Summary

The present inventors have previously identified polypeptide fragments of TGFb-1 which are immunogenic. These polypeptide fragments are disclosed in WO 2020/245264, which is herein incorporated by reference. The present inventors have now identified new immunogenic polypeptide fragments from TGFb-1.

Surprisingly, the polypeptides disclosed herein stimulate an immune response which is selective for TGFb-1. In other words, the polypeptides disclosed herein stimulate TGFb-1 selective T cells which exhibit low cross reactivity towards TGFb-2 and TGFb-3. The polypeptides in embodiments of the present disclosure are therefore expected to be particularly effective at stimulating a beneficial, selective immune response against TGFb-1-expressing cells. In particular, the polypeptides in embodiments of the present disclosure are expected to enhance the selectivity of the immune response against TGFb-1-expressing cells, without raising an immune response against TGFb-2-expressing cells or TGFb-3-expressing cells. The polypeptides in embodiments of the present disclosure are therefore expected to display low off-target toxicity. The longer polypeptides in embodiments of the present disclosure, such as those set forth in SEQ ID NOs: 7 and 10, are also expected to be particularly immunogenic as they may contain more epitopes than shorter polypeptide fragments of TGFb-1. Such peptides are also expected to have improved properties as regards ease of manufacture and formulation.

TGFb-1 is a dimeric cytokine which shares a cysteine knot structure connected together by intramolecular disulfide bonds. TGFb-1 is synthesized as a monomeric 390-amino acid precursor protein, which is referred to interchangeably as: TGFb-1 pre-protein; TGFb-1 precursor; full-length TGFb-1; pre-pro-TGFb-1. The full-length sequence of the TGFb-1 pre-protein is provided as SEQ ID NO: 1.

The TGFb-1 pre-protein monomer has a molecular weight of about 25 kDa. The TGFb-1 protein monomer has three distinct domains: the signal peptide (SP: amino acids 1-29; SEQ ID NO: 4), the latency associated peptide (LAP: amino acids 30-278; SEQ ID NO: 5) and the mature peptide (mature TGFb-1: amino acids 279-390; SEQ ID NO: 6).

The TGFb-1 SP targets the protein to a secretory pathway; the SP is cleaved off in the rough endoplasmic reticulum. TGFb-1 monomers comprising the LAP and mature TGFb-1 may dimerize in the endoplasmic reticulum via disulfide bridges between cysteine residues in the LAP (e.g. Cys 223 and Cys 225) and the mature TGFb-1 peptide (e.g. Cys 356) to form a

TGFb-1 homodimer. This TGFb-1 homodimer is referred to as the small latent complex (SLC). The SLC may be bound by so-called latent TGF-β-Binding Protein (LTBP) to form a larger complex referred to as the large latent complex (LLC). The LLC may be secreted into extracellular media (ECM). However, the presence of LAP and the LTBP prevent TGFb-1 from binding to, and activating, its extracellular receptors. Active TGFb-1 consists of a homodimer of mature TGFb-1 peptides. There are various mechanisms by which the mature TGFb-1 homodimer is released from LAP and LTBP, which include degradation of LAP by proteases, induction of conformational change in LAP by interaction with thrombospondin, and rupture of noncovalent bonds between LAP and TGFb-1.

An object of the present disclosure is the development of an immunogenic polypeptide that raises a selective immune response against TGFb-1. Another object of the present disclosure is the selective targeting of immune suppressive cells in the TME. Given that TGFb-1 is highly expressed by immune suppressive cells in the TME, selectively targeting TGFb-1 on such cells therefore allows for the depletion of immune suppressive cells in the TME.

Thus, according to one aspect, there is provided a polypeptide which is an immunogenic fragment of TGFb-1 and which comprises or consists of a sequence of at least 8 consecutive amino acids of SEQ ID NO: 5. Preferably, the polypeptide does not comprise a cysteine residue. The polypeptide may have low homology to a corresponding polypeptide sequence of TGFb-2 and/or TGFb-3. The polypeptide may have low sequence identity to a corresponding polypeptide sequence of TGFb-2 and/or TGFb-3. TGFb-2 may have the amino acid sequence of SEQ ID NO: 2. TGFb-3 may have the amino acid sequence of SEQ ID NO: 3. The polypeptide fragment may have less than about 80%, 70%, 60%, 50%, 40%, 30%, 25%, or 20% sequence identity to a corresponding polypeptide sequence of TGFb-2 and/or TGFb-3. Preferably, the polypeptide has less than about 40% sequence identity to a corresponding polypeptide sequence of TGFb-2 and/or TGFb-3. The polypeptide may comprise or consist of up to 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, or 40 consecutive amino acids of SEQ ID NO: 5. The polypeptide may comprise or consist of at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, or 40 consecutive amino acids of SEQ ID NO: 5. Preferably, the polypeptide may comprise or consist of at least 25, 26, 27, 28, 29, or 30 consecutive amino acids of SEQ ID NO: 5. More preferably, the polypeptide may comprise or consist of at least 30 consecutive amino acids of SEQ ID NO: 5. The polypeptide may comprise the amino acid

sequence of SEQ ID NO: 32. The polypeptide may comprise the amino acid sequence of any one of SEQ ID NOs: 7, 10 or 11. The polypeptide may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 7, 10, 32, 11, 14, or 15. Preferably, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 7. Preferably, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 10. In one embodiment, the polypeptide does not comprise or consist of the amino acids sequence of SEQ ID NOs: 11, 14 and/or 15. In one embodiment, the polypeptide does not consist of the amino acid sequence of SEQ ID NOs: 11, 14 and/or 15.

The polypeptide may be capable of stimulating TGFb-1 selective T cells. The TGFb-1 selective T cells may have low cross reactivity towards cells expressing and/or presenting polypeptides of TGFb-2 and/or TGFb-3. Cells expressing and/or presenting polypeptides of TGFb-2 and/or TGFb-3 may be cells in the tumor microenvironment (TME). These cells may be tumor cells. Preferably, these cells are immune suppressive cells. The cells may include cancer associated fibroblasts (CAFs), $CD8^+$ T cells, $CD4^+$ T cells, regulatory $CD4^+$ T cells, exhausted $CD8^+$ T cells, M1 tumor associated macrophages (M1_TAM), M2 tumor associated macrophages (M2_TAM), myeloid antigen presenting cells (APCmye) and/or other cells.

The measurement of cross reactivity may comprise comparing the reactivity of the TGFb-1 selective T cells to cells expressing and/or presenting a polypeptide of TGFb-1 with the reactivity of the TGFb-1 selective T cells to cells expressing and/or presenting a corresponding polypeptide from TGFb-2 or TGFb-3. The reactivity of the TGFb-1 selective T cells towards cells expressing and/or presenting the corresponding polypeptide from TGFb-2 or TGFb-3 may be less than about 50%, 40%, 30%, 20%, 10%, 5%, 1%, or 0.1% of the reactivity of the TGFb-1 selective T cells to the polypeptide of TGFb-1. Cross reactivity may be measured by IFNγ ELISPOT assay.

Embodiments of the present disclosure further provide a polynucleotide encoding a polypeptide as disclosed herein. The polynucleotide may be isolated. The polynucleotide may be comprised within a vector. The polynucleotide may be a messenger RNA (mRNA). The mRNA may comprise:

(a) an open reading frame (ORF) encoding at least one polypeptide as disclosed herein;

(b) a 5' terminal cap at the 5' end;

(c) a 5' untranslated region (UTR) which is included 5' of the ORF;

(d) a 3’ UTR which is included 3’ of the ORF; and

(e) a 3’ tailing sequence at the 3’ end.

Embodiments of the present disclosure also provide a composition comprising a polypeptide as disclosed herein and/or a polynucleotide as disclosed herein and optionally an adjuvant. The composition may further comprise at least one different polypeptide as disclosed herein; at least one different polynucleotide as disclosed herein; and/or at least one pharmaceutically acceptable diluent, carrier or preservative. 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. Preferably, the composition may be a TGFb-1 selective vaccine composition. Where the composition comprises a polynucleotide and the polynucleotide is mRNA, the composition may be formulated in a lipid nanoparticle composition. The lipid nanoparticle composition may have a mean diameter of 50-200nm.

Embodiments of the present disclosure also provide a method of treating or preventing a disease or condition in a subject, the method comprising administering to the subject a polypeptide as disclosed herein, a polynucleotide as disclosed herein, and/or a composition as disclosed herein. The method may further comprise the simultaneous or sequential administration of an additional cancer therapy, preferably an antibody. Where the disease or condition is cancer, the method may further comprise stimulation of a selective immune response to TGFb-1-expressing cancer cells.

Embodiments of the present disclosure also provide a polypeptide as disclosed herein, a polynucleotide as disclosed herein, a composition as disclosed herein, or a combination thereof for use in treating or preventing a disease or condition. The polypeptide, polynucleotide, composition, or combination thereof may be for use in combination with an additional cancer therapy, preferably an antibody. Where the disease or condition is cancer, the polypeptide, polynucleotide, composition, or combination thereof may stimulate a selective immune response to TGFb-1-expressing cancer cells.

Embodiments of the present disclosure further provide use of a polypeptide as disclosed herein, a polynucleotide as disclosed herein, a composition as disclosed herein, or a combination thereof for the manufacture of a medicament for the treatment or prevention of a disease or condition. Where the disease or condition is cancer, the use of the polypeptide, polynucleotide, composition, or combination thereof, may be for the manufacture of a medicament for stimulating a selective immune response to TGFb-1-expressing cancer cells.

The disease or condition may be characterized at least in part by inappropriate or excessive immune suppressive function of TGFb1-expressing cells. Preferably, the disease or condition is a cancer. Said cancer may be an esophageal cancer or a urothelial cancer. Said cancer may be a colorectal carcinoma, a gastric cancer, a head and neck cancer, a melanoma, a non-small-cell lung carcinoma (NSCLC) or an ovarian cancer. Said cancer may be a breast cancer, a cervical cancer, a liver cancer, or a pancreatic cancer.

The disease or condition may be a tumor and the polypeptide, polynucleotide, composition, or combination thereof, may be capable of modulating the tumor microenvironment (TME). The methods and uses in embodiments of the present disclosure may comprise modulating the TME. The modulating may comprise enhancing infiltration of the TME by T cells. Preferably, the T cells are $CD4^+$ T cells. Administering the polypeptide, polynucleotide, composition, or a combination thereof, may stimulate a selective immune response to TGFb-1-expressing cells in the TME.

Embodiments of the present disclosure also provide a method of stimulating TGFb-1 selective T cells, the method comprising contacting the T cells with a polypeptide as disclosed herein, a polynucleotide as disclosed herein, a composition as disclosed herein, or a combination thereof.

Embodiments of the present disclosure also provide an *ex vivo* method of stimulating TGFb-1 selective T cells, the method comprising contacting the T cells with a polypeptide as disclosed herein, a polynucleotide as disclosed herein, a composition as disclosed herein, or a combination thereof.

Embodiments of the present disclosure further provide the use of a polypeptide as disclosed herein, a polynucleotide as disclosed herein, a composition as disclosed herein, or a combination thereof for stimulating TGFb-1 selective T cells. The use may comprise contacting the T cells with a polypeptide as disclosed herein, a polynucleotide as disclosed herein, a composition as disclosed herein, or a combination thereof. The use may be non-therapeutic and/or *ex vivo*.

The TGFb-1 selective T cells stimulated by the peptides, polynucleotides and/or compositions as disclosed herein may have low cross reactivity towards cells expressing and/or presenting polypeptides of TGFb-2 and/or TGFb-3. Measurement of cross reactivity may comprise comparing the reactivity of the TGFb-1 selective T cells to cells expressing and/or presenting a polypeptide of TGFb-1 with the reactivity of the TGFb-1 selective T cells to cells expressing and/or presenting a corresponding polypeptide from TGFb-2 or TGFb-3.

The reactivity of the TGFb-1 selective T cells towards cells expressing and/or presenting the corresponding polypeptide from TGFb-2 or TGFb-3 may be less than about 50%, 40%, 30%, 20%, 10%, 5%, 1%, or 0.1% of the reactivity of the TGFb-1 selective T cells to the polypeptide of TGFb-1. Cross reactivity may be measured by IFNγ ELISPOT assay.

According to another aspect, there is provided a polynucleotide encoding a polypeptide as disclosed herein, which is optionally comprised within a vector, optionally wherein the polynucleotide is an mRNA comprising:

(a) an open reading frame (ORF) encoding at least one polypeptide as disclosed herein;

(b) a 5' terminal cap at the 5' end;

(c) a 5' untranslated region (UTR) which is included 5' of the ORF;

(d) a 3' UTR which is included 3' of the ORF; and

(e) a 3' tailing sequence at the 3' end.

According to another aspect, there is provided a composition comprising a polypeptide as disclosed herein; and/or a polynucleotide as disclosed herein; and optionally an adjuvant, further optionally wherein the composition further comprises at least one different polypeptide as disclosed herein; at least one different polynucleotide as disclosed herein; and/or at least one pharmaceutically acceptable diluent, carrier or preservative.

According to another aspect, there is provided 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.

According to another aspect, there is provided a method of stimulating TGFb-1 selective T cells, the method comprising contacting the T cells with a polypeptide as disclosed herein, a polynucleotide as disclosed herein, and/or a composition as disclosed herein, optionally wherein the TGFb-1 selective T cells have low cross reactivity towards cells expressing and/or presenting polypeptides of TGFb-2 and/or TGFb-3, optionally wherein measurement of cross reactivity comprises comparing the reactivity of the TGFb-1 selective T cells to cells expressing and/or presenting a polypeptide of TGFb-1 with the reactivity of the TGFb-1 selective T cells to cells expressing and/or presenting a corresponding polypeptide from TGFb-2 or TGFb-3, further optionally wherein:

(a) the reactivity of the TGFb-1 selective T cells towards cells expressing and/or presenting the corresponding polypeptide from TGFb-2 or TGFb-3 is less than about 50%,

40%, 30%, 20%, 10%, 5%, 1%, or 0.1% of the reactivity of the TGFb-1 selective T cells to the polypeptide of TGFb-1; and/or

(b) cross reactivity is measured by IFNγ ELISPOT assay.

According to another aspect, there is provided a 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 disease or condition in a subject.