the tumour microenvironment. Several studies emphasize the importance of this altered tumour arginine metabolism in the suppression of tumour-specific T-cell responses, and it was recently demonstrated that Acute Myeloid Leukemia (AML) blasts show an arginase-dependent ability to inhibit T-cell proliferation and hematopoietic stem cells. Furthermore, arginase and iNOS inhibitors reduce the suppressive activity of AML.

In mammals, two arginase isoenzymes exist: Arginase 1 and Arginase 2. The two isoenzymes catalyse the same biochemical reaction (and thus cannot be disntinguished by enzymatic assays) but differ in cellular expression, regulation and subcellular localisation.

Summary

The present inventors have previously identified a 50 amino acid region of Arginase 1 (ARG1) and Arginase 2 (ARG2) which is a "hot spot" for immunogenicity. This region corresponds to positions 161-210 of full length human ARG1 (SEQ ID NO: 20) or positions 180-229 of full length human ARG2 (SEQ ID NO: 19), or corresponding positions in murine Arginases. The region and peptides derived from it are described in WO 2018/065563.

The present inventors have also identified that a specific sub-set of polypeptides derived from the "hot spot" region of ARG1 are particularly effective at stimulating immune responses. These peptides correspond to positions 169-206 of full length human ARG1, positions 169-200 of full length human ARG1or positions 169-210 of full length human ARG1 (or corresponding positions in human ARG2 or murine ARG1). This sub-set of polypeptides is described in WO 2020/064744.

The present inventors have further identified that polypeptides derived from an entirely different region of human ARG2 are particularly effective at stimulating immune responses. This region spans the C-terminus of the transit peptide of human ARG2 (position 22 of SEQ ID NO: 19). These peptides are described in WO 2020/099582, including an immunogenic peptide designated A2L2 and corresponds to positions 2-34 of human ARG2 (SEQ ID NO: 16).

The present inventors have now identified further immunogenic polypeptides from ARG2. Unexpectedly, the polypeptides of the present invention are not located in either the "hot spot" region (i.e. amino acids 180-229 of human ARG2) or the A2L2 region (i.e. amino acids 2-34 of ARG2). The polypeptides of the present disclosure can be advantageously used to stimulate

$CD8^+$ or $CD4^+$ T cells, including cytotoxic $CD8^+$ T cells capable of lysing cancer cells (e.g. melanoma cells).

Notably, all previously observed immunogenic responses to immunogenic ARG2 polypeptides have been $CD4^+$ T cell responses. However, simultaneous activation of $CD4^+$ and $CD8^+$ epitopes has been observed to result in strong synergistic protection against tumors. Polypeptides capable of stimulating $CD8^+$ T cells, such as cytotoxic $CD8^+$ T cells, are therefore particularly advantageous.

It has also been demonstrated that ARG2-specific T cells generated using the polypeptides of the disclosure can specifically recognize and react to activated regulatory T cells (Tregs) with high ARG2 expression. The polypeptides of the disclosure thus have utility in modulating an immunosuppressive tumor microenvironment.

In one aspect, the present disclosure refers to a polypeptide which is an immunogenic fragment of human Arginase 2 (ARG2; SEQ ID NO: 19) that comprises or consists of a sequence of 9-19 consecutive amino acids of SEQ ID NO: 19 provided that said fragment does not comprise amino acids 2-34 or 180-229 of SEQ ID NO: 19.

The present disclosure provides a polypeptide which is an immunogenic fragment of human Arginase 2 (ARG2; SEQ ID NO: 19) that comprises or consists of a sequence of 9-19 consecutive amino acids of SEQ ID NO: 19 provided that said fragment does not comprise the sequence of any one of the following:

- amino acids 2-34 of SEQ ID NO: 19;
- amino acids 180 - 229 of SEQ ID NO: 19;
- amino acids 129 – 141 of SEQ ID NO: 19;
- amino acids 144 – 156 of SEQ ID NO: 19; or
- amino acids 237 – 249 of SEQ ID NO: 19.

The polypeptide may comprise or consist of a human leukocyte antigen (HLA) class I restricted epitope. The polypeptide may comprise or consist of a HLA-B8 restricted epitope. The polypeptide may be capable of stimulating $CD8^+$ T cells. The $CD8^+$ positive T cells may cytotoxic T cells. The $CD8^+$ T cells may be ARG2-specific. The $CD8^+$ positive T cells may be ARG2-specific cytotoxic T cells. The polypeptide may comprise or consist of the amino acid sequence: NLIVNPRSV (SEQ ID NO: 5).

The polypeptide may comprise or consist of a HLA class II restricted epitope. The polypeptide may be capable of stimulating $CD4^+$ T cells. The polypeptide may comprise or consist of the amino acid sequence: GLLSALDLV (SEQ ID NO: 14).

The polypeptide may have a maximum length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 amino acids. The C terminal amino acid of the polypeptide may be replaced with the corresponding amide.

In another aspect, the present disclosure refers to a polynucleotide encoding a polypeptide as disclosed herein. The polynucleotide may be comprised within a vector.

In yet another aspect, the present disclosure refers to a composition comprising a polypeptide as disclosed herein and/or a polynucleotide as disclosed herein. The composition may comprise at least one different polypeptide of the disclosure and/or at least one different polynucleotide of the disclosure. The composition may comprise at least one pharmaceutically acceptable diluent, carrier or preservative. The composition may comprise an adjuvant. The adjuvant may be selected from the group consisting of bacterial DNA based adjuvants, oil/surfactant based adjuvants, viral dsRNA based adjuvants, imidazoquinolines, and a Montanide ISA adjuvant.

Also provided by the present disclosure is a method of treating or preventing a disease or condition in a subject, the method comprising administering to the subject a polypeptide of the disclosure, a polynucleotide of the disclosure and/or a composition of the disclosure.

In yet another aspect, the present disclosure refers to a polypeptide as disclosed herein, a polynucleotide as disclosed herein and/or a composition as disclosed herein for use in the treatment or prevention of a disease or condition in a subject.

In a still further aspect, the present disclosure refers to the use of a polypeptide as disclosed herein, a polynucleotide as disclosed herein and/or a composition as disclosed herein in the manufacture of a medicament for the treatment or prevention of: (a) a disease or condition in a subject wherein the disease or condition is characterized at least in part by inappropriate or excessive immune suppressive function of ARG2, optionally wherein the excessive immune suppressive function of ARG2 is at least in part mediated by activated Treg cells expressing ARG2 and/or cancer-associated fibroblasts (CAFs) expressing ARG2; and/or cancer, optionally wherein the cancer is a melanoma (such as a malignant metastatic melanoma), chronic myeloid leukemia (CML), or pancreatic cancer.

The present disclosure further provides the use of a polypeptide of the disclosure, a polynucleotide of the disclosure and/or a composition of the disclosure for the preparation of a medicament for the treatment or prevention of a disease or condition in a subject.

The disease or condition may be characterized at least in part by inappropriate or excessive immune suppressive function of ARG2. The excessive immune suppressive function of ARG2 may be at least in part mediated by activated Treg cells expressing ARG2. The excessive immune suppressive function of ARG2 may be at least in part medicated by cancer-associated fibroblasts expressing ARG2. The disease or condition may be a cancer. The cancer may be a melanoma, (such as a malignant metastatic melanoma), chronic myeloid leukemia (CML), or pancreatic cancer.

Where the disease or condition to be treated is a cancer, the polypeptide of the disclosure, the polynucleotide of the disclosure and/or the composition of the disclosure may be used in combination with an additional cancer therapy. The additional cancer therapy may be an

immune system checkpoint inhibitor. Preferably, the immune system checkpoint inhibitor is an antibody. More preferably, the immune system checkpoint inhibitor is an anti-PD1 antibody.

The present disclosure thus provides a method of treating or preventing a cancer in a subject, the method comprising the administering to the subject a polypeptide of the disclosure, a polynucleotide of the disclosure and/or a composition of the disclosure, wherein the method further comprises the simultaneous or sequential administration of an additional cancer therapy to the subject.

The present disclosure also provides a polypeptide of the disclosure, a polynucleotide of the disclosure and/or a composition of the disclosure for use in a method of treating or preventing a cancer in a subject, the method comprising the administering to the subject a polypeptide of the disclosure, a polynucleotide of the disclosure and/or a composition of the disclosure, wherein the method further comprises the simultaneous or sequential administration of an additional cancer therapy to the subject.

The present disclosure further provides the use of a polypeptide of the disclosure, a polynucleotide of the disclosure and/or a composition of the disclosure for the preparation of a medicament for the treatment or prevention of a cancer in a subject wherein the polypeptide, polynucleotide and/or composition is to be administered to the subject simultaneously or sequentially together with an additional cancer therapy.

In a further aspect, the present disclosure refers to a method of stimulating ARG2-specific T cells, the method comprising contacting the cells with a polypeptide of the disclosure or a composition of the disclosure. The cells to be contacted may be present in a sample taken from a healthy subject or from a cancer patient. The cells to be contacted may be present in tumor sample. The ARG2-specific T cells may be $CD8^+$ positive T cells. Preferably, the ARG2-specific T cells are cytotoxic $CD8^+$ T cells.

Brief Description of the Figures

FIGURE 1 shows the identification of ARG2 peptides that elicit $CD4^+$ or $CD8^+$ T-cell responses. (A, left) IFNγ ELISPOT screening of responses to 15 different HLA-A2 predicted ARG2-derived peptides in five healthy HLA-$A2^+$ donors. $3x10^5$ cells were plated per well. Control and peptide stimulations were carried out in triplicate. Peptide-specific IFNγ secreting

CLAIMS

1. A polypeptide which is an immunogenic fragment of human Arginase 2 (ARG2; SEQ ID NO: 19) that comprises or consists of a sequence of 9-19 consecutive amino acids of SEQ ID NO: 19 provided that said fragment does not comprise amino acids 2-34 or 180-229 of SEQ ID NO: 19.

2. The polypeptide of claim 1 which comprises or consists of a human leukocyte antigen (HLA) class I restricted epitope.

3. The polypeptide of any one of claims 1 or 2 which comprises or consists of a HLA-B8 restricted epitope.

4. The polypeptide of any one of the preceding claims which is capable of stimulating $CD8^+$ T cells, optionally wherein the $CD8^+$ positive T cells are cytotoxic T cells and/or are ARG2-specific.

5. The polypeptide of any one of the preceding claims which comprises or consists of the amino acid sequence: NLIVNPRSV (SEQ ID NO: 5).

6. The polypeptide of claim 1 which comprises or consists of a HLA class II restricted epitope.

7. The polypeptide of claim 1 or 6 which is capable of stimulating $CD4^+$ T cells.

8. The polypeptide of any one of claims 1, 6 or 7 which comprises or consists of the amino acid sequence: GLLSALDLV (SEQ ID NO: 14).

9. The polypeptide of any one of the preceding claims, which has a maximum length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 amino acids and/or in which the C terminal amino acid is replaced with the corresponding amide.

10. A polynucleotide encoding a polypeptide as defined in any one of the preceding claims, optionally comprised within a vector.

11. A composition comprising a polypeptide according to any one of claims 1-9 and/or a polynucleotide according to claim 10, and optionally an adjuvant.

12. The composition of claim 11 comprising:

a. at least one different polypeptide according to any one of claims 1-9, at least one different polynucleotide according to claim 10; and/or at least one pharmaceutically acceptable diluent, carrier or preservative; and/or
b. an adjuvant selected from the group consisting of bacterial DNA based adjuvants, oil/surfactant based adjuvants, viral dsRNA based adjuvants, imidazoquinolines, and a Montanide ISA adjuvant.

13. The polypeptide of any one of claims 1-9, the polynucleotide of claim 10, and/or the composition of claim 11 or 12, for use in the treatment or prevention of a disease or condition in a subject.

14. Use of the polypeptide of any one of claims 1-9, the polynucleotide of claim 10 and/or the composition of claim 11 or 12 in the manufacture of a medicament for the treatment or prevention of:

a. a disease or condition in a subject wherein the disease or condition is characterized at least in part by inappropriate or excessive immune suppressive function of ARG2, optionally wherein the excessive immune suppressive function of ARG2 is at least in part mediated by activated Treg cells expressing ARG2 and/or cancer-associated fibroblasts (CAFs) expressing ARG2; and/or
b. cancer, optionally wherein the cancer is a melanoma (such as a malignant metastatic melanoma), chronic myeloid leukemia (CML), or pancreatic cancer.

15. The use according to claim 14 wherein the medicament is for the treatment or prevention of cancer and optionally wherein an additional anti-cancer agent is to be administered simultaneously or sequentially with the medicament to the subject, and optionally wherein the additional cancer agent is an immune system checkpoint inhibitor, preferably an antibody, more preferably an anti-PD1 antibody.

16. A method of stimulating ARG2-specific T cells, the method comprising contacting the cells with the polypeptide of any one of claims 1 to 9 or the composition of claim 11 or 12, optionally wherein:

a. the cells are present in a sample taken from a healthy subject or from a cancer patient, optionally a tumor sample; and/or

b. the ARG2-specific T cells are $CD8^+$ positive T cells, preferably cytotoxic $CD8^+$ T cells.