neutrophils and eosinophils have differences in the structures of granules and the extracellular traps that form. When neutrophils undergo NETosis, the granules disintegrate intracellularly and thus granule proteins adhere to the NETs. By contrast, most of the granules in eosinophils are intact during the process of EETosis, resulting in the generation of free extracellular granules and granule protein-free EETs. Both NETs and EETs retain histones (i.e. chromatin structure), but EETs are thicker in diameter than NETs because of less extensive protease modification of chromatin. Histone citrullination (e.g. mediated by the enzyme PAD4) is known to play a key role in NET formation, but the evidence for a similarly significant role in EET formation is inconclusive.

Given these differences, further study into EETs/EETosis is required to give a detailed understanding of the roles played in homeostasis and disease pathogenesis, and into the mechanism of EET formation and how this may be inhibited. In other words, there remains a need for compounds for the treatment or prevention of EET-associated pathologies.

Given that eosinophils and neutrophils often play a role in the underlying pathology of lung conditions there is also an ongoing need in particular for ways to target their involvement in such conditions.

Summary

There is conflicting evidence for the role of citrullination of histones in EET formation. Surprisingly the present inventors have found that an antibody that binds to citrullinated epitopes on the amino terminus of histones 2A and/or histone 4 is able to inhibit EETosis, and may thus be used in methods to inhibit EET formation. The methods may be for the treatment or prevention of an EET-associated pathology. Such pathologies may include: an eosinophilic disease of the skin; a respiratory eosinophilic disease; a gastro-intestinal eosinophilic disease; an allergic disease; or a helminth, fungal, viral, or bacterial infection. In addition, arteriosclerosis may also be treated or prevented. In another example, vasculitis may also be treated.

Given the differences between EETs and NETs, the present inventors have further found unexpectedly that it is possible to inhibit the formation of both EETs and NETs employing the same antibody that binds to citrullinated epitopes on the amino terminus of histones 2A and/or histone 4, including in the same condition. The inventors have also

found that such antibodies may be used to treat a variety of conditions including lung disorders, particularly inflammatory lung disorders, including asthma. The present inventors have also found that such antibodies may be used to treat conditions involving increased numbers of infiltrating neutrophils. The present inventors have further found that such antibodies may be used to treat conditions involving increased numbers of infiltrating eosinophils.

The inventors have further found that the approach of same antibody that binds to citrullinated epitopes on the amino terminus of histones 2A and/or histone 4 may in some instances provide a bigger effect than corticosteroids and/or a different one. Hence, the disclosure may also be used to treat individuals who do not show an adequate response to corticosteroids. For instance, it may be that the disclosure is used to treat an individual that has a condition resistant to corticosteroids. It may also be that the antibody and corticosteroid are used in combination so that the two augment each other and that represents a further preferred example. In one example, te corticosteroid is dexamethasone.

Representative antibodies that bind to citrullinated epitopes on deiminated human histone 2A and histone 4 are described in for example, WO2009147201, WO2011070172, WO2016092082, and WO2020038963. Each of these documents, and the antibodies disclosed therein (including all CDR sequences, variable region sequences and constant region sequences of both the heavy and light chains), is herein incorporated by reference. In particular, the antibodies designated RhmAb2.102, RhmAb2.108, RhmAb2.109, RhmAb2.110, RhmAb2.111 RhmAb2.112, MQ22.101, MQ22.102 and MQ22.101b/d in WO2016092082, and any antigen binding fragment thereof, are each incorporated by reference. Similarly, the antibodies disclosed with identifiers of the format hMQ22.101x/y in WO2020038963, and any antigen binding fragment thereof, are each incorporated by reference. The antibodies disclosed in WO2020038963 are particularly preferred and are discussed in more detail below. The antibody referred to in WO2020038963 as hMQ22.101f/LC41 is most preferred. This antibody may be described herein as CIT-013. The present disclosure provides:

A method of inhibiting or detecting the formation of eosinophil extracellular traps (EETs), the method comprising administering an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone

4 to a sample or a subject. The sample or subject is preferably a sample or subject in which eosinophils are present.

The method may be for the prevention or treatment of a disease or condition in a subject, and thus may comprise administering said antibody or binding fragment thereof to the subject in a prophylactically or therapeutically effective amount.

In one aspect, the present disclosure refers to the use of an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 in the manufacture of a medicament for the prevention or treatment of a disease or condition in a subject by inhibiting the formation of eosinophil extracellular traps (EETs), wherein the use comprises that said antibody or binding fragment thereof is to be administered to the subject in which eosinophils are present in a prophylactically or therapeutically effective amount, wherein said antibody or binding fragment thereof comprises: (a) the VL CDR 1 of SEQ ID NO: 9 (QSLLDADGKTY); and (b) the VH CDR 1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR 2 of SEQ ID NO: 2 (INTYSGEA), the VH CDR 3 of SEQ ID NO: 3 (LRGYTYQS FDEGGDY), the VL CDR 2 of SEQ ID NO: 4 (LVS) and the VL CDR 3 of SEQ ID NO: 5 (WQGTHFPYT).

The disease or condition which typically includes an EET-associated pathology. The disease or condition may be an eosinophilic disease or condition. Eosinophilic diseases and conditions may include: an eosinophilic disease or condition of the skin; a respiratory eosinophilic disease or condition; a gastro-intestinal eosinophilic disease or condition; an allergic disease or condition; or a helminth, fungal, viral, or bacterial infection.

Also provided is an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 for use in the above methods, particularly the above methods for the prevention or treatment of a disease or condition in a subject. Also provided is an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 for use in the manufacture of a medicament for use in the above methods for the prevention or treatment of a disease or condition in a subject. In one example, arteriosclerosis is treated. In another example, vasculitis is treated.

The method may alternatively be for the *ex vivo* inhibition or detection of EET formation in a sample. The method may be used to diagnose the presence of an EET-associated pathology.

In another aspect, the present disclosure refers to a method for the *ex vivo* inhibition or detection of EET formation in a sample, which comprises administering an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 to the sample and incubating under conditions suitable for binding to occur, wherein said antibody or binding fragment thereof comprises: (a) the VL CDR 1 of SEQ ID NO: 9 (QSLLDADGKTY); and (b) the VH CDR 1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR 2 of SEQ ID NO: 2 (INTYSGEA), the VH CDR 3 of SEQ ID NO: 3 (LRGYTYQS FDEGGDY), the VL CDR 2 of SEQ ID NO: 4 (LVS) and the VL CDR 3 of SEQ ID NO: 5 (WQGTHFPYT), optionally wherein the sample is of a body fluid obtained from a subject, such as serum or blood, and optionally wherein the sample is processed for example to isolate eosinophils.

The method may in some examples inhibit the formation of eosinophil extracellular traps (EETs) and neutrophil extracellular traps (NETs).

Also provided is a method of treating or preventing a lung disorder comprising administering an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 to a subject with said lung disorder.

In yet another aspect, the present disclosure refers to the use of an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 in the manufacture of a medicament for the treatment or prevention of a lung disorder, comprising that said antibody or binding fragment is to be administered to a subject with said lung disorder, wherein said antibody or binding fragment thereof comprises: (a) the VL CDR 1 of SEQ ID NO: 9 (QSLLDADGKTY); and (b) the VH CDR 1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR 2 of SEQ ID NO: 2 (INTYSGEA), the VH CDR 3 of SEQ ID NO: 3 (LRGYTYQS FDEGGDY), the VL CDR 2 of SEQ ID NO: 4 (LVS) and the VL CDR 3 of SEQ ID NO: 5 (WQGTHFPYT).

Brief Description of the Sequence Listing

Antibody nomenclature

CDR = complementarity-determining region.

CLAIMS

1. Use of an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 in the manufacture of a medicament for the prevention or treatment of a disease or condition in a subject by inhibiting the formation of eosinophil extracellular traps (EETs), wherein the use comprises that said antibody or binding fragment thereof is to be administered to the subject in which eosinophils are present in a prophylactically or therapeutically effective amount, wherein said antibody or binding fragment thereof comprises:

(a) the VL CDR 1 of SEQ ID NO: 9 (QSLLDADGKTY); and

(b) the VH CDR 1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR 2 of SEQ ID NO: 2 (INTYSGEA), the VH CDR 3 of SEQ ID NO: 3 (LRGYTYQS FDEGGDY), the VL CDR 2 of SEQ ID NO: 4 (LVS) and the VL CDR 3 of SEQ ID NO: 5 (WQGTHFPYT).

2. The use according to claim 1, wherein the disease or condition includes an EET-associated pathology.

3. The use according to claim 1 or 2, wherein the disease or condition is an eosinophilic disease or condition.

4. The use according to claim 3, wherein said eosinophilic disease or condition is an eosinophilic disease or condition of the skin; a respiratory eosinophilic disease or condition; a gastro-intestinal eosinophilic disease or condition; an allergic disease or condition; or a helminth, fungal, viral, or bacterial infection.

5. The use according to any one of claims 1 to 4, wherein the disease or condition is selected from: Bullous Pemphigoid, Atopic dermatitis, allergic contact dermatitis, Eosinophilic Asthma, Chronic RhinoSinusitis with Nasal Polyposis (CRSwNP), Allergic sinusitis, Allergic bronchopulmonary aspergillosis, Eosinophilic chronic rhinosinusitis, Eosinophilic Esophagitis (EoE), HyperEosinophilic Syndrome (HES), Eosinophilic Granulomatosis with PolyAngitis (EGPA), Eosinophilic otitis media (EOM), and Drug

Reaction with Eosinophilic & Systemic Symptoms (DRESS), arteriosclerosis, and vasculitis.

6. The use according to any one of claims 1 to 5, wherein the disease or condition is selected from: Eosinophilic Asthma, Chronic RhinoSinusitis with Nasal Polyposis (CRSwNP), Eosinophilic chronic rhinosinusitis, Eosinophilic otitis media (EOM), arteriosclerosis and vasculitis.

7. A method for the *ex vivo* inhibition or detection of EET formation in a sample, which comprises administering an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 to the sample and incubating under conditions suitable for binding to occur, wherein said antibody or binding fragment thereof comprises:

(a) the VL CDR 1 of SEQ ID NO: 9 (QSLLDADGKTY); and

(b) the VH CDR 1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR 2 of SEQ ID NO: 2 (INTYSGEA), the VH CDR 3 of SEQ ID NO: 3 (LRGYTYQS FDEGGDY), the VL CDR 2 of SEQ ID NO: 4 (LVS) and the VL CDR 3 of SEQ ID NO: 5 (WQGTHFPYT),

optionally wherein the sample is of a body fluid obtained from a subject, such as serum or blood, and optionally wherein the sample is processed for example to isolate eosinophils.

8. The use according to any one of claims 1 to 6, or the method according to claim 7, wherein the antibody or binding fragment thereof comprises:

a) a VL CDR1 of SEQ ID NOs: 9; and

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

OR

a) the VL CDRs of SEQ ID NOs: 16; and

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

9. The use according to any one of claims 1 to 6 or 8, or the method according to claim 7 or claim 8, wherein the antibody or binding fragment thereof comprises:

(I) a light chain variable region comprising:

a) a VL CDR1 of SEQ ID NO: 9; and

b) the VL CDR2 of SEQ ID NO: 4 and the VL CDR3 of SEQ ID NO: 5;

and

(II) a heavy chain variable region comprising:

c) the amino acid sequence of SEQ ID NO: 11 or 12; or

d) a fragment of at least 7 amino acids of (c), 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

e) a variant of (c) having at least 70% amino acid sequence identity to a sequence of (c), 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.

10. The use according to any one of claims 1 to 6 to 8 or 9, or the method according to any one of claims 7 to 9, 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: 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.

11. The use according to any one of claims 1 to 6, or 8 to 10, or the method according to any one of claims 7 to 10, wherein the antibody or binding fragment thereof 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, preferably with an affinity of at least 1nM or less.

12. The use according to any one of claims 1 to 6, or 8 to 11, or the method according to any one of claims 7 to 11, wherein (i) the antibody or binding fragment thereof is selected from the group consisting of recombinant antibodies, single chain antibodies,

single chain variable fragments (scFv), variable fragments (Fv), fragment antigen-binding regions (Fab), diabodies, and triabodies, and is preferably a full-length antibody, and/or (ii) wherein the antibody or binding fragment thereof is conjugated to an additional moiety.

13. The use according to any one of claims 1 to 6, or 8 to 12, or the method according to any one of claims 7 to 12, wherein the antibody or binding fragment thereof comprises an Fc region, such as an IgG1, IgG2, IgG3 or IgG4 region, optionally wherein the heavy chain constant region comprises SEQ ID NO: 23 or 56, and/or the light chain constant region comprises SEQ ID NO: 24.

14. The use according to any one of claims 1 to 6, or 8 to 13, or the method according to any one of claims 7 to 13, 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. The use according to any one of claims 1 to 6, or 8 to 14, or the method according to any one of claims 7 to 14, wherein the use or method inhibits the formation of eosinophil extracellular traps (EETs) and neutrophil extracellular traps (NETs) at the same time.

16. Use of an antibody or binding fragment thereof that specifically binds to a citrullinated epitope on deiminated human histone 2A and/or histone 4 in the manufacture of a medicament for the treatment or prevention of a lung disorder, comprising that said antibody or binding fragment is to be administered to a subject with said lung disorder, wherein said antibody or binding fragment thereof comprises:

(a) the VL CDR 1 of SEQ ID NO: 9 (QSLLDADGKTY); and

(b) the VH CDR 1 of SEQ ID NO: 1 (GYTFTNYG), the VH CDR 2 of SEQ ID NO: 2 (INTYSGEA), the VH CDR 3 of SEQ ID NO: 3 (LRGYTYQS

FDEGGDY), the VL CDR 2 of SEQ ID NO: 4 (LVS) and the VL CDR 3 of SEQ ID NO: 5 (WQGTHFPYT).

17. The use according to claim 16, wherein:

(a) the condition is an inflammatory lung disorder;

(b) the lung disorder is characterised by an increase in the level of neutrophils and/or eosinophils in the lungs of the subject;

(c) the use further comprises that a corticosteroid is to be administered to the subject; and/or

(d) the subject has a lung disorder which is not responsive to corticosteroids.