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**

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.

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 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. 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*.

Therefore, in one aspect, the present disclosure refers to 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 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 T, S, A or N and X3 is G or A, provided that the amino acid sequence is not QSLLDSDGKTY (SEQ ID NO: 36) or QSLVDSDGKTY (SEQ ID NO: 37); and b) at least one CDR selected from SEQ ID NOs: 1 to 5, preferably

the CDRs of SEQ ID NO: 3 and SEQ ID NO: 5, most preferably all of the CDRs of SEQ ID NOs: 1 to 5.

In another aspect, the present disclosure refers to 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 the CDRs of: a) the CDR1 of SEQ ID NOs: 13, 14, 15, 16 or 17; and b) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 or 12.

In another aspect, the present disclosure refers to a polynucleotide encoding the antibody or binding fragment thereof as disclosed herein, a cloning or expression vector comprising said polynucleotide, or a host cell comprising said cloning or expression vector.

In another aspect, the present disclosure refers to a process for the production of an antibody or 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.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody or binding fragment thereof as disclosed herein and at least one pharmaceutically acceptable diluent or carrier, and optionally other active ingredients.

In another aspect, the present disclosure refers to the antibody or binding fragment thereof as disclosed herein, or the pharmaceutical composition as disclosed herein, for use in therapy.

In yet another aspect, the present disclosure refers to a use of the antibody or binding fragment thereof as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating or preventing a NET-associated pathology, optionally 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, or another NET-associated pathology such as wound healing in diabetes, cancer, cancer metastasis, and transplant organ health *in vivo* or *ex vivo*.

The present disclosure provides:

- 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-$X_1$-D-$X_2$-D-$X_3$-KTY, wherein $X_1$ is V or L, $X_2$ is

T, S, A or N and $X_3$ is G or A, provided that the amino acid sequence is not QSLLDSDGKTY (SEQ ID NO: 36) or QSLVDSDGKTY (SEQ ID NO: 37); and

b) at least one CDR selected from SEQ ID NOs: 1 to 5.

The disclosure also provides:

- 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 the CDRs of:

a) the CDR1 of SEQ ID NOs: 13, 14, 15, 16 or 17; and

b) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 or 12.

The disclosure also provides:

- A polynucleotide encoding the antibody or binding fragment thereof as defined herein, a cloning or expression vector comprising said polynucleotide, or a host cell comprising said cloning or expression vector.

The disclosure also provides:

- A process for the production of an antibody or 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 defined herein and isolating the antibody or binding fragment thereof from said cell.

The disclosure also provides:

- A pharmaceutical composition comprising the antibody or binding fragment thereof according as defined herein and at least one pharmaceutically acceptable diluent or carrier.

The disclosure also provides:

- The antibody or binding fragment thereof as defined herein, or the pharmaceutical composition as defined herein, for use in therapy.

The disclosure also provides:

- The antibody or binding fragment thereof as defined herein, or the pharmaceutical composition as defined herein, for use in a method of treating or preventing a NET-associated pathology.

The disclosure also provides:

## CLAIMS

1. 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 VL, wherein the CDR comprises or consists of the amino acid sequence QSL-$X_1$-D-$X_2$-D-$X_3$-KTY, wherein $X_1$ is V or L, $X_2$ is T, S, A or N and $X_3$ is G or A, provided that the amino acid sequence is not QSLLDSDGKTY (SEQ ID NO: 36) or QSLVDSDGKTY (SEQ ID NO: 37); and

b) at least one CDR selected from SEQ ID NOs: 1 to 5, preferably the CDRs of SEQ ID NO: 3 and SEQ ID NO: 5, most preferably all of the CDRs of SEQ ID NOs: 1 to 5.

2. The antibody or binding fragment thereof according to any of the preceding claims, wherein the antibody or binding fragment thereof comprises:

a) one of the CDRs of SEQ ID NOs: 6, 7, 8, 9 and 10;

b) the CDRs of SEQ ID NOs: 1 to 5.

3. The antibody or binding fragment thereof according to claim 1, wherein the antibody or binding fragment thereof comprises:

a) CDR1 of VL, wherein the CDR comprises or consists of the amino acid sequence QSL-$X_1$-D-$X_2$-D-$X_3$-KTY, wherein $X_1$ is V or L, $X_2$ is T, S, A or N and $X_3$ is G or A, provided that the amino acid sequence is not QSLLDSDGKTY (SEQ ID NO: 36) or QSLVDSDGKTY (SEQ ID NO: 37), preferably wherein said CDR1 of VL is one of SEQ ID NOs: 6, 7, 8, 9 and 10;

b) at least one, preferably both, of the CDRs of SEQ ID NO: 4 and 5; and

c)

i) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 or 12;

or

ii) a fragment of at least 7 amino acids of (i), wherein the antibody or binding fragment thereof retains the ability of being specifically reactive with a citrullinated epitope on deiminated human histone 2A and/or histone 4;

or

iii) a variant of (i) having at least 70% amino acid sequence identity to a sequence of (i), wherein the antibody or binding fragment thereof retains the ability of being specifically reactive with a citrullinated epitope on deiminated human histone 2A and/or histone 4;

wherein c) is preferably i).

4. The antibody or binding fragment thereof according to any one of the preceding claims, wherein the antibody or binding fragment thereof comprises:

a) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 and the light chain variable domain amino acid sequence of SEQ ID NO: 13;

b) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 and the light chain variable domain amino acid sequence of SEQ ID NO: 14;

c) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 and the light chain variable domain amino acid sequence of SEQ ID NO: 15;

d) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 and the light chain variable domain amino acid sequence of SEQ ID NO: 16;

e) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 and the light chain variable domain amino acid sequence of SEQ ID NO: 17;

f) 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: 13;

g) 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: 14;

h) 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: 15;

i) 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; or

j) 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: 17.

5. 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 the CDRs of:

a) the CDR1 of SEQ ID NOs: 13, 14, 15, 16 or 17; and

b) the heavy chain variable domain amino acid sequence of SEQ ID NO: 11 or 12.

6. The antibody or binding fragment thereof according to any one of the preceding claims, that:

- specifically binds to a peptide selected from the group consisting of SEQ ID NOs: 18, 19, 20, 21 and 22, and binds deiminated human histone 2A and/or histone 4; and/or
- specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 with an affinity of at least 1nM or less; and/or
- is conjugated to an additional moiety; and/or
- is a full length antibody, optionally comprising an Fc region, such as an IgG1, IgG2, IgG3 or IgG4 region.

7. The antibody or binding fragment thereof according to any one of the 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), VHH antibodies, nanobodies, camelids-derived single-domain antibodies, shark IgNAR-derived single-domain antibody fragments (VNAR), diabodies, triabodies, Anticalins and aptamers.

8. The antibody or 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.

9. The antibody or binding fragment thereof according to any one of the 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.

10. A polynucleotide encoding the antibody or binding fragment thereof according to any one of claims 1 to 9, a cloning or expression vector comprising said polynucleotide, or a host cell comprising said cloning or expression vector.

11. A process for the production of an antibody or 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 10 and isolating the antibody or binding fragment thereof from said cell.

12. A pharmaceutical composition comprising the antibody or binding fragment thereof according to any one of claims 1 to 9 and at least one pharmaceutically acceptable diluent or carrier, and optionally other active ingredients.

13. The antibody or binding fragment thereof according to any one of claims 1 to 9, or the pharmaceutical composition according to claim 12, for use in therapy.

14. Use of the antibody or binding fragment thereof according to any one of claims 1 to 9, or the pharmaceutical composition according to claim 12, in the manufacture of a medicament for treating or preventing a NET-associated pathology, optionally 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, or another NET-associated pathology such as wound healing in diabetes, cancer, cancer metastasis, and transplant organ health *in vivo* or *ex vivo*.

15. The antibody, binding fragment thereof or pharmaceutical composition for use according to claim 13 or the use according to claim 14, wherein the antibody, 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.