

the world. Although these drugs represent major advances in cancer therapy, there still remain unmet medical needs for large parts of cancer patient populations that do not respond to the currently available treatments.

The patents WO 2012080351 A1, EP2651441A1, EP2946791A1, US20140322235, 5 WO2012080769A1 refer to various antibodies against BTN3A able to activate or inhibit the cytolytic function, cytokine production and proliferation of V γ 9 V δ 2 T cells. However, these murine antibodies were not suitable for therapeutic application. Indeed, for administration to human patients, it is nowadays mandatory to humanize antibodies to avoid immunogenic reactions.

10 Humanization often requires modifying amino acids in the framework regions without certainty of maintaining the potency to the same level of the original murine antibodies. This is especially true when modifying amino acids immediately adjacent to the CDR regions (see e.g Queen patent US5,585,089).

Despite the difficulty, the inventors have now selected special humanized antibodies of 15 the activating mAbs 7.2, which not only combine maintained functional properties of the mAbs 7.2 parental antibodies with predicted decreased immunogenicity for human, but also surprisingly exhibit superior developability properties, such as an improved yield in cell line production, a higher thermal stability as compared to parental murine antibody, and strong resistance to acid and heat stress. In addition, the humanized antibody mAb1 20 of the present disclosure advantageously bind to cynomolgus BTN3A and is well tolerated up to doses of 100 mg/kg/week in cynomolgus primate, thereby providing an excellent candidate for use as a drug in human therapies.

Summary

In one aspect, the present disclosure thus relates to an isolated anti-BTN3A antibody 25 comprising a variable heavy chain polypeptide VH of SEQ ID NO:1 and a variable light chain polypeptide VL of SEQ ID NO:2 or SEQ ID NO: 3. Such antibodies are humanized antibodies, in particular with predicted decreased immunogenicity with respect to their parental murine antibody. Such isolated anti-BTN3A antibody binds to human BTN3A. In particular, it binds to human BTN3A with a K_D of 10 nM or less, preferably with a K_D of 5 30 nM or less as measured by surface plasmon resonance.

In specific examples, said antibody according to the present disclosure, induces the activation of $\gamma\delta$ -T cells, typically V γ 9V δ 2 T cells, in co-culture with BTN3 expressing cells, with an EC₅₀ below 5 μ g/ml, preferably of 1 μ g/ml or below, as measured in a

degranulation assay. Consequently, said antibody induces the killing of tumor target cells independently of their tissues origin.

In specific examples, said isolated anti-BTN3A antibody comprises a mutant or chemically modified IgG1 constant region, wherein said mutant or chemically modified IgG1 constant
5 region confers no or decreased binding to Fcγ receptors when compared to a corresponding antibody with wild type IgG1 isotype constant region. Typically, said mutant IgG1 constant region is IgG1 triple mutant L247F L248E and P350S. Examples of said isolated anti-BTN3A antibody are mAb1 comprising a heavy chain of SEQ ID NO:4 and a
10 light chain of SEQ ID NO:6, or mAb2 comprising a heavy chain of SEQ ID NO:4 and a light chain of SEQ ID NO:7.

In other examples, said isolated anti-BTN3A antibody of the present disclosure is a monovalent format antibody, preferably selected from Fab or scFv antibodies.

The isolated anti-BTN3A antibody of the present disclosure may be used (i) as a therapeutic or (ii) as a diagnostic. For example, they are useful in the treatment of a
15 cancer, for example a hematologic cancer, and more specifically a lymphoma or leukaemia. In other examples, they can also be used in the treatment of solid tumors, and more specifically prostate, ovarian or endometrial cancers. Alternatively, they may be used in the treatment of infectious disorders.

In another aspect, the present disclosure refers to a use of the isolated anti-BTN3A
20 antibody as disclosed herein in the manufacture of a medicament for treating cancer.

In another aspect, the present disclosure refers to a use of the isolated anti-BTN3A antibody as disclosed herein in the manufacture of a medicament for use in a combination therapy for cancer, wherein the medicament is to be administered in combination with
25 other active ingredients, for example an anti-PD1 or anti-PD-L1 antibody, or a cytokine, such as IL-2 or IL-15 or their pegylated variants.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising an anti-BTN3A antibody, in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier, optionally comprising other active ingredients, for example an anti-PD1 or anti-PD-L1 antibody, or a cytokine, such as
30 IL-2 or IL-15. The disclosure further relates to a pharmaceutical composition comprising an anti-BTN3A antibody as described above, in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier, optionally comprising other active ingredients.

Another aspect of the present disclosure concerns a lyophilisate formulation, a pre-filled syringe or a vial comprising an anti-BTN3A antibody as disclosed herein.

In yet another aspect, the present disclosure refers to an expression vector for the recombinant production of an anti-BTN3A antibody as disclosed herein in a host cell,
5 comprising at least one nucleic acid encoding said anti-BTN3A antibody. The disclosure further relates to an expression vector for the recombinant production of an anti-BTN3A antibody as described above in a host cell, typically a mammalian host cell, such as CHO host cell, comprising at least one nucleic acid encoding said anti-BTN3A antibody. An example of such expression vector comprises at least the nucleic acids encoding the
10 heavy and light chains of mAb1 as disclosed herein. The host cell comprising such expression vectors are also described herein.

In yet another aspect, the present disclosure refers to a process for the production of an anti-BTN3A antibody of the present disclosure, comprising: (i) culturing the host cell as disclosed herein for expression of said antibody by the host cell; optionally (ii) purifying
15 said antibody; (iii) recovering the antibody.

CLAIMS

1. An isolated anti-BTN3A antibody comprising a variable heavy chain polypeptide VH of SEQ ID NO:1 and a variable light chain polypeptide VL of SEQ ID NO:2 or SEQ ID NO: 3.
- 5 2. The isolated anti-BTN3A antibody of Claim 1, which binds to human BTN3A polypeptide.
3. The isolated anti-BTN3A antibody of Claim 1 or 2, which binds to human BTN3A polypeptide with a K_D of 10 nM or less, preferably with a K_D of 5 nM or less as measured by surface plasmon resonance.
- 10 4. The isolated anti-BTN3A antibody of any one of Claims 1-3, wherein said antibody induces the activation of $V\gamma 9V\delta 2$ -T cells in co-culture with BTN3 expressing cells, with an EC_{50} below 5 μ g/ml, preferably of 1 μ g/ml or below, as measured in a degranulation assay.
- 15 5. The isolated anti-BTN3A antibody of any one of Claims 1-4, which comprises a mutant or chemically modified IgG1 constant region, wherein said mutant or chemically modified IgG1 constant region confers no or decreased binding to Fc γ receptors when compared to a corresponding antibody with wild type IgG1 isotype constant region.
- 20 6. The isolated anti-BTN3A antibody of Claim 5, wherein said mutant IgG1 constant region is IgG1 triple mutant L247F L248E and P350S.
7. The isolated anti-BTN3A antibody of any one of Claims 1-6, which is mAb1 comprising a heavy chain of SEQ ID NO:4 and a light chain of SEQ ID NO:6.
8. The isolated anti-BTN3A antibody of any one of claims 1-7, for use (i) as a therapeutic or (ii) as a diagnostic.
- 25 9. Use of the isolated anti-BTN3A antibody according to any one of Claims 1-7 in the manufacture of a medicament for treating cancer.
- 30 10. Use of the isolated anti-BTN3A antibody according to any one of Claims 1-7 in the manufacture of a medicament for use in a combination therapy for cancer, wherein the medicament is to be administered in combination with other active ingredients, for example an anti-PD1 or anti-PD-L1 antibody, or a cytokine, such as IL-2 or IL-15 or their pegylated variants.

11. A pharmaceutical composition comprising an anti-BTN3A antibody, in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier, optionally comprising other active ingredients, for example an anti-PD1 or anti-PD-L1 antibody, or a cytokine, such as IL-2 or IL-15.
- 5 12. A lyophilisate formulation, a pre-filled syringe or a vial comprising an anti-BTN3A antibody according to any one of Claims 1-7.
13. An expression vector for the recombinant production of an anti-BTN3A antibody according to any one of Claims 1-7 in a host cell, comprising at least one nucleic acid encoding said anti-BTN3A antibody.
- 10 14. An expression vector according to Claim 13, comprising at least the nucleic acids encoding the heavy and light chains of mAb1 as defined in Claim 7.
15. A host cell comprising an expression vector according to Claim 13 or 14.
16. A process for the production of an anti-BTN3A antibody of any one of Claims 1-7, comprising: (i) culturing the host cell of Claim 15 for expression of said antibody by
15 the host cell; optionally (ii) purifying said antibody; (iii) recovering the antibody.