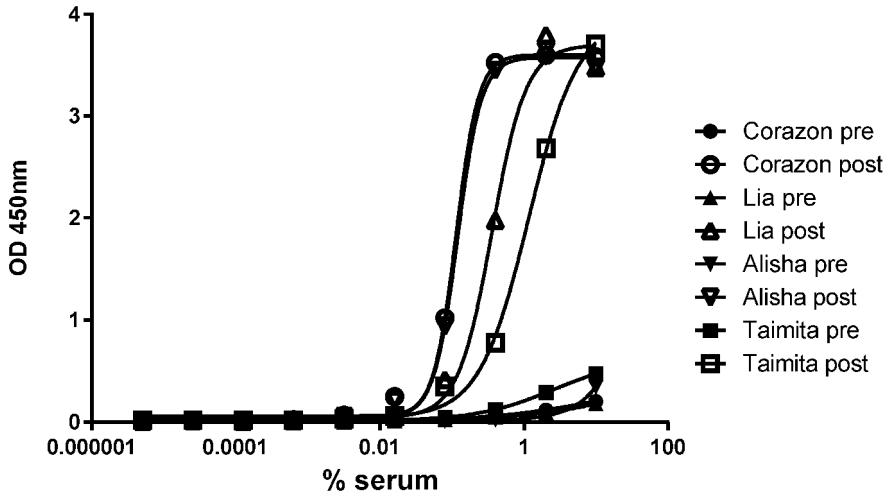
At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Fig. 1

A



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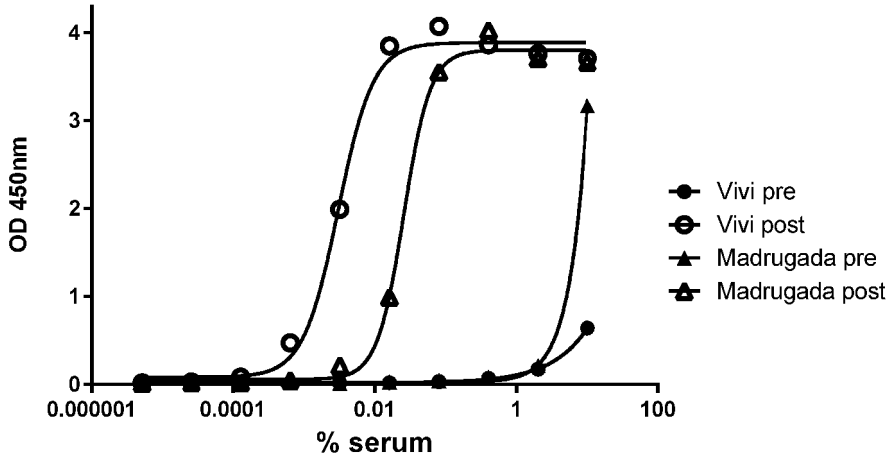
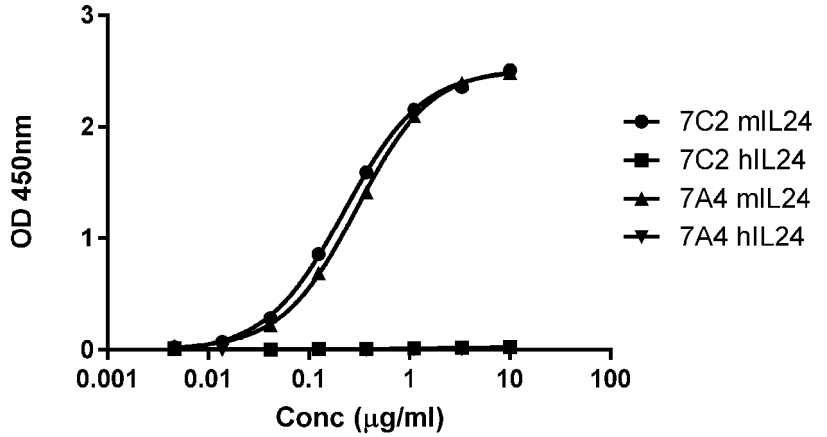


Fig. 2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2
1
0
0.001
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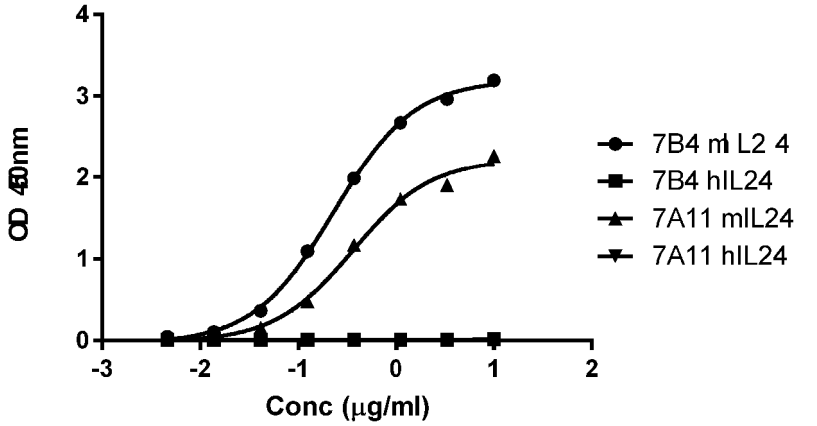
7C2 hIL24
7A4 mIL24
7A4 hIL24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3
2
1
0
-3
-2
-1
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1
2
Conc (μg/ml)
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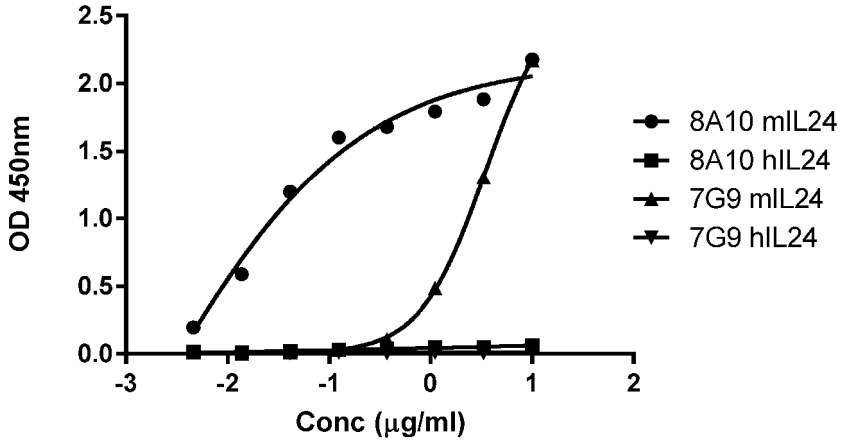
7B4 hIL24
7A11 mIL24
7A11 hIL24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1.5
1.0
0.5
0.0
-3
-2
-1
0
1
2
Conc (μg/ml)
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8A10 hIL24
7G9 mIL24
7G9 hIL24

Fig. 2 continued

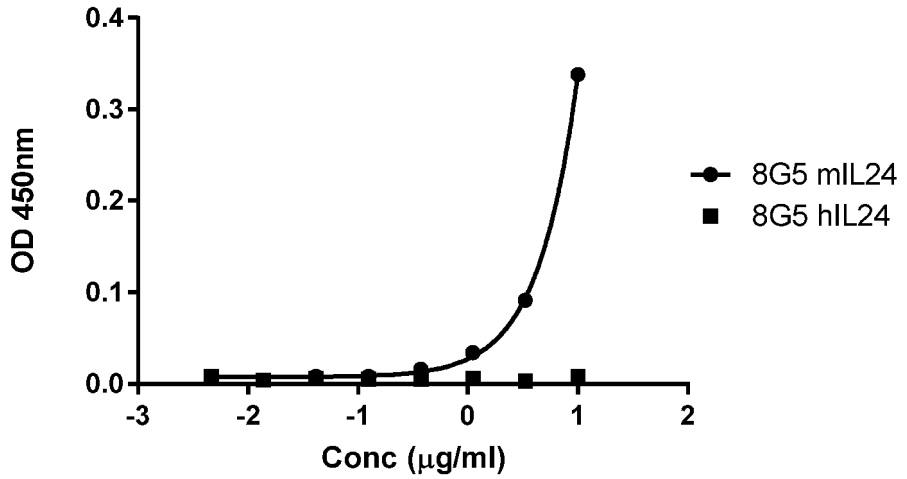
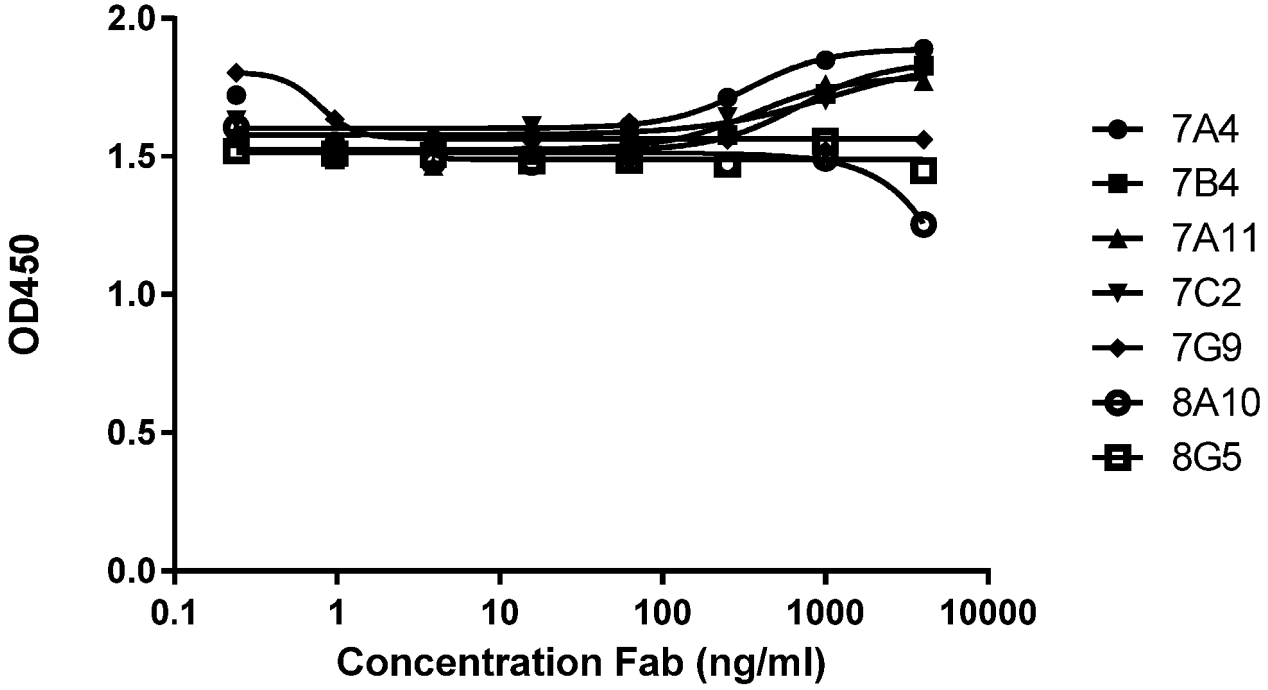


Fig. 3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0.5
0.0
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1
10
100
1000
10000
Concentration Fab (ng/ml)
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7A11
7C2
7G9
8A10
8G5

Fig. 4

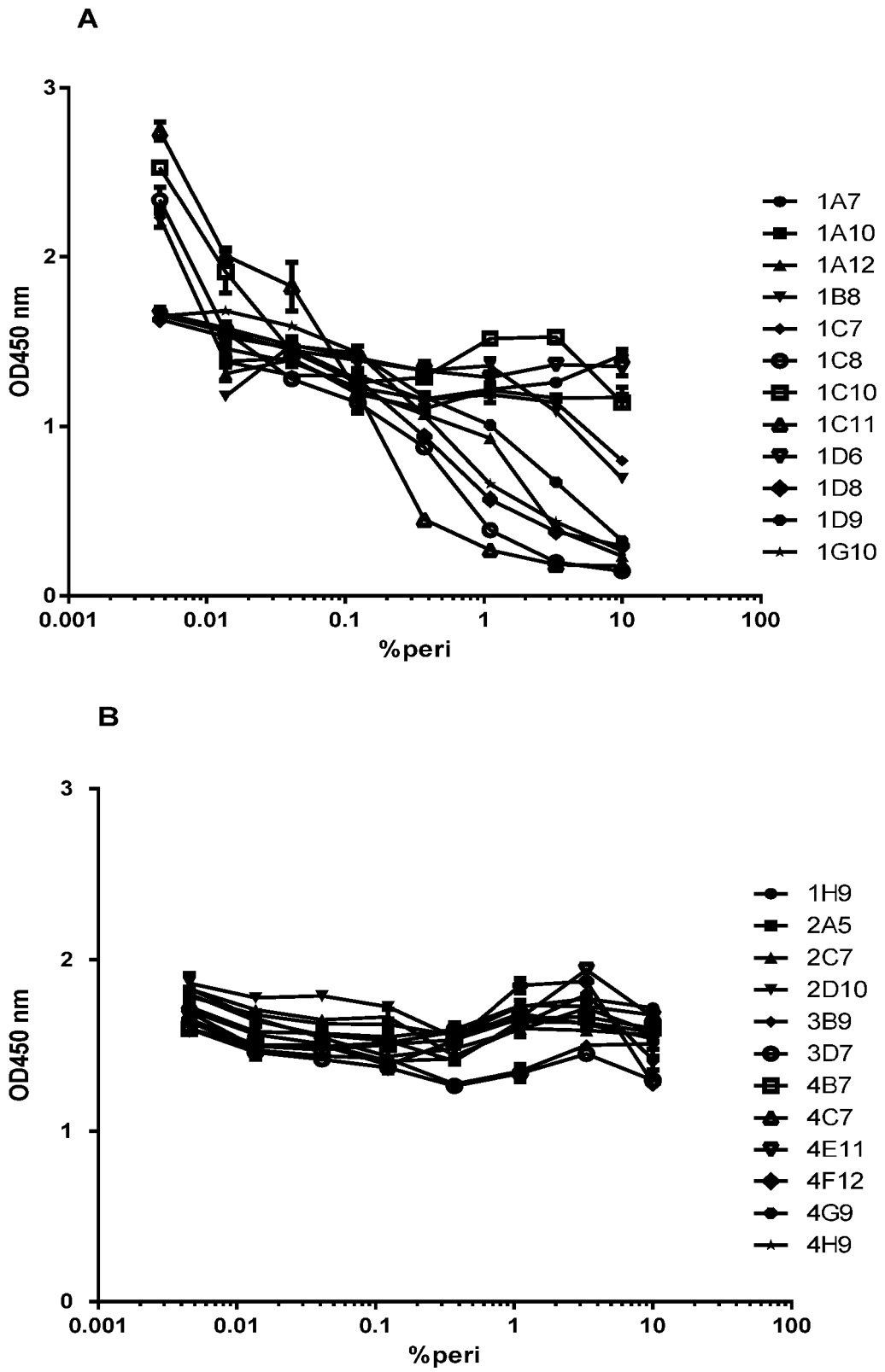
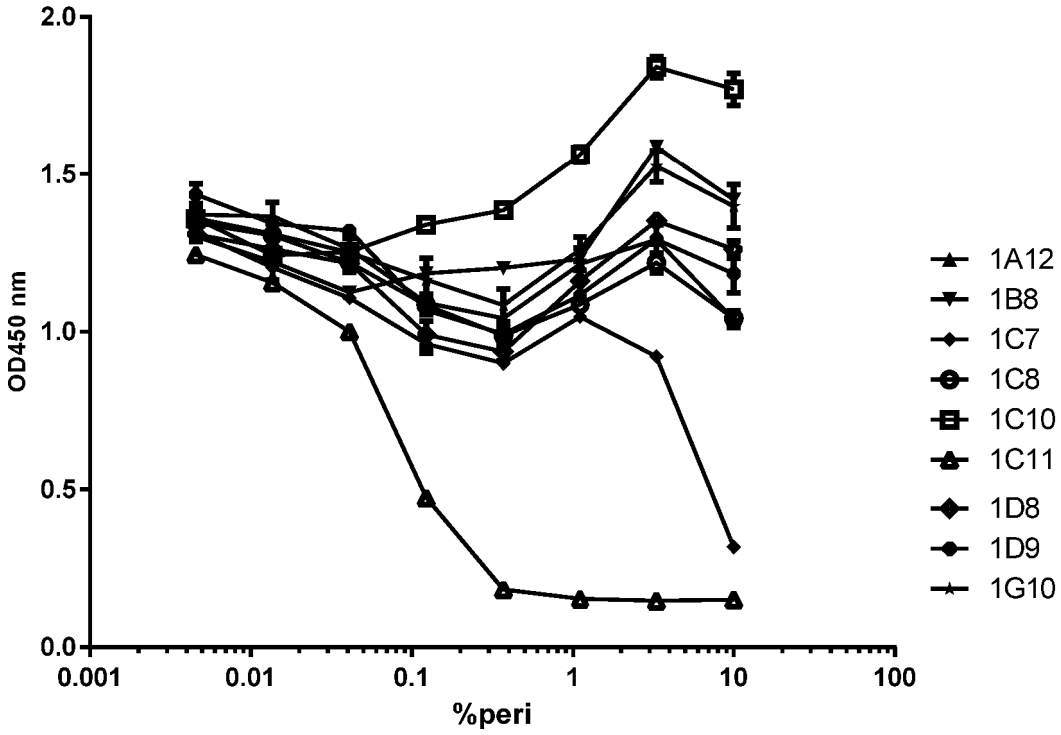

A
OD450 nm
%peri
0.001
0.01
0.1
1
10
100
0
1
2
3
1A7
1A10
1A12
1B8
1C7
1C8
1C10
1C11
1D6
1D8
1D9
1G10
B
OD450 nm
%peri
1H9
2A5
2C7
2D10
3B9
3D7
4B7
4C7
4E11
4F12
4G9
4H9

Fig. 5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1.0
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0.0
OD450 nm
0.001
0.01
0.1
1
10
100
%peri
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1B8
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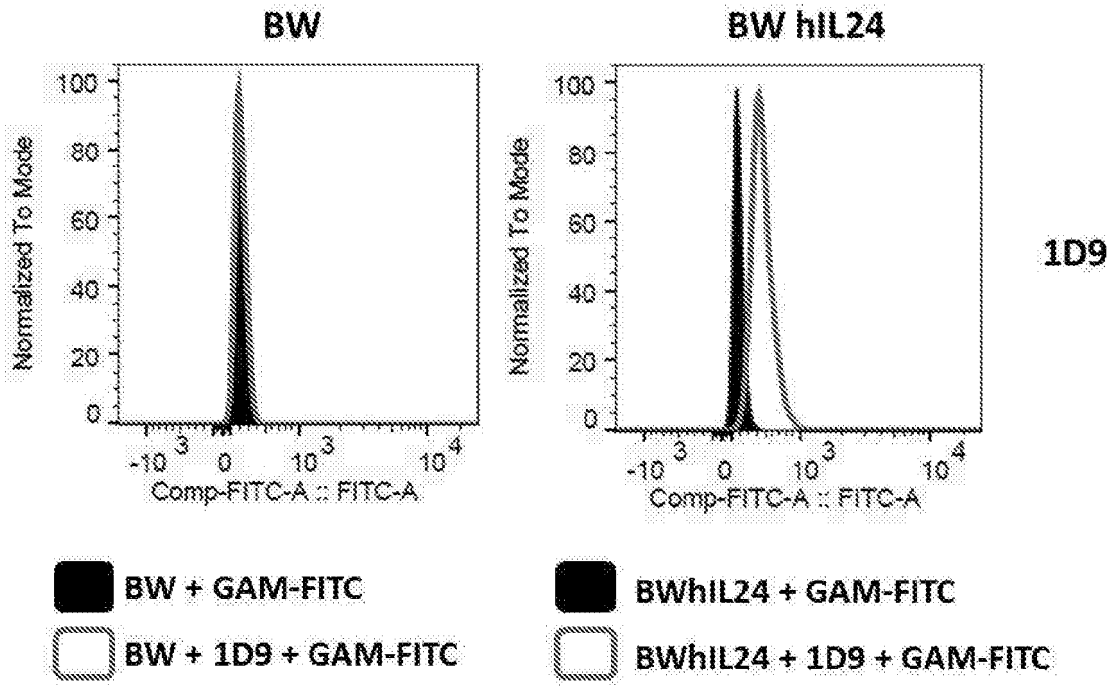
1C8
1C10
1C11
1D8
1D9
1G10

Fig. 6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BW hIL24
Normalized To Mode
100
80
60
40
20
0
-10 3
0
10 3
10 4
Comp-FITC-A :: FITC-A
1D9
BW + GAM-FITC
BW + 1D9 + GAM-FITC
BWhIL24 + GAM-FITC
BWhIL24 + 1D9 + GAM-FITC

Fig. 6 (continued)

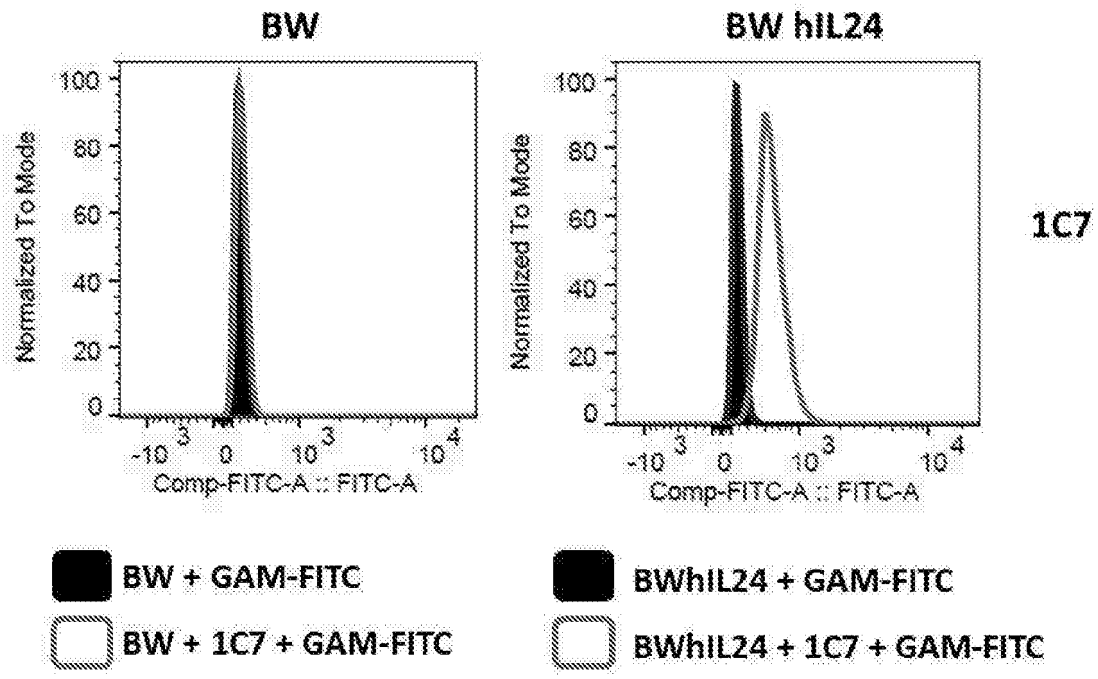


Fig. 6 (continued)

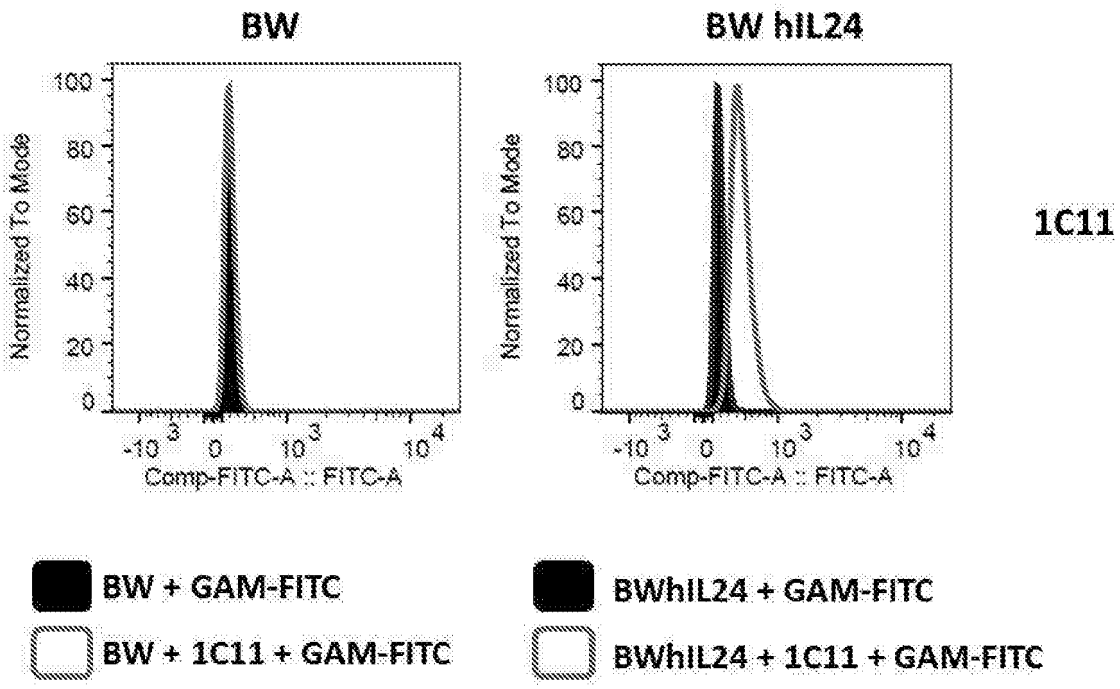


Fig. 6 (continued)

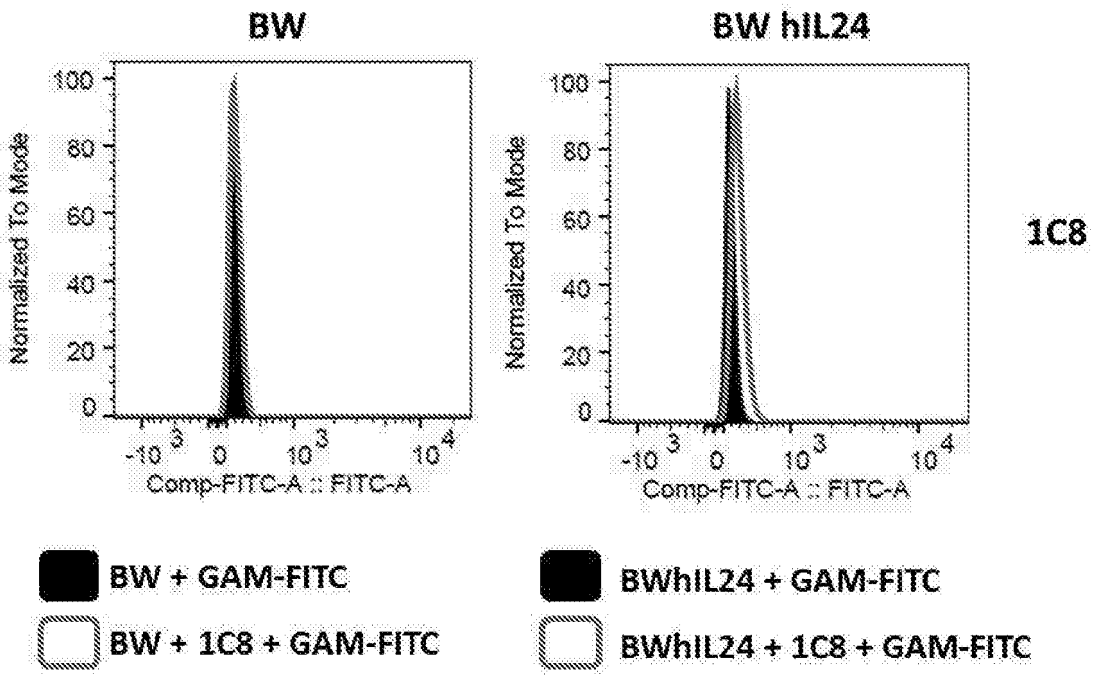
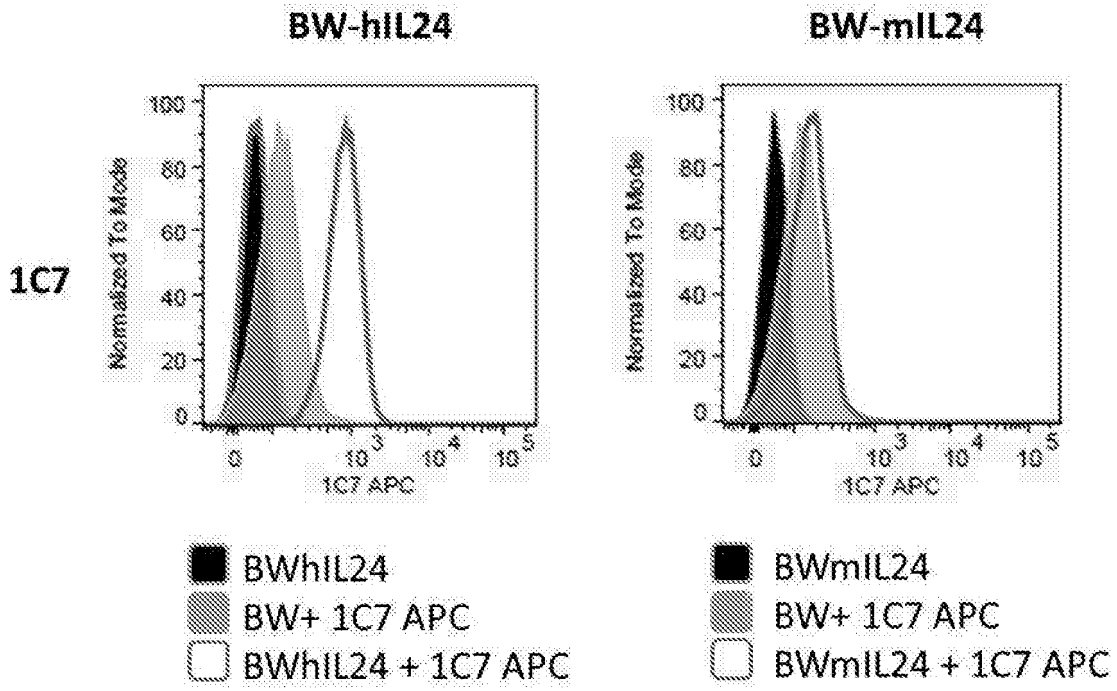


Fig. 7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BW-mIL24
1C7
Normalized To Mode
100
80
60
40
20
0
0
10^3
10^4
10^5
1C7 APC
BWhIL24
BW+ 1C7 APC
BWhIL24 + 1C7 APC
BWmIL24
BW+ 1C7 APC
BWmIL24 + 1C7 APC

Fig. 7 (continued)

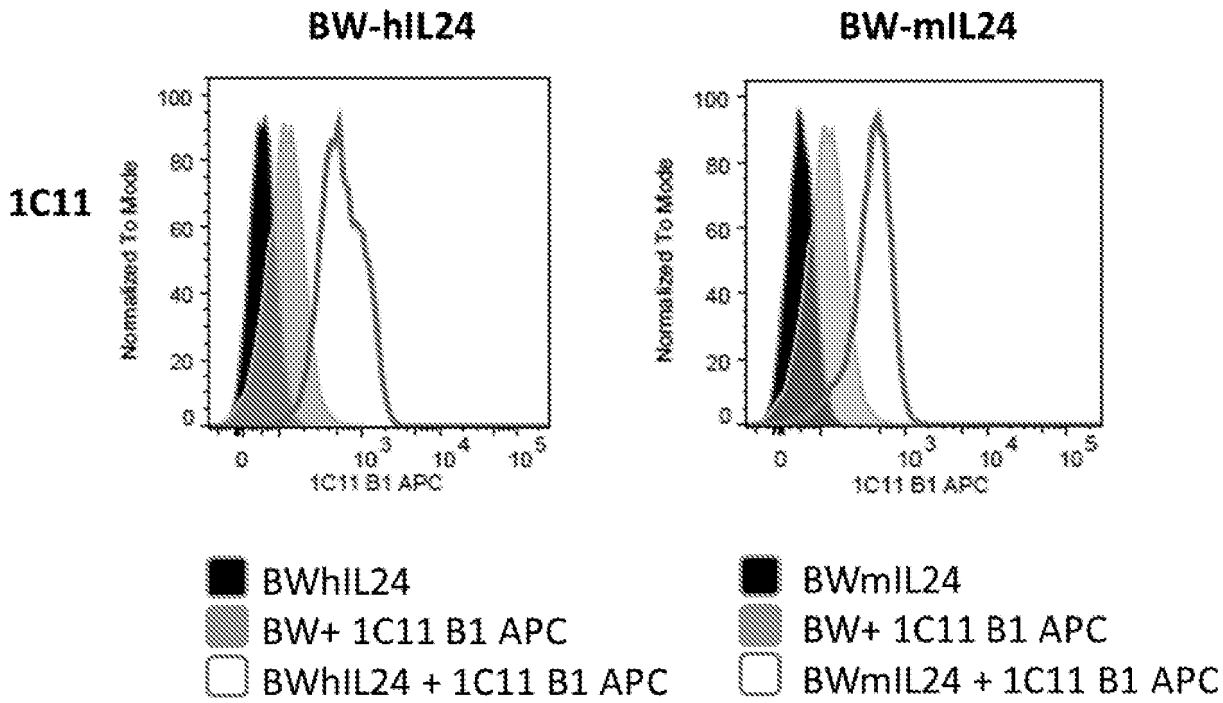
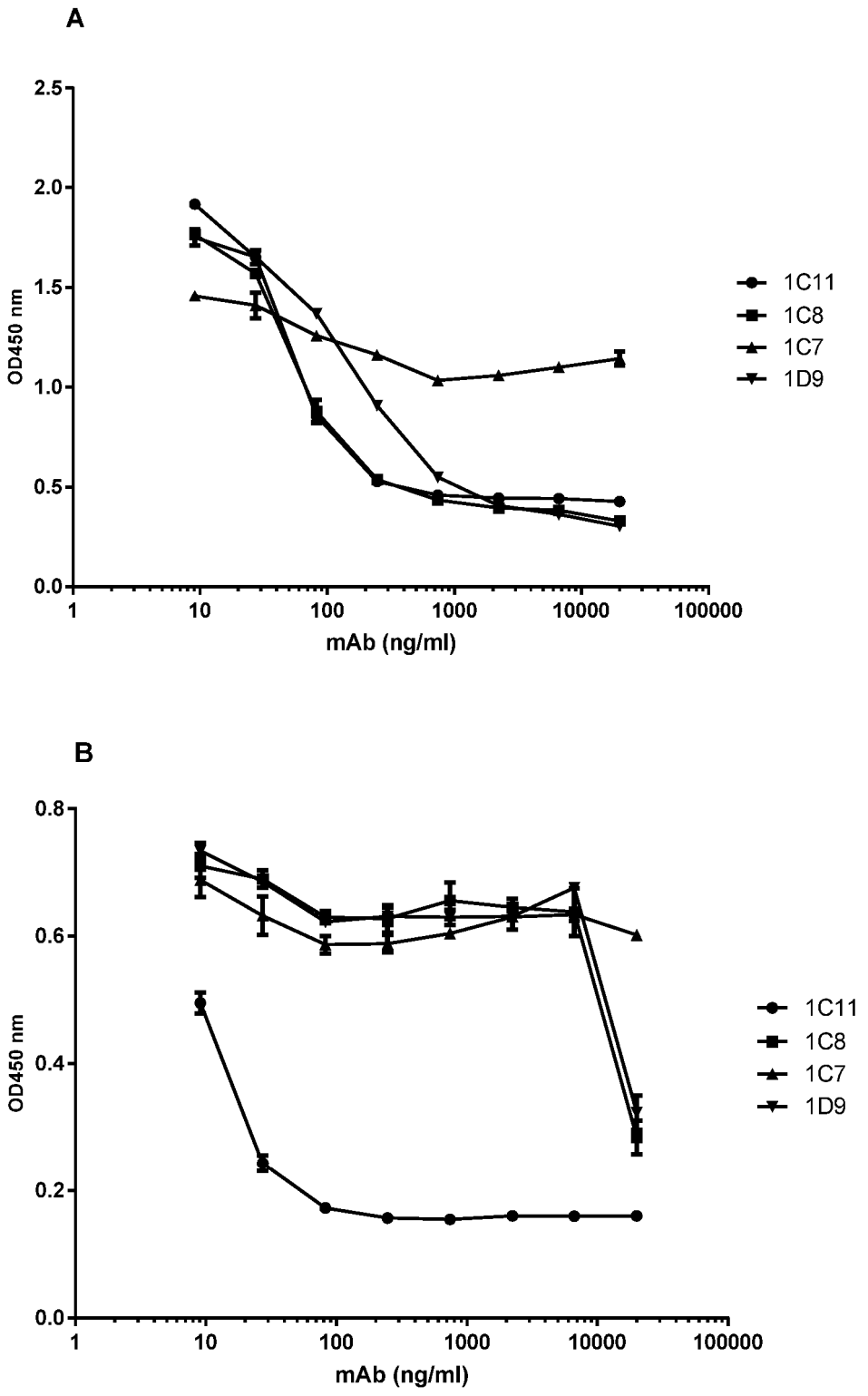


Fig. 8


A
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2.0
1.5
1.0
0.5
0.0
OD450 nm
1
10
100
1000
10000
100000
mAb (ng/ml)
1C11
1C8
1C7
1D9
B
0.8
0.6
0.4
0.2
0.0
OD450 nm
1
10
100
1000
10000
100000
mAb (ng/ml)
1C11
1C8
1C7
1D9

Fig. 9

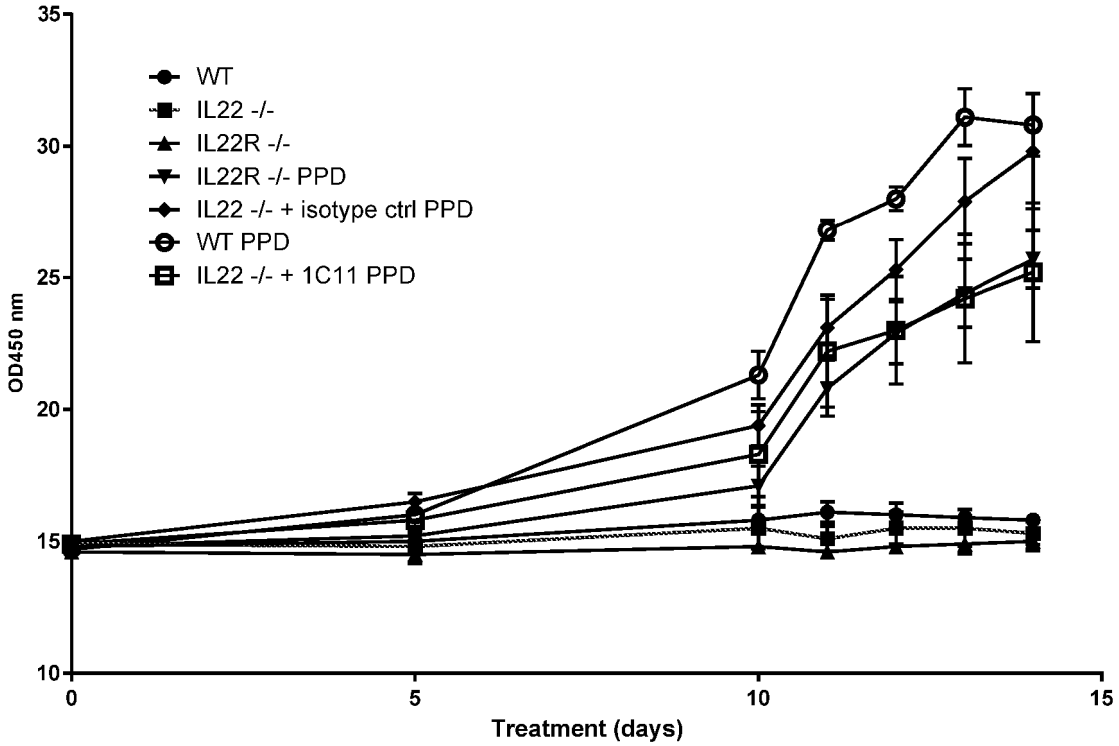
WT
IL22 -/-
IL22R -/-
IL22R -/- PPD
IL22 -/- + isotype ctrl PPD
WT PPD
IL22 -/- + 1C11 PPD
OD450 nm
35
30
25
20
15
10
0
5
10
15
Treatment (days)

Fig. 10

>hIL-24 DNA (SEQ ID NO:301)
ATGAATTTTCAACAGAGGCTGCAAAGCCTGTGGACTTTAGCCAGACCCTTCTGCCCTCCT
TTGCTGGCGACAGCCTCTCAAATGCAGATGGTTGTGCTCCCTTGCCTGGGTTTTACCCTG
CTTCTCTGGAGCCAGGTATCAGGGGCCCAGGGCCAAGAATTCCACTTTGGGCCCTGCCAA
GTGAAGGGGGTTGTTCCCCAGAAACTGTGGGAAGCCTTCTGGGCTGTGAAAGACACTATG
CAAGCTCAGGATAACATCACGAGTGCCCGGCTGCTGCAGCAGGAGGTTCTGCAGAACGTC
TCGGATGCTGAGAGCTGTTACCTTGTCCACACCCTGCTGGAGTTCTACTTGAAAACTGTT
TTCAAAAACTACCACAATAGAACAGTTGAAGTCAGGACTCTGAAGTCATTCTCTACTCTG
GCCAACAACTTTGTTCTCATCGTGTCACAACTGCAACCCAGTCAAGAAAATGAGATGTTT
TCCATCAGAGACAGTGCACACAGGCGGTTTCTGCTATTCCGGAGAGCATTCAAACAGTTG
GACGTAGAAGCAGCTCTGACCAAAGCCCTTGGGGAAGTGGACATTCTTCTGACCTGGATG
CAGAAATTCTACAAGCTC

>hIL-24 protein (SEQ ID NO:302)
MNFQQRLQSLWTLARPFCPPLLATASQMQMVVLPCLGFTLLLWSQVSGAQGQEFHFGPCQVKGVVPQKLW
EAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLVHTLLEFYLKTVFKNYHNRTVEVRTLKSFSTL
ANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLFRRAFKQLDVEAALTKALGEVDILLTWMQKFYKL

>mIL-24 DNA (SEQ ID NO:303)
ATGCTGACTGAGCCTGCCCAACTTTTTGTGCACAAGAAGAACCAGCCACCTTCACACAGC
AGCCTCCGGCTTCACTTTAGGACCCTAGCAGGAGCACTGGCCCTTTCTTCAACACAGATG
AGTTGGGGACTACAGATTCTCCCCTGCCTGAGCCTAATCCTTCTTCTTTGGAACCAAGTG
CCAGGGCTTGAGGGTCAAGAGTTCCGATTTGGGTCTTGCCAAGTGACAGGGGTGGTTCTC
CCAGAACTGTGGGAGGCCTTCTGGACTGTGAAGAACACTGTGCAAACTCAGGATGACATC
ACAAGCATCCGGCTGTTGAAGCCGCAGGTTCTGCGGAATGTCTCGGGTGCTGAGAGCTGT
TACCTTGCCCACAGCCTGCTGAAGTTCTACTTGAACACTGTTTTCAAGAACTACCACAGC
AAAATAGCCAAATTCAAGGTCTTGAGGTCATTCTCCACTCTGGCCAACAACTTCATAGTC
ATCATGTCACAACTACAGCCCAGTAAGGACAATTCCATGCTTCCCATTAGTGAGAGTGCA
CACCAGCGGTTTTTGCTGTTCCGCAGAGCATTCAAACAGTTGGATACAGAAGTCGCTTTG
GTGAAAGCCTTTGGGGAAGTGGACATTCTCCTGACCTGGATGCAGAAATTCTACCATCTC

>mIL-24 protein (SEQ ID NO:304)
MLTEPAQLFVHKKNQPPSHSSLRLHFRTLAGALALSSTQMSWGLQILPCLSLILLLWNQVPGLEGQEFRF
GSCQVTGVVLPELWEAFWTVKNTVQTQDDITSIRLLKPQVLRNVSGAESCYLAHSLLKFYLNTVFKNYHS
KIAKFKVLRSFSTLANNFIVIMSQLQPSKDNSMLPISESAHQRFLLFRRAFKQLDTEVALVKAFGEVDIL
LTWMQKFYHL

Alignment of proteins – 58% identity on protein level

```
hIL-24                1 ------------MNFQQRLQSLWTLARPFCPPLLATASQMQ--MVVLPCL
mIL-24                1 MLTEPAQLFVHKKNQPPSHSSLRLHFRTLAGALALSSTQMSWGLQILPCL

hIL-24              109 GFTLLLLWSQVSGAQGQEFHFGPCQVKGVVPQKLWEAFWAVKDTMQAQDNI
mIL-24              151 SLILLLLWNQVPGLEGQEFRFGSCQVTGVVLPELWEAFWTVKNTVQTQDDI

hIL-24              259 TSARLLQQEVLQNVSDAESCYLVHTLLEFYLKTVFKNYHNRTVEVRTLKS
mIL-24              301 TSIRLLKPQVLRNVSGAESCYLAHSLLKFYLNTVFKNYHSKIAKFKVLRS

hIL-24              409 FSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLFRRAFKQLDVEAAL
mIL-24              451 FSTLANNFIVIMSQLQPSKDNSMLPISESAHQRFLLFRRAFKQLDTEVAL

hIL-24              559 TKALGEVDILLTWMQKFYKL
mIL-24              601 VKAFGEVDILLTWMQKFYHL
```

Antibodies to IL-24

Technical Field

The present invention relates to antibodies and antigen binding fragments thereof which exhibit high affinity binding to the human IL-24 protein and also exhibit potent blocking of IL-24 signalling through the IL-22R1/IL-20R2 and/or the IL20R1/IL-20R2 receptor complex.

Background

IL-24 is a member of the IL-10 family of cytokines that is capable of signalling though two heterodimeric receptors; IL- 20R1/IL-20R2 and IL-22R1/IL-20R2. In terms of biological function, IL-24 belongs to the subgroup of IL-20-related cytokines, which are produced by immune cells such as monocytes and T lymphocytes and are involved in the maintenance of integrity of the epidermal barrier by promoting antimicrobial peptides production, chemokine expression and keratinocyte proliferation. These cytokines play redundant roles because they share common receptor chain complexes. IL-19, IL-20 and IL-24 can bind to "type I IL-20 receptor" composed of IL- 20R1 and IL-20R2. The "type II IL-20 receptor" consists of IL-22R1 and IL-20R2 and binds IL-20 and IL- 24. Finally, IL-22 signals through a complex composed of IL-22R1 subunit and IL-10R2. Despite the fact that the biological activities of IL-20-related cytokines are beneficial during wound healing or pathogen invasion, these cytokines might play a detrimental role in inflammatory skin disorders.

In vivo, IL-24 is mainly expressed by skin tissue cells during inflammatory conditions, such as psoriasis. In addition, IL-24 gene expression is elevated at the edge of excisional skin wounds. Also in other inflammatory diseases, the expression of IL-24 seems to be correlated with disease stage. In patients with active Crohn's disease, gene expression of IL-24 was found to be increased in biopsies compared to inactive disease. Skin lesions from psoriatic patients show an upregulation of the IL-20-related cytokines, and it has been demonstrated that IL-22 is implicated in keratinocyte proliferation and dedifferentiation as well as neutrophil infiltration in a mouse model of psoriasis. In addition, transgenic mice for IL-20, IL-22 and IL-24 but not IL-19 display a thickened skin due to acanthosis, demonstrating the role of IL-22R-binding cytokines in this skin inflammatory disorder. In allergic contact dermatitis (ACD), very little is known about

the role of IL-20-related cytokines. IL-22 is found in the serum of nickel-allergic patients, as well as produced by CD4+ T lymphocytes that are present in the skin of nickel-allergic patients. An increase of IL-22 expression in the skin has been reported in a murine contact hypersensitivity (CHS) model induced by trinitrochlorobenzene (TNCB). In addition, the expression of IL-19 and IL-24 is also increased in a mouse model of CHS induced by dinitrofluorobenzene (DNFB). In another model of TNCB-induced CHS, it was shown that IL-20R2 deficient mice were more affected than WT mice, suggesting that one or more of the cytokines IL-19, IL-20 and IL-24 play a protective role.

In view of the biological function of IL-24, inhibition of IL-24 signalling is postulated to be of therapeutic benefit in the treatment of dermatological conditions, such as atopic dermatitis, contact dermatitis, contact hypersensitivity, plaque psoriasis and pustular psoriasis. Accordingly, there is a need for pharmaceutical agents that are capable of inhibiting IL-24 signalling through one, or preferably both, of its native receptors.

Summary of the invention

In a first aspect of the invention there is provided an antibody, or an antigen binding fragment thereof that binds to human IL-24 and blocks the interaction between IL-24 and the IL-22R/IL-20R2 receptor complex and/or inhibits IL-24 signalling through the IL-22R/IL-20R2 receptor complex.

Preferred embodiments of the IL-24 antibodies provided herein may, as a consequence of binding to human IL-24, exhibit potent blocking of the interaction between IL-24 and the IL-22R/IL-20R2 receptor complex. The IL-24 antibodies may additionally, or alternatively, inhibit of IL-24 signalling through the IL-22R/IL-20R2 receptor complex.

Antibodies, or antigen binding fragments thereof, with these properties are referred to herein generally as "IL-24 antibodies".

The preferred IL-24 antibodies which exhibit *both* binding to human IL-24 *and* potent blocking of the interaction between human IL-24 and the human IL-22R/IL-20R2 and/or the IL-20R1/IL-20R2 receptor complex and/or potent inhibition of IL-24 signalling through the IL-22R/IL-20R2 and/or the IL-20R1/IL-20R2 receptor complex, are particularly advantageous as therapeutic agents for treatment of dermatological conditions in which IL-24 signalling through IL-22R/IL-20R2 or IL20R1/IL-20R2 plays a

role; including but not limited to atopic dermatitis, contact dermatitis, contact hypersensitivity, plaque psoriasis and pustular psoriasis.

Not all of the IL-24 antibodies described herein exhibit blocking of the interaction between human IL-24 and the human IL-22R/IL-20R2 receptor complex as a consequence of binding to IL-24. Also described herein are a number of IL-24 antibodies which exhibit high affinity binding to IL-24 but do not show significant blocking of the interaction between IL-24 and IL-22R/IL-20R2. The properties of these antibodies are described elsewhere herein. The availability of high affinity non-blocking IL-24 antibodies may enhance/extend the range of therapeutic possibilities.

Preferred embodiments of the IL-24 antibodies described herein, exhibiting high binding affinity for human IL-24, may also exhibit cross-reactivity with the murine IL-24 homolog. Cross-reactivity with murine IL-24 is advantageous in the context of human drug development, since it enables studies of IL-24 antibodies proposed for human therapeutic use to be carried out in mouse models (e.g. pre-clinical studies, investigations of disease mechanism, toxicology, etc.).

Given the relatively low homology between human IL-24 and murine IL-24 (58% sequence identity), there is no reasonable expectation that antibodies exhibiting high affinity binding to human IL-24 will also cross-react with murine IL-24. In fact, as shown in the accompanying examples, first attempts at raising cross-reactive antibodies by immunisation of llamas with murine IL-24 resulted in antibodies immunoreactive with murine IL-24, but failed to produce any antibodies cross-reactive with human IL-24. It was therefore surprising that cross-reactive antibodies could be raised by immunisation of llamas with human IL-24.

The IL-24 antibodies provided herein may be isolated or recombinantly expressed monoclonal antibodies. The IL-24 antibodies provided herein may be camelid-derived (for example llama-derived). Preferred embodiments may be a humanised (or germlined) monoclonal antibody (e.g. a humanised variant of a camelid-derived antibody), a chimeric antibody (e.g. a camelid-human chimeric antibody) or a humanised chimeric antibody (e.g. a chimeric antibody comprising humanised variants of camelid VH and VL domains and constant domains of a human antibody).

Camelid-derived IL-24 antibodies may comprise at least one hypervariable loop or complementarity determining region obtained from a VH domain or a VL domain of a species in the family *Camelidae*. In a particular embodiment, the IL-24 antibody, or

antigen binding fragment thereof, may comprise a heavy chain variable domain (VH) and light chain variable domain (VL), wherein the VH and VL domains, or one or more CDRs thereof, are camelid-derived. In particular embodiments the antibody or antigen binding fragment thereof may comprise llama VH and VL domains, or human germlined variants of llama VH and VL domains.

The camelid-derived IL-24 antibodies described herein typically exhibit VH and/or VL region amino acid sequences having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity the closest matching human antibody germline sequence.

Further preferred embodiments of the invention include humanised (or human germlined) variants of the camelid-derived IL-24 antibodies. In particular, the invention provides humanised or human germlined variants of the llama-derived IL-24 antibodies described herein.

In a further aspect of invention there is provided a chimeric camelid-human antibody which binds human IL-24, wherein the antigen-binding portions of the antibody (e.g. VH and/or VL domains or CDRs thereof) are camelid-derived and the constant regions of the antibody are derived from a human antibody. In particular, the invention provides a chimeric llama-human antibody which binds human IL-24.

In a further aspect of invention there is provided a humanised variant of a chimeric camelid-human antibody which binds human IL-24, wherein the antigen-binding portions of the antibody (e.g. VH and/or VL domains or CDRs thereof) are humanised variants of camelid-derived sequences and the constant regions of the antibody are derived from a human antibody. In particular, the invention provides a humanised variant of a chimeric llama-human antibody which binds human IL-24.

Preferred (but non-limiting) embodiments of the IL-24 antibodies, or antigen binding fragments thereof, are defined below by reference to specific structural characteristics, i.e. specified amino acid sequences of either the CDRs, or entire variable domains.

The most preferred IL-24 antibodies provided herein, which exhibit a particularly advantageous combination of properties, including high affinity binding to human IL-24, cross-reactivity with murine IL-24, and potent blocking of human IL-24-mediated cell proliferation *and* blocking of murine IL-24-mediated cell proliferation, are those based on

the antigen-binding portion of the llama-derived Fab denoted 1C11 in the accompanying examples.

Therefore, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:

H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:35 (NYWMY),

H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:37 (LITTDGGSTFYADSVKG),

H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:38 (DLGSGFSLGS),

L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144 (GGDNIGSKSAQ),

L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:167 (SDSRRPSGIPERFSGSNSG), and

L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:168 (QAWDSSANAVV).

The antibody, or antigen binding fragment, according to this aspect of the invention exhibits high affinity binding to human IL-24. Advantageously, the antibody, or antigen binding fragment, may also exhibit cross-reactivity murine IL-24. The antibody, or antigen binding fragment, may also block binding of human IL-24 to the human IL-22R/IL-20R2 receptor complex, and/or may inhibit human IL-24 signalling through the IL-22R/IL-20R2 receptor complex.

The invention also provides an IL-24 antibody or antigen binding fragment thereof based on the native VH/VL pairing of 1C11. Accordingly, there is also provided herein an antibody or antigen binding fragment thereof comprising a heavy chain variable domain (VH) comprising or consisting of an amino acid sequence selected from the group consisting of: the amino acid sequence shown as SEQ ID NO:255, germlined variants and affinity variants thereof and amino acid sequences at least 90%, 95%, 97%, 98% or 99% identical thereto, and a light chain variable domain (VL) comprising or consisting of an amino acid sequence selected from the group consisting of: the amino acid sequence shown as SEQ ID NO:283, germlined variants and affinity variants thereof and amino acid sequences at least 90%, 95%, 97%, 98% or 99% identical thereto.

In the preceding paragraph, and elsewhere herein, the structure of the antibodies/antigen binding fragments is defined on the basis of % sequence identity with a recited reference sequence (with a given SEQ ID NO). In this context, % sequence identity between two amino acid sequences may be determined by comparing these two sequences aligned in an optimum manner and in which the amino acid sequence to be compared can comprise additions or deletions with respect to the reference sequence for an optimum alignment between these two sequences. The percentage of identity is calculated by determining the number of identical positions for which the amino acid residue is identical between the two sequences, by dividing this number of identical positions by the total number of positions in the comparison window and by multiplying the result obtained by 100 in order to obtain the percentage of identity between these two sequences. Typically, the comparison window with correspond to the full length of the sequence being compared. For example, it is possible to use the BLAST program, "BLAST 2 sequences" (Tatusova et al, "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250) available on the site http://www.ncbi.nlm.nih.gov/ gorf/bl2.html, the parameters used being those given by default (in particular for the parameters "open gap penalty": 5, and "extension gap penalty": 2; the matrix chosen being, for example, the matrix "BLOSUM 62" proposed by the program), the percentage of identity between the two sequences to be compared being calculated directly by the program.

Embodiments wherein the amino acid sequence of the VH domain exhibits less than 100% sequence identity with the sequence shown as SEQ ID NO: 255 may nevertheless comprise heavy chain CDRs which are identical to HCDR1, HCDR2 and HCDR3 of SEQ ID NO:255 (SEQ ID NOs:35, 37 and 38, respectively) whilst exhibiting amino acid sequence variation within the framework regions. Likewise, embodiments wherein the amino acid sequence of the VL domain exhibits less than 100% sequence identity with the sequence shown as SEQ ID NO: 283 may nevertheless comprise heavy chain CDRs which are identical to LCDR1, LCDR2 and LCDR3 of SEQ ID NO:283 (SEQ ID NOs:144, 167 and 168, respectively) whilst exhibiting amino acid sequence variation within the framework regions.

In the foregoing preferred embodiments based on the VH and VL domains of 1C11, or variants thereof, the antibody preferably includes one or more constant domains, e.g CH1 domain, hinge region, CH2 domain and CH3 domain, of a human antibody, in

particular human IgG1, IgG2, IgG3 or IgG4. The most preferred embodiment is a human IgG4 or human IgG1. It is still further preferred for the human IgG4 or human IgG1 to be engineering to reduce or remove effector function.

The invention also provides antibodies and antigen binding fragments comprising humanised or germlined variants of VH and VL domains of 1C11, plus affinity variants and variants containing conservative amino acid substitutions, as defined herein. Specifically provided are chimeric antibodies comprising the VH and VL domains of 1C11 (SEQ ID NO:255 and SEQ ID NO:283), or human germlined variants thereof, fused to constant domains of human antibodies, in particular human IgG1, IgG2, IgG3 or IgG4. The heavy and light chain variable domains of 1C11 (SEQ ID Nos: 255 and 283), or germlined variants, affinity variants or conserved variants thereof, may be included within a conventional four-chain antibody or other antigen binding proteins, such as for example Fab, Fab', F(ab')2, bi-specific Fabs, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, a single chain variable fragment (scFv) and multispecific antibodies. The heavy chain variable domain (VH) of 1C11 (SEQ ID NO:255), or germlined variant, affinity variant or conserved variant thereof, can also be utilised as a single domain antibody.

Further IL-24 antibodies capable of blocking IL-24 signalling

Further IL-24 antibodies and antigen binding fragments provided herein, which exhibit a particularly advantageous combination of properties, including high affinity binding to human IL-24 and blocking of human IL-24-mediated cell proliferation, are those based on the antigen-binding portion of the llama-derived Fabs denoted 1C10, 1C8, 1D8, 1G10, and 1D9 in the accompanying examples.

[1C10] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:

H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:14,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:4,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:11,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:140,

L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:129, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:141.

[1C10] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:248, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:276, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1C8] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:22,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:151, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:152.

[1C8] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:250, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:278, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1D8] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:24,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:151, and

L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:148.

[1D8] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:251, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:279, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1G10] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:27,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:153, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:154.

[1G10] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:252, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:280, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1D9] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:42,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:44,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:170,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:172, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:174.

[1D9] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:256, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:284, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

In all of the foregoing embodiments according to this aspect of the invention, the antibody, or antigen binding fragment, may exhibit high affinity binding to human IL-24. The antibody, or antigen binding fragment, may also block binding of human IL-24 to the human IL-22R/IL-20R2 receptor complex, and/or may inhibit human IL-24 signalling through the IL-22R/IL-20R2 receptor complex. Advantageously, the antibody, or antigen binding fragment, may also exhibit cross-reactivity with murine IL-24.

Embodiments wherein the amino acid sequence of the VH domain exhibits less than 100% sequence identity with a defined VH domain amino acid sequence (e.g. SEQ ID NO: *x*) may nevertheless comprise heavy chain CDRs which are identical to HCDR1, HCDR2 and HCDR3 of SEQ ID NO:*x* whilst exhibiting amino acid sequence variation within the framework regions. Likewise, embodiments wherein the amino acid sequence of the VL domain exhibits less than 100% sequence identity with a defined VL domain amino acid sequence (e.g. SEQ ID NO:*y*) may nevertheless comprise light chain CDRs which are identical to LCDR1, LCDR2 and LCDR3 of SEQ ID NO:*y*, whilst exhibiting amino acid sequence variation within the framework regions.

The invention also provides antibodies and antigen binding fragments comprising humanised/germlined variants of VH and VL domains of the foregoing antibodies, plus affinity variants and variants containing conservative amino acid substitutions, as defined herein. Specifically provided are chimeric antibodies containing VH and VL domains of the llama-derived Fabs listed above, or human germlined variants thereof, fused to constant domains of human antibodies, in particular human IgG1, IgG2, IgG3 or IgG4. The heavy and light chain variable domains of the foregoing antibodies, or germlined variants, affinity variants or conserved variants thereof, may be included within a conventional four-chain antibody or other antigen binding proteins, such as for example Fab, Fab', F(ab')2, bi-specific Fabs, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, a single chain variable fragment (scFv) and multispecific

antibodies. The heavy chain variable domains, or germlined variant, affinity variant or conserved variant thereof, can also be utilised as single domain antibodies.

Non-blocking IL-24 antibodies

Further IL-24 antibodies and antigen binding fragments provided herein, which exhibit high affinity binding to human IL-24 but do not block human IL-24-mediated cell proliferation in a cell-based assay, are based on the antigen-binding portion of the llama-derived Fabs denoted 1A7, 1C7, 1A10, 1B8, 1A12, 1D6, 2C7, 2D10, 2A5, 3D7, 3B9, 4B7, 4C7, 4E11, 4F12, 4C9 and 4H9 in the accompanying examples.

[1A7] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:2,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:4,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:6,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:127,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:129, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:131.

[1A7] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:245, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:273, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1C7] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:9,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:4,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:11,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:134,

L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:135, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:136.

[1C7] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:246, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:274, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1A10] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:57,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:59,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:61,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:176,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:185, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:186.

[1A10] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:259, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:287, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1B8] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:19,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:146, and

L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:148.

[1B8] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:249, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:277, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1A12] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:22,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:155,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:151, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:157.

[1A12] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:253, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:281, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1D6] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:29,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:31,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:33,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:159,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:161, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:163.

[1D6] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:254, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:282, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[2C7] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:29,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:48,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:50,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:176,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:178, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:180.

[2C7] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:257, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:285, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[2D10] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:29,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:54,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:55,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:182,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:183, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:180.

[2D10] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:258, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:286, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[1H9] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:14,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:4,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:11,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:137,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:138, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:139.

[1H9] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:247, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:275, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[2A5] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:64,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:65,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:67,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:188,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:190, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:192.

[2A5] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:260, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:288, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[3D7] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:69,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:71,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:73,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:194,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:195, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:197.

[3D7] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:261, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:289, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[3B9] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:69,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:71,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:73,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:194,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:183, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:199.

[3B9] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:261, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:290, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[4B7/4C7/4E11] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:76,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:77,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:79,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:194,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:183, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:201.

[4B7/4C7/4E11] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:262, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:291, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[4F12] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:82,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:83,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:85,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:194,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:183, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:180.

[4F12] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:263, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:292, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[4G9/4H9] In one embodiment, the invention provides a monoclonal antibody or antigen binding fragment thereof which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:86,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:87,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:89,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:202,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:204, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:206.

[4G9] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:264, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:293, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

[4H9] The invention also provides an IL-24 antibody or antigen binding fragment thereof, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:265, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:293, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

In all of the foregoing embodiments according to this aspect of the invention, the antibody, or antigen binding fragment, may exhibit high affinity binding to human IL-24. Advantageously, the antibody, or antigen binding fragment, may also exhibit cross-reactivity with murine IL-24.

Embodiments wherein the amino acid sequence of the VH domain exhibits less than 100% sequence identity with a defined VH domain amino acid sequence (e.g. SEQ ID NO: *x*) may nevertheless comprise heavy chain CDRs which are identical to HCDR1,

HCDR2 and HCDR3 of SEQ ID NO:*x* whilst exhibiting amino acid sequence variation within the framework regions. Likewise, embodiments wherein the amino acid sequence of the VL domain exhibits less than 100% sequence identity with a defined VL domain amino acid sequence (e.g. SEQ ID NO:*y*) may nevertheless comprise light chain CDRs which are identical to LCDR1, LCDR2 and LCDR3 of SEQ ID NO:*y*, whilst exhibiting amino acid sequence variation within the framework regions.

The invention also provides antibodies and antigen binding fragments comprising humanised/germlined variants of VH and VL domains of the foregoing antibodies, plus affinity variants and variants containing conservative amino acid substitutions, as defined herein. Specifically provided are chimeric antibodies containing VH and VL domains of the llama-derived Fabs described above, or human germlined variants thereof, fused to constant domains of human antibodies, in particular human IgG1, IgG2, IgG3 or IgG4. The heavy and light chain variable domains of the foregoing antibodies, or germlined variants, affinity variants or conserved variants thereof, may be included within a conventional four-chain antibody or other antigen binding proteins, such as for example Fab, Fab', F(ab')2, bi-specific Fabs, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, a single chain variable fragment (scFv) and multispecific antibodies. The heavy chain variable domains, or germlined variant, affinity variant or conserved variant thereof, can also be utilised as single domain antibodies.

In further aspects, the invention also provides polynucleotide molecules which encode the above-listed IL-24 antibodies and antigen binding fragments thereof, in addition to expression vectors comprising the polynucleotides, host cells containing the vectors and methods of recombinant expression/production of the IL-24 antibodies.

In a still further aspect, the invention provides a pharmaceutical composition comprising any one or more of the IL-24 antibodies described above and a pharmaceutically acceptable carrier or excipient.

In a further aspect, there are provided methods of treating or preventing dermatological conditions using IL-24 antagonists, more particularly IL-24 antibodies, and even more particularly the IL-24 antibodies described herein.

In a further aspect, there are provided methods of treating or preventing inflammatory conditions using IL-24 antagonists, more particularly IL-24 antibodies, and even more particularly the IL-24 antibodies described herein.

Brief Description of the Drawings

The invention will be further understood with reference to the following experimental examples and the accompanying Figures in which:

Figure 1: Shows titration of pre- and post-immune sera of llamas immunised against IL-24. Sera of llamas immunized with hIL-24 were titrated on hIL-24 (A). Sera derived from llamas immunized with mIL-24 were titrated on mIL-24 (B).

Figure 2: Shows a titration of purified anti-mIL-24 Fab on ELISA plates coated with mIL-24 or hIL-24. Bound Fabs were detected with HRP-conjugated rabbit-anti-Myc. All clones bind to mIL-24, whereas no binding to hIL-24 is observed.

Figure 3: Shows that anti-mIL-24 Fabs (not cross-reactive with human IL-24) do not inhibit mIL-24-mediated proliferation. Dilutions of the anti-mIL-24 Fabs were incubated with mIL-24 (0.5% dilution of supernatant of HEK293 cells expressing mIL-24), and mixed with BaF3 cells expressing mIL-22R and mIL-20Rβ. After 3 days, the substrate of hexosaminidase (p-nitrophenyl-ß-D-glucosaminide) was added for 2.5 hours and the OD450nm was measured.

Figure 4: Shows inhibition of hIL-24-mediated proliferation by anti-hIL-24 Fabs. Dilutions of periplasmic extracts containing the anti-hIL-24 Fabs were incubated with hIL-24 (supernatant of HEK293 cells expressing hIL-24), and mixed with BaF3 cells expressing hIL-22R and hIL-20Rβ. After 3 days, the substrate of hexosaminidase (p-nitrophenyl-ß-D-glucosaminide) was added for 2.5 hours and the OD450nm was measured. For reasons of clarity, the results are shown on two graphs (A, B)

Figure 5: Shows inhibition of mIL-24-mediated proliferation by anti-hIL-24 Fabs. Dilutions of periplasmic extracts containing the anti-hIL-24 Fabs were incubated with mIL-24 (supernatant of HEK293 cells expressing mIL-24), and mixed with BaF3 cells

expressing mIL-22R and mIL-20Rβ. After 3 days, the substrate of hexosaminidase (p-nitrophenyl-ß-D-glucosaminide) was added for 2.5 hours and the OD450nm was measured.

Figure 6: Shows binding of anti-hIL-24 antibodies to hIL-24 expressed on BW cells. BW cells stably expressing cell surface hIL-24, or non-transfected BW cells were incubated with anti-hIL-24 antibodies. Bound anti-IL-24 was detected with goat-anti-mouse-FITC, and analysed in flow cytometry.

Figure 7: Shows binding of directly Alexa Fluor 647- conjugated mAbs on hIL-24 and mIL-24 expressing BW cells. BW cells expressing hIL-24 or mIL-24, or parental BW cells were incubated with 1/1000 dilution of the mAbs, washed and analysed in flow cytometry (APC channel). As a control, BW cells expressing IL-24 were left unstained.

Figure 8: Shows inhibition of hIL-24 or mIL-24-mediated proliferation. Dilutions of the anti-hIL-24 mAbs were incubated with hIL-24 (A) or mIL-24 (B) (supernatant of HEK293 cells expressing hIL-24 or mIL-24), and mixed with BaF3 cells expressing hIL-22R/hIL-20Rβ or mIL-22R/mIL-20Rβ, respectively. After 3 days, the substrate of hexosaminidase (p-nitrophenyl-ß-D-glucosaminide) was added for 2.5 hours and the OD450nm was measured.

Figure 9: Illustrates inhibition of PPD mediated ear swelling by blocking of IL-22 and IL-24. Ear thickness was monitored in WT, IL-22R-/-mice, and IL-22-/- mice, which received either anti-IL-24 blocking antibody 1C11 or IgG2a isotype control, sensitized and challenged with PPD (3%) solutions.

Figure 10: shows nucleotide and amino acid sequences for human and murine IL-24 and an alignment of the sequences

Detailed description of the invention

Provided herein are antibodies and antigen binding fragments thereof which exhibit high affinity binding to the human IL-24 protein and also exhibit potent blocking of IL-24 signalling through the IL-22R1/IL-20R2 and/or the IL20R1/IL-20R2 receptor

complex. Certain embodiments of the antibodies, and antigen binding fragments, also exhibit cross-reactivity with murine IL-24. The antibodies and antigen binding fragments have therapeutic utility in the treatment of dermatological conditions and inflammatory conditions associated with IL-24 signalling through the IL-22R1/IL-20R2 and/or the IL20R1/IL-20R2 receptor complex.

Definitions

"Antibody" or "Immunoglobulin"-- As used herein, the term "immunoglobulin" includes a polypeptide having a combination of two heavy and two light chains whether or not it possesses any relevant specific immunoreactivity. "Antibodies" refers to such assemblies which have significant known specific immunoreactive activity to an antigen of interest (e.g. human IL-24). The term "IL-24 antibodies" is used herein to refer to antibodies which exhibit immunological specificity for human IL-24 protein. As explained elsewhere herein, "specificity" for human IL-24 does not exclude cross-reaction with species homologues of IL-24. Antibodies and immunoglobulins comprise light and heavy chains, with or without an interchain covalent linkage between them. Basic immunoglobulin structures in vertebrate systems are relatively well understood.

The generic term "immunoglobulin" comprises five distinct classes of antibody that can be distinguished biochemically. All five classes of antibodies are within the scope of the present invention, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, immunoglobulins comprise two identical light polypeptide chains of molecular weight approximately 23,000 Daltons, and two identical heavy chains of molecular weight 53,000-70,000. The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

The light chains of an antibody are classified as either kappa or lambda (κ, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated by B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. Those skilled in the art will appreciate that heavy chains are classified as

gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (e.g., γ1 – γ 4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA, IgD or IgE, respectively. The immunoglobulin subclasses (isotypes) e.g., IgG1, IgG2, IgG3, IgG4, IgA1, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant invention.

As indicated above, the variable region of an antibody allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain of an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary antibody structure forms the antigen binding site present at the end of each arm of the Y. More specifically, the antigen binding site is defined by three complementary determining regions (CDRs) on each of the VH and VL chains.

"IL-24 protein" or "IL-24 antigen" --- As used herein, the terms "IL-24 protein" or "IL-24 antigen" or "IL-24" are used interchangeably and refer to a member of the IL-10 family of cytokines that is capable of signalling through the heterodimeric receptor complexes IL-20R1/IL-20R2 and IL-22R1/IL-20R2. The terms "human IL-24 protein" or "human IL-24 antigen" or "human IL-24" are used interchangeably to refer specifically to the human homolog, including the native human IL-24 protein naturally expressed in the human body and/or on the surface of cultured human cell lines, as well as recombinant forms and fragments thereof. Specific examples of human IL-24 include the polypeptide having the amino acid sequence shown under NCBI Reference Sequence Accession No. NG_029565, or as SEQ ID NO: 301 (Figure 10).

"Binding Site" ---As used herein, the term "binding site" comprises a region of a polypeptide which is responsible for selectively binding to a target antigen of interest (e.g. human IL-24). Binding domains comprise at least one binding site. Exemplary binding domains include an antibody variable domain. The antibody molecules of the invention may comprise a single binding site or multiple (e.g., two, three or four) binding sites.

"Derived From" ---As used herein the term "derived from" a designated protein (e.g. a IL-24 antibody or antigen-binding fragment thereof) refers to the origin of the polypeptide. In one embodiment, the polypeptide or amino acid sequence which is derived from a particular starting polypeptide is a CDR sequence or sequence related thereto. In one embodiment, the amino acid sequence which is derived from a particular starting polypeptide is not contiguous. For example, in one embodiment, one, two, three, four, five, or six CDRs are derived from a starting antibody. In one embodiment, the polypeptide or amino acid sequence which is derived from a particular starting polypeptide or amino acid sequence has an amino acid sequence that is essentially identical to that of the starting sequence, or a portion thereof wherein the portion consists of at least of at least 3-5 amino acids, 5-10 amino acids, at least 10-20 amino acids, at least 20-30 amino acids, or at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the starting sequence. In one embodiment, the one or more CDR sequences derived from the starting antibody are altered to produce variant CDR sequences, e.g. affinity variants, wherein the variant CDR sequences maintain IL-24 binding activity.

"Camelid-Derived" ---In certain preferred embodiments, the IL-24 antibody molecules of the invention comprise framework amino acid sequences and/or CDR amino acid sequences derived from a camelid conventional antibody raised by active immunisation of a camelid with IL-24 antigen. However, IL-24 antibodies comprising camelid-derived amino acid sequences may be engineered to comprise framework and/or constant region sequences derived from a human amino acid sequence (i.e. a human antibody) or other non-camelid mammalian species. For example, a human or non-human primate framework region, heavy chain portion, and/or hinge portion may be included in the subject IL-24 antibodies. In one embodiment, one or more non-camelid amino acids may be present in the framework region of a "camelid-derived" IL-24 antibody, e.g., a camelid framework amino acid sequence may comprise one or more amino acid mutations in which the corresponding human or non-human primate amino acid residue is present. Moreover, camelid-derived VH and VL domains, or humanised variants thereof, may be linked to the constant domains of human antibodies to produce a chimeric molecule, as extensively described elsewhere herein.

"Conservative amino acid substitution" --A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide may be replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

"Heavy chain portion" ---As used herein, the term "heavy chain portion" includes amino acid sequences derived from the constant domains of an immunoglobulin heavy chain. A polypeptide comprising a heavy chain portion comprises at least one of: a CH1 domain, a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. In one embodiment, an antibody or antigen binding fragment of the invention may comprise the Fc portion of an immunoglobulin heavy chain (e.g., a hinge portion, a CH2 domain, and a CH3 domain). In another embodiment, an antibody or antigen binding fragment of the invention may lack at least a portion of a constant domain (e.g., all or part of a CH2 domain). In certain embodiments, at least one, and preferably all, of the constant domains are derived from a human immunoglobulin heavy chain. For example, in one preferred embodiment, the heavy chain portion comprises a fully human hinge domain. In other preferred embodiments, the heavy chain portion comprising a fully human Fc portion (e.g., hinge, CH2 and CH3 domain sequences from a human immunoglobulin).

In certain embodiments, the constituent constant domains of the heavy chain portion are from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide may comprise a CH2 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 or IgG4 molecule. In other embodiments, the constant domains are chimeric domains comprising portions of different immunoglobulin

molecules. For example, a hinge may comprise a first portion from an IgG1 molecule and a second portion from an IgG3 or IgG4 molecule. As set forth above, it will be understood by one of ordinary skill in the art that the constant domains of the heavy chain portion may be modified such that they vary in amino acid sequence from the naturally occurring (wild-type) immunoglobulin molecule. That is, the polypeptides of the invention disclosed herein may comprise alterations or modifications to one or more of the heavy chain constant domains (CH1, hinge, CH2 or CH3) and/or to the light chain constant region domain (CL). Exemplary modifications include additions, deletions or substitutions of one or more amino acids in one or more domains.

"Chimeric" ---A "chimeric" protein comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature. The amino acid sequences may normally exist in separate proteins that are brought together in the fusion polypeptide or they may normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide. A chimeric protein may be created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship. Exemplary chimeric IL-24 antibodies include fusion proteins comprising camelid-derived VH and VL domains, or humanised variants thereof, fused to the constant domains of a human antibody, e.g. human IgG1, IgG2, IgG3 or IgG4.

"Variable region" or "variable domain" --- The terms "variable region" and "variable domain" are used herein interchangeable and are intended to have equivalent meaning. The term "variable" refers to the fact that certain portions of the variable domains VH and VL differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its target antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called "hypervariable loops" in each of the VL domain and the VH domain which form part of the antigen binding site. The first, second and third hypervariable loops of the VLambda light chain domain are referred to herein as L1(λ), L2(λ) and L3(λ) and may be defined as comprising residues 24-33 (L1(λ), consisting of 9, 10 or 11 amino acid residues), 49-53 (L2(λ), consisting of 3 residues) and 90-96 (L3(λ), consisting of 5 residues) in the VL domain (Morea et al., Methods 20:267-279 (2000)).

The first, second and third hypervariable loops of the VKappa light chain domain are referred to herein as L1(κ), L2(κ) and L3(κ) and may be defined as comprising residues 25-33 (L1(κ), consisting of 6, 7, 8, 11, 12 or 13 residues), 49-53 (L2(κ), consisting of 3 residues) and 90-97 (L3(κ), consisting of 6 residues) in the VL domain (Morea et al., Methods 20:267-279 (2000)). The first, second and third hypervariable loops of the VH domain are referred to herein as H1, H2 and H3 and may be defined as comprising residues 25-33 (H1, consisting of 7, 8 or 9 residues), 52-56 (H2, consisting of 3 or 4 residues) and 91-105 (H3, highly variable in length) in the VH domain (Morea et al., Methods 20:267-279 (2000)).

Unless otherwise indicated, the terms L1, L2 and L3 respectively refer to the first, second and third hypervariable loops of a VL domain, and encompass hypervariable loops obtained from both Vkappa and Vlambda isotypes. The terms H1, H2 and H3 respectively refer to the first, second and third hypervariable loops of the VH domain, and encompass hypervariable loops obtained from any of the known heavy chain isotypes, including γ, ε, δ, α or μ.

The hypervariable loops L1, L2, L3, H1, H2 and H3 may each comprise part of a "complementarity determining region" or "CDR", as defined below. The terms "hypervariable loop" and "complementarity determining region" are not strictly synonymous, since the hypervariable loops (HVs) are defined on the basis of structure, whereas complementarity determining regions (CDRs) are defined based on sequence variability (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD., 1983) and the limits of the HVs and the CDRs may be different in some VH and VL domains.

The CDRs of the VL and VH domains can typically be defined as comprising the following amino acids: residues 24-34 (CDRL1), 50-56 (CDRL2) and 89-97 (CDRL3) in the light chain variable domain, and residues 31-35 or 31-35b (CDRH1), 50-65 (CDRH2) and 95-102 (CDRH3) in the heavy chain variable domain; (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Thus, the HVs may be comprised within the corresponding CDRs and references herein to the "hypervariable loops" of VH and VL domains should be interpreted as also encompassing the corresponding CDRs, and vice versa, unless otherwise indicated.

The more highly conserved portions of variable domains are called the framework region (FR), as defined below. The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a β-sheet configuration, connected by the three hypervariable loops. The hypervariable loops in each chain are held together in close proximity by the FRs and, with the hypervariable loops from the other chain, contribute to the formation of the antigen-binding site of antibodies. Structural analysis of antibodies revealed the relationship between the sequence and the shape of the binding site formed by the complementarity determining regions (Chothia et al., J. Mol. Biol. 227: 799-817 (1992)); Tramontano et al., J. Mol. Biol, 215:175-182 (1990)). Despite their high sequence variability, five of the six loops adopt just a small repertoire of main-chain conformations, called "canonical structures". These conformations are first of all determined by the length of the loops and secondly by the presence of key residues at certain positions in the loops and in the framework regions that determine the conformation through their packing, hydrogen bonding or the ability to assume unusual main-chain conformations.

"CDR" ---As used herein, the term "CDR" or "complementarity determining region" means the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat et al., J. Biol. Chem. 252, 6609-6616 (1977) and Kabat et al., Sequences of protein of immunological interest. (1991), and by Chothia et al., J. Mol. Biol. 196:901-917 (1987) and by MacCallum et al., J. Mol. Biol. 262:732-745 (1996) where the definitions include overlapping or subsets of amino acid residues when compared against each other. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth for comparison. Preferably, the term "CDR" is a CDR as defined by Kabat based on sequence comparisons.

Table 1: CDR definitions

	CDR Definitions		
	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101

V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[1]Residue numbering follows the nomenclature of Kabat et al., supra

[2]Residue numbering follows the nomenclature of Chothia et al., supra

[3]Residue numbering follows the nomenclature of MacCallum et al., supra

"Framework region" --- The term "framework region" or "FR region" as used herein, includes the amino acid residues that are part of the variable region, but are not part of the CDRs (e.g., using the Kabat definition of CDRs). Therefore, a variable region framework is between about 100-120 amino acids in length but includes only those amino acids outside of the CDRs. For the specific example of a heavy chain variable domain and for the CDRs as defined by Kabat et al., framework region 1 corresponds to the domain of the variable region encompassing amino acids 1-30; framework region 2 corresponds to the domain of the variable region encompassing amino acids 36-49; framework region 3 corresponds to the domain of the variable region encompassing amino acids 66-94, and framework region 4 corresponds to the domain of the variable region from amino acids 103 to the end of the variable region. The framework regions for the light chain are similarly separated by each of the light claim variable region CDRs. Similarly, using the definition of CDRs by Chothia et al. or McCallum et al. the framework region boundaries are separated by the respective CDR termini as described above. In preferred embodiments the CDRs are as defined by Kabat.

In naturally occurring antibodies, the six CDRs present on each monomeric antibody are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding site as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the heavy and light variable domains show less inter-molecular variability in amino acid sequence and are termed the framework regions. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, these framework regions act to form a scaffold that provides for positioning the six CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding site formed by the positioned CDRs defines a surface complementary to the

epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to the immunoreactive antigen epitope. The position of CDRs can be readily identified by one of ordinary skill in the art.

"Hinge region" ---As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux et al. J. Immunol. 1998 161:4083). IL-24 antibodies comprising a "fully human" hinge region may contain one of the hinge region sequences shown in Table 2 below.

Table 2: human hinge sequences

IgG	Upper hinge	Middle hinge	Lower hinge
IgG1	EPKSCDKTHT (SEQ ID NO:305)	CPPCP (SEQ ID NO:306)	APELLGGP (SEQ ID NO:307)
IgG3	ELKTPLGDTTHT (SEQ ID NO:308)	CPRCP $(EPKSCDTPPPCPRCP)_3$ (SEQ ID NO:309)	APELLGGP (SEQ ID NO:310)
IgG4	ESKYGPP (SEQ ID NO:311)	CPSCP (SEQ ID NO:312)	APEFLGGP (SEQ ID NO:313)
IgG42	ERK (SEQ ID NO:314)	CCVECPPPCP (SEQ ID NO:315)	APPVAGP (SEQ ID NO:316)

"CH2 domain" ---As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that extends, e.g., from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system, Kabat EA et al. Sequences of Proteins of Immunological Interest. Bethesda, US Department of Health and Human Services, NIH. 1991). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2

domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

"Fragment" ---The term "fragment" refers to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen binding (i.e., specific binding to human IL-24). As used herein, the term "fragment" of an antibody molecule includes antigen-binding fragments of antibodies, for example, an antibody light chain variable domain (VL), an antibody heavy chain variable domain (VH), a single chain antibody (scFv), a F(ab')2 fragment, a Fab fragment, an Fd fragment, an Fv fragment, and a single domain antibody fragment (DAb). Fragments can be obtained, e.g., via chemical or enzymatic treatment of an intact or complete antibody or antibody chain or by recombinant means.

"Valency" --As used herein the term "valency" refers to the number of potential target binding sites in a polypeptide. Each target binding site specifically binds one target molecule or specific site on a target molecule. When a polypeptide comprises more than one target binding site, each target binding site may specifically bind the same or different molecules (e.g., may bind to different ligands or different antigens, or different epitopes on the same antigen). The subject binding molecules have at least one binding site specific for a human IL-24 molecule.

"Specificity" --The term "specificity" refers to the ability to bind (e.g., immunoreact with) a given target, e.g., IL-24. A polypeptide may be monospecific and contain one or more binding sites which specifically bind a target or a polypeptide may be multispecific and contain two or more binding sites which specifically bind the same or different targets. In one embodiment, an antibody of the invention is specific for more than one target. For example, in one embodiment, a multispecific binding molecule of the invention binds to IL-24 and a second target molecule.

"Synthetic" ---As used herein the term "synthetic" with respect to polypeptides includes polypeptides which comprise an amino acid sequence that is not naturally occurring. For example, non-naturally occurring polypeptides which are modified forms of naturally occurring polypeptides (e.g., comprising a mutation such as an addition, substitution or deletion) or which comprise a first amino acid sequence (which may or may not be naturally occurring) that is linked in a linear sequence of amino acids to a second amino acid sequence (which may or may not be naturally occurring) to which it is not naturally linked in nature.

"Engineered" ---As used herein the term "engineered" includes manipulation of nucleic acid or polypeptide molecules by synthetic means (e.g. by recombinant techniques, in vitro peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques). Preferably, the antibodies of the invention are engineered, including for example, humanized and/or chimeric antibodies, and antibodies which have been engineered to improve one or more properties, such as antigen binding, stability/half-life or effector function.

"Modified antibody" ---As used herein, the term "modified antibody" includes synthetic forms of antibodies which are altered such that they are not naturally occurring, e.g., antibodies that comprise at least two heavy chain portions but not two complete heavy chains (such as, domain deleted antibodies or minibodies); multispecific forms of antibodies (e.g., bispecific, trispecific, etc.) altered to bind to two or more different antigens or to different epitopes on a single antigen); heavy chain molecules joined to scFv molecules and the like. ScFv molecules are known in the art and are described, e.g., in US patent 5,892,019. In addition, the term "modified antibody" includes multivalent forms of antibodies (e.g., trivalent, tetravalent, etc., antibodies that bind to three or more copies of the same antigen). In another embodiment, a modified antibody of the invention is a fusion protein comprising at least one heavy chain portion lacking a CH2 domain and comprising a binding domain of a polypeptide comprising the binding portion of one member of a receptor ligand pair.

The term "modified antibody" may also be used herein to refer to amino acid sequence variants of an IL-24 antibody. It will be understood by one of ordinary skill in the art that an IL-24 antibody may be modified to produce a variant IL-24 antibody which

varies in amino acid sequence in comparison to the IL-24 antibody from which it was derived. For example, nucleotide or amino acid substitutions leading to conservative substitutions or changes at "non-essential" amino acid residues may be made (e.g., in CDR and/or framework residues). Amino acid substitutions can include replacement of one or more amino acids with a naturally occurring or non-natural amino acid.

"Humanising substitutions" ---As used herein, the term "humanising substitutions" refers to amino acid substitutions in which the amino acid residue present at a particular position in the VH or VL domain of a IL-24 antibody (for example a camelid-derived IL-24 antibody) is replaced with an amino acid residue which occurs at an equivalent position in a reference human VH or VL domain. The reference human VH or VL domain may be a VH or VL domain encoded by the human germline. Humanising substitutions may be made in the framework regions and/or the CDRs of a IL-24 antibody, defined herein.

"Humanised variants" --- As used herein the term "humanised variant" refers to a variant antibody which contains one or more "humanising substitutions" compared to a reference IL-24 antibody, wherein a portion of the reference antibody (e.g. the VH domain and/or the VL domain or parts thereof containing at least one CDR) has an amino acid sequence derived from a non-human species, and the "humanising substitutions" occur within the amino acid sequence derived from a non-human species.

"Germlined variants" --- The term "germlined variant" is used herein to refer specifically to "humanised variants" in which the "humanising substitutions" result in replacement of one or more amino acid residues present at a particular position (s) in the VH or VL domain of a IL-24 antibody (for example a camelid-derived IL-24 antibody) with an amino acid residue which occurs at an equivalent position in a reference human VH or VL domain encoded by the human germline. It is typical that for any given "germlined variant", the replacement amino acid residues substituted *into* the germlined variant are taken exclusively, or predominantly, from a single human germline-encoded VH or VL domain. The terms "humanised variant" and "germlined variant" are often used interchangeably herein. Introduction of one or more "humanising substitutions" into a camelid-derived (e.g. llama derived) VH or VL domain results in production of a

"humanised variant" of the camelid (llama)-derived VH or VL domain. If the amino acid residues substituted in are derived predominantly or exclusively from a single human germline-encoded VH or VL domain sequence, then the result may be a "human germlined variant" of the camelid (llama)-derived VH or VL domain.

"Affinity variants" --- As used herein, the term "affinity variant" refers to a variant antibody which exhibits one or more changes in amino acid sequence compared to a reference IL-24 antibody, wherein the affinity variant exhibits an altered affinity for the human IL-24 protein in comparison to the reference antibody. Typically, affinity variants will exhibit a changed affinity for human IL-24, as compared to the reference IL-24 antibody. Preferably the affinity variant will exhibit *improved* affinity for human IL-24, as compared to the reference IL-24 antibody. The improvement may be apparent as a lower K_D, for human IL-24, or a slower off-rate for human IL-24 or an alteration in the pattern of cross-reactivity with non-human IL-24 homologues. Affinity variants typically exhibit one or more changes in amino acid sequence in the CDRs, as compared to the reference IL-24 antibody. Such substitutions may result in replacement of the original amino acid present at a given position in the CDRs with a different amino acid residue, which may be a naturally occurring amino acid residue or a non-naturally occurring amino acid residue. The amino acid substitutions may be conservative or non-conservative.

"High human homology" ---An antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) will be considered as having high human homology if the VH domains and the VL domains, taken together, exhibit at least 90% amino acid sequence identity to the closest matching human germline VH and VL sequences. Antibodies having high human homology may include antibodies comprising VH and VL domains of native non-human antibodies which exhibit sufficiently high % sequence identity to human germline sequences, including for example antibodies comprising VH and VL domains of camelid conventional antibodies, as well as engineered, especially humanised or germlined, variants of such antibodies and also "fully human" antibodies.

In one embodiment the VH domain of the antibody with high human homology may exhibit an amino acid sequence identity or sequence homology of 80% or greater

with one or more human VH domains across the framework regions FR1, FR2, FR3 and FR4. In other embodiments the amino acid sequence identity or sequence homology between the VH domain of the polypeptide of the invention and the closest matching human germline VH domain sequence may be 85% or greater, 90% or greater, 95% or greater, 97% or greater, or up to 99% or even 100%.

In one embodiment the VH domain of the antibody with high human homology may contain one or more (e.g. 1 to 10) amino acid sequence mis-matches across the framework regions FR1, FR2, FR3 and FR4, in comparison to the closest matched human VH sequence.

In another embodiment the VL domain of the antibody with high human homology may exhibit a sequence identity or sequence homology of 80% or greater with one or more human VL domains across the framework regions FR1, FR2, FR3 and FR4. In other embodiments the amino acid sequence identity or sequence homology between the VL domain of the polypeptide of the invention and the closest matching human germline VL domain sequence may be 85% or greater 90% or greater, 95% or greater, 97% or greater, or up to 99% or even 100%.

In one embodiment the VL domain of the antibody with high human homology may contain one or more (e.g. 1 to 10) amino acid sequence mis-matches across the framework regions FR1, FR2, FR3 and FR4, in comparison to the closest matched human VL sequence.

Before analysing the percentage sequence identity between the antibody with high human homology and human germline VH and VL, the canonical folds may be determined, which allows the identification of the family of human germline segments with the identical combination of canonical folds for H1 and H2 or L1 and L2 (and L3). Subsequently the human germline family member that has the highest degree of sequence homology with the variable region of the antibody of interest is chosen for scoring the sequence homology. Procedures for determining the closest matching human germline, and determining % sequence identity/homology, are described in the applicant's earlier publications WO 2010/001251 and WO 2011/080350, the contents of which are incorporated herein in their entirety by reference.

Antibodies with high human homology may comprise hypervariable loops or CDRs having human or human-like canonical fold structures. In one embodiment at least one hypervariable loop or CDR in either the VH domain or the VL domain of the antibody

with high human homology may be obtained or derived from a VH or VL domain of a non-human antibody, for example a conventional antibody from a species of Camelidae, yet exhibit a predicted or actual canonical fold structure which is substantially identical to a canonical fold structure which occurs in human antibodies.

In one embodiment, both H1 and H2 in the VH domain of the antibody with high human homology exhibit a predicted or actual canonical fold structure which is substantially identical to a canonical fold structure which occurs in human antibodies.

Antibodies with high human homology may comprise a VH domain in which the hypervariable loops H1 and H2 form a combination of canonical fold structures which is identical to a combination of canonical structures known to occur in at least one human germline VH domain. It has been observed that only certain combinations of canonical fold structures at H1 and H2 actually occur in VH domains encoded by the human germline. In an embodiment H1 and H2 in the VH domain of the antibody with high human homology may be obtained from a VH domain of a non-human species, e.g. a Camelidae species, yet form a combination of predicted or actual canonical fold structures which is identical to a combination of canonical fold structures known to occur in a human germline or somatically mutated VH domain. In non-limiting embodiments H1 and H2 in the VH domain of the antibody with high human homology may be obtained from a VH domain of a non-human species, e.g. a Camelidae species, and form one of the following canonical fold combinations: 1-1, 1-2, 1-3, 1-6, 1-4, 2-1, 3-1 and 3-5.

An antibody with high human homology may contain a VH domain which exhibits both high sequence identity/sequence homology with human VH, and which contains hypervariable loops exhibiting structural homology with human VH.

It may be advantageous for the canonical folds present at H1 and H2 in the VH domain of the antibody with high human homology, and the combination thereof, to be "correct" for the human VH germline sequence which represents the closest match with the VH domain of the antibody with high human homology in terms of overall primary amino acid sequence identity. By way of example, if the closest sequence match is with a human germline VH3 domain, then it may be advantageous for H1 and H2 to form a combination of canonical folds which also occurs naturally in a human VH3 domain. This may be particularly important in the case of antibodies with high human homology which are derived from non-human species, e.g. antibodies containing VH and VL

domains which are derived from camelid conventional antibodies, especially antibodies containing humanised camelid VH and VL domains.

Thus, in one embodiment the VH domain of the IL-24 antibody with high human homology may exhibit a sequence identity or sequence homology of 80% or greater, 85% or greater, 90% or greater, 95% or greater, 97% or greater, or up to 99% or even 100% with a human VH domain across the framework regions FR1, FR2 , FR3 and FR4, and in addition H1 and H2 in the same antibody are obtained from a non-human VH domain (e.g. derived from a Camelidae species, preferably llama), but form a combination of predicted or actual canonical fold structures which is the same as a canonical fold combination known to occur naturally in the same human VH domain.

In other embodiments, L1 and L2 in the VL domain of the antibody with high human homology are each obtained from a VL domain of a non-human species (e.g. a camelid-derived VL domain), and each exhibits a predicted or actual canonical fold structure which is substantially identical to a canonical fold structure which occurs in human antibodies.

L1 and L2 in the VL domain of an antibody with high human homology may form a combination of predicted or actual canonical fold structures which is identical to a combination of canonical fold structures known to occur in a human germline VL domain. In non-limiting embodiments L1 and L2 in the VLambda domain of an antibody with high human homology (e.g. an antibody containing a camelid-derived VL domain or a humanised variant thereof) may form one of the following canonical fold combinations: 11-7, 13-7(A,B,C), 14-7(A,B), 12-11, 14-11 and 12-12 (as defined in Williams et al. J. Mol. Biol. 264:220 -32 (1996) and as shown on http://www.bioc.uzh.ch/antibody/Sequences/Germlines/VBase_hVL.html). In non-limiting embodiments L1 and L2 in the Vkappa domain may form one of the following canonical fold combinations: 2-1, 3-1, 4-1 and 6-1 (as defined in Tomlinson et al. EMBO J. 14:4628-38 (1995) and as shown on http://www.bioc.uzh.ch/antibody/Sequences/Germlines/VBase_hVK.html).

In a further embodiment, all three of L1, L2 and L3 in the VL domain of an antibody with high human homology may exhibit a substantially human structure. It is preferred that the VL domain of the antibody with high human homology exhibits both high sequence identity/sequence homology with human VL, and also that the hypervariable loops in the VL domain exhibit structural homology with human VL.

In one embodiment, the VL domain of a IL-24 antibody with high human homology may exhibit a sequence identity of 80% or greater, 85% or greater, 90% or greater, 95% or greater, 97% or greater, or up to 99% or even 100% with a human VL domain across the framework regions FR1, FR2 , FR3 and FR4, and in addition hypervariable loop L1 and hypervariable loop L2 may form a combination of predicted or actual canonical fold structures which is the same as a canonical fold combination known to occur naturally in the same human VL domain.

It is, of course, envisaged that VH domains exhibiting high sequence identity/sequence homology with human VH, and also structural homology with hypervariable loops of human VH will be combined with VL domains exhibiting high sequence identity/sequence homology with human VL, and also structural homology with hypervariable loops of human VL to provide antibodies with high human homology containing VH/VL pairings (e.g camelid-derived VH/VL pairings) with maximal sequence and structural homology to human-encoded VH/VL pairings.

Procedures for evaluating camelid-derived (e.g. llama-derived) CDRs, VH domains or VL domains for the presence of human-like canonical fold structures are described in the applicant's earlier publications WO 2010/001251 and WO 2011/080350, the contents of which are incorporated herein in their entirety by reference.

As summarised above, the invention relates, at least in part, to antibodies, and antigen binding fragments thereof, that bind to human IL-24 with high affinity. The properties and characteristics of the IL-24 antibodies, and antibody fragments, according to the invention will now be described in further detail.

IL-24 binding and affinity

The IL-24 antibodies provided herein are characterised by high affinity binding to human IL-24, and also cross-reactivity with the murine IL-24 homolog. Binding affinity for human IL-24 may be assessed using standard techniques known to persons of skill in the art.

In one embodiment, binding affinity of a Fab clone comprising a defined VH/VL pairing may be assessed using surface plasmon resonance, e.g. using the Biacore™ system. Fab clones comprising VH/VL pairings of the antibodies, and antigen binding fragments of the invention typically exhibit an off-rate for human IL-24 measured by

Biacore™ in the range of from 2 x $10^{-4\ s-1}$ to 2 x 10^{-3s-1}. An off-rate within this range may be taken as an indication that the Fab, and a corresponding bivalent mAb, exhibit high affinity binding to human IL-24. Similarly, the Fab clones comprising VH/VL pairings of the antibodies, and antigen binding fragments of the invention typically exhibit an off-rate for murine IL-24 measured by Biacore™, as described in the accompanying examples, in the range of from 2 x $10^{-4\ s-1}$ to 2 x 10^{-3s-1}. An off-rate within this range may be taken as an indication that the Fab, and a corresponding bivalent mAb, exhibit high affinity binding to murine IL-24. Therefore, Fabs that exhibit off-rates for *both* human and murine IL-24 falling within the stated ranges show high affinity binding for human IL-24, and cross-reactivity with murine IL-24. Bivalent mAbs comprising two Fabs that (individually) exhibit off-rates for human and murine IL-24 within the stated ranges are also taken to exhibit high affinity binding to human IL-24 and cross-reactivity with murine IL-24.

Binding affinity to human and murine IL-24 can also be assessed using a cell-based system as described in the accompanying examples, in which mAbs are tested for binding to mammalian cell lines that express IL-24, for example using flow cytometry.

Interaction between IL-24 and IL22R1/IL-20R2

Certain embodiments of the IL-24 antibodies provided herein may exhibit the ability to block binding of human IL-24 to the human IL-22R1/IL-20R2 receptor complex and may, as a consequence of binding to human IL-24 and blocking the interaction with the IL-22R1/IL-20R2 receptor, inhibit human IL-24 signalling through the human IL-22R1/IL-20R2 receptor complex. Alternatively, certain IL-24 antibodies which bind to human IL-24 may inhibit human IL-24 signalling through the human IL-22R1/IL-20R2 receptor complex without exhibiting significant blocking of the binding of IL-24 to IL-22R1/IL-20R2.

In this context "blocking" of the binding of IL-24 to the IL-22R1/IL-20R2 receptor complex refers to full or partial blocking. Likewise, "inhibition" of IL-24 signalling through the IL-22R1/IL-20R2 receptor complex refers to full or partial inhibition. The ability of an IL-24 antibody to (fully or partially) block IL-24 binding IL-22R1/IL-20R2 and/or (fully or partially) inhibit IL-24 signalling via IL-22R1/IL-20R2 may be demonstrated in a cell-based assay system such as, for example, the proliferation assay described in the accompanying examples. IL-24 antibodies that exhibit significant

reduction/inhibition of cell proliferation in this assay system may be score as inhibiting human IL-24 signalling through the human IL-22R1/IL-20R2 receptor complex. Such antibodies may also be referred to herein as "IL-24 antagonist" antibodies or "IL-24 neutralising" antibodies.

In certain embodiments (e.g. certain antibodies based on the VH/VL pairing of the llama-derived Fab 1C11), the antibody may also exhibit the ability to block binding of mouse IL-24 to the mouse IL-22R1/Il-20R2 receptor complex and/or inhibit mouse IL-24 signalling through the mouse IL-22R1/Il-20R2 receptor complex.

Camelid-derived IL-24 antibodies

The antibodies or antigen binding fragments thereof described herein may comprise at least one hypervariable loop or complementarity determining region obtained from a VH domain or a VL domain of a species in the family Camelidae. In particular, the antibody or antigen binding fragment may comprise VH and/or VL domains, or CDRs thereof, obtained by active immunisation of outbred camelids, e.g. llamas, with a human IL-24 antigen.

By "hypervariable loop or complementarity determining region obtained from a VH domain or a VL domain of a species in the family Camelidae" is meant that that hypervariable loop (HV) or CDR has an amino acid sequence which is identical, or substantially identical, to the amino acid sequence of a hypervariable loop or CDR which is encoded by a Camelidae immunoglobulin gene. In this context "immunoglobulin gene" includes germline genes, immunoglobulin genes which have undergone rearrangement, and also somatically mutated genes. Thus, the amino acid sequence of the HV or CDR obtained from a VH or VL domain of a Camelidae species may be identical to the amino acid sequence of a HV or CDR present in a mature Camelidae conventional antibody. The term "obtained from" in this context implies a structural relationship, in the sense that the HVs or CDRs of the IL-24 antibody embody an amino acid sequence (or minor variants thereof) which was originally encoded by a Camelidae immunoglobulin gene. However, this does not necessarily imply a particular relationship in terms of the production process used to prepare the IL-24 antibody.

Camelid-derived IL-24 antibodies may be derived from any camelid species, including *inter alia*, llama, dromedary, alpaca, vicuna, guanaco or camel.

IL-24 antibodies comprising camelid-derived VH and VL domains, or CDRs thereof, are typically recombinantly expressed polypeptides, and may be chimeric polypeptides. The term "chimeric polypeptide" refers to an artificial (non-naturally occurring) polypeptide which is created by juxtaposition of two or more peptide fragments which do not otherwise occur contiguously. Included within this definition are "species" chimeric polypeptides created by juxtaposition of peptide fragments encoded by two or more species, e.g. camelid and human.

Camelid-derived CDRs may comprise one of the CDR sequences shown in Tables 7 and 8 below.

In one embodiment the entire VH domain and/or the entire VL domain may be obtained from a species in the family Camelidae. In specific embodiments, the camelid-derived VH domain may comprise the amino acid sequence shown as SEQ ID NOs:245-265 or 266-272, whereas the camelid-derived VL domain may comprise the amino acid sequence show as SEQ ID Nos:273-293 or 294-300. The camelid-derived VH domain and/or the camelid-derived VL domain may then be subject to protein engineering, in which one or more amino acid substitutions, insertions or deletions are introduced into the camelid amino acid sequence. These engineered changes preferably include amino acid substitutions relative to the camelid sequence. Such changes include "humanisation" or "germlining" wherein one or more amino acid residues in a camelid-encoded VH or VL domain are replaced with equivalent residues from a homologous human-encoded VH or VL domain.

Isolated camelid VH and VL domains obtained by active immunisation of a camelid (e.g. llama) with a human IL-24 antigen can be used as a basis for engineering IL-24 antibodies according to the invention. Starting from intact camelid VH and VL domains, it is possible to engineer one or more amino acid substitutions, insertions or deletions which depart from the starting camelid sequence. In certain embodiments, such substitutions, insertions or deletions may be present in the framework regions of the VH domain and/or the VL domain. The purpose of such changes in primary amino acid sequence may be to reduce presumably unfavourable properties (e.g. immunogenicity in a human host (so-called humanization), sites of potential product heterogeneity and or instability (glycosylation, deamidation, isomerisation, etc.) or to enhance some other favourable property of the molecule (e.g. solubility, stability, bioavailability, etc.). In other embodiments, changes in primary amino acid sequence can be engineered in one or

more of the hypervariable loops (or CDRs) of a Camelidae VH and/or VL domain obtained by active immunisation. Such changes may be introduced in order to enhance antigen binding affinity and/or specificity, or to reduce presumably unfavourable properties, e.g. immunogenicity in a human host (so-called humanization), sites of potential product heterogeneity and or instability, glycosylation, deamidation, isomerisation, etc., or to enhance some other favourable property of the molecule, e.g. solubility, stability, bioavailability, etc.

Thus, in one embodiment, the invention provides a variant IL-24 antibody which contains at least one amino acid substitution in at least one framework or CDR region of either the VH domain or the VL domain in comparison to a camelid-derived VH or VL domain, examples of which include but are not limited to the camelid VH domains comprising the amino acid sequences shown as SEQ ID NO: 245-265 or 266-272, and the camelid VL domains comprising the amino acid sequences show as SEQ ID NO: 273-293 or 294-300.

In certain embodiments, there are provided "chimeric" antibody molecules comprising camelid-derived VH and VL domains (or engineered variants thereof) and one or more constant domains from a non-camelid antibody, for example human-encoded constant domains (or engineered variants thereof). In such embodiments it is preferred that both the VH domain and the VL domain are obtained from the same species of camelid, for example both VH and VL may be from llama (prior to introduction of engineered amino acid sequence variation). In such embodiments both the VH and the VL domain may be derived from a single animal, particularly a single animal which has been actively immunised with a human IL-24 antigen.

The invention can, in certain embodiments, encompass chimeric camelid/human antibodies, and in particular chimeric antibodies in which the VH and VL domains are of fully camelid sequence (e.g. Llama or alpaca) and the remainder of the antibody is of fully human sequence. IL-24 antibodies can include antibodies comprising "humanised" or "germlined" variants of camelid-derived VH and VL domains, or CDRs thereof, and camelid/human chimeric antibodies, in which the VH and VL domains contain one or more amino acid substitutions in the framework regions in comparison to camelid VH and VL domains obtained by active immunisation of a camelid with a human IL-24 antigen. Such "humanisation" increases the % sequence identity with human germline VH or VL domains by replacing mis-matched amino acid residues in a starting Camelidae

VH or VL domain with the equivalent residue found in a human germline-encoded VH or VL domain.

IL-24 antibodies may also be CDR-grafted antibodies in which CDRs (or hypervariable loops) derived from a camelid antibody, for example an camelid IL-24 antibody raised by active immunisation with human IL-24 protein, or otherwise encoded by a camelid gene, are grafted onto a human VH and VL framework, with the remainder of the antibody also being of fully human origin. Such CDR-grafted IL-24 antibodies may contain CDRs having the amino acid sequences shown in Tables 7 and 8 below.

Camelid-derived IL-24 antibodies include variants wherein the hypervariable loop(s) or CDR(s) of the VH domain and/or the VL domain are obtained from a conventional camelid antibody raised against human IL-24, but wherein at least one of said (camelid-derived) hypervariable loops or CDRs has been engineered to include one or more amino acid substitutions, additions or deletions relative to the camelid-encoded sequence. Such changes include "humanisation" of the hypervariable loops/CDRs. Camelid-derived HVs/CDRs which have been engineered in this manner may still exhibit an amino acid sequence which is "substantially identical" to the amino acid sequence of a camelid-encoded HV/CDR. In this context, "substantial identity" may permit no more than one, or no more than two amino acid sequence mis-matches with the camelid-encoded HV/CDR. Particular embodiments of the IL-24 antibody may contain humanised variants of the CDR sequences shown in Tables 7 and 8.

Camelid (e.g. llama) conventional antibodies provide an advantageous starting point for the preparation of antibodies with utility as human therapeutic agents due to the following factors, discussed in US 12/497,239 which is incorporated herein by reference:

1) High % sequence homology between camelid VH and VL domains and their human counterparts;

2) High degree of structural homology between CDRs of camelid VH and VL domains and their human counterparts (i.e. human-like canonical fold structures and human-like combinations of canonical folds).

The camelid (e.g. llama) platform also provides a significant advantage in terms of the functional diversity of the IL-24 antibodies which can be obtained.

The utility of IL-24 antibodies comprising camelid VH and/or camelid VL domains for human therapy can be improved still further by "humanisation" of natural camelid VH and VL domains, for example to render them less immunogenic in a human host. The overall aim of humanisation is to produce a molecule in which the VH and VL domains exhibit minimal immunogenicity when introduced into a human subject, whilst retaining the specificity and affinity of the antigen binding site formed by the parental VH and VL domains.

One approach to humanisation, so-called "germlining", involves engineering changes in the amino acid sequence of a camelid VH or VL domain to bring it closer to the germline sequence of a human VH or VL domain.

Determination of homology between a camelid VH (or VL) domain and human VH (or VL) domains is a critical step in the humanisation process, both for selection of camelid amino acid residues to be changed (in a given VH or VL domain) and for selecting the appropriate replacement amino acid residue(s).

An approach to germlining of camelid conventional antibodies has been developed based on alignment of a large number of novel camelid VH (and VL) domain sequences, typically somatically mutated VH (or VL) domains which are known to bind a target antigen, with human germline VH (or VL) sequences, human VH (and VL) consensus sequences, as well as germline sequence information available for llama pacos. This procedure, described in WO 2011/080350, contents of which are incorporated by reference, can be applied to (i) select "camelid" amino acid residues for replacement in a camelid-derived VH or VL domain or a CDR thereof, and (ii) select replacement "human" amino acid residues to substitute in, when humanising any given camelid VH (or VL) domain. This approach can be used to prepare humanised variants of camelid-derived CDRs having the amino acid sequences shown in Tables 7 and 8 and also for germlining of camelid-derived VH and VL domains having the sequences shown in Tables 9 and 10.

Format of the IL-24 antibodies

IL-24 antibodies can take various different embodiments in which both a VH domain and a VL domain are present. The term "antibody" herein is used in the broadest sense and encompasses, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific

antibodies), so long as they exhibit the appropriate immunological specificity for a human IL-24 protein. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes) on the antigen, each monoclonal antibody is directed against a single determinant or epitope on the antigen.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable domain thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, bi-specific Fab's, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, a single chain variable fragment (scFv) and multispecific antibodies formed from antibody fragments (see Holliger and Hudson, Nature Biotechnol. 23:1126-36 (2005), the contents of which are incorporated herein by reference).

In non-limiting embodiments, the IL-24 antibodies provided herein may comprise CH1 domains and/or CL domains, the amino acid sequence of which is fully or substantially human. If the IL-24 antibody is intended for human therapeutic use, it is typical for the entire constant region of the antibody, or at least a part thereof, to have fully or substantially human amino acid sequence. Therefore, one or more or any combination of the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may be fully or substantially human with respect to its amino acid sequence. Such antibodies may be of any human isotype, with human IgG4 and IgG1 being particularly preferred.

Advantageously, the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may all have fully or substantially human amino acid sequence. In the context of the constant region of a humanised or chimeric antibody, or an antibody fragment, the term "substantially human" refers to an amino acid sequence identity of at least 90%, or at least 92%, or at least 95%, or at least 97%, or at least 99% with a human constant region. The term "human amino acid sequence" in this context refers to an amino acid sequence which is encoded by a human immunoglobulin gene, which includes germline, rearranged and somatically mutated genes. Such antibodies may be of any human isotype, with human IgG4 and IgG1 being particularly preferred.

Also provided are IL-24 antibodies comprising constant domains of "human" sequence which have been altered, by one or more amino acid additions, deletions or substitutions with respect to the human sequence, excepting those embodiments where the presence of a "fully human" hinge region is expressly required.

The presence of a "fully human" hinge region in the IL-24 antibodies of the invention may be beneficial both to minimise immunogenicity and to optimise stability of the antibody.

The IL-24 antibodies provided herein may be of any isotype. Antibodies intended for human therapeutic use will typically be of the IgA, IgD, IgE IgG, IgM type, often of the IgG type, in which case they can belong to any of the four sub-classes IgG1, IgG2a and b, IgG3 or IgG4. Within each of these sub-classes it is permitted to make one or more amino acid substitutions, insertions or deletions within the Fc portion, or to make other structural modifications, for example to enhance or reduce Fc-dependent functionalities.

In non-limiting embodiments, it is contemplated that one or more amino acid substitutions, insertions or deletions may be made within the constant region of the heavy and/or the light chain, particularly within the Fc region. Amino acid substitutions may result in replacement of the substituted amino acid with a different naturally occurring amino acid, or with a non-natural or modified amino acid. Other structural modifications are also permitted, such as for example changes in glycosylation pattern (e.g. by addition or deletion of N- or O-linked glycosylation sites). Depending on the intended use of the IL-24 antibody, it may be desirable to modify the antibody of the invention with respect to its binding properties to Fc receptors, for example to modulate effector function.

In certain embodiments, the IL-24 antibodies may comprise an Fc region of a given antibody isotype which is modified in order to reduce or substantially eliminate one or more antibody effector functions naturally associated with that antibody isotype. In non-limiting embodiments, the IL-24 antibody may be substantially devoid of any antibody effector functions. In this context, "antibody effector functions" include one or more or all of antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and antibody-dependent cellular phagocytosis (ADCP).

The amino acid sequence of the Fc portion of the IL24 antibody may contain one or more mutations, such as amino acid substitutions, deletions or insertions, which have the effect of reducing one or more antibody effector functions (in comparison to a wild

type counterpart antibody not having said mutation). Several such "Fc null" mutations are known in the art of antibody engineering, for example those described in the following documents which are incorporated herein by reference: Shiels *et al.* (2001) J. Biol Chem 276 pp6591-6604; Lund *et al.,* (1996) J. Immunol 157 pp4963-4969; Lund *et al.,* (1995) FASEB. J. 9 pp115-119; Sarmay *et al.,* (1992) Mol. Immunol 29 pp633-639; Jefferis *et al.,* (1990) Mol. Immunol. 27 pp1237-1240. Non-limiting examples of "Fc null" mutations, suitable for inclusion in the IL-24 antibodies described herein, include the following mutations in the Fc domain of human IgG4 or human IgG1: N297A, N297Q, LALA (L234A, L235A), AAA (L234A, L235A, G237A), D265A, D265N or D265E (amino acid residues numbering according to the EU numbering system in human IgG1).

Cross-competing antibodies

Monoclonal antibodies or antigen-binding fragments thereof that "cross-compete" with the IL-24 antibodies disclosed herein are those that bind human IL-24 at site(s) that are identical to, or overlapping with, the site(s) at which the present IL-24 antibodies bind. Competing monoclonal antibodies or antigen-binding fragments thereof can be identified, for example, via an antibody competition assay. For example, a sample of purified or partially purified human IL-24 can be bound to a solid support. Then, an antibody compound or antigen binding fragment thereof of the present invention and a monoclonal antibody or antigen-binding fragment thereof suspected of being able to compete with such invention antibody compound are added. One of the two molecules is labelled. If the labelled compound and the unlabelled compound bind to separate and discrete sites on IL-24, the labelled compound will bind to the same level whether or not the suspected competing compound is present. However, if the sites of interaction are identical or overlapping, the unlabelled compound will compete, and the amount of labelled compound bound to the antigen will be lowered. If the unlabelled compound is present in excess, very little, if any, labelled compound will bind. For purposes of the present invention, competing monoclonal antibodies or antigen-binding fragments thereof are those that decrease the binding of the present antibody compounds to IL-24 by about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 95%, or about 99%. Details of procedures for carrying out such competition assays are well known in the art and can be found, for example, in Harlow and Lane (1988) Antibodies, A

Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pages 567-569, ISBN 0-87969-314-2. Such assays can be made quantitative by using purified antibodies. A standard curve is established by titrating one antibody against itself, i.e., the same antibody is used for both the label and the competitor. The capacity of an unlabelled competing monoclonal antibody or antigen-binding fragment thereof to inhibit the binding of the labelled molecule to the plate is titrated. The results are plotted, and the concentrations necessary to achieve the desired degree of binding inhibition are compared.

Polynucleotides encoding IL-24 antibodies

The invention also provides polynucleotide molecules encoding the IL-24 antibodies of the invention, also expression vectors containing a nucleotide sequences which encode the IL-24 antibodies of the invention operably linked to regulatory sequences which permit expression of the antigen binding polypeptide in a host cell or cell-free expression system, and a host cell or cell-free expression system containing this expression vector.

Polynucleotide molecules encoding the IL-24 antibodies of the invention include, for example, recombinant DNA molecules. The terms "nucleic acid", “polynucleotide” or a "polynucleotide molecule" as used herein interchangeably and refer to any DNA or RNA molecule, either single- or double-stranded and, if single-stranded, the molecule of its complementary sequence. In discussing nucleic acid molecules, a sequence or structure of a particular nucleic acid molecule may be described herein according to the normal convention of providing the sequence in the 5' to 3' direction. In some embodiments of the invention, nucleic acids or polynucleotides are "isolated." This term, when applied to a nucleic acid molecule, refers to a nucleic acid molecule that is separated from sequences with which it is immediately contiguous in the naturally occurring genome of the organism in which it originated. For example, an "isolated nucleic acid" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a prokaryotic or eukaryotic cell or non-human host organism. When applied to RNA, the term "isolated polynucleotide" refers primarily to an RNA molecule encoded by an isolated DNA molecule as defined above. Alternatively, the term may refer to an RNA molecule that has been purified/separated from other nucleic acids with which it would be associated in its

natural state (i.e., in cells or tissues). An isolated polynucleotide (either DNA or RNA) may further represent a molecule produced directly by biological or synthetic means and separated from other components present during its production.

For recombinant production of an IL-24 antibody according to the invention, a recombinant polynucleotide encoding it may be prepared (using standard molecular biology techniques) and inserted into a replicable vector for expression in a chosen host cell, or a cell-free expression system. Suitable host cells may be prokaryote, yeast, or higher eukaryote cells, specifically mammalian cells. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen. Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); mouse myeloma cells SP2/0-AG14 (ATCC CRL 1581; ATCC CRL 8287) or NS0 (HPA culture collections no. 85110503); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2), as well as DSM's PERC-6 cell line. Expression vectors suitable for use in each of these host cells are also generally known in the art.

It should be noted that the term "host cell" generally refers to a cultured cell line. Whole human beings into which an expression vector encoding an antigen binding polypeptide according to the invention has been introduced are explicitly excluded from the definition of a "host cell".

Antibody production

In an important aspect, the invention also provides a method of producing a IL-24 antibody of the invention which comprises culturing a host cell (or cell free expression system) containing polynucleotide (e.g. an expression vector) encoding the IL-24 antibody under conditions which permit expression of the IL-24 antibody, and recovering

the expressed IL-24 antibody. This recombinant expression process can be used for large scale production of IL-24 antibodies according to the invention, including monoclonal antibodies intended for human therapeutic use. Suitable vectors, cell lines and production processes for large scale manufacture of recombinant antibodies suitable for *in vivo* therapeutic use are generally available in the art and will be well known to the skilled person.

Therapeutic utility of IL-24 antibodies

The IL-24 antibodies provided herein have therapeutic utility in the treatment of disease conditions that benefit from inhibition of IL-24 function, including but not limited to various dermatological conditions, and inflammatory conditions. In this regard, the IL-24 antibodies provided herein are examples of a broader class of IL-24 antagonists having therapeutic utility in the treatment of said dermatological conditions and inflammatory conditions.

An exemplary IL-24 antagonist monoclonal antibody having the properties of binding IL-24 and blocking IL-24-mediated cell proliferation in a cell-based assay system has been tested in a mouse model of allergic contact dermatitis (see Example 11 below) and shown to significantly reduce ear thickening in this model. This *in vivo* study provides validation of the mode-of-action of IL-24 antagonists in the treatment of dermatological conditions, and also more broadly in the treatment of inflammatory conditions associated with, or mediated by, IL-24 signalling through the human IL-22R1/IL-20R2 receptor complex.

Therefore, in one aspect the invention provides a method of treating or preventing a dermatological condition in a human patient which comprises administering to a patient in need thereof a therapeutically effective amount of an IL-24 antagonist.

In a further aspect the invention provides an IL-24 antagonist for use in for treating or preventing a dermatological condition in a human patient.

In non-limiting embodiments the dermatological condition may be selected from the group consisting of atopic dermatitis, contact dermatitis, contact hypersensitivity, plaque psoriasis and pustular psoriasis.

The IL-24 antagonist may be an IL-24 antibody or antigen binding fragment thereof. In particular, the IL-24 antagonist is an antibody or antigen binding fragment as specifically described herein.

In a still further aspect there is provided a method of treating or preventing an inflammatory condition in a human patient which comprises administering to a patient in need thereof a therapeutically effective amount of an IL-24 antagonist.

In non-limiting embodiments the inflammatory condition may be selected from the group consisting of IBD, colitis, Endotoxemia, arthritis, rheumatoid arthritis, psoriatic arthritis, adult respiratory disease (ARD), septic shock, multiple organ failure, inflammatory lung injury such as asthma or bronchitis, bacterial pneumonia, psoriasis, eczema, ulcerative colitis and Crohn's disease.

The IL-24 antagonist may be an IL-24 antibody or antigen binding fragment thereof. In particular, the IL-24 antagonist is an antibody or antigen binding fragment as specifically described herein.

As used herein, the term "treating" or "treatment" means slowing, interrupting, arresting, controlling, ameliorating, stopping, reducing severity of a symptom, disorder, condition or disease, but does not necessarily involve a total elimination of all disease-related symptoms, conditions or disorders.

For human therapeutic use the IL-24 antagonist, including IL-24 antibodies as described herein, may be administered to a human subject in need of treatment in an "effective amount". The term "effective amount" refers to the amount or dose of an IL-24 antibody which, upon single or multiple dose administration to a human patient, provides therapeutic efficacy in the treatment of disease. Therapeutically effective amounts of the IL-24 antibody can comprise an amount in the range of from about 0.1 mg/kg to about 20 mg/kg per single dose. A therapeutic effective amount for any individual patient can be determined by the healthcare professional by monitoring the effect of the IL-24 antibody on a biomarker, or a symptom of the disease condition being treated. The amount of antibody administered at any given time point may be varied so that optimal amounts of IL-24 antibody, whether employed alone or in combination with any other therapeutic agent, are administered during the course of treatment.

Pharmaceutical compositions

The scope of the invention also includes pharmaceutical compositions, containing one or a combination of IL-24 antibodies of the invention, or antigen- binding fragments thereof, formulated with one or more a pharmaceutically acceptable carriers or excipients.

Such compositions may include one or a combination of (e.g., two or more different) IL-24 antibodies.

Techniques for formulating antibodies for human therapeutic use are well known in the art and are reviewed, for example, in Wang et al., Journal of Pharmaceutical Sciences, Vol.96, pp1-26, 2007.

Incorporation by Reference

Various publications are cited in the foregoing description and throughout the following examples, each of which is incorporated by reference herein in its entirety.

Examples

The invention will be further understood with reference to the following non-limiting experimental examples.

Example 1: Immunization of llamas

Immunizations of llamas and harvesting of peripheral blood lymphocytes (PBLs) as well as the subsequent extraction of RNA and amplification of antibody fragments were performed as described by De Haard and colleagues (De Haard H, et al., J. Bact. 187:4531-4541, 2005). Four llamas were immunized with hIL-24 (R&D Systems, cat nr. 1965-IL/CF) and two llamas with mIL-24 (R&D Systems, cat nr. 2786-ML). The llamas were immunized with weekly intramuscular injections in the neck (six times) of antigen in Freund's Incomplete Adjuvant.

Blood samples of 10 ml were collected pre- and post-immunization to investigate the immune response. The sera from the llamas were tested for the presence of antibodies against recombinant hIL-24 or mIL-24 by ELISA prior to (day 0) and after (day 40) immunization. As detection antibody a mouse anti-llama Fc 27E10 (Daley LP et al, Clin Vaccine Immunol 12:380-386, 2005) was applied, followed by HRP-conjugated donkey-anti-mouse. The results are shown in Figure 1.

Four days after the last immunization, 400 ml blood was collected for extraction of total RNA from the PBLs using a Ficoll-Paque gradient to isolate PBLs and the method described by Chomczynski P, et al., Anal. Biochem. 162: 156-159, 1987 to

prepare the RNA. In average, RNA yields of 450 µg were achieved, aliquots of which were used for random cDNA synthesis.

Example 2: Library construction

Independent VλCλ and VκCκ libraries were constructed using a two-step PCR, in which 25 cycles with non-tagged primers was done followed by 10 cycles using tagged version of these primers (De Haard H, et al., J. Biol. Chem. 274, 1999). The VHCH1 libraries were built in parallel using the same approach. The sizes of the individual libraries were between 10^8 and 10^9 cfu (Table 1). Next, the light chain from the VλCλ and VκCκ libraries were re-cloned separately in the VHCH1-expressing vector to create the "Lambda" and "Kappa" llama Fab-library respectively or alternatively VHCH1 amplicons were cloned directly in the pre-cloned light chain repertoires. The final libraries were between $3x10^8$ and $2x10^9$ cfu. Quality control of the libraries was routinely performed using PCR.

Table 1: library sizes of IL-24 immunized llamas

Library	**Size (cfu)**
Lia Kappa	3.0E+08
Lia Lambda	1.2E+09
Alisha Kappa	7.0E+08
Alisha Lambda	1.1E+09
Taimita Kappa	1.1E+09
Taimita Lambda	8.8E+08
Corazon Kappa	1.3E+09
Corazon Lambda	7.2E+08
Vivi Kappa	3.6E+08
Vivi Lambda	4.2E+08
Madrugada Kappa	2.1E+08
Madrugada Lambda	8.8E+08

Example 3: Selections and screening on murine IL-24

Three consecutive rounds of selections were done on directly coated mIL-24 using standard protocols. Elutions were done with trypsin. Individual colonies were isolated and periplasmic fractions (peris) in 96-well plates were produced by IPTG induction from all the libraries according to standard protocols. In all selections, several clones were found that were able to bind mIL-24 as determined by ELISA (Figure 2). For the binding ELISA, mIL-24 is directly coated on Maxisorp plates and binding of the Fabs is detected using an anti-myc-HRP mAb. The hit rate per selection strategy was determined and listed in Table 2. From the 188 tested clones, 61 were able to bind to mouse IL-24. Amino acid sequences of the VH and VL domains of binding llama-derived Fab clones were determined and are shown elsewhere herein (Tables 7 and 8).

Example 4: ELISA with purified mIL-24 Fabs

A selection of anti-mIL-24 Fabs was produced in larger volumes and purified using IMAC on Talon beads. Purified Fabs were titrated on Maxisorp plates coated with mIL-24 or hIL-24, and detected with anti-Myc-HRP.

All antibodies bind with high specificity to the mIL-24, whereas no binding to hIL-24 was observed (Fig 2).

Example 5: No inhibition of mIL-24 mediated cell proliferation by mIL-24 specific Fabs

To analyse the blocking of these antibodies against the mouse form of IL-24, we used a system of BaF3 cells stably transfected with mouse IL-22R/IL-20R2 complexes of receptor. These cells need mIL-24 for proliferation. The coding sequences for IL-24 were amplified by RT-PCR from RNA of mouse and human T cells stimulated with anti CD3 Ab. These cDNAs were cloned into pCEP4 plasmid (Invitrogen, Groningen, the Netherlands) under the control of the CMV promoter. Human embryonic kidney (HEK) 293-EBV nuclear Ag cells, grown in DMEM medium supplemented with 10% FCS, were transiently transfected with the human and mouse IL-24 expression construct by the Lipofectamine 2000 method (Life Technologies, Gent, Belgium). After three days of transfection, the supernatant was collected.

The conditioned supernatant of HEK293-cells expressing mIL-24, diluted to the non-saturating concentration of 0.5%, was pre-incubated for 30 minutes with 8 different

concentrations of each Fab. BaF3mIL22R/IL20Rβ ($3x10^3$), cultured in Dulbecco modified Eagle medium with fetal bovine serum (10%) and IL-3 (100 U/mL), were added and incubated for 3 days. The substrate of hexosaminidase (p-nitrophenyl-ß-D-glucosaminide) was added for 2.5 hrs, and OD at 450nm was measured as read-out. None of the tested Fabs was able to inhibit the mIL-24 dependent proliferation, indicating that these antibodies do not block the binding of the mouse IL-24 to its receptor *in vitro* (Figure 3).

Example 6: Selections and screening on hIL-24

Three consecutive rounds of selections were done on either captured recombinant biotin-hIL-24 or on directly coated hIL-24 using standard protocols. Elutions were done with trypsin.

Individual colonies were isolated and periplasmic fractions (peris) in 96-well plates were produced by IPTG induction from all the libraries according to standard protocols. In all selections, several clones were found that were able to bind biotin-hIL-24 or hIL-24 as determined by ELISA. For the binding ELISA, Biotin-hIL-24 is captured by neutravidin, and non-biotinylated hIL-24 is directly coated on Maxisorp plates and binding of the Fabs is detected using an anti-myc-HRP mAb. The hit rate per selection strategy was determined and listed in table 2. Amino acid sequences of the VH and VL domains of binding llama-derived Fab clones are shown elsewhere herein (Tables 7 and 8).

Table 2: Specific clones against hIL-24 and mIL-24

Target	Clones tested	Binders	Hit rate (%)
hIL-24	190	40	21
Biotin-hIL-24	190	79	42
mIL-24	188	61	32

Example 7: Affinity for human and mouse IL-24 of Fabs selected on human cytokine

A CM5 chip was coated with hIL-24 (R&D Systems, cat nr. 1965-IL/CF) or with mIL-24 (R&D Systems, cat nr. 2786-ML) and off-rates of the peris containing llama-

derived Fabs from the selections on hIL-24 or biotin-hIL-24 were determined using the Biacore. The results are summarized in Tables 3 and 4.

All llama-derived Fabs tested bind with apparent high affinity to human recombinant IL-24 and to mouse recombinant IL-24 in Biacore showing off-rates in the range of 2 x 10^{-4} $^{s-1}$ to 2 x 10^{-3} $^{s-1}$.

Table 3: off-rates of anti-hIL-24 Fabs

Clone	k_d (human) (s^{-1})	R0 (human)	k_d (mouse) (s^{-1})	R0 (mouse)
1A7	3.11E-04	324	5.38E-04	80.3
1C7	3.77E-04	234	1.04E-03	69
1H9	5.34E-04	233	7.03E-04	101
1C10	6.78E-04	176	1.24E-03	53
1B8	1.66E-03	208	9.29E-04	75.2
1C8	2.22E-04	200	8.92E-04	79
1D8	5.97E-04	256	1.32E-03	60.8
1G10	3.78E-04	291	8.16E-04	89.9
1A12	4.45E-04	94.7	1.30E-03	55.9
1D6	8.08E-04	215	1.50E-03	55.1
1C11	2.65E-04	311	7.32E-04	86.5
1D9	1.00E-03	243	9.90E-04	69.7
2C7	4.33E-04	219	9.68E-04	65.8
2D10	4.88E-04	188	1.08E-03	58.5
1A10	5.15E-04	208	1.55E-03	46.8
2A5	3.17E-04	81.9	1.28E-03	63.8
3D7	7.08E-04	345	8.86E-04	67.8
3B9	7.52E-04	232	1.43E-03	51.5
4B7	1.32E-03	70.6	8.76E-04	38.3
4C7	1.99E-03	67.3	1.36E-03	37.2
4E11	2.71E-03	50.9	1.90E-03	30.6
4F12	1.07E-03	108	1.35E-03	53.4

4G9	6.97E-04	171	9.79E-04	73.6
4H9	6.73E-04	125	1.49E-03	50.9

Table 4: off-rates of anti-mIL-24 Fabs

Clone	k_d (human) (s^{-1})	R_0 (human)	k_d (mouse) (s^{-1})	R_0 (mouse)
7A4	8.32E-03	7.24	6.28E-03	29.2
7A11	3.56E-03	6.46	4.37E-03	102
7B4	1.74E-04	9.19	3.17E-03	44.7
7C2	2.68E-03	8.49	1.27E-03	41.3
7G9	-1.28E-03	-1.59	0.0135	3.76
8A10	3.32E-03	12.5	1.26E-03	241
8G5	7.75E-03	8.83	9.74E-03	11.2

Example 8: Inhibition of IL-24 mediated cell proliferation by Fabs selected on human cytokine

To analyse the cross-reactivity of these antibodies against the mouse form of IL-24, a system was used of BaF3 cells stably transfected with either human or mouse IL-22R/IL-20R2 complexes of receptor. These cells need hIL-24 or mIL-24 for proliferation, respectively. The coding sequences for IL-24 were amplified by RT-PCR from RNA of mouse and human T cells stimulated with anti CD3 Ab. These cDNAs were cloned into pCEP4 plasmid (Invitrogen, Groningen, the Netherlands) under the control of the CMV promoter. Human embryonic kidney (HEK) 293-EBV nuclear Ag cells, grown in DMEM medium supplemented with 10% FCS, were transiently transfected with the human and mouse IL-24 expression construct by the Lipofectamine 2000 method (Life Technologies, Gent, Belgium). After three days of transfection, the supernatant is collected.

Conditioned supernatant of HEK293-cells expressing hIL-24 (final concentration 0.5%, non-saturating) or mIL-24 (final concentration 1%, non-saturating) was pre-incubated for 30 minutes with 8 different amounts of periplasmic extract. BaF3mIL22R/mIL20Rβ or BaF3hIL22R/hIL20Rβ ($3x10^3$), cultured in Dulbecco modified Eagle medium with fetal bovine serum (10%) and IL-3 (100 U/mL), were added

and incubated for 3 days. The substrate of hexosaminidase (p-nitrophenyl-ß-D-glucosaminide) was added for 2.5 hrs, and OD at 450nm was measured as read-out. When we added periplasmic extracts containing the different anti-hIL-24 Fabs, we observed inhibition of human IL-24 induced proliferation of these BaF3 cells with some of them (Figure 4A), meaning that these Fabs block the binding of the IL-24 to its receptor. A number of these did not antagonize proliferation (Figure 4B).

The Fabs showing antagonism in the hIL-24-mediated proliferation assay, were tested for antagonism in the same assay with mIL-24. One antibody, 1C11, was able to block activity of both hIL-24 and mIL-24 with good potency (Figure 5; Table 5).

Table 5. IC50 values for inhibition of hIL-24- or mIL-24-mediated BaF3 cell proliferation.

Clone	1A12	1C8	1C11	1D8	1D9	1G10
IC50 hIL-24 (% peri)	1.813	0.08973	0.071	0.3726	1.337	0.5162
IC50 mIL-24 (% peri)	-	-	0.066	-	-	-

Example 9: Binding of chimeric llama-mouse IL-24 mAbs to IL-24 positive cells

The VH and VL of interesting llama-derived Fab clones were fused with mouse constant IgG2a domains containing a N297A mutation, and produced as bivalent chimeric monoclonal antibodies in the system described in patent application WO 2009/145606. The chimeric llama-mouse mAbs were purified on Protein A.

To test the ability of the mAbs to bind to native IL-24 expressed by mammalian cells, BW cells were stably transfected with a human or mouse IL-24 –fusion construct. The full-length coding region of IL-24 was cloned in frame with the transmembrane domain of the human PDGFR and cloned into pDisplay (Invitrogen). The chimeric expression product allows having cells that express IL-24 protein on the cell surface. BWhIL-24 cells or parental BW cells were incubated with a 1/2500 dilution of anti-IL-24 mAb or isotype control. After washing, bound antibody was detected with goat-anti-mouse FITC, followed by flow cytometry analysis. The cells were gated based on

forward and side scatter and acquired on a FACSCalibur™ (Becton Dickinson) and analysed using FlowJo software. FACS binding profiles for mAbs 1D9, 1C7, 1C11 and 1C8 against human IL-24 expressing cells are shown in Figure 6. Antibodies 1D9, 1C7 and 1C11 are able to detect hIL-24 expressed by the BW cells.

In a second approach, anti-IL-24 antibodies 1C7 and 1C11 were directly conjugated to Alexa Fluor 647, and used to stain BW cells expressing cell surface hIL-24 or mIL-24 (Figure 7). BW cells were incubated with a 1/1000 dilution of the antibody conjugate, washed and antibody binding was determined using flow cytometry analysis. The cells were gated based on forward and side scatter and acquired on a FACSCalibur™ (Becton Dickinson) and analyzed using FlowJo software. Both antibodies detected hIL-24 on BW cells, but only mAb 1C11 was able to detect mIL-24.

Example 10: inhibition of IL-24 mediated cell proliferation by mAbs

To confirm the blocking activity of the selected antibodies against the human or mouse form of IL-24, conditioned supernatant of HEK293-cells expressing hIL-24 (final concentration 0.5%) or mIL-24 (final concentration 1%) was pre-incubated for 30 minutes with different concentrations of antibody. BaF3hIL22R/hIL20Rβ or BaF3mIL22R/mIL20Rβ (3x103) were added and incubated for 3 days. The substrate of hexosaminidase (p-nitrophenyl-ß-D-glucosaminide) was added for 3 hrs, and OD at 450nm was measured as read-out.

BaF3 cells stably expressing either human or mouse IL-22R/IL-20R2 complexes of receptor, cultured in Dulbecco modified Eagle medium with fetal bovine serum (10%) and IL-3 (100 U/mL), were incubated for 30 minutes with different concentrations of the antibodies. Antibodies 1C11, 1C8 and 1D9 were able to the hIL-24-mediated proliferation (Figure 8A). One antibody, 1C11, was able to block activity of both hIL-24 and mIL-24 (Figure 8B). The IC50 values of the antibodies are listed in Table 6.

Table 6. IC50 values of four anti-hIL-24 antibodies in the IL-24 mediated cell proliferation assay.

Clone	**IC50 hIL-24 (nM)**	**IC50 mIL-24 (nM)**
1C11	0.35	0.06
1C8	0.40	-
1C7	-	-

1D9	1.16	-

Example 11: in vivo testing of chimeric llama-mouse mAbs in a PPD-induced allergic contact dermatitis model

All mice were bred in the animal facility of the Brussels branch of the Ludwig Institute for Cancer Research under specific pathogen-free conditions. Rag2-/- BALB/c mice were originally purchased from Taconic (Ejby, Denmark). IL-20R2-/-mice were provided by U.M. Wegenka (University Medical Center, Ulm, Germany) (Wahl, C., et al. J Immunol 182(2): 802-810, 2009). Wild-type 129sv mice were originally purchased from Harlan (Horst, The Netherlands). IL-22–deficient mice were generated in the Ludwig laboratory as described previously (Kreymborg, K., et al. J Immunol 179(12): 8098-8104, 2007) . IL-22R-/- mice were generated in the Ludwig laboratory by targeting exons 1-4 in the R1 line (129X1/SvJ x 129S1) ES cells169 and backcrossed with 129sv mice for 15 generations. The targeting vector was constructed to replace the first four exons of the il22ra1 gene by a LacZ reporter cassette with a nuclear localization signal (pSKT NLS LacZ) in frame with initiation codon of il22ra1 and a neomycin-resistant cassette (PGK Neo A), which is floxed. The 5' arm of this construct consisted of a 2.8 kb fragment starting at a NheI site from the promoter and ending at the ATG initiation codon, and was inserted in frame with the LacZ gene. The 3' arm consisted of a 5.3 kb fragment that extend from the XmaI restriction site, located between exon 4 and exon 5, and the BglII site located between exon 5 and exon 6.

The IL-22-deficient mice and IL-22R-deficient mice were bred as heterozygous and littermate controls were used for in vivo experiments. IL-20R2-deficient mice were in the C57Bl/6 background and littermate mice were generated. The experiments were performed in compliance with institutional guidelines and were approved by the local ethical committee. Mice between 8 and 12 weeks of age were shaved on the back skin one day before contact hypersensitivity (CHS) triggering.

Mice were sensitized by applying a solution of para-Phenylenediamine (PPD) (CAS 106-50-03) (3% [W/V] for 129 background mice and 5% for C57Bl6 background mice) and hydrogen peroxide (3%) diluted in 4:1 Acetone:olive oil vehicle on dorsal skin (50 µl) and dorsum of the ears (25 µl/ear). Two sensitizations were performed at day 0

and day 5. At day 10, 11, 12 and 13 mice were challenged by painting dorsum of the ears (25 µl/ear) with the PPD solution. The first challenge was performed with PPD and hydrogen peroxide solution as used for the sensitization and the next challenges were performed with PPD alone in vehicle solution. Control mice received vehicle solution (including hydrogen peroxide 3% for the two sensitizations and the first challenge). All solutions were prepared freshly. Ear thickness was measured before each application of PPD and 24 hours after the last challenge with a micrometer screw (Mitutoyo). Animals receiving anti-IL24 mAb 1C11 or IgG2a isotype control were dosed 250 µg/animal intraperitoneally and 20 µg/ear intradermally at day 0 and day 5.

IL-20, IL-22 and IL-24 interact and activate the common receptor IL-22R. In the *in vivo* model we used the IL-22 deficient mice for treatment with the human – mouse IL-24 blocking mAb 1C11 to exclude the contribution of IL-22. In addition we compared the results with those obtained in the IL-22R deficient mice, which is the maximal therapeutic effect that can be achieved by completely blocking the IL-22R pathway. The results showed that IL-22-deficient mice that received anti-IL-24 antibody present a slight decrease in ear thickness compared to IL-22-deficient mice receiving control antibody (Figure 9; *$p < 0.05$). In addition, the ear thickening of IL-22-deficient mice that received anti-IL-24 antibody is similar to the thickening of IL-22R-deficient mice.

Table 7: sequence of IL-24 specific llama-derived VH; frameworks and CDRs

Fab	Framework 1	SEQ ID	H-CDR1	SEQ ID
1A7	QVQLQESGPGLVKPSQTLSLTCTVSGGSIT	1	TGTYSWS	2
1C7	EVQLQESGPGLVKPSQTLSLTCTVSGDSIT	8	SSYYHYS	9
1H9	EVQVQESGPGLVKPSQTLSLTCTVSGGSIT	13	TGTYSWS	14
1C10	EVQLQESGPGLVKPSQTLSLTCTVSGGSIT	15	TGTYSWS	14
1B8	QLQLVESGGALVQPGGSLRLSCAASGFTFD	16	DYAMS	17
1C8	QLQLVESGGALVQPGGSLRLSCAASGFTFD	16	DYAMS	17
1D8	EVQLVESGGALVQPGGSLRLSCAASGFTFD	23	DYAMS	17
1G10	ELQLVESGGALVQPGGSLRLSCAASGFTFD	26	DYAMS	17
1A12	EVQLVESGGALVQPGGSLRLSCAASGFTFD	23	DYAMS	17
1D6	EVQLVESGGGLVQPGGSLRLSCVASGFTFD	28	DYGMS	29
1C11	ELQLVESGGGSVQPGGSLRLSCAASGFTFS	34	NYWMY	35
1D9	QLQVVESGGGLVQPGGSLRLSCVASGFTFG	40	DYAMS	17
2C7	QLQVVESGGGLVQPGGSLRLSCAASGFTFD	46	DYGMS	29
2D10	EVQLVQPGGSLRLSCAASGFTFD	52	DYGMS	29
1A10	EVQLVQPGAELRNPGASVKVSCKASGYTFT	56	SYYID	57
2A5	QVQLQESGGGLVQPGGSLRLSCAASGFTFS	63	SDAMS	64
3D7	QLQLVESGGGLVQPGGSLRLSCAASGFTFG	68	SYDMS	69

3B9	QLQLVESGGGLVQPGGSLRLSCAASGFTFG	68	SYDMS	69
4B7	QVQLVESGGGLVQPGGSLRLSCAASGFTFS	75	RKWMY	76
4C7	QVQLVESGGGLVQPGGSLRLSCAASGFTFS	75	RKWMY	76
4E11	QVQLVESGGGLVQPGGSLRLSCAASGFTFS	75	RKWMY	76
4F12	QLQLVESGGGLVQPGGSLRLSCAASGFTFS	81	GYWMY	82
4G9	QLQLVESGGGLVQPGGSLRLSCAASGFTFS	81	GYAMS	86
4H9	QLQLVESGGGLVQPGGSLRLSCAASGFTFS	81	GYAMS	86
7C2	EVQVQESGPGLVKPSQTLSLTCTVSGGSIT	13	TSYSAWS	91
7A4	ELQLVESGGGLVQPGGSLRLSCAASGFTFS	95	SYAMS	96
7B4	ELQLVESGGGLVQPGGSLRLSCVASGFTFD	100	DYAMN	101
7A11	ELQLVESGGGLVQPGGSLRLSCAASGFTNGFTFD	105	DYGMS	29
8A10	QVQLVESGPGLVKPSQTLYLTCTVSGGSIT	110	TAYYAWN	111
7G9	QVQRQESGGGLVQPGGSLRLSCAASGFTFS	116	SHVMA	117
8G5	EVQLVESGGGLVQPGGSLRVSCAASGFTFS	121	SYYMN	122

Fab	Framework 2	SEQ ID	H-CDR2	SEQ ID
1A7	WIRQPPGKGLEWMG	3	VIAYDGSTYYSPSLKS	4
1C7	WIRQPPGKGLEWMG	3	VIAYDGSTYYSPSLKS	4
1H9	WIRQPPGKGLEWMG	3	VIAYDGSTYYSPSLKS	4
1C10	WIRQPPGKGLEWMG	3	VIAYDGSTYYSPSLKS	4
1B8	WVRQAPGKGLEWVS	18	HISGNGESTFYAESMKG	19
1C8	WVRQAPGKGLEWVS	18	HISGNGFSTFYAESMKG	22
1D8	WVRQAPGKGLEWVS	18	HISGNGHSTFYAESMKG	24
1G10	WVRQAPGKGLEWVS	18	HISGNGRSTFYAESVKG	27
1A12	WVRQAPGKGLEWVS	18	HISGNGFSTFYAESMKG	22
1D6	WVRQAPGKGLEWVS	30	FITWNGGITYYAESMKG	31
1C11	WVRQAPGKGLEWLS	36	LITTDGGSTFYADSVKG	37
1D9	WVRQAPGKGLEWIA	41	GITWNGNTYYPDSLEG	42
2C7	WVRHSPGKGLEWVS	47	TISWNGGVTYYAASVKG	48
2D10	WVRHSPGKGLEWVS	53	TISWNGGVTYYAASMKG	54
1A10	WVRQAPGQGLEWMG	58	RIDPEDGGTKYAQKFQG	59
2A5	WVRQAPGKGLEWVS	18	SINSGGGSTNYADSVKG	65
3D7	WVRQAPGKGPEWVS	70	AINSGGRSTYYADSVKG	71
3B9	WVRQAPGKGPEWVS	70	AINSGGRSTYYADSVKG	71
4B7	WVRQAPGKGLEWVS	18	VINTGGGSTYYSDSVKG	77
4C7	WVRQAPGKGLEWVS	18	VINTGGGSTYYSDSVKG	77
4E11	WVRQAPGKGLEWVS	18	VINTGGGSTYYSDSVKG	77
4F12	WVRQAPGKGLEWVS	18	VINTREGSTYYADSVKG	83
4G9	WVRQAPGKGLEWVS	18	GINSDGGNTYHVDSVKG	87
4H9	WVRQAPGKGLEWVS	18	GINSDGGNTYHVDSVKG	87
7C2	WIRQPPGKGLEWMG	3	LIAYDGSTYYSPSLKS	92
7A4	WVRQAPGKGLEWVS	18	SINSGGDRLHYADSVKG	97
7B4	WVRQAPGKGLEWVS	18	SISWDGGSTDVAESMKG	102
7A11	WVRQVPGKGLEWVS	106	SISWTGERTYYAESVKG	107
8A10	WIRQPPGKGLEWMG	3	AIAYDGSTYYSPSLKS	112
7G9	WVRQAPGKGLEWVS	18	AINGGGGTTSYADSVKG	118
8G5	WVRQAPGKGLEWVS	18	AVSTGGGSTYYADSVKG	123

Fab	Framework 3	SEQ ID	H-CDR3	SEQ ID
1A7	RTSISRDTSKNQFSLQLSSVT PEDTAVYYCAR	5	NNVAAPGY	6
1C7	RTSISRDTSKNQFSLQLNSVT PEDTAVYYCAR	10	DDVAAPGY	11
1H9	RTSISRDTSKNQFSLQLSSVT PEDTAVYYCAR	5	DDVAAPGY	11
1C10	RTSISRDTSKNQFSLQLSSVT PEDTAVYYCAR	5	DDVAAPGY	11
1B8	RATISRDNAKNTLYLQMNSLK SEDTAVYYCAK	20	DDYGSGLYAY	21
1C8	RATISRDNAKNTLYLQMNSLK SEDTAVYYCAK	20	DDYGSGLYAY	21
1D8	RFTISRDNAKNTLYLQMNSLK SEDTAVYYCAK	25	DDYGSGLYAY	21
1G10	RFTISRDNAKNTLYLQMNSLK SEDTAVYYCAK	25	DDYGSGLYAY	21
1A12	RATISRDNAKNTLYLQMNSLK SEDTAVYYCAK	20	DDYGSGLYAY	21
1D6	RFTISRDNAKNTLYLQMDSLK SEDTAVYYCAK	32	DSDPSIATPYFGS	33
1C11	RFTISRDNAKNTLYLQMNSLK SEDTAVYYCAK	25	DLGSGFSLGS	38
1D9	RFTISRDNAKNTLSLQMNSLK SEDTAVYYCAK	43	GGIGATVFGS	44
2C7	RFTISRDNAKNTVYLQMNSLK PEDTAVYYCAK	49	EIITVQALRAAIAFDS	50
2D10	RFTISRDNAKNTVYLQMNSLK PEDTAVYYCAK	49	EIITVQAMRAAIAFDS	55
1A10	RVTFTADTSTSTAYVELSSLR SEDTAVYYCAR	60	KGERAYGSSWYSIYDYGMDY	61
2A5	RFTISRDNAKNTLYLQMNSLK PEDTAVYYCAE	66	SDYGYGYGLAYTYDY	67
3D7	RFTISRDNAKNTLYLQMNSLK PEDTAVYYCAT	72	RGSGGSFGS	73
3B9	RFTISRDNAKNTLYLQMNSLK PEDTAVYYCAT	72	RGSGGSFGS	73
4B7	RFTISRDNAKNTLYLQMNSLK SEDTAVYYCAN	78	GGAELQPARFGGIWSPDRSLDA	79
4C7	RFTISRDNAKNTLYLQMNSLK SEDTAVYYCAN	78	GGAELQPARFGGIWSPDRSLDA	79
4E11	RFTISRDNAKNTLYLQMNSLK SEDTAVYYCAN	78	GGAELQPARFGGIWSPDRSLDA	79
4F12	RFTISRDLAKNTLYLQMNSLK SEDTAVYYCAN	84	GGAELQPTTYGGLWSPDRSLDA	85
4G9	RFTISRDNAKNTVYLQMNSLK PEDTAVYYCAT	88	TIVPGY	89
4H9	RFTISRDNAKNTVYLQMNSLK PEDTAVYYCAT	88	TIVPGY	89
7C2	RTSISRDTSKNQFSLQLSSVT PEDTAVYYCAS	93	DSYYSGSYYSGSYYYTA	94
7A4	RFTISRDNAKNTLYLQMNSLK PEDTAVYYCAK	98	YGSPNMALMDWRVDFDS	99
7B4	RFTIVRDNAKNTLYLQMNSLK SEDTAVYYCAK	103	SLYSDPSYDY	104
7A11	RFTIFRDNAKNMLYLQMNSLK SEDTAVYYCAK	108	ALMGEYDY	109

8A10	RSSISRDTSKNQFSLQLSSVT PEDTAVYYCAE	113	AGGAGWGPTFFY	114
7G9	RFTISRDNAKNTLYLQMNSMK SEDTAVYYCAN	119	SLWPYQGS	120
8G5	RFTISRDNAKNTLYLQMNSLK PEDTALYYCVR	124	GDVRGTYDSYGY	125

Fab	FRAMEWORK 4	SEQ ID
1A7	WGQGTRGTGSWV	7
1C7	WGQGTQVTVSS	12
1H9	WGQGTQVTVSS	12
1C10	WGQGTQVTVSS	12
1B8	WGQGTQVTVSS	12
1C8	WGQGTQVTVSS	12
1D8	WGQGTQVTVSS	12
1G10	WGQGTQVTVSS	12
1A12	WGQGTQVTVSS	12
1D6	WGQGTQVTVSS	12
1C11	WGQGTQVTVSA	39
1D9	WGQGTQVTISS	45
2C7	WDQGTQVTVSS	51
2D10	WDQGTQVTVSS	51
1A10	WGKGTLVTVSS	62
2A5	WGQGTQVTVSS	12
3D7	WGQGTRVTVSS	74
3B9	WGQGTRVTVSS	74
4B7	WGQGTLVTVSS	80
4C7	WGQGTLVTVSS	80
4E11	WGQGTLVTVSS	80
4F12	WGQGTLVTVSS	80
4G9	WVQGTQVTVSS	90
4H9	WGQGTQVTVSS	12
7C2	WGQGTQVTVSS	12
7A4	WGQGTQVTVSS	12
7B4	WGQGTQVTVSS	12
7A11	WGQGTQVTVSS	12
8A10	WGQGAQVTVSS	115
7G9	WGQGTQVTVSS	12
8G5	WGQGTQVTVSS	12

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Table 8: sequence of IL-24 specific llama-derived VL; frameworks and CDRs

Fab	Framework 1	SEQ ID	L-CDR1	SEQ ID
1A7	QAVVTQEPSLSVSPGGTVTLTC	126	GLSSGSVTTSNYPG	127
1C7	QTVVTQEPSLSVSPGGTVTLTC	133	GLSSGPVTTSNYPG	134
1H9	QAVVTQEPSLSVSPGGTVTLTC	126	GLSSESVTTSNYPG	137
1C10	QAVVTQEPSLSVSPGGTVTLTC	126	GLSSGSVTTGNYPG	140
1B8	SYELTQSPSVSVALRQTAKITC	143	GGDNIGSKSAQ	144
1C8	SYELTQSPSVSVALKQTAKITC	149	GGDNIGSKSAQ	144

1D8	SYELTQSPSVSVALRQTAKITC	143	GGDNIGSKSAQ	144
1G10	SYELTQSPSVSVALRQTAKITC	143	GGDNIGSKSAQ	144
1A12	SYELTQSPSVSVALRQTAKITC	143	GGDNIGSKSVQ	155
1D6	EIVLTQSPSSVTASVGEKVTINC	158	KSSQSVVSGSNQKSYLN	159
1C11	SYELTQSPSVSVAQRQTAKITC	165	GGDNIGSKSAQ	144
1D9	SSALTQPSAVSVSLGQTARIAC	169	QGDSIGNYGAN	170
2C7	HSAVTQPSAVSVSLGQTARITC	175	QGDNFGSYYAS	176
2D10	SSALTQPSAVSVSLGQTARITC	181	QGGNFGNYYAN	182
1A10	HSAVTQPSAVSVSLGQMARITC	184	QGDNFGSYYAS	176
2A5	EIVMTQSPSSLSASLGDRVTITC	187	QASQSISSYLA	188
3D7	LPAVNHPSALSVSLGQMAKITC	193	QGGNFGSYYAS	194
3B9	QPVLNQPSAVSVSLGQMAKITC	198	QGGNFGSYYAS	194
4B7	NFMLTQPSAVSVSLGQTARITC	200	QGGNFGSYYAS	194
4C7	NFMLTQPSAVSVSLGQTARITC	200	QGGNFGSYYAS	194
4E11	NFMLTQPSAVSVSLGQTARITC	200	QGGNFGSYYAS	194
4F12	NFMLTQPSAVSVSLGQTARITC	200	QGGNFGSYYAS	194
4G9	QTVVTQEPSLSVSPGGTVTLTC	133	GLSSGSVTSSNYPH	202
4H9	QTVVTQEPSLSVSPGGTVTLTC	133	GLSSGSVTSSNYPH	202
7C2	DVVLTQTPGSLSVVPGESASISC	207	KASQSLVHTDGKTYLS	208
7A4	DIVMTQSPSSMTASTGEKVTINC	213	KSSRSVLYSANQKNYLA	214
7B4	DVVLTQTPGSLSVVPGESASISC	207	KASQSLVYIDGKTYLS	220
7A11	SYELTQPPSVSGSPGKTVTISC	224	AGTSSDVGYGNYVS	225
8A10	QAVLTQPPSVSGSPGKTVTISC	230	AGTSSDVGYGNYVS	225
7G9	QSALTQPSAVSVSLGQTARITC	233	EGGSLLGNYGTN	234
8G5	DIVMTQSPSSLSASLGDRVTITC	239	QASQSISTELS	240

Fab	Framework 2	SEQ ID	L-CDR2	SEQ ID
1A7	WFQQTPGQAPRTLIY	128	STSSRHSGVPSRFSGSISG	129
1C7	WFQQTPGQAPRTLIY	128	STGSRHSGVPSRFSGSISG	135
1H9	WFQQTPGQAPRTLIY	128	STISRHSGVPSRFSGSISG	138
1C10	WFQQTPGQAPRTLIY	128	STSSRHSGVPSRFSGSISG	129
1B8	WYQRKPGQAPVMVIY	145	SDNRRPSGIPERFSGSNSG	146
1C8	WYQQKPGQAPVLVIY	150	ADSRRPSGIPERFSGSNSG	151
1D8	WYQQKPGQAPVLVIY	150	ADSRRPSGIPERFSGSNSG	151
1G10	WYQQKPGQAPVLVIY	150	ADSRRPSGIPGRFSGSNSG	153
1A12	WYQQKPGQAPVLVIY	150	ADSRRPSGIPERFSGSNSG	151
1D6	WYQQRPGQSPRLLIY	160	SASTQESGIPDRFSGSGST	161
1C11	WYQQKPGQAPVMVIY	166	SDSRRPSGIPERFSGSNSG	167
1D9	WYQQKPGQAPALVIY	171	DDDSRTSGIPERFSGSRSG	172
2C7	WYQQNPGQAPKLVIY	177	KESERPSGIPERFSGSVSG	178
2D10	WYQQKPGQAPVLVIY	150	KDSERPSGIPERFSGSSSG	183
1A10	WYQQKPGQAPVLVIY	150	KDSDRPSGIPERFSGSTSG	185
2A5	WYQQKPGQAPNLLIY	189	GAASLQTGVPSRFSGSGSG	190
3D7	WYQQKPGQAPVLVIY	150	KDRERPSGIPERFSGSSSG	195
3B9	WYQQKPGQAPVLVIY	150	KDSERPSGIPERFSGSSSG	183
4B7	WYQQKPGQAPVLVIY	150	KDSERPSGIPERFSGSSSG	183
4C7	WYQQKPGQAPVLVIY	150	KDSERPSGIPERFSGSSSG	183
4E11	WYQQKPGQAPVLVIY	150	KDSERPSGIPERFSGSSSG	183
4F12	WYQQKPGQAPVLVIY	150	KDSERPSGIPERFSGSSSG	183

4G9	WYLQTPGQAPRLVIY	203	NTNSRRSGVPSRFSGSISG	204
4H9	WYLQTPGQAPRLVIY	203	NTNSRRSGVPSRFSGSISG	204
7C2	WLLQKPGQRPQLVIY	209	QVSNRGSGVPDRFTGSGSG	210
7A4	WYQQKPGQSPRLLIY	215	WASTRESGVPDRFSGSGST	216
7B4	WLLQKPGQRPQLLIY	221	QVSNRGSGVPDRFSGSGSG	222
7A11	WYQQLPGMAPKLLIY	226	DVNKRASGIADRFSGSKSG	227
8A10	WYQQLPGMAPKLLIY	226	EANIRASGIADRFSGSKSG	231
7G9	WHQQKPGQAPILLIY	235	SDNIRPSGIPVSFSGSRSG	236
8G5	WYQQKPGQTPKLLIY	241	GASRLQTGVPSRFSGSGSG	242

Fab	Framework 3	SEQ ID	L-CDR3	SEQ ID
1A7	NKAALTITGAQPEDEADYYC	130	ALDIGSYTM	131
1C7	NKAALTITGAQPEDEADYYC	130	ALDIGSYAV	136
1H9	NKAALTITGAQPEDEADYYC	130	ALDIGSYTV	139
1C10	NKAALTITGAQPEDEADYYC	130	ALDIGSYVV	141
1B8	NTATLTISGAQAEDEADYYC	147	QVWDSSANAAV	148
1C8	NTATLTISGAQAEDEADYYC	147	QVWDSSAAV	152
1D8	NTATLTISGAQAEDEADYYC	147	QVWDSSANAAV	148
1G10	NTATLTISGAQAEDEADYYC	147	QVWDVSAAV	154
1A12	NTATLTSSGTQAEDEADYYC	156	QVWDSSVAV	157
1D6	TDFTLTISSVQPEDAAVYYC	162	QQAYSAPLS	163
1C11	NTATLTISGAQAEDEADYYC	147	QAWDSSANAVV	168
1D9	GTATLSISGAQAEDEADYYC	173	HSADSSNYFLSV	174
2C7	DTATLTISGAQAEDEADYYC	179	QSGSSSDNAV	180
2D10	DTATLTISGAQAEDEADYYC	179	QSGSSSDNAV	180
1A10	DTATLTISGAQAEDEADYYC	179	QSGSSSGNAVV	186
2A5	TSFTLTISGLEAEDAGTYYC	191	QQYYSANS	192
3D7	DTATLTISGTQAEDEADYYC	196	ESGTSSHNAV	197
3B9	DTATLTISGAQAEDEADYYC	179	QSGTSSHNAV	199
4B7	DTATLTISGAQAEDEADYYC	179	QSGSSSDAV	201
4C7	DTATLTISGAQAEDEADYYC	179	QSGSSSDAV	201
4E11	DTATLTISGAQAEDEADYYC	179	QSGSSSDAV	201
4F12	DTATLTISGAQAEDEADYYC	179	QSGSSSDNAV	180
4G9	IKAALTITGARPEDEADYYC	205	ALYMGSRSNNAV	206
4H9	IKAALTITGARPEDEADYYC	205	ALYMGSRSNNAV	206
7C2	TDFTLKISGVKAEDAGVYYC	211	AQAIYYPRT	212
7A4	TDFTLTISSFQPEDAAVYYC	217	QQGVSSPVT	218
7B4	TDFTLKISGVKAEDAGVYYC	211	AQPLYYPYS	223
7A11	NTASLTISGLQSEDEADYYC	228	ASYTSRDNAV	229
8A10	NTASLTISGLQSEDEADYYC	228	ASYRSSNNYV	232
7G9	GTATLTISGVRAEDEADYYC	237	QSLDSSANPM	238
8G5	TSFTLAISGLEAEDLGTYYC	243	LQDYAWPLT	244

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Fab	FRAMEWORK 4	SEQ ID
1A7	FGGGTHLTVL	132
1C7	FGGGTHLTVL	132
1H9	FGGGTHLTVL	132
1C10	FGGGTKLTVL	142

Fab	FRAMEWORK 4	SEQ ID
1B8	FGGGTHLTVL	132
1C8	FGGGTHLTVL	132
1D8	FGGGTHLTVL	132
1G10	FGGGTHLTVL	132
1A12	FGGGTHLTVL	132
1D6	FGSGTRLEIK	164
1C11	FGGGTHLTVL	132
1D9	FGGGTHLTVL	132
2C7	FGGGTHLTVL	132
2D10	FGGGTHLTVL	132
1A10	FGGGTHLTVL	132
2A5	FGSGTRLEIK	164
3D7	FGGGTHLTVL	132
3B9	FGGGTHLTVL	132
4B7	FGGGTHLTVL	132
4C7	FGGGTHLTVL	132
4E11	FGGGTHLTVL	132
4F12	FGGGTHLTVL	132
4G9	FGGGTKLTVL	142
4H9	FGGGTKLTVL	142
7C2	FGQGTRLEIK	213
7A4	FGQGTKVELK	219
7B4	FGSGTRLEIK	164
7A11	FGGGTHLTVL	132
8A10	FGGGTHLTVL	132
7G9	FGGGTHLTVL	132
8G5	FGQGTKVELK	219

Table 9: Full Length llama-derived VH

Fab	Full Length Sequence of VH domain	SEQ ID
1A7	QVQLQESGPGLVKPSQTLSLTCTVSGGSITTGTYSWSWIRQPPGKGLEWMG VIAYDGSTYYSPSLKSRTSISRDTSKNQFSLQLSSVTPEDTAVYYCARNNV AAPGYWGQGTRGTGSWV	245
1C7	EVQLQESGPGLVKPSQTLSLTCTVSGDSITSSYYHYSWIRQPPGKGLEWMG VIAYDGSTYYSPSLKSRTSISRDTSKNQFSLQLNSVTPEDTAVYYCARDDV AAPGYWGQGTQVTVSS	246
1H9	EVQVQESGPGLVKPSQTLSLTCTVSGGSITTGTYSWSWIRQPPGKGLEWMG VIAYDGSTYYSPSLKSRTSISRDTSKNQFSLQLSSVTPEDTAVYYCARDDV AAPGYWGQGTQVTVSS	247
1C10	EVQLQESGPGLVKPSQTLSLTCTVSGGSITTGTYSWSWIRQPPGKGLEWMG VIAYDGSTYYSPSLKSRTSISRDTSKNQFSLQLSSVTPEDTAVYYCARDDV AAPGYWGQGTQVTVSS	248
1B8	QLQLVESGGALVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSHI SGNGESTFYAESMKGRATISRDNAKNTLYLQMNSLKSEDTAVYYCAKDDYG SGLYAYWGQGTQVTVSS	249
1C8	QLQLVESGGALVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSHI SGNGFSTFYAESMKGRATISRDNAKNTLYLQMNSLKSEDTAVYYCAKDDYG SGLYAYWGQGTQVTVSS	250

Fab	Full Length Sequence of VH domain	SEQ ID
1D8	EVQLVESGGALVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSHI SGNGHSTFYAESMKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKDDYG SGLYAYWGQGTQVTVSS	251
1G10	ELQLVESGGALVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSHI SGNGRSTFYAESVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKDDYG SGLYAYWGQGTQVTISS	252
1A12	EVQLVESGGALVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSHI SGNGFSTFYAESMKGRATISRDNAKNTLYLQMNSLKSEDTAVYYCAKDDYG SGLYAYWGQGTQVTVSS	253
1D6	EVQLVESGGGLVQPGGSLRLSCVASGFTFDDYGMSWVRQAPGKGLEWVSFI TWNGGITYYAESMKGRFTISRDNAKNTLYLQMDSLKSEDTAVYYCAKDSDP SIATPYFGSWGQGTQVTVSS	254
1C11	ELQLVESGGGSVQPGGSLRLSCAASGFTFSNYWMYWVRQAPGKGLEWLSLI TTDGGSTFYADSVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKDLGS GFSLGSWGQGTQVTVSA	255
1D9	QLQVVESGGGLVQPGGSLRLSCVASGFTFGDYAMSWVRQAPGKGLEWIAGI TWNGNTYYPDSLEGRFTISRDNAKNTLSLQMNSLKSEDTAVYYCAKGGIGA TVFGSWGQGTQVTISS	256
2C7	QLQVVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRHSPGKGLEWVSTI SWNGGVTYYAASVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAKEIIT VQALRAAIAFDSWDQGTQVTVSS	257
2D10	EVQLVQPGGSLRLSCAASGFTFDDYGMSWVRHSPGKGLEWVSTISWNGGVT YYAASMKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAKEIITVQAMRAA IAFDSWDQGTQVTVSS	258
1A10	EVQLVQPGAELRNPGASVKVSCKASGYTFTSYYIDWVRQAPGQGLEWMGRI DPEDGGTKYAQKFQGRVTFTADTSTSTAYVELSSLRSEDTAVYYCARKGER AYGSSWYSIYDYGMDYWGKGTLVTVSS	259
2A5	QVQLQESGGGLVQPGGSLRLSCAASGFTFSSDAMSWVRQAPGKGLEWVSSI NSGGGSTNYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAESDYG YGYGLAYTYDYWGQGTQVTVSS	260
3D7	QLQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI NSGGRSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATRGSG GSFGSWGQGTRVTVSS	261
3B9	QLQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI NSGGRSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATRGSG GSFGSWGQGTRVTVSS	261
4B7	QVQLVESGGGLVQPGGSLRLSCAASGFTFSRKWMYWVRQAPGKGLEWVSVI NTGGGSTYYSDSVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCANGGAE LQPARFGGIWSPDRSLDAWGQGTLVTVSS	262
4C7	QVQLVESGGGLVQPGGSLRLSCAASGFTFSRKWMYWVRQAPGKGLEWVSVI NTGGGSTYYSDSVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCANGGAE LQPARFGGIWSPDRSLDAWGQGTLVTVSS	262
4E11	QVQLVESGGGLVQPGGSLRLSCAASGFTFSRKWMYWVRQAPGKGLEWVSVI NTGGGSTYYSDSVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCANGGAE LQPARFGGIWSPDRSLDAWGQGTLVTVSS	262
4F12	QLQLVESGGGLVQPGGSLRLSCAASGFTFSGYWMYWVRQAPGKGLEWVSVI NTREGSTYYADSVKGRFTISRDLAKNTLYLQMNSLKSEDTAVYYCANGGAE LQPTTYGGLWSPDRSLDAWGQGTLVTVSS	263
4G9	QLQLVESGGGLVQPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSGI NSDGGNTYHVDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATTIVP GYWVQGTQVTVSS	264
4H9	QLQLVESGGGLVQPGGSLRLSCAASGFTFSGYAMSWVRQAPGKGLEWVSGI NSDGGNTYHVDSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATTIVP GYWGQGTQVTVSS	265
7C2	EVQVQESGPGLVKPSQTLSLTCTVSGGSITTSYSAWSWIRQPPGKGLEWMG LIAYDGSTYYSPSLKSRTSISRDTSKNQFSLQLSSVTPEDTAVYYCASDSY YSGSYYSGSYYYTAWGQGTQVTVSS	266

Fab	Full Length Sequence of VH domain	SEQ ID
7A4	ELQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSI NSGGDRLHYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAKYGSP NMALMDWRVDFDSWGQGTQVTVSS	267
7B4	ELQLVESGGGLVQPGGSLRLSCVASGFTFDDYAMNWVRQAPGKGLEWVSSI SWDGGSTDVAESMKGRFTIVRDNAKNTLYLQMNSLKSEDTAVYYCAKSLYS DPSYDYWGQGTQVTVSS	268
7A11	ELQLVESGGGLVQPGGSLRLSCAASGFTNGFTFDDYGMSWVRQVPGKGLEW VSSISWTGERTYYAESVKGRFTIFRDNAKNMLYLQMNSLKSEDTAVYYCAK ALMGEYDYWGQGTQVTVSS	269
8A10	QVQLVESGPGLVKPSQTLYLTCTVSGGSITTAYYAWNWIRQPPGKGLEWMG AIAYDGSTYYSPSLKSRSSISRDTSKNQFSLQLSSVTPEDTAVYYCAEAGG AGWGPTFFYWGQGAQVTVSS	270
7G9	QVQRQESGGGLVQPGGSLRLSCAASGFTFSSHVMAWVRQAPGKGLEWVSAI NGGGGTTSYADSVKGRFTISRDNAKNTLYLQMNSMKSEDTAVYYCANSLWP YQGSWGQGTQVTVSS	271
8G5	EVQLVESGGGLVQPGGSLRVSCAASGFTFSSYYMNWVRQAPGKGLEWVSAV STGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCVRGDVR GTYDSYGYWGQGTQVTVSS	272

Table 10: Full Length llama-derived VL

Fab	Full Length Sequence	SEQ ID
1A7	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTTSNYPGWFQQTPGQAPRTLIY STSSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALDIGSYTMFGG GTHLTVL	273
1C7	QTVVTQEPSLSVSPGGTVTLTCGLSSGPVTTSNYPGWFQQTPGQAPRTLIY STGSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALDIGSYAVFGG GTHLTVL	274
1H9	QAVVTQEPSLSVSPGGTVTLTCGLSSESVTTSNYPGWFQQTPGQAPRTLIY STISRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALDIGSYTVFGG GTHLTVL	275
1C10	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTTGNYPGWFQQTPGQAPRTLIY STSSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCALDIGSYVVFGG GTKLTVL	276
1B8	SYELTQSPSVSVALRQTAKITCGGDNIGSKSAQWYQRKPGQAPVMVIYSDN RRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCQVWDSSANAAVFGGG THLTVL	277
1C8	SYELTQSPSVSVALKQTAKITCGGDNIGSKSAQWYQQKPGQAPVLVIYADS RRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCQVWDSSAAVFGGGTH LTVL	278
1D8	SYELTQSPSVSVALRQTAKITCGGDNIGSKSAQWYQQKPGQAPVLVIYADS RRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCQVWDSSANAAVFGGG THLTVL	279
1G10	SYELTQSPSVSVALRQTAKITCGGDNIGSKSAQWYQQKPGQAPVLVIYADS RRPSGIPGRFSGSNSGNTATLTISGAQAEDEADYYCQVWDVSAAVFGGGTH LTVL	280
1A12	SYELTQSPSVSVALRQTAKITCGGDNIGSKSVQWYQQKPGQAPVLVIYADS RRPSGIPERFSGSNSGNTATLTSSGTQAEDEADYYCQVWDSSVAVFGGGTH LTVL	281
1D6	EIVLTQSPSSVTASVGEKVTINCKSSQSVVSGSNQKSYLNWYQQRPGQSPR LLIYSASTQESGIPDRFSGSGSTTDFTLTISSVQPEDAAVYYCQQAYSAPL SFGSGTRLEIK	282

Fab	Full Length Sequence	SEQ ID
1C11	SYELTQSPSVSVAQRQTAKITCGGDNIGSKSAQWYQQKPGQAPVMVIYSDS RRPSGIPERFSGSNSGNTATLTISGAQAEDEADYYCQAWDSSANAVVFGGG THLTVL	283
1D9	SSALTQPSAVSVSLGQTARIACQGDSIGNYGANWYQQKPGQAPALVIYDDD SRTSGIPERFSGSRSGGTATLSISGAQAEDEADYYCHSADSSNYFLSVFGG GTHLTVL	284
2C7	HSAVTQPSAVSVSLGQTARITCQGDNFGSYYASWYQQNPGQAPKLVIYKES ERPSGIPERFSGSVSGDTATLTISGAQAEDEADYYCQSGSSSDNAVFGGGT HLTVL	285
2D10	SSALTQPSAVSVSLGQTARITCQGGNFGNYYANWYQQKPGQAPVLVIYKDS ERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDNAVFGGGT HLTVL	286
1A10	HSAVTQPSAVSVSLGQMARITCQGDNFGSYYASWYQQKPGQAPVLVIYKDS DRPSGIPERFSGSTSGDTATLTISGAQAEDEADYYCQSGSSSGNAVVFGGG THLTVL	287
2A5	EIVMTQSPSSLSASLGDRVTITCQASQSISSYLAWYQQKPGQAPNLLIYGA ASLQTGVPSRFSGSGSGTSFTLTISGLEAEDAGTYYCQQYYSANSFGSGTR LEIK	288
3D7	LPAVNHPSALSVSLGQMAKITCQGGNFGSYYASWYQQKPGQAPVLVIYKDR ERPSGIPERFSGSSSGDTATLTISGTQAEDEADYYCESGTSSHNAVFGGGT HLTVL	289
3B9	QPVLNQPSAVSVSLGQMAKITCQGGNFGSYYASWYQQKPGQAPVLVIYKDS ERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGTSSHNAVFGGGT HLTVL	290
4B7	NFMLTQPSAVSVSLGQTARITCQGGNFGSYYASWYQQKPGQAPVLVIYKDS ERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDAVFGGGTH LTVL	291
4C7	NFMLTQPSAVSVSLGQTARITCQGGNFGSYYASWYQQKPGQAPVLVIYKDS ERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDAVFGGGTH LTVL	291
4E11	NFMLTQPSAVSVSLGQTARITCQGGNFGSYYASWYQQKPGQAPVLVIYKDS ERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDAVFGGGTH LTVL	291
4F12	NFMLTQPSAVSVSLGQTARITCQGGNFGSYYASWYQQKPGQAPVLVIYKDS ERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDNAVFGGGT HLTVL	292
4G9	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPHWYLQTPGQAPRLVIY NTNSRRSGVPSRFSGSISGIKAALTITGARPEDEADYYCALYMGSRSNNAV FGGGTKLTVL	293
4H9	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPHWYLQTPGQAPRLVIY NTNSRRSGVPSRFSGSISGIKAALTITGARPEDEADYYCALYMGSRSNNAV FGGGTKLTVL	293
7C2	EVQVQESGPGLVKPSQTLSLTCTVSGGSITTSYSAWSWIRQPPGKGLEWMG LIAYDGSTYYSPSLKSRTSISRDTSKNQFSLQLSSVTPEDTAVYYCASDSY YSGSYYSGSYYYTAWGQGTQVTVSS	294
7A4	ELQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSI NSGGDRLHYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAKYGSP NMALMDWRVDFDSWGQGTQVTVSS	295
7B4	ELQLVESGGGLVQPGGSLRLSCVASGFTFDDYAMNWVRQAPGKGLEWVSSI SWDGGSTDVAESMKGRFTIVRDNAKNTLYLQMNSLKSEDTAVYYCAKSLYS DPSYDYWGQGTQVTVSS	296
7A11	ELQLVESGGGLVQPGGSLRLSCAASGFTNGFTFDDYGMSWVRQVPGKGLEW VSSISWTGERTYYAESVKGRFTIFRDNAKNMLYLQMNSLKSEDTAVYYCAK ALMGEYDYWGQGTQVTVSS	297
8A10	QVQLVESGPGLVKPSQTLYLTCTVSGGSITTAYYAWNWIRQPPGKGLEWMG AIAYDGSTYYSPSLKSRSSISRDTSKNQFSLQLSSVTPEDTAVYYCAEAGG AGWGPTFFYWGQGAQVTVSS	298

Fab	Full Length Sequence	SEQ ID
7G9	QVQRQESGGGLVQPGGSLRLSCAASGFTFSSHVMAWVRQAPGKGLEWVSAI NGGGGTTSYADSVKGRFTISRDNAKNTLYLQMNSMKSEDTAVYYCANSLWP YQGSWGQGTQVTVSS	299
8G5	EVQLVESGGGLVQPGGSLRVSCAASGFTFSSYYMNWVRQAPGKGLEWVSAV STGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCVRGDVR GTYDSYGYWGQGTQVTVSS	300

NB: The VL domains of clones ID6, 2A5, 7C2, 7A4, 7B4 and 8G5 are of the kappa isotype; VL domains of all other clones are of the lambda isotype.

Claims

1. A monoclonal antibody or antigen binding fragment thereof that binds to human IL-24 and which blocks human IL-24 binding to the IL-22R/IL-20R2 receptor complex and/or inhibits human IL-24 signalling through the IL-22R/IL-20R2 receptor complex.

2. The monoclonal antibody or antigen binding fragment according to claim 1 which is cross-reactive with murine IL-24.

3. The antibody or antigen binding fragment according to claim 1 or claim 2 which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:35 (NYWMY),
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:37 (LITTDGGSTFYADSVKG),
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:38 (DLGSGFSLGS),
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144 (GGDNIGSKSAQ),
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:167 (SDSRRPSGIPERFSGSNSG), and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:168 (QAWDSSANAVV).

4. The antibody or antigen binding fragment according to claim 3, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:255, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:283, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

5. The antibody or antigen binding fragment according to claim 1 which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:14,

H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:4,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:11,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:140,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:129, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:141.

6. The antibody or antigen binding fragment according to claim 5, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:248, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:276, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

7. The antibody or antigen binding fragment according to claim 1 which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:22,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:151, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:152.

8. The antibody or antigen binding fragment according to claim 7, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:250, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:278, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

9. The antibody or antigen binding fragment according to claim 1 which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:24,

H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:151, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:148.

10. The antibody or antigen binding fragment according to claim 9, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:251, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:279, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

11. The antibody or antigen binding fragment according to claim 1 which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:27,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:21,
L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:144,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:153, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:154.

12. The antibody or antigen binding fragment according to claim 11, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:252, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:280, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

13. The antibody or antigen binding fragment according to claim 1 which comprises a heavy chain variable domain comprising H-CDR1, H-CDR2 and H-CDR3, and a light chain variable domain comprising L-CDR1, L-CDR2 and L-CDR3, wherein:
H-CDR1 comprises the amino acid sequence shown as SEQ ID NO:17,
H-CDR2 comprises the amino acid sequence shown as SEQ ID NO:42,
H-CDR3 comprises the amino acid sequence shown as SEQ ID NO:44,

L-CDR1 comprises the amino acid sequence shown as SEQ ID NO:170,
L-CDR2 comprises the amino acid sequence shown as SEQ ID NO:172, and
L-CDR3 comprises the amino acid sequence shown as SEQ ID NO:174.

14. The antibody or antigen binding fragment according to claim 13, wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:256, or a sequence at least 90%, 95%, 97% or 99% identical thereto, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:284, or a sequence at least 90%, 95%, 97% or 99% identical thereto.

15. A monoclonal antibody or antigen binding fragment thereof that binds to the same epitope on human IL-24 as the antibody or antigen binding fragment of any one of claims 1 to 14, or that cross-competes for binding to human IL-24 with the antibody or antigen binding fragment of any one of claims 1 to 14.

16. An antibody or antigen binding fragment according to any one of any one of the preceding claims which comprises one or more constant domains derived from a human immunoglobulin.

17. The antibody or antigen binding fragment of claim 16 which comprises one or more constant domains of human IgG1, human IgG2, human IgG3 or human IgG4.

18. The antibody or antigen binding fragment of claim 17 which comprises the CH1 domain, hinge region, CH2 domain and CH3 domain of human IgG1 or human IgG4.

19. The antibody or antigen binding fragment of any one of claims 1 to 18 which is substantially devoid of effector functions.

20. An isolated polynucleotide which encodes the antibody or antigen binding fragment of any one of the preceding claims.

21. An expression vector comprising the polynucleotide of claim 20 operably linked to regulatory sequences which permit expression of the antigen binding polypeptide in a host cell or cell-free expression system.

22. A host cell or cell-free expression system containing the expression vector of claim 21.

23. A method of producing a recombinant antibody or antigen binding fragment thereof which comprises culturing the host cell or cell free expression system of claim 22 under conditions which permit expression of the antibody or antigen binding fragment and recovering the expressed antibody or antigen binding fragment.

24. A pharmaceutical composition comprising an antibody or antigen binding fragment according to any one of claims 1 to 19 and at least one pharmaceutically acceptable carrier or excipient.

25. A method of treating or preventing a dermatological condition in a human patient which comprises administering to a patient in need thereof a therapeutically effective amount of an IL-24 antagonist.

26. The method of claim 25 wherein the dermatological condition is selected from the group consisting of atopic dermatitis, contact dermatitis, contact hypersensitivity, plaque psoriasis and pustular psoriasis.

27. The method of claim 25 or claim 26 wherein the IL-24 antagonist is an IL-24 antibody or antigen binding fragment thereof.

28. The method of claim 27 wherein the IL-24 antagonist is an antibody or antigen binding fragment according to any one of claims 1 to 19.

29. An IL-24 antagonist for use in for treating or preventing a dermatological condition in a human patient.

30. An Il-24 antagonist for use according to claim 29, wherein the dermatological condition is selected from the group consisting of atopic dermatitis, contact dermatitis, contact hypersensitivity, plaque psoriasis and pustular psoriasis.

31. An IL-24 antagonist for use according to claim 29 or claim 30 wherein the IL-24 antagonist is an IL-24 antibody or antigen binding fragment thereof.

32. An IL-24 antagonist for use according to claim 31 wherein the IL-24 antagonist is an antibody or antigen binding fragment according to any one of claims 1 to 19.

33. A method of treating or preventing an inflammatory condition in a human patient which comprises administering to a patient in need thereof a therapeutically effective amount of an IL-24 antagonist.

34. The method of claim 33 wherein the inflammatory condition is selected from the group consisting of IBD, colitis, Endotoxemia, arthritis, rheumatoid arthritis, psoriatic arthritis, adult respiratory disease (ARD), septic shock, multiple organ failure, inflammatory lung injury such as asthma or bronchitis, bacterial pneumonia, psoriasis, eczema, ulcerative colitis and Crohn's disease.

35. The method of claim 33 or claim 34 wherein the IL-24 antagonist is an IL-24 antibody or antigen binding fragment thereof.

36. The method of claim 35 wherein the IL-24 antagonist is an antibody or antigen binding fragment according to any one of claims 1 to 19.

37. An IL-24 antagonist for use in for treating or preventing an inflammatory condition in a human patient.

38. An Il-24 antagonist for use according to claim 37, wherein the inflammatory condition is selected from the group consisting of IBD, colitis, Endotoxemia, arthritis, rheumatoid arthritis, psoriatic arthritis, adult respiratory disease (ARD), septic shock,

multiple organ failure, inflammatory lung injury such as asthma or bronchitis, bacterial pneumonia, psoriasis, eczema, ulcerative colitis and Crohn's disease.

39. An IL-24 antagonist for use according to claim 37 or claim 38 wherein the IL-24 antagonist is an IL-24 antibody or antigen binding fragment thereof.

40. An IL-24 antagonist for use according to claim 39 wherein the IL-24 antagonist is an antibody or antigen binding fragment according to any one of claims 1 to 19.

Intellectual Property Office

Application No:	GB1507228.3	**Examiner:**	Richard Sewards
Claims searched:	1-24; and 28, 32, 36 and 40 in part	**Date of search:**	27 February 2017

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X,Y	X: 1-24; Y; 28, 32, 36 & 40	Molecular Human Reproduction (2013); Vol 19, pp 655-664, "Estrogen promotes the growth of decidual...", Shao et al See p 656 col 1 para 3, p 658, Fig 2
X,Y	X: 1-24; Y; 28, 32, 36 & 40	Molecular Therapy (2005); VOl 11, pp 724-733, "mda-7/IL24 kills pancreatic cancer cells ...", Chada et al See pp 727-730 "MDA-7 Protein Kills Pancreatic Cancer Cells and Triggers Apoptosis" & Figs 6B & 6C
X,Y	X: 1-24; Y; 28, 32, 36 & 40	Cancer Immunology Immunotherapy (2007); Vol 56, pp 205-215, "Human interleukin 24 (MDA-7/IL-24) protein...", Zheng et al See p 206 "Cell culture and reagents", p 210 "IL-24 requires binding to its IL-20 receptors to induce apoptosis, Fig 4A
Y	28, 32, 36 & 40	Immunology (2005); Vol 114, pp 166-170, "Interleukin-24 and its receptors", Wang & Liang See whole document
Y	36 & 40	US 2005/0142108 A (GRUNIG ET AL) see paras 9-14, 87-89 & 117

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

A61K; C07K

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, BIOSIS, MEDLINE, CAS-ONLINE, BLASTP

Intellectual Property Office

International Classification:

Subclass	Subgroup	Valid From
C07K	0016/24	01/01/2006
A61K	0039/395	01/01/2006
A61P	0017/00	01/01/2006
A61P	0017/06	01/01/2006
A61P	0029/00	01/01/2006
A61P	0037/08	01/01/2006