

Antibodies that bind to citrullinated epitopes on deiminated human histone 2A and histone 4 are described in WO2009147201, WO2011070172 and WO2016092082.

Summary of the invention

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The present inventors have created improved antibodies that bind to citrullinated epitopes on the amino terminus of histones 2A and/or histone 4. These antibodies can be used to treat diseases or pathologies associated with citrullination, such as NET-associated pathologies and inflammatory conditions.

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The present inventors have created antibodies that show improved properties over the therapeutic antibodies disclosed in WO2009147201, WO2011070172 and WO2016092082. The inventors discovered, by accelerated stability testing and Mass Spectrometry analyses, that isomerisation of certain amino acid residues in the

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Complementarity-Determining Region 1 (CDR1) of the light chain of the antibodies disclosed in WO2009147201, WO2011070172 and WO2016092082 resulted in a reduction of the binding affinity of the antibodies for the tested histone-derived peptides over time. The inventors then conducted a thorough analysis of CDR1 light chain mutants to solve the isomerisation problem, whilst attempting to retain the binding properties of the antibody.

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Several attempts resulted in antibodies with reduced binding affinity for the target peptides.

Finally, the inventors were successful in identifying a group of mutations in CDR1 of the light chain that removed the isomerisation issue, whilst maintaining the binding properties of the original antibody. Surprisingly, the mutant antibodies showed improved properties over the original antibodies both *in vitro* and *in vivo*.

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Therefore, in one aspect, the present disclosure refers to an antibody or antigen-binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4, wherein the antibody or antigen-binding fragment thereof comprises: a) the VL CDR1 of SEQ ID NO: 9 (QSLLDADGKTY); and b) the VH CDR1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR2 of SEQ ID NO: 2 (INTYSGEA), the VH CDR3 of SEQ ID NO: 3 (LRGYTYQS FDEGGDY), the VL CDR2 of SEQ ID NO: 4 (LVS) and the VL CDR3 of SEQ ID NO: 5 (WQGTHFPYT).

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- In another aspect, the present disclosure refers to a polynucleotide encoding the antibody or antigen-binding fragment thereof as disclosed herein, a cloning or expression vector comprising said polynucleotide, or a host cell comprising said cloning or expression vector.
- 5 In another aspect, the present disclosure refers to a process for the production of an antibody or antigen-binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4, comprising culturing the host cell as disclosed herein and isolating the antibody or binding fragment thereof from said cell.
- 10 In another aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody or antigen-binding fragment thereof as disclosed herein and at least one pharmaceutically acceptable diluent or carrier, and optionally other active ingredients.
- 15 In another aspect, the present disclosure refers to the antibody or antigen-binding fragment thereof as disclosed herein, or the pharmaceutical composition as disclosed herein, for use in therapy.
- 20 In yet another aspect, the present disclosure refers to a use of the antibody or antigen-binding fragment thereof as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a NET-associated pathology, wherein the NET-associated pathology is systemic lupus erythematosus (SLE), lupus, sepsis, vasculitis, inflammatory arthritis, rheumatoid arthritis and osteoarthritis,
- 25 psoriasis, Alzheimer's disease, autoimmune hepatitis, juvenile idiopathic arthritis, Sjögren's disease, Anti-phospholipid Syndrome, Bechet's disease, spondylitis, spondyloarthropathy, multiple system atrophy, Parkinson's disease, Lewy body dementia asthma, allergic rhinovirus exacerbated asthma, allergic asthma, cystic fibrosis, fibrosis and idiopathic pulmonary fibrosis, dry eye disease, uveitis, nongranulomatous uveitis,
- 30 granulomatous uveitis, dermatitis, atopic dermatitis, COPD, bronchitis, wound healing in diabetes, cancer, cancer metastasis, or transplant organ health *in vivo*.
- The present disclosure provides:
- 35 - An antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4, wherein the antibody or binding fragment thereof comprises:
- a) CDR1 of the light chain variable domain (VL), wherein the CDR comprises or consists of the amino acid sequence QSL-X₁-D-X₂-D-X₃-KTY, wherein X₁ is V or L, X₂ is

CLAIMS

1. An antibody or antigen-binding fragment thereof that specifically binds to a
citrullinated epitope on deiminated human histone 2A and/or histone 4, wherein the
5 antibody or antigen-binding fragment thereof comprises:
- a) the VL CDR1 of SEQ ID NO: 9 (QSLLDADGKTY); and
 - b) the VH CDR1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR2 of SEQ ID NO: 2
(INTYSGEA), the VH CDR3 of SEQ ID NO: 3 (LRGYTYQS FDEGGDY), the VL CDR2
of SEQ ID NO: 4 (LVS) and the VL CDR3 of SEQ ID NO: 5 (WQGTHFPYT).
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2. The antibody or antigen-binding fragment thereof according to claim 1, wherein the
antibody or antigen-binding fragment thereof comprises:
- a) VL CDR1 of SEQ ID NO: 9;
 - b) VL CDR2 of SEQ ID NO: 4 and VL CDR3 of SEQ ID NO: 5; and
 - 15 c) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 or 12.
3. The antibody or antigen-binding fragment thereof according to claim 1 or 2,
wherein the antibody or antigen-binding fragment thereof comprises:
- a) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 and the
20 light chain variable domain amino acid sequence of SEQ ID NO: 16; or
 - b) the heavy chain variable domain amino acid sequence of SEQ ID NO: 12 and the
light chain variable domain amino acid sequence of SEQ ID NO: 16.
4. The antibody or antigen-binding fragment thereof according to any one of the
25 preceding claims, selected from the group consisting of recombinant antibodies, single
chain antibodies, single chain variable fragments (scFv), variable fragments (Fv), fragment
antigen-binding regions (Fab), single-domain antibodies (sdAb), camelids-derived single-
domain antibodies, shark IgNAR-derived single-domain antibody fragments (VNAR),
diabodies, triabodies, Anticalins and aptamers.
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5. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, which is a full-length antibody, optionally comprising an Fc region selected from an IgG1, IgG2, IgG3 or IgG4 region.
- 5 6. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, which comprises a heavy chain constant region comprising SEQ ID NO: 23 or 56, and/or a light chain constant region comprising SEQ ID NO: 24.
7. The antibody or antigen-binding fragment thereof according to any one of the
10 preceding claims, wherein the antibody comprises the heavy chain variable domain amino acid sequence of SEQ ID NO: 11, the light chain variable domain amino acid sequence of SEQ ID NO: 16, the heavy chain constant region amino acid sequence of SEQ ID NO: 23 or 56, and the light chain constant region amino acid sequence of SEQ ID NO: 24.
- 15 8. The antibody or antigen-binding fragment thereof according to any one of the preceding claims conjugated to an additional moiety.
9. A polynucleotide encoding the antibody or antigen-binding fragment thereof according to any one of claims 1 to 7, a cloning or expression vector comprising said
20 polynucleotide, or a host cell comprising said cloning or expression vector.
10. A process for the production of an antibody or antigen-binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4, comprising culturing the host cell of claim 9 and isolating the antibody or
25 antigen-binding fragment thereof from said cell.
11. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof according to any one of claims 1 to 8 and at least one pharmaceutically acceptable diluent or carrier, and optionally other active ingredients.
- 30 12. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 8, or the pharmaceutical composition according to claim 11, for use in therapy.

13. Use of the antibody or antigen-binding fragment thereof according to any one of claims 1 to 8, or the pharmaceutical composition according to claim 11, in the manufacture of a medicament for treating a NET-associated pathology, wherein the NET-associated pathology is systemic lupus erythematosus (SLE), lupus, sepsis, vasculitis, inflammatory arthritis, rheumatoid arthritis and osteoarthritis, psoriasis, Alzheimer's disease, autoimmune hepatitis, juvenile idiopathic arthritis, Sjögren's disease, Anti-phospholipid Syndrome, Bechet's disease, spondylitis, spondyloarthropathy, multiple system atrophy, Parkinson's disease, Lewy body dementia asthma, allergic rhinovirus exacerbated asthma, allergic asthma, cystic fibrosis, fibrosis and idiopathic pulmonary fibrosis, dry eye disease, uveitis, nongranulomatous uveitis, granulomatous uveitis, dermatitis, atopic dermatitis, COPD, bronchitis, wound healing in diabetes, cancer, cancer metastasis, or transplant organ health *in vivo*.
14. The antibody, antigen-binding fragment thereof or pharmaceutical composition for use according to claim 12 or the use according to claim 13, wherein the antibody, antigen-binding fragment thereof or pharmaceutical composition is to be administered by a parenteral route of administration such as an intravenous, subcutaneous, intraocular, intramuscular, intradermal, intraperitoneal, spinal route or by injection or infusion; or by another administration route such as rectal, oral, ocular, topical, epidermal, mucosal, local, peritumoral, juxtatumoral, intratumoral, to the resection margin of tumors, intralesional, perilesional, by intra cavity infusion, intravesicle administration, or by inhalation.