

CLAIMS

1. An isolated antigen binding protein (ABP) which specifically binds to the extra cellular domain (ECD) of leukocyte immunoglobulin-like receptor subfamily B1 (LILRB1) and LILRB2 protein and wherein the isolated ABP comprises at least one, preferably 3 or 6, complementarity determining regions (CDRs) and is capable of inhibiting the binding of LILRB1 and LILRB2 to a natural ligand thereof, preferably wherein the natural ligand of LILRB1 and/or LILRB2 is HLA-G), wherein the ABP does not bind to, or binds with less affinity to, LILRA1 and LILRA3.

2. The isolated ABP of claim 1, wherein:

- the ABP binds to the ECD of LILRB1 and LILRB2 with at least 5 times higher, or preferably 10 times higher binding affinity compared the binding of the ABP to an ECD of LILRA1 and/or LILRA3, preferably wherein the ABP binding affinity is determined by bio-layer interferometry (BLI); and/or
- the ABP binds to the ECD of LILRB1 and/or LILRB2 with a binding dissociation constant (KD) that is at least 2 times lower, preferably 3 times lower, more preferably 5 times lower, most preferably 10 times lower, compared to the binding KD of the ABP to an ECD of LILRA1 and/or LILRA3, wherein the KD is determined by bio-layer interferometry (BLI), preferably under the conditions set out in example 3; and/or
- the ABP binds to the ECD of LILRB1 and/or LILRB2 with a binding dissociation constant (KD) that is at least 2 times lower, preferably 3 times lower, more preferably 5 times lower, most preferably 10 times lower, compared to the binding KD of the ABP to an ECD of LILRA1 and/or LILRA3, wherein the KD is determined by bio-layer interferometry (BLI), preferably under the conditions set out in example 3.

3. The isolated ABP of claim 1 or 2, wherein the ABP competes for binding to an ECD of LILRB1 and/or LILRB2, or to ECD of the variant of LILRB1 and/or LILRB2, with an endogenous LILRB1 and/or LILRB2 ligand or receptor, preferably wherein said endogenous LILRB1 and/or LILRB2 ligand or receptor is an HLA-G protein or a variant of HLA-G.

4. The isolated ABP of claim 3, wherein the ABP is capable of inhibiting the binding of HLA-G protein or a variant thereof to LILRB1 and/or LILRB2 protein or a variant thereof with an IC₅₀ of less than 100nM, less than 50nM, or preferably 20nM or less, such as 15nM or less, or less than 10nM, as determined by ELISA.

5. The isolated ABP of any one of claims 1 to 4, wherein the ABP is an antibody, or an antigen binding fragment thereof, composed of at least one, preferably two, antibody heavy chain sequences, and at least one, preferably two, antibody light chain sequences, wherein at least one, preferably both, of the antibody heavy chain sequences and at least one, preferably both, of the antibody light chain sequences comprise CDR1 to CDR3 sequences in a combination selected from any of the following combinations of heavy and/or light chain CDRs, CDRs-A-001 to CDRs-A-048:

ABP Combination (ID)	Heavy Chain CDR1 to CDR3 (SEQ ID NO)			Light Chain CDR1 to CDR3 (SEQ ID NO)		
	1	2	3	5	6	7
CDRs-A-001	1	2	3	5	6	7

CDRs-A-002	9	10	11	13	14	15
CDRs-A-003	17	18	19	21	22	23
CDRs-A-004	25	26	27	29	30	31
CDRs-A-005	33	34	35	37	38	39
CDRs-A-006	41	42	43	45	46	47
CDRs-A-007	49	50	51	53	54	55
CDRs-A-008	57	58	59	61	62	63
CDRs-A-009	65	66	67	69	70	71
CDRs-A-010	73	74	75	77	78	79
CDRs-A-011	81	82	83	85	86	87
CDRs-A-012	89	90	91	93	94	95
CDRs-A-013	97	98	99	101	102	103
CDRs-A-014	105	106	107	109	110	111
CDRs-A-015	113	114	115	117	118	119
CDRs-A-016	121	122	123	125	126	127
CDRs-A-017	129	130	131	133	134	135
CDRs-A-018	137	138	139	141	142	143
CDRs-A-019	145	146	147	149	150	151
CDRs-A-020	153	154	155	157	158	159
CDRs-A-021	161	162	163	165	166	167
CDRs-A-022	169	170	171	173	174	175
CDRs-A-023	177	178	179	181	182	183
CDRs-A-024	185	186	187	189	190	191
CDRs-A-025	193	194	195	197	198	199
CDRs-A-026	201	202	203	205	206	207
CDRs-A-027	209	210	211	213	214	215
CDRs-A-028	217	218	219	221	222	223
CDRs-A-029	225	226	227	229	230	231
CDRs-A-030	233	234	235	237	238	239
CDRs-A-031	241	242	243	245	246	247

CDRs-A-032	249	250	251	253	254	255
CDRs-A-033	257	258	259	261	262	263
CDRs-A-034	265	266	267	269	270	271
CDRs-A-035	273	274	275	277	278	279
CDRs-A-036	281	282	283	285	286	287
CDRs-A-037	289	290	291	293	294	295
CDRs-A-038	297	298	299	301	302	303
CDRs-A-039	305	306	307	309	310	311
CDRs-A-040	313	314	315	317	318	319
CDRs-A-041	321	322	323	325	326	327
CDRs-A-042	329	330	331	333	334	335
CDRs-A-043	337	338	339	341	342	343
CDRs-A-044	345	346	347	349	350	351
CDRs-A-045	353	354	355	357	358	359
CDRs-A-046	361	362	363	365	366	367
CDRs-A-047	369	370	371	373	374	375
CDRs-A-048	377	378	379	381	382	383

in each case independently, optionally with no more than three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to these sequences.

6. The isolated ABP of any one of claims 1 to 5,

comprising a heavy chain variable domain sequence comprising:

- a heavy chain CDR1 of SEQ ID NO: 353, optionally with no more than three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence; and
- a heavy chain CDR2 of SEQ ID NO: 354, optionally with no more than three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence; and
- a heavy chain CDR3 of SEQ ID NO: 355, optionally with no more than three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence; and

and comprising a light chain variable domain sequence comprising

- a light chain CDR1 of SEQ ID NO: 357, optionally with no more than three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence; and
- a light chain CDR2 of SEQ ID NO: 358, optionally with no more than three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence; and
- a light chain CDR3 of SEQ ID NO: 359; optionally with no more than three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence.

7. The isolated ABP of any one of claims 1 to 6, comprising an antibody heavy chain variable domain sequence of SEQ ID No: 356, optionally with no more than ten, nine, eight, seven, six, five, four, three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence; and comprising an antibody light chain variable domain sequence of SEQ ID No: 360, optionally with no more than ten, nine, eight, seven, six, five, four, three or two, preferably no more than one, amino acid substitution(s), insertion(s) or deletion(s) compared to this sequence.

8. The isolated ABP of any one of claims 1 to 7, characterized by:

- a. specifically binding to LILRB1 and LILRB2, wherein the ABP is characterized by binding the same epitope on LILRB1 and LILRB2 as an antibody of Table 1, preferably A-001, A-003, A-010 or A-045; and/or
- b. not binding to, or binding with less affinity to, LILRA1; and/or
- c. not binding to, or binding with less affinity to, LILRA3; and/or
- d. when bound to LILRB1 and/or LILRB2 on a human monocyte decrease M2-like polarization of monocyte; and/or
- e. when bound to LILRB1 on a human macrophage induces phagocytosis of the cell; and/or
- f. when bound to human LILRB1 and/or LILRB2 on a human monocyte or macrophage reduces expression of markers of M2-like macrophages, such as CD163, CD206 or CD209; and/or
- g. when bound to human LILRB1 and/or LILRB2 on a human monocyte or macrophage increases expression of markers of M1-like macrophages, such as CD86; and/or
- h. when bound to human LILRB1 and/or LILRB2 on a human monocyte or macrophage induces secretion of pro-inflammatory cytokines, such as increases secretion GM-CSF, IFN γ and IL-9; preferably independent of the presence of LILRA1 and/or LILRA3.

9. The isolated ABP of claim 8, characterized by not binding to, or binding with less affinity to, LILRA1 and LILRA2 compared to the binding affinity of the ABP to LILRB1 and LILRB2.

10. The isolated ABP of any one of claims 1 to 9, wherein the ABP is an inhibitor or antagonist of LILRB1 and LILRB2, preferably of an interaction of LILRB1 and LILRB2 with a natural ligand thereof, such as HLA-G.

11. The isolated ABP of any one of claims 1 to 10, wherein:

- the ABP when bound to LILRB1 or LILRB2 on a macrophage reduces the polarization into an M2 like macrophage or repolarize an M2 like macrophage; and/or
- the ABP when bound to LILRB1 or LILRB2 on a macrophage reduces the polarization into an M2 like macrophage or repolarize an M2 like macrophage into an M1 like macrophage.

12. **An isolated ABP** which competes with an ABP as recited in any one of claims 1 to 11 for binding to the ECD of the LILRB1 and/or LILRB2 protein and is able to inhibit the binding of the LILRB1 and/or LILRB2 protein or the variant thereof to a natural ligand of LILRB1 and/or LILRB2.

13. The isolated ABP of any one of claims 1 to 12 that (i) inhibits a binding of a natural ligand of LILRB1 and/or LILRB2 to LILRB1 and/or LILRB2, wherein such ligand is preferably HLA-G, and/or (ii) reduces development of immune-suppressive phenotypes, for example in a tumour microenvironment.

14. The isolated ABP of any one of claims 1 to 13 that is an antibody or an antigen binding fragment thereof, wherein the antibody is a monoclonal antibody, or wherein the antigen binding fragment is a fragment of a monoclonal antibody.
- 5 15. **An isolated nucleic acid** encoding for an ABP, or for an antigen binding fragment or a monomer of an ABP, wherein the ABP is one recited in any one of claims 1 to 14.
- 10 16. **A nucleic acid construct (NAC)**, comprising a nucleic acid of claim 15, optionally with one or more additional features permitting the expression of the encoded ABP or component of said ABP in a cell, such as in a host cell.
- 15 17. The NAC of claim 16, comprising two constructs, one of which includes a nucleic acid encoding the heavy antibody chain, the other of which includes a nucleic acid encoding the light antibody chain, such that expression from both constructs can generate a complete antibody molecule.
18. **A recombinant host cell** comprising a nucleic acid recited in claim 15, or a NAC recited in claim 16 or 17.
- 20 19. **A pharmaceutical composition** comprising: (i) an ABP recited in any one of claims 1 to 14, or (ii) a nucleic acid recited in claim 15, or (iii) a NAC recited in claim 16 or 17, or (iv) a recombinant host cell of 18, and a pharmaceutically acceptable carrier, stabiliser and/or excipient.
- 25 20. **Use of a product in the manufacture of a medicament for treating a disease, disorder or condition**, wherein the product is selected from the list consisting of: (i) an ABP recited in any one of claims 1 to 14, or (ii) a nucleic acid recited in claim 15, or (iii) a NAC recited in claim 16 or 17, or (iv) a recombinant host cell of 18, and (iv) a pharmaceutical composition of claim 19.
- 30 21. The use of claim 20, wherein the medicament is for treating a proliferative disorder, preferably a cancer, and wherein cells involved in the proliferative disorder express a natural ligand of LILRB1 and/or LILRB2, and wherein such cells induce an immune-suppressive phenotype in immune cells, such as macrophages, preferably of the subject.
- 35 22. The use of claim 20 or 21, wherein the medicament is for enhancing an immune response in a mammalian subject to be treated, preferably for promoting immune-activation of a humoral or cell-mediated immune response in the subject, such as promoting pro-inflammatory polarization of macrophages towards an M1 phenotype, for example for treating a proliferative disease, such as a cancer disease, or for treating an infectious disease.
- 40 23. The use of any one of claims 20 to 22, wherein the product has any one or any combination of the following characteristics:
- a. specific binding to human LILRB1 and/or (preferably and) LILRB2 (e.g. comprising the amino acid sequence of SEQ ID NO: 353 (LILRB1) and SEQ ID NO: 358 (LILRB2)), e.g., with a KD 50 nM or less, or of 20nM or less; more preferably of 10nM or less or 5nM or less; and
 - b. lack of specific binding to a LILRA protein, such as preferably LILRA1 and/or LILRA3; and

- c. stimulates T cell activation, e.g., in a mixed lymphocyte reaction (MLR) assay, as measured by increased T cell proliferation or IFN-gamma secretion; and
 - d. stimulates differentiation or activation of monocytes into macrophages, e.g., stimulates differentiation of monocytes into pro-inflammatory macrophages, e.g., as shown in an assay described in the Examples; and
 - e. inhibits binding of LILRB1 and/or (preferably and) LILRB2 to HLA-A and HLA-B, preferably to HLA-G; and
 - f. has a binding profile as shown in **Table 2**; and
 - g. promotes pro-inflammatory polarization of macrophages towards M1 macrophages; and
 - h. does not induce or trigger basophil activation; and
 - i. induces secretion of proinflammatory cytokines in macrophages, such as GM-CSF, IFNg and IL-9; and
 - j. induces phagocytosis.
- 24.** The use of any one of claims 20 to 23, wherein the ABP is characterized by binding to an epitope of an extracellular domain of LILRB1 and LILRB2 which is bound by the antibody clone A-045.
- 25.** The use of any one of claims 20 to 24, wherein the medicament antagonizes immune-suppressive macrophage polarization in the subject, such as a polarization into M2 macrophages, and thereby enhances cell-mediated immune responses against cells involved with the disease.
- 26. A method of inducing phagocytosis in a cell**, preferably a macrophage, the method comprising a step of contacting the macrophage with an ABP of any one of claims 1 to 14.
- 27. A method of modulating macrophage polarization**, the method comprising a step of contacting a target cell, such as a monocyte or macrophage, with an ABP of any one of claims 1 to 14, and thereby inducing or enhancing M1 like macrophage phenotype in said target cell.
- 28.** The method of claim 27, wherein:
- macrophage polarization is modulated in the presence of cells expressing LILRA1 and/or LILRA3; and/or
 - the modulation is a repolarization of an M2 like phenotype into an M1 like phenotype.