

regulated by surface receptors, chemokines and cytokines. V γ 9/V δ 2 T cells are activated by nonpeptidic phosphorylated isoprenoid pathway metabolites, referred to as phosphoagonists (PAg).

5 Development of antibodies targeting BTN2A1, and activating immune cells, notably more than one immune cell compartment, such as macrophage, NK and/or $\gamma\delta$ T cells, in particular V γ 9/V δ 2 T cells, may be particularly relevant notably in cancer therapy and for the treatment of infectious diseases.

SUMMARY

10 The present disclosure describes for the first-time antibodies binding to BTN2A (notably binding the BTN2A1 isoform - *e.g.*, the human BTN2A1 polypeptide) that exhibit at least one of the following properties:

- i. inhibit the polarization of monocytes towards M2 macrophages,
- ii. induce reversion of M2 macrophages towards anti-tumoral M1 macrophages,
- iii. trigger NK cell activation directly,
- 15 iv. enhance NK cell-mediated cytotoxicity.

Thus, in one aspect, the present disclosure refers to an anti-butyrophilin-2A1 (BTN2A1) antibody characterized in that it has at least one of the following functions: i.it inhibits the polarization of monocytes towards M2 macrophages, ii. it induces reversion of M2 macrophages towards anti-tumoral M1 macrophages. In particular, an antibody of the
20 present disclosure exhibits at least one of the properties i) and ii), and at least one of properties iii) and iv), more particularly, an antibody of the present disclosure exhibits properties i) to iv).

Such antibodies according to the present disclosure can thus

- favor an anti-tumoral microenvironment by shifting macrophage phenotype and
25 functionality towards pro-inflammatory M1 macrophages, leading to the secretion of pro-inflammatory cytokines, and/or
- trigger NK cell activation directly and thus further reinforce their cytolytic activity.

Such antibodies as per the present disclosure therefore represent powerful tools that can be used in various strategies for cancer therapy.

30 In specific examples, anti-BTN2A antibodies of the present disclosure compete for binding to BTN2A with any one of:

- the reference murine antibody mAb 101G5 comprising (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:19 and (ii) a light chain variable region comprising the amino acid sequence of SEQ ID NO:20, or

Most particular anti-BTN2A antibodies according to such embodiment may typically compete for binding to BTN2A with the reference murine antibody mAb 107G3 comprising (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 and (ii) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

5 More specifically, such anti-BTN2A antibodies may comprise:

- a heavy chain variable region CDR1 comprising SEQ ID NO:3, a heavy chain variable region CDR2 comprising SEQ ID NO:4, a heavy chain variable region CDR3 comprising SEQ ID NO:5, a light chain variable region CDR1 comprising SEQ ID NO:6, a light chain variable region CDR2 comprising SEQ ID NO:7, and a light chain variable region CDR3 comprising SEQ ID NO:8, or
- 10 - a heavy chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:2, or
- 15 - a heavy chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:2.

20 In some examples of the present disclosure antibodies of the present disclosure have specificity for BTN2A1.

In specific examples, anti-BTN2A antibodies of the present disclosure are human, chimeric or humanized antibodies.

25 In another aspect, the present disclosure refers to a nucleic acid molecule, which encodes a heavy chain and/or a light chain of the anti-BTN2A1 antibody as disclosed herein. The present disclosure also encompasses nucleic acid molecules encoding a heavy chain and/or a light chain of the anti-BTN2A antibodies as described above.

30 In yet another aspect, the present disclosure refers to a host cell comprising the nucleic acid as disclosed herein. The present disclosure also pertains to a host cell comprising such nucleic acids, in particular for use in the manufacturing of any one of the anti-BTN2A antibodies as described above.

Another example of the present disclosure describes anti-BTN2A as described above for use in therapy, notably for the treatment of cancers or infectious diseases. Thus, in a further aspect, the present disclosure refers to the anti-BTN2A1 antibody as disclosed

herein, for use in therapy. In another aspect, the present disclosure refers to use of the anti-BTN2A1 antibody as disclosed herein, in the manufacture of a medicament for treating a solid cancer.

Typically, cancers as per the present disclosure includes blood cancers and solid cancers
5 including cervical carcinomas such as squamous cell carcinoma and endocervical adenocarcinoma, ovarian carcinoma, skin cancers including squamous cell carcinoma

and melanoma, lung cancers including lung small cell carcinoma, prostate cancers, colon cancers, pancreatic cancers and endometrial carcinomas and more specifically: squamous cell carcinoma and endocervical adenocarcinoma, squamous cell carcinoma, ovarian carcinoma, lung small cell carcinoma, prostate cancers, colon cancers, pancreatic
5 cancers and endometrial carcinomas.

The present disclosure also describes a pharmaceutical composition comprising an anti-BTN2A antibody as described above and at least a pharmaceutically acceptable carrier. Thus, in yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the anti-BTN2A1 antibody as disclosed herein, and at least a pharmaceutically
10 acceptable carrier.

The present disclosure further describes a method for activating an immune response in a subject, comprising administering to the subject an effective amount of an anti-BTN2A as disclosed herein.

DETAILED DESCRIPTION OF THE INVENTION

15 Definitions

As used herein the term "BTN2" has its general meaning in the art and refers to human BTN2 polypeptides including either BTN2A1 of SEQ ID NO:17 or BTN2A2 of SEQ ID NO:18.

SEQ ID NO:17: BTN2A isoform 1 precursor (*Homo sapiens*):

20 MESAAALHFSRPASLLLLLLSLCALVSAQFIVVGPTDPILATVGENTTLRCHLSPEKNAED
MEVRWFRSQFSPAVFVYKGGREERTEEQMEEYRGRTTFVSKDISRGSVALVIHNITAQEN
GTYRCYFQEGRSYDEAILHLVVAGLGSKPLISMRGHEDGGIRLECISRGWYPKPLTVWR
DPYGGVAPALKEVSMPDADGLFMVTTAVIIRDKSVRNMSCSINNTLLGQKKESVIFIPESF
MPSVSPCAVALPIIVVILMIPIAVCIYWINKLQKEKKILSGEKEFERETREIALKELEKERVQK
25 EEELQVKEKLQEELRWRRFTLHAVDVVLDPDTAHPDLFLSEDRRSVRRCPFRHLGESVP
DNPERFDSQPCVLGRESFASGKHYYEVEVENVIEWTVGVCRDSVERKGEVLLIPQNGF
WTLEMHKGQYRAVSSPDRILPLKESLCRVGVFLDYEAGDVSFYNMRDRSHIYTCPRSAF
SVPVRPFFRLGCEDSPIFICPALTGANGVTVP EEGTLT LHRVGTHQSL

SEQ ID NO:18: BTN2A isoform 2 precursor (*Homo sapiens*):

30 MEPAAALHFSLPASLLLLLLLLLLLLSLCALVSAQFTVVGPANPILAMVGENTTLRCHLSPEKN
AEDMEVRWFRSQFSPAVFVYKGGREERTEEQMEEYRGRITFVSKDINRGSVALVIHNVT
QENGIYRCYFQEGRSYDEAILRLVVAGLGSKPLIEIKAQEDGSIWLECISGGWYPEPLTV

degranulation (Figure 1c and 2), and leading to the identification of the reference mAb 107G3. Sequencing of VH and VL regions of these subclones was performed (see Table 11 below).

5 Anti-BTN2A1 107G3 antibody induces V γ 9V δ 2-T cell degranulation against different cancer cell targets

Purified V γ 9/V δ 2 T cells were expanded from PBMCs of healthy donors and co-cultured with different cancer cell lines, including Daudi (Burkitt's lymphoma), Jurkat (acute T cell leukemia), L-IPC (pancreatic adenocarcinoma) and MDA-MB-134 (breast carcinoma) as target cells, with or without anti-BTN2A1 107G3 hybridoma culture supernatant. As shown in Figure 2 and Table 3, the addition of anti-BTN2A1 107G3 hybridoma supernatant lead to an induction of the cytolytic function of V γ 9/V δ 2 T cells, as measured by the percentage of CD107+ degranulating cells, compared to co-cultures with target cells alone, or in the presence of control hybridoma culture medium. PMA/ionomycin treatment of V γ 9/V δ 2 T cells lead to a maximum induction of their cytolytic function independently of the target cell, as expected.

The percentage of CD107+ cells induced by anti-BTN2A1 107G3 hybridoma supernatant ranged from $71.1 \pm 7.4\%$ in Daudi cells to $17.1 \pm 2.9\%$ in MDA-MB-134 cells vs. $24.9 \pm 4.7\%$ and $4.9 \pm 0.4\%$, respectively, in co-cultures with control hybridoma culture medium. In co-cultures of V γ 9/V δ 2 T cells, with all of the cancer cell lines tested as targets, anti-BTN2A1 107G3 induced between 2-fold and 8-fold more V γ 9/V δ 2 T cell degranulation compared to the same co-cultures in the presence of control hybridoma culture medium.

Purified anti-BTN2A1 mAb 107G3 displayed an EC₅₀ of 0.77 μ g/mL (95%IC 0.32 – 13.22 μ g/mL) for the induction of V γ 9/V δ 2 T cell degranulation, as depicted by the percentage of CD107+ cells, co-cultured with Daudi cells as targets in the presence of increasing concentrations (0 to 18 μ g/ml) of the anti-BTN2A1 107G3 mAb (Figure 2b).

Table 3

	Anti-BTN2A1 107G3	Control hybridoma supernatant
EC ₅₀ on HEK-293T BTN2 KO + BTN2A1	0.32 (95%IC 0.21-0.46) μ g/mL	not applicable

CLAIMS

1. An anti-butyrophilin-2A1 (BTN2A1) antibody characterized in that it has at least one of the following functions:
 - it inhibits the polarization of monocytes towards M2 macrophages,
 - it induces reversion of M2 macrophages towards anti-tumoral M1 macrophages.
2. The anti-BTN2A1 antibody of claim 1, which has in addition, at least one of the following functions:
 - it triggers NK cell activation directly,
 - it enhances NK cell mediated cytotoxicity.
3. The anti-BTN2A1 antibody of claim 1, which competes for binding to BTN2A1 with
 - a reference antibody mAb 107G3 comprising (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 and (ii) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2, or
 - a reference antibody mAb 101G5 comprising (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:19 and (ii) a light chain variable region comprising the amino acid sequence of SEQ ID NO:20.
4. The antibody of any one of claim 1 to 3, which binds an epitope comprising residues located in positions: 65, 68, 69, 72, 78; 84, 85, 95, 97, and 100 of SEQ ID NO:17.
5. The antibody of any one of claim 1 to 3, which binds an epitope comprising residues located in positions 212, 213, 218, 220, 224, and 229 of SEQ ID NO:17.
6. The antibody of any one of claim 1 to 5, which does not cross-react with the human BTN3A isoforms, and/or which cross-reacts with the cynomolgus BTN2A1 ortholog.

7. The anti-BTN2A1 antibody of any one of the preceding claims, comprising:
- a heavy chain variable region CDR1 comprising SEQ ID NO:3, a heavy chain variable region CDR2 comprising SEQ ID NO:4, a heavy chain variable region CDR3 comprising SEQ ID NO:5, a light chain variable region CDR1 comprising SEQ ID NO:6, a light chain variable region CDR2 comprising SEQ ID NO:7, and a light chain variable region CDR3 comprising SEQ ID NO:8, or
 - a heavy chain variable region CDR1 comprising SEQ ID NO:21, a heavy chain variable region CDR2 comprising SEQ ID NO:22, a heavy chain variable region CDR3 comprising SEQ ID NO:23, a light chain variable region CDR1 comprising SEQ ID NO:24, a light chain variable region CDR2 comprising SEQ ID NO:25 and a light chain variable region CDR3 comprising SEQ ID NO:26.
8. The anti-BTN2A1 antibody of claim 7, which comprises:
- a heavy chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:2, or
 - a heavy chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:19 and a light chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:20.
9. The anti-BTN2A1 antibody of any one of claims 1-8, wherein said antibody further has at least one of the following functions:
- it activates secretion of cytolytic molecules of V γ 9V δ 2 T cells,
 - it activates the cytolytic function of V γ 9V δ 2 T cells, and/or
 - it activates the proliferation of V γ 9V δ 2 T cells.
10. The anti-BTN2A1 antibody of claim 9, which competes for binding to BTN2A1 with the reference murine antibody mAb 107G3 comprising (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 and (ii) a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.
11. The anti-BTN2A1 antibody of any one of claim 9 or 10, which comprises:

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- a heavy chain variable region CDR1 comprising SEQ ID NO:3, a heavy chain variable region CDR2 comprising SEQ ID NO:4, a heavy chain variable region CDR3 comprising SEQ ID NO:5, a light chain variable region CDR1 comprising SEQ ID NO:6, a light chain variable region CDR2 comprising SEQ ID NO:7, and a light chain variable region CDR3 comprising SEQ ID NO:8

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12. The anti-BTN2A1 antibody of claim 11, which comprises:
- a heavy chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising a sequence having at least 90 % identity with the amino acid sequence of SEQ ID NO:2.

13. The anti-BTN2A1 antibody of any one of claims 9-12, having specificity for the human BTN2A1 isoform.

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14. The anti-BTN2A1 antibody of any one of claims 1-13, which is a human, chimeric or humanized antibody.

15. A nucleic acid molecule, which encodes a heavy chain and/or a light chain of the anti-BTN2A1 antibody of any one of claims 1-14.

16. A host cell comprising the nucleic acid of claim 15.

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17. The anti-BTN2A1 antibody of any one of claims 1-14, for use in therapy.

18. Use of the anti-BTN2A1 antibody of any one of claims 1-14, in the manufacture of a medicament for treating a solid cancer.

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19. A pharmaceutical composition comprising the anti-BTN2A1 antibody of any one of claims 1-14, and at least a pharmaceutically acceptable carrier.