

THERAPIES FOR PREVENTING OR TREATING INFLAMMATORY EYE DISEASE

FIELD OF THE INVENTION

The present invention relates to a combination therapy for preventing or treating an inflammatory eye disease. The present invention also relates to CD3 inhibitors, polynucleotides, cells, vectors, kits, products, and pharmaceutical compositions for preventing or treating an inflammatory eye disease.

BACKGROUND TO THE INVENTION

Chronic inflammation in the eye can lead to cumulative damage that eventually causes significant vision loss. Chronic non-infectious uveitis is a sight-threatening intraocular inflammation that accounts for 10% of blindness in the working-age population and has a disproportionately large economic burden (see e.g. Joltikov, K.A. and Lobo-Chan, A.M., 2021. *Frontiers in Medicine*, 8:695904). Uveitis may include intraocular inflammation that affects the uvea and adjacent structures, such as the cornea, vitreous humor, retina, and optic nerve. Most commonly, uveitis is idiopathic, but can be linked to infection, malignancy, or underlying inflammatory conditions such as spondyloarthritis, sarcoidosis, juvenile idiopathic arthritis (JIA), inflammatory bowel disease, rheumatoid arthritis, tubulointerstitial nephritis, and other autoinflammatory diseases (see e.g. Rosenbaum, J.T., et al., 2019. *Seminars in Arthritis and Rheumatism*, 49(3), pp. 438-445).

Currently, the first line treatment for non-infectious uveitis is corticosteroids, which can be administered topically, periocularly, intraocularly, or systemically. However, there are issues associated with this treatment option. Systemic administration of corticosteroids has a number of well-known side effects that can lead to adverse events, and while local administration of corticosteroids can reduce the concentrations required, there is a need for repeat injections as the ocular concentration of the drug declines over time. Due to the recurrent nature of the condition, patients may need to be maintained on continual systemic corticosteroids treatment, which can lead to numerous adverse effects (see e.g. Valenzuela, R.A., et al., 2020. *Frontiers in Pharmacology*, 11:655). Local treatment options, including intraocular steroid-based implants and intravitreal injection, have not significantly improved the clinical landscape. Whilst effective at reducing recurrence in milder disease, these are associated with significant adverse events, such as cataracts and glaucoma.

Immunosuppressant therapy (IMT) is an alternative to corticosteroid therapy, including antimetabolites, calcineurinic inhibitors, and alkylating agents. When conventional

corticosteroids and IMT fail, biological agents and biologics such as TNF inhibitors, IL-1 blockers, and anti-CD20 may be used.

- However, these agents are associated with adverse events. For example, adverse effects associated with TNF inhibitors include development of autoimmune diseases, increased risk of infection, reactions at the injection site, increased risk of malignancy and worsening of demyelinating disorders (see e.g. Valenzuela, R.A., et al., 2020. *Frontiers in Pharmacology*, 11:655). Moreover, there remains up to 40% incidence of recurrent flares despite repeated, systemic dosing of TNF inhibitors. Thus, there is an unmet need for consistent control of disease in patients.
- Thus, there is a demand for new approaches for treating or preventing inflammatory eye diseases, such as uveitis.

SUMMARY OF THE INVENTION

The present inventors have developed therapies for treating or preventing inflammatory eye diseases, such as uveitis, in which TNF inhibitors and CD3 inhibitors are delivered to the eye.

- The inventors have surprisingly demonstrated that a combination therapy comprising intraocular administration of a TNF inhibitor and a CD3 inhibitor (for example each administered in the form of a vector encoding the same) may have increased efficacy compared to a TNF inhibitor monotherapy. The combination therapy may therefore represent an improved approach to prevent or treat inflammatory eye disease compared to the monotherapy. Dual targeting of cytokine and cellular mediators of inflammation may increase efficacy and response rate.

- The inventors have also surprisingly demonstrated that vectors encoding a transgene under the control of an inflammation-inducible promoter may allow for inflammation-inducible expression of the transgene in the eye. When expression of a TNF inhibitor and/or a CD3 inhibitor is coupled to an inflammation-inducible promoter, the combination therapy may therefore provide an adaptable and responsive dose level to prevent or treat inflammatory eye disease. Such a combination therapy may prevent re-occurrence of inflammation and/or maintain inflammation at a sub-clinical level, thereby preventing cumulative damage, whilst reducing the occurrence of adverse events imparted by long term systemic immunosuppression.

In one aspect, the present invention provides a combination of (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

The combination of the present invention may be provided in any suitable form. For example, in the form of a bicistronic or multicistronic vector, two separate vectors, or a product, kit or pharmaceutical composition comprising the combination.

5 In one aspect, the present invention provides a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

Suitably, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element. In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence encoding a 2A self-cleaving peptide. In some embodiments, the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are each operably linked to the same promoter. In some embodiments, the vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a TNF inhibitor; a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a CD3 inhibitor. In some embodiments, the vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a CD3 inhibitor; a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a TNF inhibitor.

20 In another aspect, the present invention provides a kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

In another aspect, the present invention provides a pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

25 In another aspect, the present invention provides a product comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor, as a combined preparation for simultaneous, separate or sequential use in therapy.

30 In another aspect, the present invention provides a kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

In another aspect, the present invention provides a pharmaceutical composition comprising:
(a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same;
and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

- 5 In another aspect, the present invention provides a product comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, as a combined preparation for simultaneous, separate or sequential use in therapy.

In preferred embodiments, the TNF inhibitor is an anti-TNF antibody or a fragment thereof.

- 10 Any suitable anti-TNF antibody or fragment thereof may be used. In some embodiments, the TNF inhibitor is any of adalimumab or a fragment thereof, infliximab or a fragment thereof, golimumab or a fragment thereof, or certolizumab or a fragment thereof. In some embodiments, the TNF inhibitor is adalimumab or a fragment thereof, or infliximab or a fragment thereof. In some embodiments, the TNF inhibitor is an anti-TNF antibody fragment.
- 15 Any suitable anti-TNF antibody fragment may be used. In some embodiments, the anti-TNF antibody fragment is an antigen-binding fragment (Fab), a fragment antibody (F(ab')₂), a single chain antibody (scFv), or a single-domain antibody (sdAb). In some embodiments, the anti-TNF antibody fragment is an antigen-binding fragment (Fab).

- In some embodiments, the TNF inhibitor is adalimumab or a fragment thereof. In some
- 20 embodiments, the TNF inhibitor is an antigen binding fragment (Fab) of adalimumab. In some embodiments, the TNF inhibitor is an anti-TNF antibody or a fragment thereof comprising one or more CDR regions selected from SEQ ID NOs: 16 to 21 or derivatives thereof comprising one amino acid substitution. In some embodiments, the TNF inhibitor is an anti-TNF antibody or a fragment thereof comprising CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2,
- 25 LCDR3 comprising or consisting of SEQ ID NOs: 16, 17, 18, 19, 20 and 21 respectively, or derivatives thereof comprising one amino acid substitution. In some embodiments, the TNF inhibitor is an anti-TNF antibody or a fragment thereof comprising a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 22 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 23. In some
- 30 embodiments, the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 62 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 63. The nucleotide sequence encoding the heavy chain and the nucleotide sequence encoding the light chain may be connected via a linker sequence. Suitably, the linker sequence encodes a 2A self-cleaving peptide, and/or an enzymatically
- 35 cleavable peptide motif. In some embodiments, the linker sequence encodes a 2A self-

cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9. The nucleotide sequence encoding the heavy chain and/or the nucleotide sequence encoding the light chain may each be operably linked to a signal sequence. In some embodiments, the signal sequence encodes a signal peptide selected from any of: a Human Growth Hormone (HGH) signal peptide, an interleukin-2 (IL-2) signal peptide, a CD5 signal peptide, an immunoglobulin Kappa light chain signal peptide, a trypsinogen signal peptide, a serum albumin signal peptide, and a prolactin signal peptide. In some embodiments, the nucleotide sequence encoding a TNF inhibitor encodes an anti-TNF antibody or a fragment comprising or consisting of a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 22, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 23. In some embodiments, the nucleotide sequence encoding a TNF inhibitor encodes an anti-TNF antibody or a fragment comprising or consisting of an amino acid sequence having at least 70% identity to SEQ ID NO: 66. In some embodiments, the nucleotide sequence encoding a TNF inhibitor comprises or consists of: a nucleotide sequence having at least 70% identity to SEQ ID NO: 62, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 63. In some embodiments, the nucleotide sequence encoding a TNF inhibitor comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 67.

In preferred embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof. Any suitable anti-CD3 antibody or fragment thereof may be used. In some embodiments, the CD3 inhibitor is any of foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, oteelixizumab or a fragment thereof, muromonab-CD3 or a fragment thereof, T3/4.A or a fragment thereof, YTH12.5 or a fragment thereof, or HuM291 or a fragment thereof. In some embodiments, the CD3 inhibitor is foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, or oteelixizumab or a fragment thereof.

In some embodiments, the CD3 inhibitor is an anti-CD3 antibody fragment. Any suitable anti-CD3 antibody fragment may be used. In some embodiments, the anti-CD3 antibody fragment is an antigen-binding fragment (Fab), a fragment antibody (F(ab')₂), a single chain antibody (scFv), or a single-domain antibody (sdAb). In some embodiments, the anti-CD3 antibody fragment is an antigen-binding fragment (Fab). In some embodiments, the CD3 inhibitor is an antigen binding fragment (Fab) of foralumab, teplizumab, visilizumab, or oteelixizumab.

In some embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof comprising one or more CDR regions selected from: (a) SEQ ID NOs: 70 to 75 or derivatives thereof comprising one amino acid substitution; (b) SEQ ID NOs: 81 to 86 or derivatives thereof comprising one amino acid substitution; (c) SEQ ID NOs: 92 to 97 or derivatives thereof comprising one amino acid substitution; or (d) SEQ ID NOs: 103 to 108 or derivatives thereof comprising one amino acid substitution. In some embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof comprising: (a) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 70, 71, 72, 73, 74 and 75 respectively, or derivatives thereof comprising one amino acid substitution; (b) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 81, 82, 83, 84, 85 and 86 respectively, or derivatives thereof comprising one amino acid substitution; (c) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 92, 93, 94, 95, 96 and 97 respectively, or derivatives thereof comprising one amino acid substitution; or (d) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 103, 104, 105, 106, 107 and 108 respectively, or derivatives thereof comprising one amino acid substitution. In some embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof comprising: (a) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 76 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110. In some embodiments: (a) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 125 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 126; (b) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 127 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 128; (c) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 129 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 130; or (d) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 131 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 132. The nucleotide sequence encoding the heavy chain and the nucleotide sequence encoding the light chain may be connected via a linker sequence.

Suitably, the linker sequence encodes a 2A self-cleaving peptide, and/or an enzymatically cleavable peptide motif. In some embodiments, the linker sequence encodes a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9. The nucleotide sequence encoding the heavy chain and/or the nucleotide sequence encoding the light chain may each be operably linked to a signal sequence. In some embodiments, the signal sequence encodes a signal peptide selected from any of: a Human Growth Hormone (HGH) signal peptide, an interleukin-2 (IL-2) signal peptide, a CD5 signal peptide, an immunoglobulin Kappa light chain signal peptide, a trypsinogen signal peptide, a serum albumin signal peptide, and a prolactin signal peptide. In some embodiments, the nucleotide sequence encoding a CD3 inhibitor encodes an anti-CD3 antibody or a fragment comprising or consisting of: (a) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 76, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110. In some embodiments, the nucleotide sequence encoding a CD3 inhibitor encodes an anti-CD3 antibody or a fragment comprising or consisting of: (a) an amino acid sequence having at least 70% identity to SEQ ID NO: 133; (b) an amino acid sequence having at least 70% identity to SEQ ID NO: 135; (c) an amino acid sequence having at least 70% identity to SEQ ID NO: 137; or (d) an amino acid sequence having at least 70% identity to SEQ ID NO: 139. In some embodiments, the nucleotide sequence encoding a CD3 inhibitor comprises or consists of: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 125, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 126; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 127, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 128; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 129, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 130.

NO: 130; (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 131, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 132. In some embodiments, the nucleotide sequence encoding a CD3 inhibitor comprises or consists of: (a) a nucleotide
5 sequence having at least 70% identity to SEQ ID NO: 134; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 136; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 138; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 140.

In preferred embodiments, the nucleotide sequence encoding a TNF inhibitor is operably
10 linked to an inflammation-inducible promoter and/or the nucleotide sequence encoding a CD3 inhibitor is operably linked to an inflammation-inducible promoter. In some embodiments, the nucleotide sequence encoding a TNF inhibitor is operably linked to an inflammation-inducible promoter and the nucleotide sequence encoding a CD3 inhibitor is operably linked to an inflammation-inducible promoter. In some embodiments, the nucleotide sequence encoding a
15 TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are operably linked to the same inflammation-inducible promoter. Any suitable inflammation-inducible promoter may be used. Suitably, the inflammation-inducible promoter comprises one or more inflammation-inducible transcription factor binding motif selected from: an AP-1 transcription factor binding motif; a NF- κ B transcription factor binding motif; an IRF transcription factor binding motif; a
20 STAT transcription factor binding motif; and a NFAT transcription factor binding motif or any combination thereof. In some embodiments, the inflammation-inducible promoter comprises one or more AP-1 binding motif and/or one or more NF- κ B binding motif. In some embodiments, the inflammation-inducible promoter comprises two or more AP-1 binding motifs and/or two or more NF- κ B binding motifs, three or more AP-1 binding motifs and/or
25 three or more NF- κ B binding motifs, four or more AP-1 binding motifs and/or four or more NF- κ B binding motifs, or five or more AP-1 binding motifs and/or five or more NF- κ B binding motifs. In some embodiments, the inflammation-inducible promoter comprises at least one AP-1 binding motif coupled to at least one NF- κ B binding motif. In some embodiments, the inflammation-inducible promoter comprises five AP-1 binding motifs coupled to five NF- κ B
30 binding motifs. Suitably, an AP-1 binding motif comprises or consists of SEQ ID NO: 151, or comprises or consists of any of SEQ ID NOs: 152-154 or derivatives thereof comprising one nucleotide substitution. Suitably, a NF- κ B binding motif comprises or consists of SEQ ID NO: 155, or comprises or consists of SEQ ID NO: 156 or a derivative thereof comprising two or fewer nucleotide substitutions. In some embodiments, the inflammation-inducible promoter
35 comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 157.

The vector(s) may comprise any other suitable vector elements. The vector(s) may comprise a polyadenylation sequence. Suitably, the polyadenylation sequence is selected from any of: a bovine growth hormone (bGH) polyadenylation sequence, a SV40 polyadenylation sequence, and a rabbit beta-globin polyadenylation sequence. In some embodiments, the polyadenylation sequence comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 163. The vector(s) may comprise a woodchuck hepatitis post-transcriptional regulatory element (WPRE). In some embodiments, the WPRE comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 164. The vector(s) may comprise an intron. Suitably, the intron is selected from a beta-globin intron or a SV40 intron. In some embodiments, the intron comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 165.

In preferred embodiments, the vector is a viral vector. Any suitable viral vector may be used. Suitably, the viral vector is any of a parvoviral vector, preferably an adeno-associated virus (AAV) vector, an adenoviral vector, a herpes simplex viral vector, an anelloviral vector, a retroviral vector or a lentiviral vector. In preferred embodiments, the vector is an adeno-associated virus (AAV) vector. In preferred embodiments, the vector is an AAV vector particle. The AAV vector particle may be pseudotyped to confer ocular tissue tropism. Suitably, the AAV vector particle comprises AAV2 capsid proteins or AAV2 capsid variant proteins, optionally wherein the AAV2 capsid variant is selected from any of: AAV2.tYF, AAV2.7m8, R100, AAV2.GL and AAV2.NN. The vector may comprise one or more inverted terminal repeats (ITRs).

In another aspect, the present invention provides a cell comprising (i) the vector of the present invention; and/or (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor. The cell may be an isolated cell.

In another aspect, the present invention provides a kit for the production of (i) the vector of the present invention; and/or (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor, and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

In another aspect, the present invention provides a pharmaceutical composition comprising (i) the vector of the present invention; (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor, and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor; or (iii) the cell of the present invention. The vector(s) or cell may be in combination with a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, the present invention provides a vector according to the present invention, a cell according to the present invention, and/or a pharmaceutical composition according to the present invention, for use as a medicament.

5 In another aspect, the present invention provides use of a vector according to the present invention, a cell according to the present invention, or a pharmaceutical composition according to the present invention, for the manufacture of a medicament.

In another aspect, the present invention provides a method comprising administering a vector according to the present invention, a cell according to the present invention, or a pharmaceutical composition according to the present invention, to a subject in need thereof.

10 In another aspect, the present invention provides a vector for use in preventing or treating an inflammatory eye disease, wherein the vector comprises: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

In another aspect, the present invention provides use of a vector in the manufacture of a medicament for preventing or treating an inflammatory eye disease, wherein the vector
15 comprises: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

In another aspect, the present invention provides a method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a vector to a subject in need thereof, wherein the vector comprises: (a) a nucleotide sequence encoding a TNF
20 inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

In another aspect, the present invention provides a vector according to the present invention, a cell according to the present invention, and/or a pharmaceutical composition according to the present invention, for use in preventing or treating an inflammatory eye disease.

25 In another aspect, the present invention provides use of a vector according to the present invention, a cell according to the present invention, or a pharmaceutical composition according to the present invention, for the manufacture of a medicament for preventing or treating an inflammatory eye disease.

In another aspect, the present invention provides a method of preventing or treating an inflammatory eye disease comprising administering a vector according to the present
30 invention, a cell according to the present invention, or a pharmaceutical composition according to the present invention, to a subject in need thereof.

The inflammatory eye disease may be any inflammatory eye disease. Suitably, the inflammatory eye disease is uveitis. The vector(s) or pharmaceutical composition may be administered in response to relapse of an inflammatory eye disease, particularly wherein the inflammatory eye disease is uveitis.

- 5 The vector(s) or pharmaceutical composition may be administered by any suitable route. Suitably, the vector(s) or pharmaceutical composition are administered intraocularly. In some embodiments, the vector(s) or pharmaceutical composition are administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection. In some embodiments, the vector(s) or pharmaceutical composition are administered via intravitreal injection.
- 10 The vector(s) or pharmaceutical composition may be administered in any suitable regimen. Suitably, the vector(s) or pharmaceutical composition are administered as a single dose. Suitably, the vector(s) are administered at a dose of at least about $1E10$ vg/mL, at least about $1E11$ vg/mL, at least about $1E12$ vg/mL, or at least about $5E12$ vg/mL. Suitably, the vector(s) are administered at a dose of at least about $1E9$ vg/eye, at least about $1E10$ vg/eye, or at
- 15 least about $1E11$ vg/eye. Suitably, the vector(s) are administered at a dose of about $1E9$ vg/eye to about $5E12$ vg/eye.

In another aspect, the present invention provides a kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

- 20 In another aspect, the present invention provides a pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

- In another aspect, the present invention provides a product comprising: (a) a TNF inhibitor, or
- 25 a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, as a combined preparation for simultaneous, separate or sequential use in preventing or treating an inflammatory eye disease.

- In another aspect, the present invention provides a method for preventing or treating an
- 30 inflammatory eye disease, wherein the method comprises administering to a subject in need thereof: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

Suitably, (a) the TNF inhibitor or polynucleotide encoding the same may be administered in the form of a vector comprising a nucleotide sequence encoding a TNF inhibitor and/or a pharmaceutical composition comprising the TNF inhibitor or polynucleotide encoding the same; and/or (b) the CD3 inhibitor or polynucleotide encoding the same may be administered
5 in the form of a vector comprising a nucleotide sequence encoding a CD3 inhibitor and/or a pharmaceutical composition comprising the CD3 inhibitor or polynucleotide encoding the same.

The inflammatory eye disease may be any inflammatory eye disease. Suitably, the inflammatory eye disease is uveitis. The TNF inhibitor or polynucleotide encoding the same
10 and/or the CD3 inhibitor or polynucleotide encoding the same may be administered in response to relapse of an inflammatory eye disease, particularly wherein the inflammatory eye disease is uveitis.

The TNF inhibitor or polynucleotide encoding the same and the CD3 inhibitor or polynucleotide encoding the same may be administered by any suitable route and in any suitable regimen.
15 Suitably, the TNF inhibitor or polynucleotide encoding the same and/or the CD3 inhibitor or polynucleotide encoding the same is administered intraocularly. In some embodiments, the TNF inhibitor or polynucleotide encoding the same and/or the CD3 inhibitor or polynucleotide encoding the same is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection. In some embodiments, the TNF inhibitor or polynucleotide encoding
20 the same and/or the CD3 inhibitor or polynucleotide encoding the same is administered via intravitreal injection.

The present inventors have also developed therapies for treating or preventing inflammatory eye diseases, such as uveitis, in which anti-cell activation CD3 inhibitors are delivered to the eye.

25 The inventors have surprisingly demonstrated that a vector encoding a CD3 inhibitor under the control of an inflammation-inducible promoter may allow for inflammation-inducible expression of a CD3 inhibitor in the eye. When expression of the CD3 inhibitor is coupled to an inflammation-inducible promoter, the therapy may therefore provide an adaptable and responsive dose level to prevent or treat inflammatory eye disease. Such a therapy may
30 prevent re-occurrence of inflammation and/or maintain inflammation at a sub-clinical level, thereby preventing cumulative damage, whilst reducing the occurrence of adverse events imparted by long term systemic immunosuppression.

The inventors have also surprisingly demonstrated that intraocular administration of a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, may be

used to suppress ocular inflammation. Such a therapy may therefore be suitable to prevent or treat inflammatory eye disease.

In one aspect, the present invention provides a CD3 inhibitor, a polynucleotide comprising a nucleotide sequence encoding a CD3 inhibitor, or a vector comprising a nucleotide sequence
5 encoding a CD3 inhibitor.

In preferred embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof. Any suitable anti-CD3 antibody or fragment thereof may be used. In some embodiments, the CD3 inhibitor is any of foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, otelexizumab or a fragment thereof, muromonab-CD3 or a
10 fragment thereof, T3/4.A or a fragment thereof, YTH12.5 or a fragment thereof, or HuM291 or a fragment thereof. In some embodiments, the CD3 inhibitor is foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, or otelexizumab or a fragment thereof.

In some embodiments, the CD3 inhibitor is an anti-CD3 antibody fragment. Any suitable anti-
15 CD3 antibody fragment may be used. In some embodiments, the anti-CD3 antibody fragment is an antigen-binding fragment (Fab), a fragment antibody (F(ab')₂), a single chain antibody (scFv), or a single-domain antibody (sdAb). In some embodiments, the anti-CD3 antibody fragment is an antigen-binding fragment (Fab). In some embodiments, the CD3 inhibitor is an antigen binding fragment (Fab) of foralumab, teplizumab, visilizumab, or otelexizumab.

20 In some embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof comprising one or more CDR regions selected from: (a) SEQ ID NOs: 70 to 75 or derivatives thereof comprising one amino acid substitution; (b) SEQ ID NOs: 81 to 86 or derivatives thereof comprising one amino acid substitution; (c) SEQ ID NOs: 92 to 97 or derivatives thereof comprising one amino acid substitution; or (d) SEQ ID NOs: 103 to 108 or derivatives
25 thereof comprising one amino acid substitution. In some embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof comprising: (a) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 70, 71, 72, 73, 74 and 75 respectively, or derivatives thereof comprising one amino acid substitution; (b) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ
30 ID NOs: 81, 82, 83, 84, 85 and 86 respectively, or derivatives thereof comprising one amino acid substitution; (c) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 92, 93, 94, 95, 96 and 97 respectively, or derivatives thereof comprising one amino acid substitution; or (d) CDR regions HCDR1, HCDR2, HCDR3,

LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 103, 104, 105, 106, 107 and 108 respectively, or derivatives thereof comprising one amino acid substitution.

In some embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof comprising: (a) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 76 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110.

In some embodiments: (a) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 125 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 126; (b) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 127 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 128; (c) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 129 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 130; or (d) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 131 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 132. The nucleotide sequence encoding the heavy chain and the nucleotide sequence encoding the light chain may be connected via a linker sequence. Suitably, the linker sequence encodes a 2A self-cleaving peptide, and/or an enzymatically cleavable peptide motif. In some embodiments, the linker sequence encodes a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9. The nucleotide sequence encoding the heavy chain and/or the nucleotide sequence encoding the light chain may each be operably linked to a signal sequence. In some embodiments, the signal sequence encodes a signal peptide selected from any of: a Human Growth Hormone (HGH) signal peptide, an interleukin-2 (IL-2) signal peptide, a CD5 signal peptide, an immunoglobulin Kappa light chain signal peptide, a trypsinogen signal peptide, a serum albumin signal peptide, and a prolactin signal peptide.

In some embodiments, the nucleotide sequence encoding a CD3 inhibitor encodes an anti-CD3 antibody or a fragment comprising or consisting of: (a) a heavy chain comprising or

consisting of a sequence with at least 70% identity to SEQ ID NO: 76, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109, optionally a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110. In some embodiments, the nucleotide sequence encoding a CD3 inhibitor encodes an anti-CD3 antibody or a fragment comprising or consisting of: (a) an amino acid sequence having at least 70% identity to SEQ ID NO: 133; (b) an amino acid sequence having at least 70% identity to SEQ ID NO: 135; (c) an amino acid sequence having at least 70% identity to SEQ ID NO: 137; or (d) an amino acid sequence having at least 70% identity to SEQ ID NO: 139.

In some embodiments, the nucleotide sequence encoding a CD3 inhibitor comprises or consists of: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 125, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 126; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 127, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 128; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 129, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 130; (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 131, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 132. In some embodiments, the nucleotide sequence encoding a CD3 inhibitor comprises or consists of: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 134; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 136; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 138; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 140.

In preferred embodiments, the nucleotide sequence encoding a CD3 inhibitor is operably linked to an inflammation-inducible promoter. Any suitable inflammation-inducible promoter may be used. Suitably, the inflammation-inducible promoter comprises one or more inflammation-inducible transcription factor binding motif selected from: an AP-1 transcription factor binding motif; a NF- κ B transcription factor binding motif; an IRF transcription factor binding motif; a STAT transcription factor binding motif; and a NFAT transcription factor binding motif or any combination thereof.

In some embodiments, the inflammation-inducible promoter comprises one or more AP-1 binding motif and/or one or more NF- κ B binding motif. In some embodiments, the inflammation-inducible promoter comprises two or more AP-1 binding motifs and/or two or more NF- κ B binding motifs, three or more AP-1 binding motifs and/or three or more NF- κ B binding motifs, four or more AP-1 binding motifs and/or four or more NF- κ B binding motifs, five or more AP-1 binding motifs and/or five or more NF- κ B binding motifs. In some embodiments, the inflammation-inducible promoter comprises at least one AP-1 binding motif coupled to at least one NF- κ B binding motif. In some embodiments, the inflammation-inducible promoter comprises five AP-1 binding motifs coupled to five NF- κ B binding motifs. Suitably, an AP-1 binding motif comprises or consists of SEQ ID NO: 151, or comprises or consists of any of SEQ ID NOs: 152-154 or derivatives thereof comprising one nucleotide substitution. Suitably, a NF- κ B binding motif comprises or consists of SEQ ID NO: 155, or comprises or consists of SEQ ID NO: 156 or a derivative thereof comprising two or fewer nucleotide substitutions. In some embodiments, the inflammation-inducible promoter comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 157.

In some embodiments, the vector comprises: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 159; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 160; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 161; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 162.

The vector may comprise any other suitable vector elements. The nucleotide sequence encoding the CD3 inhibitor may be operably linked to a polyadenylation sequence. Suitably, the polyadenylation sequence is selected from any of: a bovine growth hormone (bGH) polyadenylation sequence, a SV40 polyadenylation sequence, and a rabbit beta-globin polyadenylation sequence. In some embodiments, the polyadenylation sequence comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 163. The nucleotide sequence encoding the CD3 inhibitor may be operably linked to a woodchuck hepatitis post-transcriptional regulatory element (WPRE). In some embodiments, the WPRE comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO:

164. The nucleotide sequence encoding the CD3 inhibitor may be operably linked to an intron. Suitably, the intron is selected from a beta-globin intron or a SV40 intron. In some embodiments, the intron comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 165.

- 5 In preferred embodiments, the vector is a viral vector. Any suitable viral vector may be used. Suitably, the viral vector is any of a parvoviral vector, preferably an adeno-associated virus (AAV) vector, an adenoviral vector, a herpes simplex viral vector, an anelloviral vector, a retroviral vector or a lentiviral vector.

- 10 In preferred embodiments, the vector is an adeno-associated virus (AAV) vector. In preferred embodiments, the vector is an AAV vector particle. The AAV vector particle may be pseudotyped to confer ocular tissue tropism. Suitably, the AAV vector particle comprises AAV2 capsid proteins or AAV2 capsid variant proteins, optionally wherein the AAV2 capsid variant is selected from any of: AAV2.tYF, AAV2.7m8, R100, AAV2.GL and AAV2.NN. The vector may comprise one or more inverted terminal repeats (ITRs).

- 15 In some embodiments, the vector comprises or consists of: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 183; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 184; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 185; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 186.

- 20 In another aspect, the present invention provides a vector comprising or consisting of: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 183; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 184; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 185; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 186. The vector may be a viral vector. The vector may be an AAV vector.

- 25 In another aspect, the present invention provides a cell comprising the vector of the present invention. The cell may be an isolated cell.

In another aspect, the present invention provides a kit for the production of the vector of the present invention.

- 30 In another aspect, the present invention provides a pharmaceutical composition comprising the vector of the present invention or the cell of the present invention. The vector or cell may be in combination with a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, the present invention provides a vector according to the present invention, a cell according to the present invention, and/or a pharmaceutical composition according to the present invention, for use as a medicament.

- 5 In another aspect, the present invention provides use of a vector according to the present invention, a cell according to the present invention, or a pharmaceutical composition according to the present invention, for the manufacture of a medicament.

In another aspect, the present invention provides a method comprising administering a vector according to the present invention, a cell according to the present invention, or a pharmaceutical composition according to the present invention, to a subject in need thereof.

- 10 In another aspect, the present invention provides a vector for use in preventing or treating an inflammatory eye disease, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.

- In another aspect, the present invention provides use of a vector in the manufacture of a medicament for preventing or treating an inflammatory eye disease, wherein the vector
15 comprises a nucleotide sequence encoding a CD3 inhibitor.

In another aspect, the present invention provides a method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a vector to a subject in need thereof, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.

- 20 In another aspect, the present invention provides a vector according to the present invention, or a pharmaceutical composition according to the present invention, for use in preventing or treating an inflammatory eye disease.

- In another aspect, the present invention provides use of a vector according to the present invention, or a pharmaceutical composition according to the present invention, for the
25 manufacture of a medicament for preventing or treating an inflammatory eye disease.

In another aspect, the present invention provides a method of preventing or treating an inflammatory eye disease comprising administering a vector according to the present invention, or a pharmaceutical composition according to the present invention, to a subject in need thereof.

In another aspect, the present invention provides a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, for use in preventing or treating an inflammatory eye disease.

5 In another aspect, the present invention provides use of a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, in the manufacture of a medicament for preventing or treating an inflammatory eye disease.

10 In another aspect, the present invention provides a method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, to a subject in need thereof.

The CD3 inhibitor or polynucleotide may be administered in the form of a vector comprising a nucleotide sequence encoding a CD3 inhibitor and/or a pharmaceutical composition comprising the CD3 inhibitor or polynucleotide.

15 The inflammatory eye disease may be any inflammatory eye disease. Suitably, the inflammatory eye disease is uveitis. The CD3 inhibitor, polynucleotide, vector or pharmaceutical composition may be administered in response to relapse of an inflammatory eye disease, particularly wherein the inflammatory eye disease is uveitis.

20 The CD3 inhibitor, polynucleotide, vector or pharmaceutical composition may be administered by any suitable route. Suitably, the CD3 inhibitor, polynucleotide, vector or pharmaceutical composition is administered intraocularly. In some embodiments, the CD3 inhibitor, polynucleotide, vector or pharmaceutical composition is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection. In some embodiments, the CD3 inhibitor, polynucleotide, vector or pharmaceutical composition is administered via intravitreal injection.

25 The CD3 inhibitor, polynucleotide, vector or pharmaceutical composition may be administered in any suitable regimen. Suitably, the CD3 inhibitor, polynucleotide, vector or pharmaceutical composition is administered as a single dose. Suitably, the vector is administered at a dose of at least about 1×10^{10} vg/mL, at least about 1×10^{11} vg/mL, at least about 1×10^{12} vg/mL, or at least about 5×10^{12} vg/mL. Suitably, the vector is administered at a dose of at least about 1×10^9 vg/eye, 30 at least about 1×10^{10} vg/eye, or at least about 1×10^{11} vg/eye. Suitably, the vector is administered at a dose of about 1×10^9 vg/eye to about 5×10^{12} vg/eye.

BRIEF DESCRIPTION OF DRAWING

Figure 1: Local administration of an anti-TNF antibody suppresses Experimental Autoimmune Uveoretinitis (EAU)

B10.RIII mice were immunized for Experimental Autoimmune Uveoretinitis (EAU) and eyes monitored using Topical Endoscopic Fundal Imaging (TEFI) from day 10 onward to select experimental mice displaying clinically evident disease. Groups of mice were injected via intravitreal route with 15 µg infliximab or vehicle control (EAU) on day 10. Eyes were enucleated (day 14), and retinal infiltrate characterized. **(A)** Representative fundus images, **(B)** clinical disease scores and **(C)** flow cytometric analysis of total CD45+ cell numbers from single eyes at day 14. **P < 0.005; Data presented as means +/- SEM, representative of two independent experiments.

Figure 2: Therapeutic vector design

Schematic showing the vector organization of CMV.Infliximab Fab (expression under control of a constitutive CMV promoter) and AP1-NFkB.Infliximab Fab (expression under control of an inflammation-inducible promoter comprised of 5 repeated AP1 and NFkB binding sites). The heavy and light chains of Infliximab Fab are separated by a self-cleaving 2A peptide.

Figure 3: Constitutive therapeutic transgene expression

(A) HEK-293T (standard cell line for AAV development) or ARPE-19 (ocular cell line) cells were transduced with AAV2.CMV.Infliximab or AAV.CMV.NULL vectors [MOI 1E5vg/cell], and culture supernatants assayed using a clinical IFX ELISA kit. Detectable expression of Infliximab Fab from both cell types was evident by 72hrs (~30ng/ml). ****P < 0.0001. Data presented as means +/- SEM. **(B)** AAV2.CMV.Infliximab or AAV.CMV.NULL was administered by intravitreal (IVT) injection at 5E12 vg/ml into eyes of healthy B10.RIII mice. At 4wks post-AAV, mice were killed, eyes dissected and supernatants (retina and vitreous) assayed using the clinical IFX ELISA kit. Detectable expression of *in vivo* Infliximab Fab (~1.5ng/ml) observed in eyes receiving the 5E12 vg/ml dose. *P<0.05. Data presented as means +/- SEM, with each data point representing a single eye.

Figure 4: *In vitro* inducible transgene expression

HEK-293T cells transduced with AAV2.AP1-NFkB.EGFP (reporter vector) or AAV2.AP1-NFkB.Infliximab (therapeutic vector) were stimulated with reCL-1b (2ng/mL). **(A)** Activation leads to visible GFP expression at 24hrs, increasing in intensity by 72hrs, with no GFP signal observed with AAV2.AP1-NFkB.NULL (control vector). Images captured on EVOS FL, 10X magnification. **(B)** Stimulation results in a rapid induction of Infliximab Fab expression (8hrs), accumulation reaching ~20ng/ml at 72hrs. ****P < 0.0001; Data presented as means +/- SEM.

Figure 5: *In vivo* inducible transgene expression

AAV2.AP1-NFkB.EGFP (reporter) or AAV.AP1-NFkB.NULL (control) at a 5E12 vg/ml dose was administered by intravitreal (IVT) injection to C57BL/6J mice. **(A)** At 4wks post-AAV injection, mice were immunized to induce experimental autoimmune uveoretinitis (EAU), and imaged to monitor onset of ocular inflammation. At day 14 EAU, representative fundus and OCT images demonstrate clear clinical signs of disease (perivascular sheathing and vitreous infiltrate), and induction of GFP expression. In mice that only received AAV (no EAU), no clinical signs of disease or expression of the GFP transgene are observed. **(B)** Groups of mice were injected with AAV2.AP1-NFkB.Infliximab or AAV2.AP1-NFkB.NULL in the contralateral eye, and EAU induced at 4wks post-AAV. At 3wks post-EAU, mice were killed, eyes dissected and supernatants (retina and vitreous) assayed using the clinical IFX ELISA kit. Detectable expression of the Infliximab Fab is only observed in EAU eyes receiving the therapeutic vector and not the control. *P<0.05. Data presented as means +/- SEM, with each data point representing a single eye.

Figure 6: *In vivo* evaluation of constitutive therapeutic transgene efficacy

To demonstrate efficacy of the constitutive therapeutic transgene, groups of mice were injected with AAV7m8.CMV.Infliximab or AAV7m8.CMV.NULL in the contralateral eye, followed by intravitreal administration of recombinant human TNF (rec_hTNF) at 4wks post-AAV. At 18hrs (peak of inflammatory response to rec_hTNF), representative fundus and OCT images demonstrate increased inflammation (vitreous infiltrate) in the control (NULL) vs infliximab eyes **(A)**. In mice that only received AAV, no clinical signs of disease are observed. At 18hrs mice were killed, eyes dissected prepared for flow cytometric analysis to determine absolute numbers of Ly6C+ monocytes (predominant infiltrate in this model) from single eyes. A significant reduction in number of monocytes in eyes receiving the therapeutic vector and not the control **(B)**, this effect is further highlighted further with paired (contralateral eye) analysis **(C)**. **Wilcoxon signed rank test; *P<0.05 Wilcoxon matched pairs analysis.

Figure 7: Constitutive expression of other anti-TNF biologics *in vitro* and *in vivo*

HEK-293T cells were transfected with the huTNFR1-hulgG plasmid, and culture supernatants assayed using an anti-human TNF antibody ELISA kit. Detectable expression of huTNFR1-hulgG was evident by 48hrs **(A)**. HEK-293T cells were transduced with AAV7m8.CMV.huTNFR1-hulgG or AAV7m8.CMV.NULL vectors [MOI 1E5vg/cell], and culture supernatants assayed using an anti-human TNF antibody ELISA kit. Detectable expression was evident by 72hrs (~30ng/ml) **(B)**. ****P < 0.0001. Data presented as means +/- SEM. AAV7m8.CMV.huTNFR1-hulgG or AAV7m8.CMV.NULL was administered by intravitreal (IVT)

injection at range of doses [2E8 or 2E9 vg/eye] into the eyes of healthy C57BL/6J mice. At 4wks post-AAV, mice were killed, eyes dissected and supernatants (retina and vitreous) assayed using the anti-human TNF antibody ELISA kit. Detectable expression of huTNFR1-hulgG at ~4ng/ml and 9ng/ml was observed in eyes receiving 2E8 and 2E9 vg/eye doses respectively (C). ns – not significant; One way ANOVA; **P<0.05. Data presented as means +/- SEM, with each data point representing a single eye.

Figure 8: Bioactivity of constitutively expressed anti-TNF transgenes *in vitro*

HEK-BLUE TNF reporter cells were transfected with AAV.CMV.huTNFR1-hulgG, AAV.CMV.huTNFR1-mslgG, AAV.CMV.msTNFR1-mslgG, AAV.CMV.ADALIMUMAB plasmids or AAV2.CMV.Infliximab for 48hrs. Cells were then stimulated with recombinant human TNF or mouse TNF (0.5ng/ml) for further 24 hours and NFkB activation assessed. All the TNFR1 antibody-like plasmid constructs inhibited huTNF- and msTNF-mediated activation in the reporter cell line compared to NULL or recTNF alone (A). For the monoclonal Fab based anti-TNF biologics, plasmid expression of Adalimumab Fab (B) and AAV-mediated Infliximab Fab expression (8C) both inhibit activation with huTNF.

Figure 9: Evaluation of expression and bioactivity of an inducible anti-TNF transgene *in vitro*

HEK-293T cells were transduced with AAV7m8.AP1-NFkB.huTNFR1-hulgG or AAV7m8.AP1-NFkB.NULL vectors [MOI 1E5vg/cell] and stimulated with IL-1b (2ng/ml). Stimulation results in robust induction of huTNFR1-hulgG expression by 24hrs, accumulation reaching ~25ng/ml at 48hrs (A). One-way ANOVA; **P<0.0001. Data presented as means +/- SEM. Conditioned media was “spiked” with recombinant human or mouse TNF (final concentration 10ng/ml), and incubated with HEK-BLUE reporter cells for 24hrs. In response to both huTNF or mTNF stimulation alone, or with conditioned media from AAV7m8.AP1-NFkB.NULL, NFkB activation is robustly induced in the reporter cells. When supplemented with conditioned media from cells transduced with AAV7m8.AP1-NFkB.huTNFR1-hulgG and stimulated with IL-1b, NFkB activation in the reporter cells is completely suppressed (B). One-way ANOVA; **p<0.05 ***p<0.001; Data presented as means +/- SEM.

Figure 10: Evaluation of expression and bioactivity of an inducible anti-TNF transgene *in vivo*

B10.RIII mice were injected with AAV7m8.AP1-NFkB.huTNFR1-hulgG, and EAU induced at 4wks post-AAV. At day 19 post-EAU, when mild to moderate clinical signs of inflammation (not yet peak disease) were observed, mice were killed, eyes dissected and supernatants

(retina and vitreous) assayed using the anti-human TNF antibody ELISA. Clinical disease drove detectable expression of the huTNFR1-huIgG in EAU eyes receiving the therapeutic vector and not the control (AAV only) **(A)**. Mice were injected with AAV7m8.AP1-NFkB.huTNFR1-huIgG and AAV7m8.AP1-NFkB.NULL (contralateral eye control) at 2E9vg/eye. At 4wks post-AAV, mice received bilateral administration of recombinant human TNF (rec_hTNF), 18hrs later were killed, eyes dissected and ocular supernatants (retina and vitreous) assayed for huTNFR1-huIgG expression. Acute activation elicits a significant increase in expression of the huTNFR1-huIgG compared to the control **(B)**. ****P<0.0001. Data presented as means +/- SEM, with each data point representing a single eye.

10 **Figure 11: Evaluation of inducible therapeutic transgene efficacy *in vivo***

B10.RIII mice were injected with AAV7m8.AP1-NFkB.huTNFR1-IgG or AAV7m8.CMV.NULL [2E9 vg/eye] in contralateral eyes, and then immunized for EAU at 4wks post-AAV. At day 11, representative fundus and OCT images demonstrate increased inflammation in the eyes receiving the control NULL vector **(A)**. The contralateral eyes of the same three animals, which received the inducible therapeutic vector, showed substantially reduced clinical inflammation, both peri-vascular sheathing and vitreous infiltrate. At this time-point mice were killed, eyes dissected prepared for flow cytometric analysis to determine absolute numbers of CD45+ (all leukocytes), CD3+ (lymphocytes), CD4+ (Th T cells) and CD11b+ (macrophages and monocytes) populations from single eyes **(B)**. We observed a trend of reduction in immune cell infiltrates with AAV7m8.AP1-NFkB.huTNFR1-IgG compared to AAV7m8.CMV.NULL.

Figure 12: Efficacy of AAV.CMV.msTNFR1-msIgG in EAU

C57BL/6J mice injected with AAV7m8.CMV.msTNFR1-msIgG in one eye, and with AAV7m8.CMV.NULL in contralateral eye, were immunized for Experimental Autoimmune Uveoretinitis (EAU) at 4wks post-AAV. **(A)** At day 18, clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) only in the eyes receiving the NULL vector; contralateral eyes receiving the therapeutic transgene appear normal with no clinical signs of disease. **(B)** FACS analysis of immune cell infiltrate from individual eyes (n=21 eyes/group combined from 2 independent experiments), demonstrates a significant reduction in CD45+ cells treated with AAV7m8.CMV.msTNFR1-msIgG compared to AAV7m8.CMV.NULL, confirmed through paired analysis which shows reduced lymphocyte (CD3+) and myeloid (CD11b+) cell counts [***p=0.0002, n=21, two tailed test].

Figure 13: Evaluation of anti-CD3 Fab antibody to reduce inflammation in the EAU model

Mice received a single intravitreal injection of 6 μ g Anti-CD3 Fab, with vehicle (PBS) administered to the contralateral eye. At day 14 (peak disease in the B10.RIII EAU model), mice were imaged and then killed, eyes dissected and immune cell infiltrate immunophenotyped using FACS. (A) Representative clinical images (Fundus and OCT) demonstrate clinical inflammation in control (PBS) eyes compared to eyes receiving anti-CD3 Fab. (B) FACS analysis of immune cell infiltrate from individual eyes demonstrates substantially reduced CD45+ cell counts [One-way ANOVA, ** p=0.0021, n=6].

Figure 14: Therapeutic vector design

Schematic showing the organization of vectors comprising constitutive (CMV promotor) or inflammation-inducible promoter (AP1-NFkB; inflammation responsive promoter comprised of x5 repeated AP1 & NFkB binding sites) expressing: (A) TNFRI-IgG; (B) Anti-CD3 Fab; (C) TNFRI-IgG and Anti-CD3 Fab.

Figure 15: Evaluation of constitutive anti-CD3 Fab transgene expression & bioactivity *in vitro*

(A) SDS-PAGE image showing HA-positive 22kD protein band (lanes 1-3), which corresponds to the expected light chain fragment of the anti-CD3 Fab (which includes the HA-tag sequence on the C-terminus). No band was detected in supernatants collected from AAV.CMV.NULL transfected cells (lanes 4-6). (B) CD4+ T cell proliferation response by FACS. Incubation of splenocytes with anti-CD3 FAB antibody (2c11b; positive control) demonstrates suppressed proliferation, in a dose-dependent manner (0.01-1 μ g/ml). Compared to control (media only) or supernatant from AAV.NULL transfected HEK293T cells, conditioned media from AAV.CMV.anti-CD3 FAB also significantly reduces CD4+ proliferation.

Figure 16: Evaluation of combined targeting therapy (AAV.CMV.msTNFRI-msIgG + AAV.CMV.anti-CD3 Fab) to suppress inflammation in EAU

C57BL/6J mice were injected with (i) AAV7m8.CMV.msTNFRI-msIgG [2E9 vg/eye] or (ii) AAV "MIX" containing AAV7m8.CMV.msTNFRI-msIgG [2E9 vg/eye] + AAV7m8.CMV.anti-CD3 FAB [2E9 vg/eye] in one eye, with AAV7m8.CMV.NULL [4E9 vg/eye] in contralateral eye, and were then immunized for EAU at 4wks post-AAV. (A) At day 18, clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) only in the eyes receiving the NULL vector; contralateral eyes receiving either the combined "MIX" therapeutic transgenes or AAV.CMV.TNFRI vector only appear normal with no clinical signs of disease. FACS analysis of immune cell infiltrate from individual eyes of the (B) AAV.TNFRI only treatment group, with 31% reduction in the average (mean) number of CD45+ cells/eye [**p=0.0039,

n=9, two tailed test]; and (C) AAV "MIX" treatment group, with 63% reduction in the average (mean) number of CD45+ cells [***p=0.0005, n=13, two tailed test].

Figure 17: Evaluation of constitutive anti-CD3 Fab transgene expression & bioactivity from ARPE-19 (ocular cell line) *in vitro*

- 5 (A) A non-reducing SDS-PAGE image showing HA-positive protein band (lanes 1-3), which corresponds to the expected light chain fragment of the anti-CD3 Fab (which includes the HA-tag sequence on the C-terminus). No band was detected in supernatants collected from AAV.CMV.NULL transfected cells (lanes 4-6). (B) CD4+ T cell proliferation response by FACS. Incubation of splenocytes with anti-CD3 FAB antibody (2c11b; positive control) demonstrates
- 10 suppressed proliferation, in a dose-dependent manner (0.005-5ug/ml). Compared to control (media only) or supernatant from AAV.NULL transfected ARPE-19 cells, conditioned media from AAV.CMV.anti-CD3 Fab also significantly reduces CD4+ proliferation, comparable to 0.05ug/ml 2c11b control.

Figure 18: Evaluation of inducible anti-CD3 Fab transgene expression *in vitro*

- 15 ARPE-19 cells were transduced with AAV7m8.AP1-NFkB.anti-CD3 Fab or AAV7m8.AP1-NFkB.NULL vectors [MOI 1E5vg/cell] and stimulated with IL-1b (2ng/ml). Conditioned media was collected at 0, 24 & 48hrs, and concentrated for SDS-PAGE. Stimulation results in robust induction of anti-CD3 Fab expression evident at 24hrs (lanes 4-6) and 48hrs (lanes 1-3), shown by HA-positive protein band, corresponding to the expected light chain fragment of the
- 20 anti-CD3 Fab (which includes the HA-tag sequence on the C-terminus). No band is observed at 0hrs (lanes 7-9) or NULL (lanes 10-11).

Figure 19: Efficacy of AAV.CMV.anti-CD3 Fab in EAU

- C57BL/6J mice were injected in both eyes with AAV7m8.CMV.anti-CD3 Fab (n=7 eyes) or AAV7m8.CMV.NULL (n=3 eyes) [Vector dose: 4E9 vg/eye], and were immunized for
- 25 Experimental Autoimmune Uveoretinitis (EAU) at 4wks post-AAV. (A) At day 18, clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) in the eyes receiving the NULL vector; eyes receiving the therapeutic transgene show only mild clinical signs of disease. (B) FACS analysis of immune cell infiltrate from individual eyes (n=3-7 eyes/group), demonstrates lower numbers of CD45+ and CD3+ cell populations treated with
- 30 AAV7m8.CMV.anti-CD3 Fab compared to AAV7m8.CMV.NULL. (C) AAV7m8.CMV.2c11b treatment promotes an exhausted CD3+ phenotype, shown by increased expression of CTLA-4 and TIM-3.

Figure 20: Evaluation of constitutive expression of two transgenes (TNFRI-IgG and Anti-CD3 Fab) from bi-cistronic plasmid *in vitro*

ARPE-19 cells were transfected with AAV.CMV.anti-CD3 Fab, AAV.CMV.NULL or AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab plasmids (1ug/ml), and incubated for 72hrs. (A) Conditioned media (CM) was collected and “spiked” with recombinant mouse TNF (final concentration 0.1ng/ml), before incubation with HEK-BLUE reporter cells for 24hrs, for NFkB activation assessment. CM from AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab (Bi-cistronic plasmid) inhibited mTNF-mediated activation in the reporter cell line compared to NULL or recTNF alone, indicating expression of bioactive msTNFRI-msIgG transgene from the bi-cistronic plasmid. One-way ANOVA; ****p<0.0001; Data presented as means +/- SEM. (B) CM from AAV.CMV.anti-CD3 Fab, AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab or AAV.CMV.NULL was resolved by SDS-PAGE using non-reducing conditions. Image shows HA-positive protein band (light chain fragment of the anti-CD3 Fab) present in the AAV.CMV.anti-CD3 Fab and AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab samples. No band was detected in supernatants collected from AAV.CMV.NULL. This demonstrates expression of the anti-CD3 Fab transgene from the bi-cistronic plasmid.

Figure 21: Evaluation of constitutive expression of two transgenes (TNFRI-IgG and Anti-CD3 Fab) from bi-cistronic AAV *in vitro*

HEK293T or ARPE-19 cells were transduced with AAV7m8.CMV.msTNFRI-IgG, AAV7m8.CMV.anti-CD3 Fab, AAV7m8.CMV.NULL or AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab vectors [MOI 1E5vg/cell], and incubated for 48hrs. (A) Conditioned media (CM) was collected and “spiked” with recombinant mouse TNF (final concentration 0.1ng/ml), before incubation with HEK-BLUE reporter cells for 24hrs, and NFkB activity assessed. CM from AAV7m8.CMV.msTNFRI-msIgG and AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab transfected HEK293T or ARPE-19 inhibited mTNF-mediated activation in the reporter cell line compared to NULL, and confirms expression of bioactive msTNFRI-msIgG transgene from bi-cistronic AAV. One-way ANOVA; ****p<0.0001; Data presented as means +/- SEM. (B) HEK293T CM from AAV.CMV.anti-CD3 Fab, AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab or AAV.CMV.NULL was resolved by SDS-PAGE (non-reducing conditions). Image shows HA-positive protein band (light chain fragment of the anti-CD3 Fab) present in the AAV.CMV.anti-CD3 Fab and AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab samples only. No band was detected in supernatants collected from AAV.CMV.NULL. This demonstrates expression of the anti-CD3 Fab transgene from the bi-cistronic AAV vector.

Figure 22: Evaluation of combined targeting therapy (AAV.CMV.msTNFRI-msIgG + AAV.CMV.anti-CD3 Fab) to maintain reduced inflammation post-peak EAU

C57BL/6J mice were injected with AAV "MIX" containing AAV7m8.CMV.msTNFRI-msIgG [2E9 vg/eye] + AAV7m8.CMV.anti-CD3 Fab [2E9 vg/eye] in one eye, with AAV7m8.CMV.NULL [4E9 vg/eye] in contralateral eye, and were then immunized for EAU at 4wks post-AAV. **(A)** At day 28, clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) in eyes receiving the NULL vector; contralateral eyes receiving the combined "MIX" therapeutic transgenes only show mild clinical signs of disease. **(B)** FACS analysis of immune cell infiltrate from individual eyes of the AAV "MIX" vs NULL eyes, shows 34% reduction in the average (mean) number of CD45+ cells [$**p=0.005$, $n=7$, paired t test].

Figure 23: Evaluation of bi-cistronic AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab to suppress inflammation in EAU

C57BL/6J mice injected with AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab in one eye, and with AAV7m8.CMV.NULL in contralateral eye, were immunized for Experimental Autoimmune Uveoretinitis (EAU) at 4wks post-AAV. **(A)** At day 18, clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) only in the eyes receiving the NULL vector; contralateral eyes receiving the therapeutic transgenes appear normal with no clinical signs of disease. **(B)** FACS analysis of immune cell infiltrate from individual eyes ($n=8$ eyes/group), demonstrates a significant reduction in CD45+ cells treated with AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab compared to AAV7m8.CMV.NULL, confirmed through paired analysis which shows reduced lymphocyte (CD3+) and myeloid (CD11b+) cell counts [$*p=0.05$; $**p=0.005$, $n=8$, two tailed test].

Figure 24: Bicistronic plasmids

Plasmid maps showing the organization of the bi-cistronic constructs comprising **(A)** constitutive (CMV promoter) or **(B)** inflammation-inducible promoter (AP1-NFkB; inflammation responsive promoters for expression of TNFRI-IgG and Anti-CD3 Fab transgenes.

DETAILED DESCRIPTION

Various preferred features and embodiments of the present invention will now be described by way of non-limiting examples.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes", "containing", or "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or steps. The terms "comprising", "comprises" and "comprised of" also include the term "consisting of".

- 5 Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation and amino acid sequences are written left to right in amino to carboxy orientation, respectively.

10 The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that such publications constitute prior art to the claims appended hereto. All publications mentioned in the specification are herein incorporated by reference.

15 This disclosure is not limited by the exemplary methods and materials disclosed herein, and any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of this disclosure. The skilled person will understand that they can combine all features of the invention disclosed herein without departing from the scope of the invention as disclosed.

Combinations

20 The present invention provides a combination of: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

25 The combination of the present invention may be provided in any suitable form. In some embodiments, (a) the TNF inhibitor or polynucleotide encoding the same is in the form of a vector comprising a nucleotide sequence encoding a TNF inhibitor; and/or (b) the CD3 inhibitor or polynucleotide encoding the same may be in the form of a vector comprising a nucleotide sequence encoding a CD3 inhibitor. In some embodiments, (a) the TNF inhibitor or polynucleotide encoding the same is in the form of a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) the CD3 inhibitor or polynucleotide encoding the same may be in the form of a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

30 The combination of the present invention may be provided in the form of a bicistronic or multicistronic vector, two separate vectors, or a product, kit or pharmaceutical composition comprising the combination. In some embodiments, the combination of the present invention may be provided in the form of a bicistronic or multicistronic vector encoding a TNF inhibitor and a CD3 inhibitor. In other embodiments, the combination of the present invention may be

provided in the form of a vector encoding a TNF inhibitor and a vector encoding a CD3 inhibitor. In other embodiments, the combination of the present invention may be provided in the form of a product, kit, or pharmaceutical comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

Antibodies and antibody fragments

In some embodiments, (a) the TNF inhibitor comprises or consists of an anti-TNF antibody or a fragment thereof; and/or (b) the CD3 inhibitor comprises or consists of an anti-CD3 antibody or a fragment thereof. In preferred embodiments, (a) the TNF inhibitor comprises or consists of an anti-TNF antibody or a fragment thereof; and (b) the CD3 inhibitor comprises or consists of an anti-CD3 antibody or a fragment thereof.

In preferred embodiments, (a) the TNF inhibitor is an anti-TNF antibody or a fragment thereof; and/or (b) the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof. In more preferred embodiments, (a) the TNF inhibitor is an anti-TNF antibody or a fragment thereof; and (b) the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof.

Antibodies are glycoproteins belonging to the immunoglobulin superfamily. Antibodies are typically made of basic structural units, each with two heavy chains and two light chains. An antibody may recognise an antigen via the fragment antigen-binding (Fab) variable region. The fragment crystallizable region (Fc region) is the tail region of an antibody that may allow antibodies to activate the immune system. The hinge region is a stretch of heavy chains linking the Fab and Fc regions.

The "heavy chain" and "light chain" may each comprise a variable domain and one or more constant domains. For example, in IgG antibodies, a heavy chain comprises a variable domain (VH) and three constant domains (CH1, CH2, and CH3) and a light chain comprises a variable domain (VL) and one constant domain (CL). In some embodiments, for example in an antigen-binding fragment (Fab), a heavy chain comprises one variable domain (VH) and one constant domain (CH1). In some embodiments, for example in an antigen-binding fragment (Fab), a light chain comprises one variable domain (VL) and one constant domain (CL).

"Heavy chain variable region" or "VH" refers to the fragment of the heavy chain of an antibody that contains three CDRs interposed between flanking stretches known as framework regions, which form a scaffold to support the CDRs. "Light chain variable region" or "VL" refers to the fragment of the light chain of an antibody that contains three CDRs interposed between framework regions.

“Complementarity determining region” or “CDR” with regard to an antibody or antigen-binding fragment thereof refers to a highly variable loop in the variable region of the heavy chain or the light chain of an antibody. CDRs can interact with the antigen conformation and largely determine binding to the antigen. The heavy chain variable region and the light chain variable region each contain 3 CDRs (heavy chain CDRs 1, 2 and 3 and light chain CDRs 1, 2 and 3, numbered from the amino to the carboxy terminus).

The CDRs of the variable regions of a heavy and light chain of an antibody can be predicted from the heavy and light chain variable region sequences of the antibody, using prediction software available in the art, e.g. using the Abysis algorithm, the IMGT/V-QUEST software, or AbRSA (see e.g. Lefranc et al, 2009 NAR 37:D1006-D1012; Lefranc 2003, Leukemia 17: 260-266; and Li, L., et al., 2019. Protein Science, 28(8), pp.1524-1531). CDR regions identified by any algorithm are considered to be equally suitable for use in the invention.

CDRs may vary in length, depending on the numbering scheme and the antibody from which they are predicted and between the heavy and light chains. Thus, the three heavy chain CDRs of an intact antibody may be of different lengths (or may be of the same length) and the three light chain CDRs of an intact antibody may be of different lengths (or may be of the same length). A CDR for example, may range from 2 or 3 amino acids in length to 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length. Particularly, a CDR may be from 3-14 amino acids in length, e.g. at least 3 amino acids and less than 15 amino acids. Any suitable numbering scheme may be used to predict the CDRs, such as Kabat, Chothia, or IMGT numbering schemes (see e.g. Dondelinger, M., et al., 2018. Frontiers in immunology, 9, p.2278). Suitably, the CDRs are predicted using the Kabat numbering scheme

In preferred embodiments, (a) the anti-TNF antibody or fragment thereof is an anti-TNF antibody fragment; and/or (b) the anti-CD3 antibody or fragment thereof is an anti-CD3 antibody fragment. In more preferred embodiments, (a) the anti-TNF antibody or fragment thereof is an anti-TNF antibody fragment; and (b) the anti-CD3 antibody or fragment thereof is an anti-CD3 antibody fragment. An “antibody fragment” may be a fragment of an antibody, or a genetically engineered product of one of more fragments of an antibody, which fragment is involved in binding with the target molecule (e.g. TNF or CD3). Examples include an antigen-binding fragment (Fab), a fragment antibody (F(ab')₂), a variable region (Fv), a single chain antibody (scFv), a single-domain antibody (sdAb), and a camelid antibody (VHH). The use of an antibody fragment may be advantageous because it may reduce inflammation associated with the Fc region.

In some embodiments, the antibody fragment is an antigen-binding fragment (Fab), a fragment antibody (F(ab')₂), a single chain antibody (scFv), or a single-domain antibody (sdAb).

In preferred embodiments, the antibody fragment is an antigen-binding fragment (Fab). "Antigen-binding fragment" (Fab) refers to a region on an antibody that binds to antigens. It is
5 composed of one constant and one variable region of each of the heavy and the light chain.

In other embodiments, the antibody fragment is a fragment antibody (F(ab')₂). "Fragment antibody" (F(ab')₂) refers to a region on an antibody that remains following digestion of the Fc region while leaving intact some of the hinge region.

In other embodiments, the antibody fragment is a single chain antibody (scFv). "Single chain
10 antibody" (scFv) refers to an engineered antibody consisting of a light chain variable region and a heavy chain variable region connected to one another directly or via a peptide linker sequence. The peptide linker sequence is usually about 10 to 25 amino acids in length, rich in glycine for flexibility, and serine or threonine for solubility. The peptide linker sequence can either connect the N-terminus of the heavy chain variable region with the C-terminus of the
15 light chain variable region, or vice versa.

In other embodiments, the antibody fragment is a single-domain antibody (sdAb). "Single-domain antibody" (sdAb), also known as a nanobody, refers to an antibody fragment consisting of a single monomeric variable antibody domain. Accordingly, a sdAb may be a heavy chain variable region (VH) or a light chain variable region (VL). Examples of single-domain
20 antibodies include, but are not limited to, VHH fragments, and VNAR fragments. Single-domain antibodies may also be generated by splitting the dimeric variable domains from common IgG molecules into monomers.

The present invention includes "variants" of the CDR regions described below. The term "variant" as used herein is defined below in the section "Variants, derivatives and fragments".
25 It will be appreciated that one or more amino acid substitutions may be made in the CDRs whilst retaining the antigen-binding ability. For instance, the CDR variants may comprise 3 or fewer amino acid substitutions, e.g. 3 amino acid substitutions, 2 amino acid substitutions or 1 amino acid substitution. In particular, in some embodiments the CDR variants comprise one amino acid substitution and retain the antigen-binding ability. The variant may be a variant
30 with at least 80% or 90% identity to the CDR. Examples of antibodies, and fragments and/or derivatives thereof that can be used in the invention are further described below.

Signal peptides

The TNF inhibitor and/or CD3 inhibitor may be operably linked to one or more signal peptides.

In some embodiments, the TNF inhibitor and/or the CD3 inhibitor is operably linked to one or more signal peptides. In some embodiments, the TNF inhibitor and the CD3 inhibitor is operably linked to one or more signal peptides.

5 A "signal peptide" may refer to a short peptide that directs the insertion of proteins into the membrane of the endoplasmic reticulum. Signal peptides are typically N-terminal extensions of newly synthesized secretory and membrane proteins that are 16 to 30 amino acid residues in length and comprised of a hydrophilic, usually positively charged N-terminal region, a central hydrophobic domain, and a C-terminal region with the cleavage site for signal peptidase. Besides these common characteristics, signal peptides do not share sequence similarity and
10 some are more than 50 amino acid residues long (see e.g. Kapp, K., et al., 2009. Protein transport into the endoplasmic reticulum, pp.1-16).

The TNF inhibitor and/or CD3 inhibitor may be operably linked to any suitable signal peptide(s). The SPdb signal peptide database is a repository of experimentally determined and computationally predicted signal peptides (see e.g. Choo, K.H., et al., 2005. BMC
15 bioinformatics, 6(1), pp.1-8). Suitable signal peptides include, a Human Growth Hormone (HGH) signal peptide, an interleukin-2 (IL-2) signal peptide, a CD5 signal peptide, an immunoglobulin Kappa light chain signal peptide, a trypsinogen signal peptide, a serum albumin signal peptide, or a prolactin signal peptide.

In some embodiments, the signal peptide is selected from one or more of: a Human Growth
20 Hormone (HGH) signal peptide, an interleukin-2 (IL-2) signal peptide, a CD5 signal peptide, an immunoglobulin Kappa light chain signal peptide, a trypsinogen signal peptide, a serum albumin signal peptide, and a prolactin signal peptide. In some embodiments, the signal peptide is a Human Growth Hormone (HGH) signal peptide.

In some embodiments, the TNF inhibitor is operably linked to one or more Human Growth
25 Hormone (HGH) signal peptide. In some embodiments, the TNF inhibitor is operably linked to one or more signal peptide comprising or consisting of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 1. In some embodiments the TNF inhibitor is operably linked to one or more signal peptide comprising or
30 consisting of the amino acid sequence of SEQ ID NO: 1.

In some embodiments, the CD3 inhibitor is operably linked to one or more Human Growth Hormone (HGH) signal peptide. In some embodiments, the CD3 inhibitor is operably linked to one or more signal peptide comprising or consisting of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at

least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 1. In some embodiments the CD3 inhibitor is operably linked to one or more signal peptide comprising or consisting of the amino acid sequence of SEQ ID NO: 1.

In some embodiments, (a) the TNF inhibitor is operably linked to one or more Human Growth Hormone (HGH) signal peptide; and (b) the CD3 inhibitor is operably linked to one or more Human Growth Hormone (HGH) signal peptide. In some embodiments, (a) the TNF inhibitor is operably linked to one or more signal peptide comprising or consisting of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 1; and (b) the CD3 inhibitor is operably linked to one or more signal peptide comprising or consisting of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 1. In some embodiments, (a) the TNF inhibitor is operably linked to one or more signal peptide comprising or consisting of the amino acid sequence of SEQ ID NO: 1; and (b) the CD3 inhibitor is operably linked to one or more signal peptide comprising or consisting of the amino acid sequence of SEQ ID NO: 1.

MATGSRTSLLLLAFGLLCLPWLQEGSA

Example HGH signal peptide (SEQ ID NO: 1)

Example nucleotide sequences

The TNF inhibitor and/or CD3 inhibitor may be encoded by any suitable nucleotide sequence.

In some embodiments, (a) the nucleotide sequence encoding the TNF inhibitor; and/or (b) the nucleotide sequence encoding the CD3 inhibitor is codon-optimised, for example codon-optimised for expression in humans. In some embodiments, (a) the nucleotide sequence encoding the TNF inhibitor is codon-optimised, for example codon-optimised for expression in humans; and (b) the nucleotide sequence encoding the CD3 inhibitor is codon-optimised, for example codon-optimised for expression in humans. Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same token, it is possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available. Codon usage tables are known in the art for mammalian cells (e.g. humans), as well as for a variety of other organisms.

Suitably, a nucleotide sequence encoding a Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a heavy chain (e.g. VH + CH1); a linker sequence; a second signal sequence; and a nucleotide encoding a light chain. Suitably, a nucleotide sequence encoding a Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a light chain; a linker sequence; a second signal sequence; and a nucleotide encoding a heavy chain (e.g. VH + CH1).

Signal sequences

The first signal sequence and the second signal sequence may encode the same signal peptide or different signal peptides. In some embodiments, the first signal sequence and the second signal sequence encode the same signal peptide. The first signal sequence and the second signal sequence may be any signal sequence described herein.

In some embodiments, the first signal sequence and/or the second signal sequence encode any of: a Human Growth Hormone (HGH) signal peptide, an interleukin-2 (IL-2) signal peptide, a CD5 signal peptide, an immunoglobulin Kappa light chain signal peptide, a trypsinogen signal peptide, a serum albumin signal peptide, and a prolactin signal peptide.

In some embodiments, the first signal sequence and/or the second signal sequence encode a Human Growth Hormone (HGH) signal peptide. In some embodiments, the first signal sequence and the second signal sequence encode a Human Growth Hormone (HGH) signal peptide.

In some embodiments, the first signal sequence and/or the second signal sequence comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 2-5. In some embodiments, the first signal sequence and/or the second signal sequence comprises or consists of the nucleotide sequence of any of SEQ ID NOs: 2-5.

atggcaacaggtagtcgaaccagcctattactggccttcggtctcctgtgtctgccttggt
tcaagagggtctgc
Example HGH signal sequence (SEQ ID NO: 2)

atggccacgggaagtgcgacttccttactactcgcccttggtcttctttgcttgccatggct
ccaggagggtagtgca
Example HGH signal sequence (SEQ ID NO: 3)

atggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttggt
gcaagagggcagcgcc
Example HGH signal sequence (SEQ ID NO: 4)

atggctaccggcagcagaaccagcctgctgctggcattcggccttctgtgcctgccttggt
gcaagagggctctgcc

Example HGH signal sequence (SEQ ID NO: 5)

Linker sequences

- 5 The linker sequence may comprise one or more cleavage sites. As used herein, “cleavage sites” may include nucleotide sequences encoding specific peptide sequences at which site-specific proteases may cleave or cut the peptide (also known as enzymatically cleavable peptide motifs) and nucleotide sequences encoding self-cleaving peptides.

10 In preferred embodiments, the linker sequence encodes a cleavage site. In some embodiments, the linker sequence encodes a self-cleaving peptide and/or an enzymatically cleavable peptide motif.

In some embodiments, the linker sequence encodes a 2A self-cleaving peptide. 2A self-cleaving peptides are a class of 18–22 aa-long peptides, which can induce ribosomal skipping during translation of a protein in a cell. Suitable 2A self-cleaving peptides include T2A, P2A, 15 E2A and F2A, or derivatives thereof.

In some embodiments, the linker sequence encodes a 2A self-cleaving peptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 6-9. In some embodiments, the linker sequence encodes a 2A self-cleaving peptide comprising or 20 consisting of any of SEQ ID NOs: 6-9.

In some embodiments, the linker sequence encodes a 2A self-cleaving peptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 6. In some 25 embodiments, the linker sequence encodes a 2A self-cleaving peptide comprising or consisting of SEQ ID NO: 6.

ATNFSLLKQAGDVEENPGP

Example P2A peptide sequence (SEQ ID NO: 6)

EGRGSLLTCGDVEENPGP

Example T2A peptide sequence (SEQ ID NO: 7)

30 QCTNYALLKLAGDVESNPGP

Example E2A peptide sequence (SEQ ID NO: 8)

VKQTLNFDLLKLAGDVESNPGP

Example F2A peptide sequence (SEQ ID NO: 9)

In some embodiments, the linker sequence comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 10-11.

In some embodiments, the linker sequence comprises any of SEQ ID NOs: 10-11.

5 gctacgaatttttcattgctcaagcaagcgggagatgtggaggagaaccctggcccc
Example P2A nucleotide sequence (SEQ ID NO: 10)

gctaccaacttcagcctcctgaaacaggccggcgatgtggaggaaaaccctggacct
Example P2A nucleotide sequence (SEQ ID NO: 11)

The linker sequence may comprise any other suitable nucleotide sequences, for example
10 nucleotide sequences which aid expression. Suitably, the linker sequence may comprise a furin site and/or a fusion protein linker sequence.

In some embodiments, the linker sequence comprises a furin site. Furin is a protease enzyme that may cleave at a conserved polybasic RNRR site. Suitably, the furin site encodes RKRR.

In some embodiments, the linker sequence comprises fusion protein linker sequence. Fusion
15 protein linker sequences may join two protein domains together. Suitably, the fusion protein linker sequence encodes SGSG.

In some embodiments, the linker sequence encodes from 5' to 3': a furin site, a fusion protein linker sequence, and a 2A self-cleaving peptide.

In some embodiments, the linker sequence encodes an amino acid having at least 70%, at
20 least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 12. In some embodiments, the linker sequence encodes an amino acid comprising or consisting of SEQ ID NO: 12.

RKRRSGSGATNFSLLKQAGDVEENPGP
Example linker peptide sequence (SEQ ID NO: 12)

25 In some embodiments, the linker sequence comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 13-14. In some embodiments, the linker sequence comprises or consists of any of SEQ ID NOs: 13-14.

30 aggaagcggcggttccgggagcggggctacgaatttttcattgctcaagcaagcgggagatgt
ggaggagaaccctggcccc
Example linker sequence (SEQ ID NO: 13)

agaaagcgggagaagcgggaagcgggtgctaccaacttcagcctcctgaaacaggccggcgatgt
ggaggaaaaccctggacct
35 Example linker sequence (SEQ ID NO: 14)

Other sequences

The nucleotide sequence encoding may comprise any other suitable sequences. For example, the TNF inhibitor or CD3 inhibitor may be linked to a HA tag for detection. Suitably, an HA tag may comprise or consist of SEQ ID NO: 15.

5 YPYDVDPDYA
 Example HA tag (SEQ ID NO: 15)

TNF inhibitors

10 The combination of the present invention comprises a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

As used herein, a “TNF inhibitor” may be any protein or oligonucleotide that suppresses an inflammatory response to TNF. Tumour necrosis factor (TNF) is also known as cachexin or cachectin, and may also be known as tumour necrosis factor alpha (TNF- α). TNF is synthesized as a transmembrane protein (mTNF) and cleaved to soluble TNF (sTNF). There are two types of TNF, which are very closely related, TNF-alpha and TNF-beta. The activities of both TNFs are mediated through binding to the TNF receptors, TNFR1 and TNFR2. The binding of TNF may activate several signalling pathways, including transcription factor activation, proteases, and protein kinases. This signalling may lead to activation of the target cell leading to the inflammatory and immune response by releasing several cytokines and apoptotic pathway initiation (see Gerriets, V., et al., 2021. “Tumor necrosis factor inhibitors”. In StatPearls).

Example TNF inhibitors include adalimumab, infliximab, golimumab, certolizumab pegol, etanercept, XPro1595, XENP345, R1antTNF, Atrosab, and Atrosimab (see e.g. Lis, K., Kuzawińska, O. and Bałkowiec-Iskra, E., 2014. AMS, 10(6), p.1175; and Fischer, R., et al., 2020. Frontiers in cell and developmental biology, 8, p.401). Suitably, a TNF inhibitor may inhibit TNF activity by directly binding to TNF. For example, a TNF inhibitor may be an anti-TNF antibody or fragment thereof (e.g. adalimumab, infliximab, golimumab, certolizumab), or comprise the TNF-binding domain of a TNFR receptor (e.g. etanercept). Alternatively, a TNF inhibitor may inhibit TNF activity by binding to a TNF receptor. For example, a TNF inhibitor may be a TNF mutein (e.g. XPro1595, XENP345, R1antTNF) or may be an anti-TNFR antibody or fragment thereof (e.g. Atrosab and Atrosimab).

In preferred embodiments, the TNF inhibitor is a human-specific TNF inhibitor, i.e. a protein or oligonucleotide that suppresses an inflammatory response to human TNF. In some

embodiments, the TNF inhibitor is an anti-human TNF antibody or a fragment thereof (e.g. adalimumab, infliximab, golimumab, certolizumab, or fragments thereof).

Suitably, a TNF inhibitor may suppress an inflammatory response by binding to TNF α . For example, a TNF inhibitor may be an anti-TNF α antibody (e.g. adalimumab, infliximab, 5 golimumab, certolizumab) or a fragment thereof. In some embodiments, the TNF inhibitor is an anti-human TNF α antibody or a fragment thereof (e.g. adalimumab, infliximab, golimumab, certolizumab, or fragments thereof).

Anti-TNF antibody or fragment thereof

In some embodiments, the TNF inhibitor comprises or consists of an anti-TNF antibody or a 10 fragment thereof. In preferred embodiments, the TNF inhibitor is an anti-TNF antibody or a fragment thereof.

The anti-TNF antibody may be a monoclonal antibody (mAb) or a fragment thereof. In some embodiments, the TNF inhibitor is a humanized anti-TNF mAb or a fragment thereof. In some embodiments, the TNF inhibitor is a human anti-TNF mAb or a fragment thereof.

15 Suitable anti-TNF antibodies are known in the art. Moreover, anti-TNF antibodies, and fragments and/or derivatives thereof, can be prepared using methods known by those of skill in the art. Such methods include phage display, methods to generate human or humanized antibodies, or methods using transgenic animal or plant engineered to produce human antibodies. Phage display libraries of partially or fully synthetic antibodies are available and 20 can be screened for an antibody or fragment thereof that can bind to the target molecule. Phage display libraries of human antibodies are also available. Once identified, the amino acid sequence or polynucleotide sequence encoding for the antibody (or fragment and/or derivative thereof) can be isolated and/or determined. The sequence of the antibody can be used to design suitable fragments and/or derivatives thereof.

25 In preferred embodiments, the anti-TNF antibody or fragment thereof is an anti-TNF antibody fragment. An "anti-TNF antibody fragment" may be a fragment of an anti-TNF antibody, or a genetically engineered product of one of more fragments of the anti-TNF antibody, which fragment is involved in binding with TNF. In some embodiments, the anti-TNF antibody fragment is an antigen-binding fragment (Fab), a fragment antibody (F(ab')₂), a single chain 30 antibody (scFv), or a single-domain antibody (sdAb). In preferred embodiments, the anti-TNF antibody fragment is an antigen-binding fragment (Fab).

The TNF inhibitor may comprise at least one CDR (e.g. HCDR3), which can be predicted from an anti-TNF antibody (or a variant of such a predicted CDR e.g. a variant with one, two or

three amino acid substitutions). It will be appreciated that molecules containing three or fewer CDR regions (e.g. a single CDR or even a part thereof) may be capable of retaining the antigen-binding activity of the antibody from which the CDR is derived. Molecules containing two CDR regions are described in the art as being capable of binding to a target antigen, e.g. in the form of a minibody (see e.g. Vaughan and Sollazzo, 2001, *Combinational Chemistry & High Throughput Screening*, 4, 417-430). Molecules containing a single CDR have been described which can display strong binding activity to target (see e.g. Nicaise et al, 2004, *Protein Science*, 13: 1882-91).

The TNF inhibitor may comprise one or more variable heavy chain CDRs, e.g. one, two or three variable heavy chain CDRs. Alternatively, or additionally, the TNF inhibitor may comprise one or more variable light chain CDRs, e.g. one, two or three variable light chain CDRs. The TNF inhibitor may comprise three heavy chain CDRs and/or three light chain CDRs (and more particularly a heavy chain variable region comprising three CDRs and/or a light chain variable region comprising three CDRs) wherein at least one CDR, preferably all CDRs, may be from an anti-TNF antibody, or may be selected from one of the CDR sequences provided below.

The TNF inhibitor may comprise any combination of variable heavy and light chain CDRs, e.g. one variable heavy chain CDR together with one variable light chain CDR, two variable heavy chain CDRs together with one variable light chain CDR, two variable heavy chain CDRs together with two variable light chain CDRs, three variable heavy chain CDRs together with one or two variable light chain CDRs, one variable heavy chain CDR together with two or three variable light chain CDRs, or three variable heavy chain CDRs together with three variable light chain CDRs. Preferably, the TNF inhibitor comprises three variable heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and/or three variable light chain CDRs (LCDR1, LCDR2 and LCDR3).

The one or more CDRs present within the TNF inhibitor may not all be from the same antibody, as long as the domain has the binding activity described above. Thus, one CDR may be predicted from the heavy or light chains of an anti-TNF antibody, whilst another CDR present may be predicted from a different anti-TNF antibody. In this instance, it may be preferred that CDR3 be predicted from an anti-TNF antibody. Particularly however, if more than one CDR is present in the TNF inhibitor, it is preferred that the CDRs are predicted from anti-TNF antibodies. A combination of CDRs may be used from different antibodies, particularly from antibodies that bind to the same desired region or epitope.

In a preferred embodiment, the TNF inhibitor comprises three CDRs predicted from the variable heavy chain sequence of an anti-TNF antibody and/or three CDRs predicted from the variable light chain sequence of an anti-TNF antibody.

5 Examples of antibodies, and fragments and/or derivatives thereof that can be used in the invention are further described below. The TNF inhibitor may comprise or consist of an amino acid sequence comprising the CDRs described herein and substitutions, variations, modifications, replacements, deletions and/or additions of one or more amino acid residues may occur in the framework region. The derivatives described herein may retain TNF-binding ability. The derivatives may be capable of binding TNF to at least 10%, at least 20%, at least 10
10 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the level of the corresponding reference amino acid sequence.

TNF-binding affinity may be determined by equilibrium binding constants (KD), which may be determined by any suitable assay e.g. surface plasmon resonance (see e.g. Shealy, D.J., et al., 2010. mAbs, 2(4), pp. 428-439). Suitably, an anti-TNF antibody or a fragment thereof may
15 bind to soluble TNF with a binding affinity of about 1000 pM or less, about 900 pM or less, about 800 pM or less, about 700 pM or less, about 600 pM or less, about 500 pM or less, about 400 pM or less, about 300 pM or less, about 200 pM or less, or about 100 pM or less, for example as determined by surface plasmon resonance. Suitably, an anti-TNF antibody or a fragment thereof may bind to soluble TNF with a binding affinity of from about 0.1 pM to
20 about 1000 pM, from about 1 pM to about 1000 pM, from about 10 pM to about 1000 pM, 0.1 pM to about 100 pM, from about 1 pM to about 100 pM, or from about 10 pM to about 100 pM, for example as determined by surface plasmon resonance. Suitably, an anti-TNF antibody or a fragment thereof may bind to transmembrane TNF with a binding affinity of about 10000 pM or less, about 9000 pM or less, about 8000 pM or less, about 7000 pM or less, about 6000 pM
25 or less, about 5000 pM or less, or about 4000 pM or less, for example as determined by surface plasmon resonance.

In some embodiments, the TNF inhibitor is selected from any of: adalimumab, or a fragment and/or derivative thereof; infliximab, or a fragment and/or derivative thereof; golimumab, or a fragment and/or derivative thereof; and certolizumab or a fragment and/or derivative thereof.

30 In some embodiments, the TNF inhibitor is selected from: adalimumab, or a fragment and/or derivative thereof; and infliximab, or a fragment and/or derivative thereof.

In some embodiments, the TNF inhibitor is selected from any of: an adalimumab fragment or derivative thereof; an infliximab fragment or derivative thereof; a golimumab fragment or derivative thereof; and a certolizumab fragment or derivative thereof.

In some embodiments, the TNF inhibitor is selected from any of: an adalimumab fragment or derivative thereof; and an infliximab fragment or derivative thereof.

Adalimumab

In preferred embodiments, the TNF inhibitor is adalimumab, or a fragment and/or derivative thereof. In some embodiments, the TNF inhibitor is an adalimumab Fab, an adalimumab F(ab')₂, an adalimumab scFv, an adalimumab sdAb, or a derivative thereof. In some embodiments, the TNF inhibitor is an adalimumab Fab, or a derivative thereof.

Adalimumab (Humira®) is a recombinant, fully human IgG1 monoclonal antibody that binds specifically to TNF, thereby neutralizing the activity of the cytokine. The skilled person would be able to generate adalimumab derivatives using conservative mutations and/or knowledge of the mechanism of adalimumab inhibition of TNF (see e.g. Hu, S., et al., 2013. Journal of Biological Chemistry, 288(38), pp.27059-27067). For example, the following adalimumab variants have been shown to have comparable KD values to wild-type adalimumab: L178K, L178N, Q160N, L116N, T118N, A122N, Q179N, L183N, and T199N (see e.g. Reslan, M., et al., 2020. International Journal of Biological Macromolecules, 158, pp.189-196).

In some embodiments, the TNF inhibitor is a fragment of adalimumab, or a derivative thereof. In some embodiments, the TNF inhibitor is an adalimumab Fab, an adalimumab F(ab')₂, an adalimumab scFv, or an adalimumab sdAb, or a derivative thereof. In some embodiments, the TNF inhibitor is an adalimumab Fab, or a derivative thereof.

Suitable adalimumab Fab derivatives include those described in Yoshikawa, M., et al., 2022. The Journal of Biochemistry, mvac040; and Nakamura, H., et al., 2020. Biological and Pharmaceutical Bulletin, 43(3), pp.418-423. Suitably, an adalimumab Fab derivative may be selected from one or more of: H:K137C-L:I117C, H:K137C-L:F209C, H:S138C-L:F116C, H:S140C-L:S114C, and H:V177C-L:Q160C.

In some embodiments, the TNF inhibitor is, or is derived from, an anti-TNF antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 16-21, or variants thereof. In other words, in some embodiments the TNF inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 16-21, or variants thereof.

In some embodiments, the TNF inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 16 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 17 or a variant thereof, and/or

a HCDR3 having an amino acid sequence of SEQ ID NO: 18 or a variant thereof;
and/or

- (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 19 or a variant thereof, a
LCDR2 having an amino acid sequence of SEQ ID NO: 20 or a variant thereof, and/or
5 a LCDR3 having an amino acid sequence of SEQ ID NO: 21 or a variant thereof.

In some embodiments, the TNF inhibitor comprises a HCDR2 having an amino acid sequence
of SEQ ID NO: 17 or a variant thereof and/or a LCDR2 having an amino acid sequence of
SEQ ID NO: 20 or a variant thereof. For adalimumab, CDRs L2 and H2 contribute to the
majority of the interactions with the antigen (see Hu, S., et al., 2013. Journal of Biological
10 Chemistry, 288(38), pp.27059-27067).

In some embodiments, the TNF inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 16 or a variant thereof, a
HCDR2 having an amino acid sequence of SEQ ID NO: 17 or a variant thereof, and a
HCDR3 having an amino acid sequence of SEQ ID NO: 18 or a variant thereof; and/or
15 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 19 or a variant thereof, a
LCDR2 having an amino acid sequence of SEQ ID NO: 20 or a variant thereof, and a
LCDR3 having an amino acid sequence of SEQ ID NO: 21 or a variant thereof.

In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the
heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least
20 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at
least 99% identity, or 100% identity to SEQ ID NO: 22.

In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the
light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%,
at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least
25 99% identity, or 100% identity to SEQ ID NO: 23.

In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light
chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at
least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%,
at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 22, and the light chain
30 comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least
85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity
or 100% identity to SEQ ID NO: 23.

In some embodiments, the TNF inhibitor comprises or consists of:

5 (i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 22, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 16 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 17 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 18 or a variant thereof; and/or

10 (ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 23, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 19 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 20 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 21 or a variant thereof.

15 In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 24 or 25.

20 In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 26.

25 In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 24, and the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 26.

In some embodiments, the TNF inhibitor comprises or consists of:

30 (i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 24, wherein the amino acid sequence comprises a HCDR1 having an amino acid

sequence of SEQ ID NO: 16, a HCDR2 having an amino acid sequence of SEQ ID NO: 17, and a HCDR3 having an amino acid sequence of SEQ ID NO: 18; and/or

- (ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 26, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 19, a LCDR2 having an amino acid sequence of SEQ ID NO: 20, and a LCDR3 having an amino acid sequence of SEQ ID NO: 21.

Name	Example sequence
Adalimumab HCDR1	TFDDYA (SEQ ID NO: 16)
Adalimumab HCDR2	TWNSGHID (SEQ ID NO: 17)
Adalimumab HCDR3	VSYLSTASSL (SEQ ID NO: 18)
Adalimumab LCDR1	GIRNYLA (SEQ ID NO: 19)
Adalimumab LCDR2	YAASTLQ (SEQ ID NO: 20)
Adalimumab LCDR3	RYNRA (SEQ ID NO: 21)
Adalimumab VH	EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSA ITWNSGHIDYADSVEGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAKVS YLSTASSLDYWGQGTLLTVS (SEQ ID NO: 22)
Adalimumab VL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRPYTFGQ GTKVEIK (SEQ ID NO: 23)
Adalimumab VH + CH1	EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSA ITWNSGHIDYADSVEGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAKVS YLSTASSLDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQ TYICNVNHKPSNTKVDKKV (SEQ ID NO: 24)
Adalimumab heavy chain	EVQLVESGGGLVQPGRSRLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSA ITWNSGHIDYADSVEGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAKVS YLSTASSLDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG K (SEQ ID NO: 25)
Adalimumab light chain	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISLQPEDVATYYCQRYNRPYTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC (SEQ ID NO: 26)

Infliximab

In some embodiments, the TNF inhibitor is infliximab, or a fragment and/or derivative thereof. In some embodiments, the TNF inhibitor is an infliximab Fab, an infliximab F(ab')₂, an infliximab scFv, an infliximab sdAb, or a derivative thereof. In some embodiments, the TNF inhibitor is an infliximab Fab, or a derivative thereof.

- 5 Infliximab (Remicade®) is a chimeric monoclonal antibody to human TNF. It binds to both soluble and transmembrane forms of TNF at picomolar concentrations. The skilled person would be able to generate infliximab derivatives using conservative mutations and/or knowledge of the mechanism of infliximab inhibition of TNF (see e.g. Liang, S., et al., 2013. Journal of Biological Chemistry, 288(19), pp.13799-13807).

- 10 In some embodiments, the TNF inhibitor is a fragment of infliximab, or a derivative thereof. In some embodiments, the TNF inhibitor is an infliximab Fab, an infliximab F(ab')₂, an infliximab scFv, or an infliximab sdAb, or a derivative thereof. In some embodiments, the TNF inhibitor is an infliximab Fab, or a derivative thereof.

- 15 In some embodiments, the TNF inhibitor is, or is derived from, an anti-TNF antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 27-32, or variants thereof. In other words, in some embodiments the TNF inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 27-32, or variants thereof.

In some embodiments, the TNF inhibitor comprises:

- 20 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 27 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 28 or a variant thereof, and/or a HCDR3 having an amino acid sequence of SEQ ID NO: 29 or a variant thereof; and/or
- 25 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 30 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 31 or a variant thereof, and/or a LCDR3 having an amino acid sequence of SEQ ID NO: 32 or a variant thereof.

In some embodiments, the TNF inhibitor comprises:

- 30 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 27 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 28 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 29 or a variant thereof; and/or

(ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 30 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 31 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 32 or a variant thereof.

In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 33.

In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 34.

In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 33, and the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 34.

In some embodiments, the TNF inhibitor comprises or consists of:

(i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 33, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 27 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 28 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 29 or a variant thereof; and/or

(ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 34, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 30 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 31 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 32 or a variant thereof.

In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least

75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 35 or 36.

In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 37.

In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 35, and the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 37.

In some embodiments, the TNF inhibitor comprises or consists of:

- (i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 35, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 27, a HCDR2 having an amino acid sequence of SEQ ID NO: 28, and a HCDR3 having an amino acid sequence of SEQ ID NO: 29; and/or
- (ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 37, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 30, a LCDR2 having an amino acid sequence of SEQ ID NO: 31, and a LCDR3 having an amino acid sequence of SEQ ID NO: 32.

Name	Example sequence
Infiximab HCDR1	IFSNHW (SEQ ID NO: 27)
Infiximab HCDR2	RSKSINSATH (SEQ ID NO: 28)
Infiximab HCDR3	NYYGSTY (SEQ ID NO: 29)
Infiximab LCDR1	FVGSSIH (SEQ ID NO: 30)
Infiximab LCDR2	KYASESM (SEQ ID NO: 31)
Infiximab LCDR3	QSHSW (SEQ ID NO: 32)
Infiximab VH	EVKLEESGGGLVQPGGSMKLSQVSGFIFSNHWMNWVRQSPEKGLEWVAE IRSKSINSATHYAESVKGRTISRDDSKSAVYLQMTDLRTEDTGYYCSR NYYGSTYDYWGQGTTLTVSS (SEQ ID NO: 33)
Infiximab VL	DILLTQSPAILSVPGERVVSFSCRASQFVGSSIHWHYQQRRTNGSPRLLIK ASEMSGIPSRFSGSGSGTDFTLSTNTVESEDIADYYCQQSHSWPFTFGS GTNLEVK (SEQ ID NO: 34)

Infliximab VH + CH1	EVKLEESGGGLVQPGGSMKLSKVASGFIFSNHWMNWVRQSPEKGLEWVAE IRSKSINSATHYAESVKGRTISRDDSKSAVYLQMTDLRTEDTGVIYCSR NYYGSTYDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFSSSLGTQT YICNVNHKPSNTKVDKKV (SEQ ID NO: 35)
Infliximab heavy chain	EVKLEESGGGLVQPGGSMKLSKVASGFIFSNHWMNWVRQSPEKGLEWVAE IRSKSINSATHYAESVKGRTISRDDSKSAVYLQMTDLRTEDTGVIYCSR NYYGSTYDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVFSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPETVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGDSFFLYSKLTVDKSRWQQGNVSCFVSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 36)
Infliximab light chain	DILLTQSPAILSVPGERVFSFSCRASQFVGSSIHWHYQORTNGSPRLIKY ASEMSGIPSRFSGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGS GTNLEVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC (SEQ ID NO: 37)

Golimumab

In some embodiments, the TNF inhibitor is golimumab, or a fragment and/or derivative thereof.

- In some embodiments, the TNF inhibitor is a golimumab Fab, a golimumab F(ab')₂, a
 5 golimumab scFv, a golimumab sdAb, or a derivative thereof. In some embodiments, the TNF
 inhibitor is a golimumab Fab, or a derivative thereof.

- Golimumab (Simponi®) is a human IgG1 TNF antagonist monoclonal antibody. The skilled
 person would be able to generate golimumab derivatives using conservative mutations and/or
 knowledge of the mechanism of golimumab inhibition of TNF (see e.g. Shealy, D.J., et al.,
 10 2010. MAb, 2(4), pp. 428-439).

In some embodiments, the TNF inhibitor is a fragment of golimumab, or a derivative thereof.

In some embodiments, the TNF inhibitor is a golimumab Fab, a golimumab F(ab')₂, a
 golimumab scFv, or a golimumab sdAb, or a derivative thereof. In some embodiments, the
 TNF inhibitor is a golimumab Fab, or a derivative thereof.

- 15 In some embodiments, the TNF inhibitor is, or is derived from an anti-TNF antibody (e.g. is a
 Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions,
 selected from SEQ ID NOs: 38-43, or variants thereof. In other words, in some embodiments
 the TNF inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 38-43, or
 variants thereof.

- 20 In some embodiments, the TNF inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 38 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 39 or a variant thereof, and/or a HCDR3 having an amino acid sequence of SEQ ID NO: 40 or a variant thereof; and/or
- 5 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 41 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 42 or a variant thereof, and/or a LCDR3 having an amino acid sequence of SEQ ID NO: 43 or a variant thereof.

In some embodiments, the TNF inhibitor comprises:

- 10 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 38 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 39 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 40 or a variant thereof; and/or
- (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 41 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 42 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 43 or a variant thereof.

- 15 In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 44.

- In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the
- 20 light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 45.

- In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at
- 25 least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 44, and the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 45.

- 30 In some embodiments, the TNF inhibitor comprises or consists of:

5 (i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 44, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 38 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 39 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 40 or a variant thereof; and/or

10 (ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 45, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 41 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 42 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 43 or a variant thereof.

15 In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 46 or 47.

20 In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 48.

25 In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 46, and the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 48.

In some embodiments, the TNF inhibitor comprises or consists of:

30 (i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 46, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 38, a HCDR2 having an amino acid sequence of SEQ ID NO: 39, and a HCDR3 having an amino acid sequence of SEQ ID NO: 40; and/or

(ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 48, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 41, a LCDR2 having an amino acid sequence of SEQ ID NO: 42, and a LCDR3 having an amino acid sequence of SEQ ID NO: 43.

5

Name	Example sequence
Golimumab HCDR1	IFSSYA (SEQ ID NO: 38)
Golimumab HCDR2	SYDGSNKK (SEQ ID NO: 39)
Golimumab HCDR3	NYYYYGM (SEQ ID NO: 40)
Golimumab LCDR1	SVYSYLA (SEQ ID NO: 41)
Golimumab LCDR2	YDASNRA (SEQ ID NO: 42)
Golimumab LCDR3	QRSNW (SEQ ID NO: 43)
Golimumab VH	QVQLVESGGGVVQPGRSLRLSCAASGFI FSSYAMHWVRQAPGNGLEWVAF MSYDGSNKKYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDR GIAAGGNYYYYGMDVWGQGT TTVTVSS (SEQ ID NO: 44)
Golimumab VL	EIVLTQSPATLSLSPGERATLSCRASQSVSYLA WYQQKPGQAPRLLIYD ASNRATGIPARFSGSGSGTDFTLT ISSLEPEDFAVYYCQQRSNWPPFTFG PGTKVDIK (SEQ ID NO: 45)
Golimumab VH + CH1	QVQLVESGGGVVQPGRSLRLSCAASGFI FSSYAMHWVRQAPGNGLEWVAF MSYDGSNKKYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDR GIAAGGNYYYYGMDVWGQGT TTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS SLGTQTYICNVNHKPSNTKVDKKV (SEQ ID NO: 46)
Golimumab heavy chain	QVQLVESGGGVVQPGRSLRLSCAASGFI FSSYAMHWVRQAPGNGLEWVAF MSYDGSNKKYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDR GIAAGGNYYYYGMDVWGQGT TTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTEPVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMEALHNHYTQKSL SLSPGK (SEQ ID NO: 47)
Golimumab light chain	EIVLTQSPATLSLSPGERATLSCRASQSVSYLA WYQQKPGQAPRLLIYD ASNRATGIPARFSGSGSGTDFTLT ISSLEPEDFAVYYCQQRSNWPPFTFG PGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLLLEGINENNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSITLTLTKADYEKHKVY ACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 48)

Certolizumab

In some embodiments, the TNF inhibitor is certolizumab, or a fragment and/or derivative thereof. In some embodiments, the TNF inhibitor is a certolizumab Fab, a certolizumab F(ab')₂,
 10 a certolizumab scFv, a certolizumab sdAb, or a derivative thereof. In some embodiments, the TNF inhibitor is a certolizumab Fab, or a derivative thereof.

Certolizumab is a humanized antigen-binding fragment (Fab') of a monoclonal antibody, that is usually administered in a form that is conjugated to polyethylene glycol (Cimzia®). The

skilled person would be able to generate certolizumab derivatives using conservative mutations and/or knowledge of the mechanism of certolizumab inhibition of TNF (see e.g. Lee, J.U., et al., 2017. International journal of molecular sciences, 18(1), p.228).

In some embodiments, the TNF inhibitor is a fragment of certolizumab, or a derivative thereof.

- 5 In some embodiments, the TNF inhibitor is a certolizumab Fab, a certolizumab F(ab')₂, a certolizumab scFv, or a certolizumab sdAb, or a derivative thereof. In some embodiments, the TNF inhibitor is a certolizumab Fab, or a derivative thereof.

- 10 In some embodiments, the TNF inhibitor is, or is derived from an anti-TNF antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 49-54, or variants thereof. In other words, in some embodiments the TNF inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 49-54, or variants thereof.

In some embodiments, the TNF inhibitor comprises:

- 15 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 49 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 50 or a variant thereof, and/or a HCDR3 having an amino acid sequence of SEQ ID NO: 51 or a variant thereof; and/or
- (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 52 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 53 or a variant thereof, and/or
- 20 a LCDR3 having an amino acid sequence of SEQ ID NO: 54 or a variant thereof.

In some embodiments, the TNF inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 49 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 50 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 51 or a variant thereof; and/or
- 25 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 52 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 53 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 54 or a variant thereof.

- In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least
- 30 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 55.

In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 56.

- 5 In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 55, and the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 56.

In some embodiments, the TNF inhibitor comprises or consists of:

- 15 (i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 55, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 49 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 50 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 51 or a variant thereof; and/or
- 20 (ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 56, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 52 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 53 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 54 or a variant thereof.

- 25 In some embodiments, the TNF inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 57 or 58.

- 30 In some embodiments, the TNF inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 59.

- In some embodiments, the TNF inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 57, and the
- 5 light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 59.

In some embodiments, the TNF inhibitor comprises or consists of:

- 10 (i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 57, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 49, a HCDR2 having an amino acid sequence of SEQ ID NO: 50, and a HCDR3 having an amino acid sequence of SEQ ID NO: 51; and/or
- 15 (ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 59, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 49, a LCDR2 having an amino acid sequence of SEQ ID NO: 50, and a LCDR3 having an amino acid sequence of SEQ ID NO: 51.

Name	Example sequence
Certolizumab HCDR1	VFTDYG (SEQ ID NO: 49)
Certolizumab HCDR2	NTYIGEPI (SEQ ID NO: 50)
Certolizumab HCDR3	GYRSYAM (SEQ ID NO: 51)
Certolizumab LCDR1	NVGTNVA (SEQ ID NO: 52)
Certolizumab LCDR2	YSASFLY (SEQ ID NO: 53)
Certolizumab LCDR3	QYNIY (SEQ ID NO: 54)
Certolizumab VH	EVQLVESGGGLVQPGGSLRLSCAASGYVFTDYG MNWVRQAPGKGLEWMGW INTYIGEPIYADSVKGRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCARGY RSYAMDYWGQGTTLTVSS (SEQ ID NO: 55)
Certolizumab VL	DIQMTQSPSSLSASVGRVTITCKASQNVGTNVAWYQQKPGKAPKALIYS ASFLYSGVPYRFGSGSGTDFTLTISLQPEDFATYYCQQYNIYPLTFGQ GTKVEIK (SEQ ID NO: 56)
Certolizumab VH + CH1	EVQLVESGGGLVQPGGSLRLSCAASGYVFTDYG MNWVRQAPGKGLEWMGW INTYIGEPIYADSVKGRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCARGY RSYAMDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI CNVNHKPSNTKVDKKV (SEQ ID NO: 57)
Certolizumab heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGYVFTDYG MNWVRQAPGKGLEWMGW INTYIGEPIYADSVKGRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCARGY RSYAMDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCAA (SEQ ID NO: 58)

Certolizumab light chain	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYS ASFLYSGVPYRFGSGSGTDFTLTISSLQPEDFATYYCQQYNIYPLTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC (SEQ ID NO: 59)
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Other TNF inhibitors

In some embodiments, the TNF inhibitor comprises the TNF-binding domain of a TNF receptor (TNFR).

- 5 TNF signals through two receptors (TNFR1 and TNFR2) that share a similar structural arrangement with an N-terminal extracellular domain (ECD) composed of four cysteine-rich domains (CRDs), an α -helical transmembrane domain and a cytoplasmic domain. The two receptors are most divergent in the cytoplasmic domain, where TNFR1 has a death domain that is absent from TNFR2 (see e.g. Bodmer, J.L., et al. Trends in biochemical sciences, 27(1), pp.19-26)

In some embodiments, the TNF inhibitor comprises a soluble form of a TNF receptor (TNFR). In some embodiments, the TNF inhibitor comprises a soluble form of TNFR1 or TNFR2. In some embodiments, the TNF inhibitor comprises or consists a soluble form of TNFR2.

- 15 The TNF-binding domain and/or soluble form of a TNF receptor may be fused to any suitable domain. Suitably, the TNF-binding domain and/or soluble form of a TNF receptor may be coupled to an Fc domain (e.g. the Fc portion of human IgG1).

Etanercept

In some embodiments, the TNF inhibitor is etanercept or a derivative thereof.

- 20 Etanercept (Enbrel®) is a fusion protein, consisting of a TNFR2 domain coupled to the Fc portion of human IgG1. The skilled person would be able to generate etanercept derivatives using conservative mutations and/or knowledge of the mechanism of etanercept inhibition of TNF (see e.g. Lamanna, W.C., et al., 2017. Scientific reports, 7(1), pp.1-8).

- 25 In some embodiments, the TNF inhibitor comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 60. In some embodiments, the TNF inhibitor comprises or consists of the amino acid sequence of SEQ ID NO: 60.

5 LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
 QLWNWVPECLSCGSRCSDDQVETQACTREQNRICTCRPGWYCALSKEGCRLCAPLRKCRPG
 FGVARPGTETSDVCKPCAPGTFSTNTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
 MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLPMGPSPPAEGSTGDEPKSCDKTHTCPP
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLF
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Example etanercept sequence (SEQ ID NO: 60)

10 Other fusion proteins

In some embodiments, the TNF inhibitor is a fusion protein, consisting of a TNFR1 extracellular domain coupled to the Fc portion of human IgG1.

15 In some embodiments, the TNF inhibitor comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 61. In some embodiments, the TNF inhibitor comprises or consists of the amino acid sequence of SEQ ID NO: 61.

20 MGLSTVPDLLLPLVLELLVGIYPSGVIGLVPHLGDREKRDSVCPQGYIHPQNNICCTKC
 HKGTLYLNDPCPGPGQDTCRECESGSFTASENHLRHCLSCSKCRKEMGQVEISSCTVDRDTV
 CGCRKNQYRHYWSENLFQCFNCSLCLNGTVHLSCQEKQNTVCTCHAGFFLRENECVSCSNCK
 KSLECTKLCLPQIENVKGTEDSGTDTKHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
 VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
 KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
 25 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
 SPGK

Example fusion protein sequence (SEQ ID NO: 61)

TNF-specific aptamers

Other suitable TNF inhibitors include TNF-specific DNA aptamers, and TNF-specific RNA aptamers (see e.g. Lai, W.Y., et al., 2019. Theranostics, 9(6), p.1741; and Yan, X., et al., 2004. 30 Genomics, Proteomics & Bioinformatics, 2(1), pp.32-42.).

Aptamers are single-stranded DNA- or RNA-based synthetic oligonucleotides, which adopt complex three-dimensional conformations that allow them to bind and interact with a wide variety of targets with high affinity and specificity.

35 In some embodiments, the TNF inhibitor is a TNF-specific aptamer. In some embodiments, the TNF inhibitor is a TNF-specific DNA aptamer. In some embodiments, the TNF inhibitor is a TNF-specific RNA aptamer.

Example nucleotide sequences

The TNF inhibitor may be encoded by any suitable nucleotide sequence. In some embodiments, the nucleotide sequence is codon-optimised, for example codon-optimised for expression in humans.

- 5 Suitably, a nucleotide sequence encoding an anti-TNF Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a heavy chain (e.g. VH + CH1); a linker sequence; a second signal sequence; and a nucleotide encoding a light chain. Suitably, a nucleotide sequence encoding an anti-TNF Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a light chain; a linker sequence; a second signal sequence; and a
- 10 nucleotide encoding a heavy chain (e.g. VH + CH1).

Heavy chains and light chains

The nucleotide encoding a heavy chain (e.g. VH + CH1) and the nucleotide encoding a light chain may encode any heavy chain (e.g. VH + CH1) and light chain combination described herein. For example, the nucleotide sequence encoding an anti-TNF Fab may comprise from

- 15 5' to 3':
- (a) a first signal sequence; a nucleotide encoding an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an adalimumab light chain, or a derivative thereof;
 - 20 (b) a first signal sequence; a nucleotide encoding an infliximab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an infliximab light chain, or a derivative thereof;
 - (c) a first signal sequence; a nucleotide encoding a golimumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a golimumab light chain, or a derivative thereof;
 - 25 (d) a first signal sequence; a nucleotide encoding a certolizumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a certolizumab light chain, or a derivative thereof;
 - (e) a first signal sequence; a nucleotide encoding an adalimumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide
 - 30 encoding an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof;

- (f) a first signal sequence; a nucleotide encoding an infliximab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an infliximab heavy chain (e.g. VH + CH1), or a derivative thereof;
- (g) a first signal sequence; a nucleotide encoding a golimumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a golimumab heavy chain (e.g. VH + CH1), or a derivative thereof; or
- (h) a first signal sequence; a nucleotide encoding a certolizumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a certolizumab heavy chain (e.g. VH + CH1), or a derivative thereof.
- 10 In some embodiments, the nucleotide sequence encoding an anti-TNF Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an adalimumab light chain, or a derivative thereof. In some embodiments,
- 15 the nucleotide sequence encoding an anti-TNF Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding an adalimumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof.

In some embodiments, the nucleotide sequence encoding an adalimumab heavy chain (e.g. VH + CH1) comprises or consists of a nucleotide sequence having at least 70%, at least 75%,

20 at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 62. In some embodiments, the nucleotide sequence encoding an adalimumab heavy chain (e.g. VH + CH1) comprises or consists of SEQ ID NO: 62.

25 gaggtgcaactggtggaaagcggcggcgccctggtccagcctggaaggtccctgagactgag
ctgtgccgccagcggcctttaccttcgacgactacgccatgcactgggtgcgccaggccctg
gcaagggcctggaatgggtctccgccatcacctggaatagcggccacatcgactacgccgat
agcgtggaaggcagattcaccatcagccgggacaacgccaagaactctctgtatctgcaaat
gaacagcctgcgggctgaagatacagccgtgtactattgcgccaaagtgagctacctctcca
ccgccagcagcctggactattggggacagggcacccctggtgaccgtgtctagcgccctccaca
30 aagggcccttctgtgtttccactggctccaagctccaaaagcacatctggaggaaccgctgc
cctgggctgcctggttaaggactacttccccgagcctgtgaccgtgagctggaacagcggcg
ccctgacatctggtgttcataccttccctgcccgttctgcaatcttctggactctacagcctg
tcttctgtggtgaccgtgcccagcagcagccttggaaacacagacctacatctgcaatgtgaa
ccacaagcctagcaacaccaaggtggacaagaaggtg

35 Example nucleotide sequence encoding an adalimumab heavy chain (VH + CH1) (SEQ ID NO: 62)

In some embodiments, the nucleotide sequence encoding an adalimumab light chain comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 63. In some embodiments, nucleotide
 5 sequence encoding an adalimumab light chain comprises or consists of SEQ ID NO: 63.

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gacatccagatgacccagagcccttcctcactgagcgccagcgtgggacagagtgactat
tacatgcagagccagccaaggcatccggaactacctggcctggtatcagcagaagcccgga
aagcccctaagctgctgatctacgccgccagcaccctgcaaagcggcgtgcctagcagattc
agcggctcaggctctggcactgatttcaccctgaccatctcctctctgcaacctgaggacgt
10 ggccacatactactgccagagatacaacagagccccatacacctttggccagggcacaaaag
tggaatcaagagaaccgtggccgctcccagtggtgttcattctccccccagtgatgagcag
ctgaagtccggcacagcctctgtcgtgtgcctgctgaacaacttctaccccagagaggccaa
ggtgcagtggaaggtggataatgccctgcaaagcggcaacagccaggagagcgtgacagagc
aggacagcaaggacagcacctacagcctctctagcacactgaccctgagcaaggccgactac
15 gagaagcacaaggtgtacgcatgcgaggtgacccaccagggcctgagcagtcctgtgaccaa
gagcttcaaccggggcgagtgt

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Example nucleotide sequence encoding an adalimumab light chain
 (SEQ ID NO: 63)

In some embodiments, the nucleotide sequence encoding an anti-TNF Fab may comprise
 20 from 5' to 3': a first signal sequence; a nucleotide encoding an infliximab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an infliximab light chain, or a derivative thereof. In some embodiments, the nucleotide sequence encoding an anti-TNF Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding an infliximab light chain, or a derivative thereof; a linker
 25 sequence; a second signal sequence; and a nucleotide encoding an infliximab heavy chain (e.g. VH + CH1), or a derivative thereof.

In some embodiments, the nucleotide sequence encoding an infliximab heavy chain (e.g. VH + CH1) comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%,
 30 at least 99% identity, or 100% identity to SEQ ID NO: 64. In some embodiments, nucleotide sequence encoding an infliximab heavy chain (e.g. VH + CH1) comprises or consists of SEQ ID NO: 64.

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gagggtgaaactggaggaaagtggaggaggcctcggttcaaccaggcggcctccatgaagctgtc
gtgtgtggcatctggcttcattctcagcaatcactggatgaactgggtcaggcaatctcctg
35 agaaggggctagagtgggtggcgagatccgctcaaaatcaatcaattccgccacacattat
gcagagtcagtaaaaggcggttcaccatttctagagatgacagcaaaagcgccgtgtacct
ccagatgaccgacctgcgaacagaggacactggggtctactactgctcccggaactactatg
gctccacctatgactactggggccaagggaccacattgacagtatcctcagcctccactaaa
ggtccttcagtgtttccgctggctccctcctccaaaagtacgtcaggcggcaccgctgctct
40 gggctgtctggtgaaggattacttcctgaacctgtgactgtttcctggaacagtgaggcct
tgacttcaggagtgccacacatttccggcagtgctccagagcagtggtctctattccctaagc

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agtgtagtgtaccgtgccctctagcagcctcggaaccagacatacatctgcaatgtcaatca
caagccaagcaatacaaaaagtggaagaaggtt

Example nucleotide sequence encoding an infliximab heavy chain
(VH + CH1) (SEQ ID NO: 64)

- 5 In some embodiments, the nucleotide sequence encoding an infliximab light chain comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 65. In some embodiments, nucleotide sequence encoding an infliximab light chain comprises or consists of SEQ ID NO: 65.

10 gacatttctctgacccagtcctctgctatcttgtctgtctccccggagagcgcgtctcctt
ctcttgacagagcttcccagtttgtgggcagcagcattcactggtatcagcagagaacaaatg
gatcaccaaggcttttgatcaagtatgcttcagaaagcatgagtgggataccatccaggttt
agtgggaagtggctctggtactgacttcactctctctataaacacgggtggaaagcgaagatat
15 tgctgactattactgtcagcaaaagccatagctggccatttacttttgatcagggaaccaacc
tggaagtcaagagaactgtggcgcgccttcgggtttttatcttcccccatctgatgaacag
ctgaagagcgggtacagccagtgtagtgtgcctgctcaacaacttctaccctagagaagccaa
ggtgcagtgggaaggtcgacaatgcattacagagcgggaacagccaggaaagtgttactgagc
aggatagcaaggacagcacctactctctgtctagcacactcactttgtctaaagcagattat
20 gagaaacataaaagtttatgcctgtgaagttacccaccaggggcctgagcagtcctcgtcaccaa
gtctttcaaccgcggggagtg

Example nucleotide sequence encoding an infliximab light chain
(SEQ ID NO: 65)

Example anti-TNF Fab sequences

- 25 In some embodiments, the nucleotide sequence encoding an anti-TNF Fab encodes an amino acid sequence comprising or consisting of from 5' to 3': an amino acid sequence having at least 70% identity to SEQ ID NO: 1, an amino acid sequence having at least 70% identity to SEQ ID NO: 22, an amino acid sequence having at least 70% sequence identity to any of SEQ ID NOs: 6-9, an amino acid sequence having at least 70% identity to SEQ ID NO: 1, and an amino acid sequence having at least 70% identity to SEQ ID NO: 23.
- 30 In some embodiments, the nucleotide sequence encoding an anti-TNF Fab encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 66. In some embodiments, the nucleotide sequence encoding an anti-TNF Fab encodes the amino acid sequence of SEQ ID NO: 66.

35 MATGSRTSLLLAFLGLLCLPWLQEGSAEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHW
VRQAPGKGLEWVSAITWNSGHIDYADSVGEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAK
VSYLSTASSLDYWGGQTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVRKR
40 RSGSGATNFSLKQAGDVEENPGPMATGSRTSLLLAFLGLLCLPWLQEGSADIQMTQSPSSLS
ASVGRVITITCRASQGI RNYLAWYQQKPGKAPKLLIYAASLTLSGVPSRFSGSGSGTDFTLT

ISSLPEDVATYYCQRYNRPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
 NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEEKHKVYACEVTH
 QGLSSPVTKSFNREGEC

Example adalimumab Fab amino acid sequence (SEQ ID NO: 66)

- 5 In some embodiments, the nucleotide sequence encoding an anti-TNF Fab comprises or consists of from 5' to 3': a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, a nucleotide sequence having at least 70% identity to SEQ ID NO: 62, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, and a nucleotide sequence
 10 having at least 70% identity to SEQ ID NO: 63.

- In some embodiments, the nucleotide sequence encoding an anti-TNF Fab comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 67. In some embodiments, the nucleotide sequence encoding
 15 an anti-TNF Fab comprises or consists of SEQ ID NO: 67.

atggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtcgtgccttggt
 gcaagagggcagcgccgaggtgcaactgggtggaaagcgccggcgccctgggtccagcctggaa
 ggtccctgagactgagctgtgccgccagcggtttaccttcgacgactacgccatgcactgg
 gtgcccaggccctggcaaggcctggaatgggtctccgccatcacctggaatagcggcca
 20 catcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaagaact
 ctctgtatctgcaaatgaacagcctgcggtgaagatacagccgtgtactattgcgcaaaa
 gtgagctacctctccaccgccagcagcctggactattggggacagggcaccctggtgaccgt
 gtctagcgcctccacaaaggcccttctgtgtttccactggctccaagctccaaaagcacat
 ctggaggaaccgctgcccctgggtgctgctggttaaggactacttccccgagcctgtgaccgtg
 25 agctggaacagcgccgcccgtgacatctggtgttcataccttccctgcccgttctgcaatcttc
 tggactctacagcctgtcttctgtggtgaccgtgccagcagcagccttggaacacagacct
 acatctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcgg
 agaagcggaaagcgtgctaccaacttcagcctcctgaaacagggcggcgatgtggaggaaaa
 ccctggacctatggctaccgagcagaaccagcctgctgctggcattcggccttctgtgcc
 30 tgccttggtgcaagagggctctgccgacatccagatgaccagagcccttccctactgagc
 gccagcgtgggacagagtgactattacatgcagagccagccaaggcatccggaactacct
 ggcttggtatcagcagaagcccgcaaaagcccctaagctgctgatctacgccgacagaccc
 tgcaaaagcggcgtgcctagcagattcagcggctcaggctctggcactgatttcaccctgacc
 atctcctctctgcaacctgaggacgtggccacatactactgccagagatacaacagagcccc
 35 atacacctttggccagggcacaaaagtggaaatcaagagaaccgtggccgctcccagtggtg
 tcatcttccccccagtgatgagcagctgaagtccggcacagcctctgtcgtgtgcctgctg
 aacaacttctacccagagaggccaaggtgcagtggaaaggtggataatgccttgcaaaagcgg
 caacagccaggagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagca
 cactgaccctgagcaaggccgactacgagaagcacaaggtgtacgcatgcagggtgacccac
 40 cagggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgt

Example adalimumab Fab nucleotide sequence (SEQ ID NO: 67)

In some embodiments, the nucleotide sequence encoding an anti-TNF Fab encodes an amino acid sequence comprising or consisting of from 5' to 3': an amino acid sequence having at

least 70% identity to SEQ ID NO: 1, an amino acid sequence having at least 70% identity to SEQ ID NO: 33, an amino acid sequence having at least 70% sequence identity to any of SEQ ID NOs: 6-9, an amino acid sequence having at least 70% identity to SEQ ID NO: 1, and an amino acid sequence having at least 70% identity to SEQ ID NO: 34.

- 5 In some embodiments, the nucleotide sequence encoding an anti-TNF Fab encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 68. In some embodiments, the nucleotide sequence encoding an anti-TNF Fab encodes the amino acid sequence of SEQ ID NO: 68.

10 MATGSRTSLLLAFGLLCLPWLQEGSAEVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNW
VRQSPEKGLEWVAEIRSKSINSATHYAESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYC
SRNYYGSTYDWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVRKRR
15 SGGGATNFSLLKQAGDVEENPGPMATGSRTSLLLAFGLLCLPWLQEGSADILLTQSPAILSV
SPGERVSFSCRASQFVGSSIHWHYQQRRTNGSPRLLIKYASEMSGIPSRFSGSGSGTDFTLISI
NTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Example infliximab Fab amino acid sequence (SEQ ID NO: 68)

- 20 In some embodiments, the nucleotide sequence encoding an anti-TNF Fab comprises or consists of from 5' to 3': a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, a nucleotide sequence having at least 70% identity to SEQ ID NO: 64, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, and a nucleotide sequence
25 having at least 70% identity to SEQ ID NO: 65.

- In some embodiments, the nucleotide sequence encoding an anti-TNF Fab comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 69. In some embodiments, the nucleotide sequence encoding
30 an anti-TNF Fab comprises or consists of SEQ ID NO: 69.

atggcaacaggtagtcgaaccagcctattactggccttcggtctcctgtgtctgccttggt
tcaagagggctctgctgaggtgaaactggaggaaagtggaggaggcctcggtcaaccaggcg
gctccatgaagctgtcgtgtgtggcatctggcttcattcagcaatcactggatgaactgg
gtcaggcaatctcctgagaaggggctagagtggtggcgagatccgctcaaatcaatcaa
35 ttcgcccacacattatgcagagtcagtaaaaggcggttcaccatttctagagatgacagca
aaagcgccgtgtacctccagatgaccgacctgcaacagaggacactggggtctactactgc
tcccggaactactatggctccacctatgactactggggccaagggaaccacattgacagtatc
ctcagcctccactaaaggtccttcagtgtttccgctggctccctcctccaaaagtacgtcag
gcggcaccgctgctctggctgtctggtgaaggattactccctgaacctgtgactgtttcc
40 tggaaacagtggagccttgacttcaggaggtccacacatttccggcagtgctccagagcagtgg

5 tctctattccctaagcagtgtagtgaccgtgccctctagcagcctcggaacccagacataca
 tctgcaatgtcaatcacaagccaagcaatacaaaagtggaagaagggttaggaagcggcgt
 tccgggagcggggctacgaatttttcatgtctcaagcaagcgggagatgtggaggagaaccc
 10 tggcccatggccacgggaagtcggacttcttactactcgccttttggtcttcttctgttc
 catggctccaggagggtagtcagacattctcctgacccagtccttctgtctgtc
 tcccccgagagcgcgtctccttctcttgcagagcttcccagtttggtgggcagcagcattca
 ctggtatcagcagagaacaaatggatcaccaaggcttttgatcaagtatgcttcagaaagca
 tgagtgggataccatccaggttttagtggaagtggctctggtactgacttcactctctctata
 aacacggtggaagcgaagatattgctgactattactgtcagcaaagccatagctggccatt
 15 tacttttggatcagggaccaacctggaagtcaagagaactgtggccgcgccttcggttttta
 ttttcccccatctgatgaacagctgaagagcgggtacagccagtgtagtgtgcctgtcgaac
 aacttctaccctagagaagccaaggtgcagtggaaggtcgacaatgcattacagagcgggaa
 cagccaggaaagtgttactgagcaggatagcaaggacagcacctactctctgtctagcacac
 tcactttgtctaaagcagattatgagaaacataaagtttatgcctgtgaagttaccaccag
 20 ggctgagcagtcctcgtcaccaagtcctttcaaccgcgaggagtgc

Example infliximab Fab nucleotide sequence (SEQ ID NO: 69)

CD3 inhibitors

The combination of the present invention comprises a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

- 20 The present invention also provides CD3 inhibitors, and polynucleotides and vectors comprising a nucleotide sequence encoding the same. The present inventors have surprisingly shown that intraocular administration of a CD3 inhibitor, or a polynucleotide or vector comprising a nucleotide sequence encoding the same, may be used to prevent or treat an inflammatory eye disease.
- 25 As used herein, a "CD3 inhibitor" may refer to any protein or oligonucleotide that suppresses T-cell activation by interacting with the T-cell receptor (TCR)-CD3 complex. CD3 (cluster of differentiation 3) is a protein complex and T cell co-receptor. In mammals, the CD3 complex contains a CD3 γ chain, a CD3 δ chain, and two CD3 ϵ chains. These chains associate with the T-cell receptor (TCR) and the CD3 ζ chain to generate an activation signal in T lymphocytes
- 30 (see e.g. Kuhns, M.S. et al., 2006. Deconstructing the form and function of the TCR/CD3 complex. *Immunity*, 24(2), pp.133-139).

- Example CD3 inhibitors include foralumab, teplizumab, visilizumab, oteelixumab, muromonab-CD3, T3/4.A, YTH12.5, HuM291, 145-2C11, G4.18, 17A2, Dow2, 20-2b2, blinatumomab, tebentafusp, CD3-specific DNA aptamers, and CD3-specific RNA aptamers
- 35 (see e.g. Kuhn, C. and Weiner, H.L., 2016. *Immunotherapy*, 8(8), pp.889-906; Sugita, S., et al., 2017. *Arthritis Research & Therapy*, 19, pp.1-12; Menon, A.P., et al., 2023. *Cancers*, 15(4), p.1189; and Renders, L. and Valerius, T., 2003. *Clinical & Experimental Immunology*, 133(3), pp.307-309).

In preferred embodiments, the CD3 inhibitor is a human-specific CD3 inhibitor, i.e. a protein or oligonucleotide that suppresses T-cell activation by binding to human CD3. In some embodiments, the CD3 inhibitor is an anti-human CD3 antibody or a fragment thereof (e.g. foralumab, teplizumab, visilizumab, otelexizumab, muromonab-CD3, T3/4.A, YTH12.5, HuM291, or fragments thereof).

Suitably, a CD3 inhibitor may suppress T-cell activation by binding to a CD3 γ chain, a CD3 δ chain, and/or a CD3 ϵ chain. In some embodiments, a CD3 inhibitor may inhibit CD3 activity by binding to a CD3 ϵ chain. For example, a CD3 inhibitor may be an anti-CD3 ϵ antibody (e.g. foralumab, teplizumab, visilizumab, otelexizumab, or muromonab-CD3) or a fragment thereof.

10 In some embodiments, the CD3 inhibitor is an anti-human CD3 ϵ antibody or a fragment thereof (e.g. foralumab, teplizumab, visilizumab, otelexizumab, and muromonab-CD3, or fragments thereof).

Anti-CD3 antibody or fragment thereof

In some embodiments, the CD3 inhibitor comprises or consists of an anti-CD3 antibody or a fragment thereof. In preferred embodiments, the CD3 inhibitor is an anti-CD3 antibody or a fragment thereof.

The anti-CD3 antibody may be a monoclonal antibody (mAb) or a fragment thereof. In some embodiments, the CD3 inhibitor is a humanized anti-CD3 mAb or a fragment thereof. In some embodiments, the CD3 inhibitor is a human anti-CD3 mAb or a fragment thereof.

20 Suitable anti-CD3 antibodies are known in the art. Moreover, anti-CD3 antibodies, and fragments and/or derivatives thereof, can be prepared using methods known by those of skill in the art. Such methods include phage display, methods to generate human or humanized antibodies, or methods using transgenic animal or plant engineered to produce human antibodies. Phage display libraries of partially or fully synthetic antibodies are available and can be screened for an antibody or fragment thereof that can bind to the target molecule.

25 Phage display libraries of human antibodies are also available. Once identified, the amino acid sequence or polynucleotide sequence encoding for the antibody (or fragment and/or derivative thereof) can be isolated and/or determined. The sequence of the antibody can be used to design suitable fragments and/or derivatives thereof.

30 In preferred embodiments, the anti-CD3 antibody or fragment thereof is an anti-CD3 antibody fragment. An "anti-CD3 antibody fragment" may be a fragment of an anti-CD3 antibody, or a genetically engineered product of one or more fragments of the anti-CD3 antibody, which fragment is involved in binding with CD3. In some embodiments, the anti-CD3 antibody

fragment is an antigen-binding fragment (Fab), a fragment antibody (F(ab')₂), a single chain antibody (scFv), or a single-domain antibody (sdAb). In preferred embodiments, the anti-CD3 antibody fragment is an antigen-binding fragment (Fab).

5 The CD3 inhibitor may comprise at least one CDR (e.g. HCDR3), which can be predicted from an anti-CD3 antibody (or a variant of such a predicted CDR e.g. a variant with one, two or three amino acid substitutions). It will be appreciated that molecules containing three or fewer CDR regions (e.g. a single CDR or even a part thereof) may be capable of retaining the antigen-binding activity of the antibody from which the CDR is derived. Molecules containing two CDR regions are described in the art as being capable of binding to a target antigen, e.g.
10 in the form of a minibody (see e.g. Vaughan and Sollazzo, 2001, *Combinational Chemistry & High Throughput Screening*, 4, 417-430). Molecules containing a single CDR have been described which can display strong binding activity to target (see e.g. Nicaise et al, 2004, *Protein Science*, 13: 1882-91).

The CD3 inhibitor may comprise one or more variable heavy chain CDRs, e.g. one, two or
15 three variable heavy chain CDRs. Alternatively, or additionally, the CD3 inhibitor may comprise one or more variable light chain CDRs, e.g. one, two or three variable light chain CDRs. The CD3 inhibitor may comprise three heavy chain CDRs and/or three light chain CDRs (and more particularly a heavy chain variable region comprising three CDRs and/or a light chain variable region comprising three CDRs) wherein at least one CDR, preferably all CDRs, may be from
20 an anti-CD3 antibody, or may be selected from one of the CDR sequences provided below.

The CD3 inhibitor may comprise any combination of variable heavy and light chain CDRs, e.g. one variable heavy chain CDR together with one variable light chain CDR, two variable heavy chain CDRs together with one variable light chain CDR, two variable heavy chain CDRs together with two variable light chain CDRs, three variable heavy chain CDRs together with
25 one or two variable light chain CDRs, one variable heavy chain CDR together with two or three variable light chain CDRs, or three variable heavy chain CDRs together with three variable light chain CDRs. Preferably, the CD3 inhibitor comprises three variable heavy chain CDRs (HCDR1, HCDR2 and HCDR3) and/or three variable light chain CDRs (LCDR1, LCDR2 and LCDR3).

30 The one or more CDRs present within the CD3 inhibitor may not all be from the same antibody, as long as the domain has the binding activity described above. Thus, one CDR may be predicted from the heavy or light chains of an anti-CD3 antibody, whilst another CDR present may be predicted from a different anti-CD3 antibody. In this instance, it may be preferred that CDR3 be predicted from an anti-CD3 antibody. Particularly however, if more than one CDR is

present in the CD3 inhibitor, it is preferred that the CDRs are predicted from anti-CD3 antibodies. A combination of CDRs may be used from different antibodies, particularly from antibodies that bind to the same desired region or epitope.

5 In a preferred embodiment, the CD3 inhibitor comprises three CDRs predicted from the variable heavy chain sequence of an anti-CD3 antibody and/or three CDRs predicted from the variable light chain sequence of an anti-CD3 antibody.

10 Examples of antibodies, and fragments and/or derivatives thereof that can be used in the invention are further described below. The CD3 inhibitor may comprise or consist of an amino acid sequence comprising the CDRs described herein and substitutions, variations, modifications, replacements, deletions and/or additions of one or more amino acid residues may occur in the framework region. The derivatives described herein may retain CD3-binding ability. The derivatives may be capable of binding CD3 to at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the level of the corresponding reference amino acid sequence.

15 CD3-binding affinity may be determined by equilibrium binding constants (KD), which may be determined by any suitable assay e.g. surface plasmon resonance (see e.g. Kjer-Nielsen, L., et al., 2004. PNAS, 101(20), pp.7675-7680). Suitably, an anti-CD3 antibody or a fragment thereof may bind to human CD3 with a binding affinity of about 10 μ M or less, 1 μ M or less, 100 nM or less, 10 nM or less, 1000 pM or less, about 900 pM or less, about 800 pM or less, 20 about 700 pM or less, about 600 pM or less, about 500 pM or less, about 400 pM or less, about 300 pM or less, about 200 pM or less, or about 100 pM or less, for example as determined by surface plasmon resonance. Suitably, an anti-CD3 antibody or a fragment thereof may bind to human CD3 with a binding affinity of from about 0.1 pM to about 10 μ M, from about 0.1 pM to about 1 μ M, from about 0.1 pM to about 100 nM, from about 0.1 pM to 25 about 10 nM, or from about 0.1 pM to about 1000 pM, for example as determined by surface plasmon resonance. Suitably, an anti-CD3 antibody or a fragment thereof may bind to human CD3 with a binding affinity of from about 1 pM to about 10 μ M, from about 1 pM to about 1 μ M, from about 1 pM to about 100 nM, from about 1 pM to about 10 nM, or from about 1 pM to about 1000 pM, for example as determined by surface plasmon resonance. Suitably, an 30 anti-CD3 antibody or a fragment thereof may bind to human CD3 with a binding affinity of from about 10 pM to about 10 μ M, from about 10 pM to about 1 μ M, from about 10 pM to about 100 nM, from about 10 pM to about 10 nM, or from about 10 pM to about 1000 pM, for example as determined by surface plasmon resonance. Suitably, an anti-CD3 antibody or a fragment thereof may bind to human CD3 with a binding affinity of from about 100 pM to about 10 μ M, 35 from about 100 pM to about 1 μ M, from about 100 pM to about 100 nM, from about 100 pM to

about 10 nM, or from about 100 pM to about 1000 pM, for example as determined by surface plasmon resonance.

In some embodiments, the CD3 inhibitor is selected from any of: foralumab, or a fragment and/or derivative thereof; teplizumab, or a fragment and/or derivative thereof; visilizumab, or
5 a fragment and/or derivative thereof; otelixizumab or a fragment and/or derivative thereof; muromonab-CD3, or a fragment and/or derivative thereof; T3/4.A or a fragment and/or derivative thereof; YTH12.5, or a fragment and/or derivative thereof; and HuM291, or a fragment and/or derivative thereof.

In some embodiments, the CD3 inhibitor is selected from any of: foralumab, or a fragment
10 and/or derivative thereof; teplizumab, or a fragment and/or derivative thereof; visilizumab, or a fragment and/or derivative thereof; otelixizumab or a fragment and/or derivative thereof; and muromonab-CD3 or a fragment and/or derivative thereof.

In some embodiments, the CD3 inhibitor is selected from any of: foralumab, or a fragment and/or derivative thereof; teplizumab, or a fragment and/or derivative thereof; visilizumab, or
15 a fragment and/or derivative thereof; and otelixizumab or a fragment and/or derivative thereof.

In some embodiments, the CD3 inhibitor is selected from: foralumab, or a fragment and/or derivative thereof; and teplizumab, or a fragment and/or derivative thereof.

In some embodiments, the CD3 inhibitor is foralumab, or a fragment and/or derivative thereof.

In some embodiments, the CD3 inhibitor is selected from any of: a foralumab fragment or derivative thereof; a teplizumab fragment or derivative thereof; a visilizumab fragment or derivative thereof; a otelixizumab fragment or derivative thereof; a muromonab-CD3 fragment or derivative thereof; a T3/4.A fragment or derivative thereof; a YTH12.5 fragment or derivative thereof; and a HuM291 fragment or derivative thereof.
20

In some embodiments, the CD3 inhibitor is selected from any of: a foralumab fragment or derivative thereof; a teplizumab fragment or derivative thereof; a visilizumab fragment or derivative thereof; a otelixizumab fragment or derivative thereof; and a muromonab-CD3 fragment or derivative thereof.
25

In some embodiments, the CD3 inhibitor is selected from any of: a foralumab fragment or derivative thereof; a teplizumab fragment or derivative thereof; a visilizumab fragment or derivative thereof; and a otelixizumab fragment or derivative thereof.
30

In some embodiments, the CD3 inhibitor is selected from any of: a foralumab fragment or derivative thereof; and a teplizumab fragment or derivative thereof.

In some embodiments, the CD3 inhibitor is a foralumab fragment or derivative thereof.

Foralumab

- 5 In some embodiments, the CD3 inhibitor is foralumab, or a fragment and/or derivative thereof. In some embodiments, the CD3 inhibitor is a foralumab Fab, a foralumab F(ab')₂, a foralumab scFv, a foralumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a foralumab Fab, or a derivative thereof.

10 Foralumab is a fully human IgG1 monoclonal antibody that binds specifically to CD3ε, thereby suppressing T-cell activation. The heavy chain constant regions are mutated at two amino acid positions, L234A and L235E (see e.g. Ilan, Y., et al., 2018. Clinical & Experimental Immunology, 193(3), pp.275-283). The skilled person would be able to generate foralumab derivatives using conservative mutations and/or knowledge of the mechanism of foralumab binding to CD3.

- 15 In some embodiments, the CD3 inhibitor is a fragment of foralumab, or a derivative thereof. In some embodiments, the CD3 inhibitor is a foralumab Fab, a foralumab F(ab')₂, a foralumab scFv, or a foralumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a foralumab Fab, or a derivative thereof.

20 In some embodiments, the CD3 inhibitor is, or is derived from, an anti-CD3 antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 70-75, or variants thereof. In other words, in some embodiments the CD3 inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 70-75, or variants thereof.

In some embodiments, the CD3 inhibitor comprises:

- 25 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 70 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 71 or a variant thereof, and/or a HCDR3 having an amino acid sequence of SEQ ID NO: 72 or a variant thereof; and/or
- 30 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 73 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 74 or a variant thereof, and/or a LCDR3 having an amino acid sequence of SEQ ID NO: 75 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 70 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 71 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 72 or a variant thereof; and/or
- 5 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 73 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 74 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 75 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 76.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 77.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 76, and the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 77.

In some embodiments, the CD3 inhibitor comprises or consists of:

- 25 (i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 76, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 70 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 71 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 72 or a variant thereof; and/or
- 30 (ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 77, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ

ID NO: 73 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 74 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 75 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 78 or 79.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 80.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 78, and the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 80.

- In some embodiments, the CD3 inhibitor comprises or consists of:
- (i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 78, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 70, a HCDR2 having an amino acid sequence of SEQ ID NO: 71, and a HCDR3 having an amino acid sequence of SEQ ID NO: 72; and/or
 - (ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 80, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 73, a LCDR2 having an amino acid sequence of SEQ ID NO: 74, and a LCDR3 having an amino acid sequence of SEQ ID NO: 75.

Name	Example sequence
Foralumab HCDR1	GYGMH (SEQ ID NO: 70)
Foralumab HCDR2	VIWYDGSKKYVDSVKG (SEQ ID NO: 71)
Foralumab HCDR3	QMGYWTFDL (SEQ ID NO: 72)

Foralumab LCDR1	RASQSVSSYLA (SEQ ID NO: 73)
Foralumab LCDR2	DASNRAT (SEQ ID NO: 74)
Foralumab LCDR3	QQRSNWPPLT (SEQ ID NO: 75)
Foralumab VH	QVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWVAV IWYDGSKKYYVDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARQM GYWHFDLWGRGTLTV (SEQ ID NO: 76)
Foralumab VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD ASN RATGIPARFSGSGSGTDFTLT ISSLEPEDFAVYYCQQRSNWPPLTFG GGTKVEIK (SEQ ID NO: 77)
Foralumab VH + CH1	QVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWVAV IWYDGSKKYYVDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARQM GYWHFDLWGRGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRV (SEQ ID NO: 78)
Foralumab heavy chain	QVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWVAV IWYDGSKKYYVDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARQM GYWHFDLWGRGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPEAEAGGSPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 79)
Foralumab light chain	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD ASN RATGIPARFSGSGSGTDFTLT ISSLEPEDFAVYYCQQRSNWPPLTFG GGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC (SEQ ID NO: 80)

Teplizumab

In some embodiments, the CD3 inhibitor is teplizumab, or a fragment and/or derivative thereof. In some embodiments, the CD3 inhibitor is a teplizumab Fab, a teplizumab F(ab')₂, a
5 teplizumab scFv, a teplizumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a teplizumab Fab, or a derivative thereof.

Teplizumab is a recombinant, humanized IgG1 monoclonal antibody that binds specifically to CD3ε, thereby suppressing T-cell activation. The heavy chain constant regions are mutated at two amino acid positions, L234A and L235A (see e.g. Sherry, N., et al., 2011. The Lancet,
10 378(9790), pp.487-49). Teplizumab is a humanized version of the mouse monoclonal OKT3 antibody, retaining the same binding region of OKT3 but with amino acids at positions 234 and 235 of the human IgG1 Fc changed to alanine resulting in decreased Fc binding (see e.g. Masharani, U.B. and Becker, J., 2010. Expert opinion on biological therapy, 10(3), pp.459-

465). The skilled person would be able to generate teplizumab derivatives using conservative mutations and/or knowledge of the mechanism of teplizumab binding to CD3.

In some embodiments, the CD3 inhibitor is a fragment of teplizumab, or a derivative thereof. In some embodiments, the CD3 inhibitor is a teplizumab Fab, a teplizumab F(ab')₂, a
5 teplizumab scFv, or a teplizumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a teplizumab Fab, or a derivative thereof.

In some embodiments, the CD3 inhibitor is, or is derived from, an anti-CD3 antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 81-86, or variants thereof. In other words, in some embodiments
10 the CD3 inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 81-86, or variants thereof.

In some embodiments, the CD3 inhibitor comprises:

(i) a HCDR1 having an amino acid sequence of SEQ ID NO: 81 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 82 or a variant thereof, and/or
15 a HCDR3 having an amino acid sequence of SEQ ID NO: 83 or a variant thereof; and/or

(ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 84 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 85 or a variant thereof, and/or
a LCDR3 having an amino acid sequence of SEQ ID NO: 86 or a variant thereof.

20 In some embodiments, the CD3 inhibitor comprises:

(i) a HCDR1 having an amino acid sequence of SEQ ID NO: 81 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 82 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 83 or a variant thereof; and/or

(ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 84 or a variant thereof, a
25 LCDR2 having an amino acid sequence of SEQ ID NO: 85 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 86 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at
30 least 99% identity, or 100% identity to SEQ ID NO: 87.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 88.

- 5 In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 87, and the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 88.

In some embodiments, the CD3 inhibitor comprises or consists of:

- 15 (i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 87, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 81 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 82 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 83 or a variant thereof; and/or

- 20 (ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 88, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 84 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 85 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 86 or a variant thereof.

- 25 In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 89 or 90.

- 30 In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 91.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 89, and the

5 light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 91.

In some embodiments, the CD3 inhibitor comprises or consists of:

- 10 (i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 89, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 81, a HCDR2 having an amino acid sequence of SEQ ID NO: 82, and a HCDR3 having an amino acid sequence of SEQ ID NO: 83; and/or
- 15 (ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 91, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 84, a LCDR2 having an amino acid sequence of SEQ ID NO: 85, and a LCDR3 having an amino acid sequence of SEQ ID NO: 86.

Name	Example sequence
Teplizumab HCDR1	RYTMH (SEQ ID NO: 81)
Teplizumab HCDR2	YINPSRGYTNYNQKVVD (SEQ ID NO: 82)
Teplizumab HCDR3	YYDDHYCLDY (SEQ ID NO: 83)
Teplizumab LCDR1	SASSSVSYMN (SEQ ID NO: 84)
Teplizumab LCDR2	DTSKLAS (SEQ ID NO: 85)
Teplizumab LCDR3	QQWSSNPFT (SEQ ID NO: 86)
Teplizumab VH	QVQLVQSGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKVVDKDRFTISRDNKNTAFLQMDSLRPEDTGVYFCARYY DDHYCLDYWGQGTPTVTVS (SEQ ID NO: 87)
Teplizumab VL	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQTPGKAPKRWIYDT SKLASGVPSRFSGSGSDYFTFTISLQPEDATYYCQQWSSNPFTFGQG TKLQIT (SEQ ID NO: 88)
Teplizumab VH + CH1	QVQLVQSGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKVVDKDRFTISRDNKNTAFLQMDSLRPEDTGVYFCARYY DDHYCLDYWGQGTPTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTY ICNVNHNKPSNTKVDKKV (SEQ ID NO: 89)
Teplizumab heavy chain	QVQLVQSGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKVVDKDRFTISRDNKNTAFLQMDSLRPEDTGVYFCARYY DDHYCLDYWGQGTPTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD

	YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHTQKSLSLSPGK (SEQ ID NO: 90)
Teplizumab light chain	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDT SKLASGVPSRFSGSGSGTDYFTFTISSLPEDIATYYCQQWSSNPFTFGQG TKLQITRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC (SEQ ID NO: 91)

Visilizumab

In some embodiments, the CD3 inhibitor is visilizumab, or a fragment and/or derivative thereof.
In some embodiments, the CD3 inhibitor is a visilizumab Fab, a visilizumab F(ab')₂, a
5 visilizumab scFv, a visilizumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a visilizumab Fab, or a derivative thereof.

Visilizumab is a recombinant, humanized IgG2 monoclonal antibody that binds specifically to CD3ε, thereby suppressing T-cell activation. The heavy chain constant regions are mutated at two amino acid positions, V234A and V237A (see e.g. Carpenter, P.A., et al., 2002.
10 Blood, 99(8), pp.2712-2719). The skilled person would be able to generate visilizumab derivatives using conservative mutations and/or knowledge of the mechanism of visilizumab binding to CD3.

In some embodiments, the CD3 inhibitor is a fragment of visilizumab, or a derivative thereof.
In some embodiments, the CD3 inhibitor is a visilizumab Fab, a visilizumab F(ab')₂, a
15 visilizumab scFv, or a visilizumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a visilizumab Fab, or a derivative thereof.

In some embodiments, the CD3 inhibitor is, or is derived from, an anti-CD3 antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 92-97, or variants thereof. In other words, in some embodiments
20 the CD3 inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 92-97, or variants thereof.

In some embodiments, the CD3 inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 92 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 93 or a variant thereof, and/or

a HCDR3 having an amino acid sequence of SEQ ID NO: 94 or a variant thereof;
and/or

- (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 95 or a variant thereof, a
LCDR2 having an amino acid sequence of SEQ ID NO: 96 or a variant thereof, and/or
5 a LCDR3 having an amino acid sequence of SEQ ID NO: 97 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 92 or a variant thereof, a
HCDR2 having an amino acid sequence of SEQ ID NO: 93 or a variant thereof, and a
HCDR3 having an amino acid sequence of SEQ ID NO: 94 or a variant thereof; and/or
10 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 95 or a variant thereof, a
LCDR2 having an amino acid sequence of SEQ ID NO: 96 or a variant thereof, and a
LCDR3 having an amino acid sequence of SEQ ID NO: 97 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the
heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least
15 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at
least 99% identity, or 100% identity to SEQ ID NO: 98.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the
light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%,
at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least
20 99% identity, or 100% identity to SEQ ID NO: 99.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light
chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at
least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%,
at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 98, and the light chain
25 comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least
85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity
or 100% identity to SEQ ID NO: 99.

In some embodiments, the CD3 inhibitor comprises or consists of:

- (i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%,
30 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 98, wherein
the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ

ID NO: 92 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 93 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 94 or a variant thereof; and/or

- 5 (ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 99, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 95 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 96 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 97 or a variant thereof.

- 10 In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 100 or 101.

- 15 In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 102.

- 20 In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 100, and the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 102.

- 25 In some embodiments, the CD3 inhibitor comprises or consists of:

- 30 (i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 100, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 92, a HCDR2 having an amino acid sequence of SEQ ID NO: 93, and a HCDR3 having an amino acid sequence of SEQ ID NO: 94; and/or

(ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 102, wherein the amino acid sequence comprises a LCDR1 having an amino acid

sequence of SEQ ID NO: 95, a LCDR2 having an amino acid sequence of SEQ ID NO: 96, and a LCDR3 having an amino acid sequence of SEQ ID NO: 97.

Name	Example sequence
Visilizumab HCDR1	SYTMH (SEQ ID NO: 92)
Visilizumab HCDR2	YINPRSGYTHYNQKLKD (SEQ ID NO: 93)
Visilizumab HCDR3	SAYYDYDGFAY (SEQ ID NO: 94)
Visilizumab LCDR1	SASSSVSYMN (SEQ ID NO: 95)
Visilizumab LCDR2	DTSKLAS (SEQ ID NO: 96)
Visilizumab LCDR3	QQWSSNPPT (SEQ ID NO: 97)
Visilizumab VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGY INPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSED TAVYYCARSA YYDYDGFAYWGQGLTVTVS (SEQ ID NO: 98)
Visilizumab VL	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAPKRLIYDT SKLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQWSSNPPTFGGG TKVEIK (SEQ ID NO: 99)
Visilizumab VH + CH1	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGY INPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSED TAVYYCARSA YYDYDGFAYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQT YTCNVDPKPSNTKVDKTV (SEQ ID NO: 100)
Visilizumab heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGY INPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSED TAVYYCARSA YYDYDGFAYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQT YTCNVDPKPSNTKVDKTVVERKCCVECPAPPAAPSVFLFPPKPKDTL MISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVELTVVHQDWLNGKEYCKVSNKGLPAPIEKTISKTKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSD GSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLSPSK (SEQ ID NO: 101)
Visilizumab light chain	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAPKRLIYDT SKLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQWSSNPPTFGGG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC (SEQ ID NO: 102)

Otelixizumab

- 5 In some embodiments, the CD3 inhibitor is otelixizumab, or a fragment and/or derivative thereof. In some embodiments, the CD3 inhibitor is a otelixizumab Fab, a otelixizumab F(ab')₂, a otelixizumab scFv, a otelixizumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a otelixizumab Fab, or a derivative thereof.

Otelixizumab is a recombinant, chimeric and humanized IgG1 monoclonal antibody that binds specifically to CD3ε, thereby suppressing T-cell activation. The heavy chain constant regions

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are mutated at N297A (see e.g. Aronson, R., et al, 2014. Diabetes care, 37(10), pp.2746-2754). The skilled person would be able to generate otelixizumab derivatives using conservative mutations and/or knowledge of the mechanism of otelixizumab binding to CD3.

In some embodiments, the CD3 inhibitor is a fragment of otelixizumab, or a derivative thereof.

- 5 In some embodiments, the CD3 inhibitor is a otelixizumab Fab, a otelixizumab F(ab')₂, a otelixizumab scFv, or a otelixizumab sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a otelixizumab Fab, or a derivative thereof.

- 10 In some embodiments, the CD3 inhibitor is, or is derived from, an anti-CD3 antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 103-108, or variants thereof. In other words, in some embodiments the CD3 inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 103-108, or variants thereof.

In some embodiments, the CD3 inhibitor comprises:

- 15 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 103 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 104 or a variant thereof, and/or a HCDR3 having an amino acid sequence of SEQ ID NO: 105 or a variant thereof; and/or
- 20 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 106 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 107 or a variant thereof, and/or a LCDR3 having an amino acid sequence of SEQ ID NO: 108 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises:

- 25 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 103 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 104 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 105 or a variant thereof; and/or
- (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 106 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 107 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 108 or a variant thereof.

- 30 In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least

80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 109.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%,
 5 at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 110.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%,
 10 at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 109, and the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 110.

In some embodiments, the CD3 inhibitor comprises or consists of:

- 15 (i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 109, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 103 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 104 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ
 20 ID NO: 105 or a variant thereof; and/or
- (ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 110, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 106 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID
 25 NO: 107 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 108 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at
 30 least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 111 or 112.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least

75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 113.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 111, and the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 113.

10 In some embodiments, the CD3 inhibitor comprises or consists of:

(i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 111, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 103, a HCDR2 having an amino acid sequence of SEQ ID NO: 104, and a HCDR3 having an amino acid sequence of SEQ ID NO: 105; and/or

(ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 113, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 106, a LCDR2 having an amino acid sequence of SEQ ID NO: 107, and a LCDR3 having an amino acid sequence of SEQ ID NO: 108.

Name	Example sequence
Otelixizumab HCDR1	SFPMA (SEQ ID NO: 103)
Otelixizumab HCDR2	TISTSGGRITYRDSVKG (SEQ ID NO: 104)
Otelixizumab HCDR3	FRQYSGGFDY (SEQ ID NO: 105)
Otelixizumab LCDR1	TLSSGNIENNYVH (SEQ ID NO: 106)
Otelixizumab LCDR2	DDDKRPD (SEQ ID NO: 107)
Otelixizumab LCDR3	HSYVSSFNV (SEQ ID NO: 108)
Otelixizumab VH	EVQLLESGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVST ISTSGGRITYRDSVKGRTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFR QYSGGFDYWGQGT LTVTS (SEQ ID NO: 109)
Otelixizumab VL	DIQLTQPN SVSTSLGSTVKLSCTLSSGNIENNYVHWYQLYEGRSPTTMIY DDDKRPDGV PDRFSGSIDRSSNSAFLTIHNVAIEDAIFYCHSYVSSFNV FGGGTKLTVL (SEQ ID NO: 110)
Otelixizumab VH + CH1	EVQLLESGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVST ISTSGGRITYRDSVKGRTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFR QYSGGFDYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD

	YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKV (SEQ ID NO: 111)
Otelixizumab heavy chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFFMAWVRQAPGKGLEWVST ISTSGGRYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFR QYSGGFDYWGGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGK (SEQ ID NO: 112)
Otelixizumab light chain	DIQLTQPNVSTSLGSTVKLSCTLSSGNIENNYVHWYQLYEGRSPTTMIY DDDKRPDGVPDRFSGSIDRSSNSAFLTIHNVAIEDEAIYFCHSYVSSFNV FGGGTKLTVLRQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVT HEGSTVEKTVAPTECS (SEQ ID NO: 113)

Muromonab-CD3

In some embodiments, the CD3 inhibitor is muromonab-CD3, or a fragment and/or derivative thereof. In some embodiments, the CD3 inhibitor is a muromonab-CD3 Fab, a muromonab-CD3 F(ab')₂, a muromonab-CD3 scFv, a muromonab-CD3 sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a muromonab-CD3 Fab, or a derivative thereof.

Muromonab-CD3 is a recombinant, murine monoclonal antibody that binds specifically to CD3ε, thereby suppressing T-cell activation (see e.g. Wilde, M.I. and Goa, K.L., 1996. Drugs, 51, pp.865-894). The skilled person would be able to generate muromonab-CD3 derivatives using conservative mutations and/or knowledge of the mechanism of muromonab-CD3 binding to CD3 (see e.g. Kjer-Nielsen, L., et al., 2004. PNAS, 101(20), pp.7675-7680).

In some embodiments, the CD3 inhibitor is a fragment of muromonab-CD3, or a derivative thereof. In some embodiments, the CD3 inhibitor is a muromonab-CD3 Fab, a muromonab-CD3 F(ab')₂, a muromonab-CD3 scFv, or a muromonab-CD3 sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a muromonab-CD3 Fab, or a derivative thereof.

In some embodiments, the CD3 inhibitor is, or is derived from, an anti-CD3 antibody (e.g. is a Fab, F(ab')₂, scFv, or sdAb) wherein the antibody comprises one or more CDR regions, selected from SEQ ID NOs: 114-119, or variants thereof. In other words, in some embodiments the CD3 inhibitor comprises one or more CDR regions, selected from SEQ ID NOs: 114-119, or variants thereof.

In some embodiments, the CD3 inhibitor comprises:

- (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 114 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 115 or a variant thereof, and/or a HCDR3 having an amino acid sequence of SEQ ID NO: 116 or a variant thereof; and/or
- 5 (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 117 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 118 or a variant thereof, and/or a LCDR3 having an amino acid sequence of SEQ ID NO: 119 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises:

- 10 (i) a HCDR1 having an amino acid sequence of SEQ ID NO: 114 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 115 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 116 or a variant thereof; and/or
- (ii) a LCDR1 having an amino acid sequence of SEQ ID NO: 117 or a variant thereof,
- 15 a LCDR2 having an amino acid sequence of SEQ ID NO: 118 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 119 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at

20 least 99% identity, or 100% identity to SEQ ID NO: 120.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 121.

25 In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 120, and the light chain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least

30 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 121.

In some embodiments, the CD3 inhibitor comprises or consists of:

(i) a heavy chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 120, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 114 or a variant thereof, a HCDR2 having an amino acid sequence of SEQ ID NO: 115 or a variant thereof, and a HCDR3 having an amino acid sequence of SEQ ID NO: 116 or a variant thereof; and/or

(ii) a light chain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 121, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 117 or a variant thereof, a LCDR2 having an amino acid sequence of SEQ ID NO: 118 or a variant thereof, and a LCDR3 having an amino acid sequence of SEQ ID NO: 119 or a variant thereof.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 122 or 123.

In some embodiments, the CD3 inhibitor comprises or consists of a light chain, wherein the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 124.

In some embodiments, the CD3 inhibitor comprises or consists of a heavy chain and a light chain, wherein the heavy chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 122, and the light chain comprises or consists of an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity or 100% identity to SEQ ID NO: 124.

In some embodiments, the CD3 inhibitor comprises or consists of:

(i) a heavy chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 122, wherein the amino acid sequence comprises a HCDR1 having an amino acid sequence of SEQ ID NO: 114, a HCDR2 having an amino acid sequence of SEQ ID NO: 115, and a HCDR3 having an amino acid sequence of SEQ ID NO: 116; and/or

(ii) a light chain comprising or consisting of an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 124, wherein the amino acid sequence comprises a LCDR1 having an amino acid sequence of SEQ ID NO: 117, a LCDR2 having an amino acid sequence of SEQ ID NO: 118, and a LCDR3 having an amino acid sequence of SEQ ID NO: 119.

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Name	Example sequence
Muromonab-CD3 HCDR1	RYTMH (SEQ ID NO: 114)
Muromonab-CD3 HCDR2	YINPSRGYTNYNQKFKD (SEQ ID NO: 115)
Muromonab-CD3 HCDR3	YYDDHYCLDY (SEQ ID NO: 116)
Muromonab-CD3 LCDR1	SASSSVSYMN (SEQ ID NO: 117)
Muromonab-CD3 LCDR2	DTSKLAS (SEQ ID NO: 118)
Muromonab-CD3 LCDR3	QQWSSNPFT (SEQ ID NO: 119)
Muromonab-CD3 VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLE WIGYINPSRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSA VYYCARYYDDHYCLDYWGQGTTLTVS (SEQ ID NO: 120)
Muromonab-CD3 VL	QIVLTQSPAIMASAPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRW IYDTSKLASGVPAPFRGSGSGTSYSLTISGMEADAATYYCQQWSS NPFTFGSGTKLEIN (SEQ ID NO: 121)
Muromonab-CD3 VH + CH1	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLE WIGYINPSRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSA VYYCARYYDDHYCLDYWGQGTTLTVSSAKTTAPSVYPLAPVCGGTT GSSVTLGCLVKGYFPEPVTLTWNHSGSLSSGVHTFPAVLQSDLYTSL SSVTVTSSSTWPSQSITCNVAHPASSTKVDKKI (SEQ ID NO: 122)
Muromonab-CD3 heavy chain	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLE WIGYINPSRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSA VYYCARYYDDHYCLDYWGQGTTLTVSSAKTTAPSVYPLAPVCGGTT GSSVTLGCLVKGYFPEPVTLTWNHSGSLSSGVHTFPAVLQSDLYTSL SSVTVTSSSTWPSQSITCNVAHPASSTKVDKKIEPRPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 123)
Muromonab-CD3 light chain	QIVLTQSPAIMASAPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRW IYDTSKLASGVPAPFRGSGSGTSYSLTISGMEADAATYYCQQWSS NPFTFGSGTKLEINRADTAPTVISIFPPSSEQLTSGGASVVCFLNMF YPKDINVKKIDGSRQNGVLNSWTDQDSKDSTYSMSSTLTTLTKDE YERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO: 124)

Other anti-CD3 antibodies

Other suitable anti-CD3 antibodies, and fragments and/or derivatives thereof, are known in the art.

In some embodiments, the CD3 inhibitor is T3/4.A, or a fragment and/or derivative thereof. In some embodiments, the CD3 inhibitor is a T3/4.A Fab, a T3/4.A F(ab')₂, a T3/4.A scFv, a T3/4.A sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a T3/4.A Fab, or a derivative thereof.

- 5 T3/4.A (also known as CLB-T3/4.A) is a murine IgA mAb specific for human CD3. T3/4.A is non-mitogenic for human peripheral blood mononuclear cells (PBMC) *in vitro*. The mAb was developed as a switch variant antibody, derived from an originally IgG1-producing hybridoma (see e.g. Meijer, R.T., et al., 2003. *Clinical & Experimental Immunology*, 133(3), pp.485-492).

In some embodiments, the CD3 inhibitor is YTH12.5, or a fragment and/or derivative thereof.

- 10 In some embodiments, the CD3 inhibitor is a YTH12.5 Fab, a YTH12.5 F(ab')₂, a YTH12.5 scFv, a YTH12.5 sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a YTH12.5 Fab, or a derivative thereof.

YTH12.5 a rat CD3 antibody which has been humanized to provide a aglycosylated IgG1 antibody (e.g. CAMPATH 3) (see e.g. Friend, P.J., et al., 1999. *Transplantation*, 68(11), pp.1632-1637).

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In some embodiments, the CD3 inhibitor is HuM291, or a fragment and/or derivative thereof. In some embodiments, the CD3 inhibitor is a HuM291Fab, a HuM291F(ab')₂, a HuM291scFv, a HuM291sdAb, or a derivative thereof. In some embodiments, the CD3 inhibitor is a HuM291Fab, or a derivative thereof.

- 20 HuM291 is a humanized IgG2 antibody that was engineered to have less mitogenic activity in humans compared with murine anti-CD3 antibodies (see e.g. Norman, D.J., et al., 2000. *Transplantation*, 70(12), pp.1707-1712).

Other CD3 inhibitors

- Other suitable CD3 inhibitors include CD3 T-cell engagers, CD3-specific DNA aptamers, and
25 CD3-specific RNA aptamers (see e.g. Menon, A.P., et al., 2023. *Cancers*, 15(4), p.1189).

CD3 T-cell engagers

In some embodiments, the CD3 inhibitor is a CD3 T-cell engager or a fragment and/or derivative thereof.

- T-cell engagers are designed to bind to a target antigen expressed on a cancer cell and to a
30 trigger molecule on T cells, such as CD3. Typically, T-cell engagers are based on bispecific monoclonal antibodies (bsAbs). A bispecific T-cell engager (BiTE) is a sub-class of T-cell

engaging bsAbs with promising clinical results in the treatment of cancers (see e.g. Huehls, A.M., et al., 2015. Immunology and cell biology, 93(3), pp.290-296; Zhou, S., et al., 2021. Biomarker Research, 9(1), p.38; and Tian, Z., et al., 2021. Journal of Hematology & Oncology, 14(1), pp.1-18). Examples of CD3 T-cell engagers include blinatumomab, tebentafusp, talquetamab, mosunetuzumab, glofitamab, epcoritamab, odronextamab, teclistamab, cevostamab, and lotetuzumab.

In some embodiments, the CD3 inhibitor is a CD3-binding fragment from a CD3 T-cell engager, or a derivative thereof. For example, the CD3-binding fragment may be a single-chain variable fragment (scFv).

10 *CD3-specific aptamers*

In some embodiments, the CD3 inhibitor is a CD3-specific aptamer. In some embodiments, the CD3 inhibitor is a CD3-specific DNA aptamer. In some embodiments, the CD3 inhibitor is a CD3-specific RNA aptamer.

A DNA aptamer selected against the human CD3 ϵ complex by ligand-guided selection (LIGS) has been described and US. Patent Application No. 17/629,943 describes RNA aptamers selected against recombinant CD3 ϵ/γ and CD3 ϵ/δ proteins (see e.g. Menon, A.P., et al., 2023. Cancers, 15(4), p.1189).

Example nucleotide sequences

The CD3 inhibitor may be encoded by any suitable nucleotide sequence. In some embodiments, the nucleotide sequence is codon-optimised, for example codon-optimised for expression in humans.

In preferred embodiments, the CD3 inhibitor is an anti-CD3 antigen-binding fragment (Fab). Suitably, a nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a heavy chain (e.g. VH + CH1); a linker sequence; a second signal sequence; and a nucleotide encoding a light chain. Suitably, a nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a light chain; a linker sequence; a second signal sequence; and a nucleotide encoding a heavy chain (e.g. VH + CH1).

Heavy chains and light chains

The nucleotide encoding a heavy chain (e.g. VH + CH1) and the nucleotide encoding a light chain may encode any heavy chain (e.g. VH + CH1) and light chain combination described

herein. For example, the nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3':

- 5 (a) a first signal sequence; a nucleotide encoding a foralumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a foralumab light chain, or a derivative thereof;
- (b) a first signal sequence; a nucleotide encoding a teplizumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a teplizumab light chain, or a derivative thereof;
- 10 (c) a first signal sequence; a nucleotide encoding a visilizumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a visilizumab light chain, or a derivative thereof;
- (d) a first signal sequence; a nucleotide encoding an otelexizumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an otelexizumab light chain, or a derivative thereof;
- 15 (e) a first signal sequence; a nucleotide encoding a muromonab-CD3 heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a muromonab-CD3 light chain, or a derivative thereof;
- (f) a first signal sequence; a nucleotide encoding a foralumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a foralumab heavy chain (e.g. VH + CH1), or a derivative thereof;
- 20 (g) a first signal sequence; a nucleotide encoding a teplizumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a teplizumab heavy chain (e.g. VH + CH1), or a derivative thereof;
- (h) a first signal sequence; a nucleotide encoding a visilizumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a visilizumab heavy chain (e.g. VH + CH1), or a derivative thereof;
- 25 (i) a first signal sequence; a nucleotide encoding an otelexizumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding an otelexizumab heavy chain (e.g. VH + CH1), or a derivative thereof; or

(j) a first signal sequence; a nucleotide encoding a muromonab-CD3 light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a muromonab-CD3 heavy chain (e.g. VH + CH1), or a derivative thereof.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a foralumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a foralumab light chain, or a derivative thereof. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a foralumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a foralumab heavy chain (e.g. VH + CH1), or a derivative thereof.

In some embodiments, the nucleotide sequence encoding a foralumab heavy chain (e.g. VH + CH1) comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 125. In some embodiments, the nucleotide sequence encoding a foralumab heavy chain (e.g. VH + CH1) comprises or consists of SEQ ID NO: 125.

caggtgcagttggtggagagcggcgggcggtggtgcagccggcgagcctgcggctgtc
 ttgtgccgctagcggattcaagttctctggctacggcatgcactgggtgcgccaggcacctg
 gcaagggcctggagtggtggcctgatctggtacgacggctccaagaaatattacgtggat
 tctgtgaaggagcgtttacaatctcccgcgacaatagcaagaacaccctgtatctgcagat
 gaactccctgagggccgaggacacagccgtgtattactgcgcccggcagatgggctactggc
 actttgatctgtggggcagaggcaccctggtgacagtgtctagcgccagcaccaaggggct
 agcgtgttccccctggccccctcttccaagtcaacctccgggggcaccgcccctgggctg
 cctggtgaaagactattttctgagcctgtgacagtgtcttggaaactccggggccctgactt
 ctggcgtgcatactttccagcagtgctgcagtcctccgggctgtactctctgagctccgtg
 gtgactgtgcctagcagctctctggggacacagacctacatctgtaatgtgaaccacaaacc
 cagcaataccaaggtggacaagagagtgtaga

Example nucleotide sequence encoding a foralumab heavy chain (VH + CH1) (SEQ ID NO: 125)

In some embodiments, the nucleotide sequence encoding a foralumab light chain comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 126. In some embodiments, nucleotide sequence encoding a foralumab light chain comprises or consists of SEQ ID NO: 126.

gagattgtgctgacccagagcccagctaccctgagcctgtccccggcgagcggggccacact
 gagctgcagagcctcacagagcgtgtcctcctacctggccttggtaccagcagaagccaggcc
 aggcctcctagactgctgatttacgacgcctcaaacagagccaccggcattccccgaagattc
 agcggctctggatctgggacagacttcacactgaccattagcagcctggagcccaggattt

5 tgccgtgtattactgtcagcagagatcaaactggccacctctgacatttggcggggggacca
 aggtggaaattaaaagaaccgtggccgcccccaagcgtgttcattctttccccccagcgacgag
 cagctgaaatccgggactgcctctgtggtgtgcctgctgaacaacttttaccgaagagaagc
 caaggtgcagtggaaggtggacaatgccctgcagagtggaaattcccaggagtctgtgaccg
 agcaggatagcaaagacagcacatactcactgagctccaccctgaccctgagcaaggccgac
 tacgagaagcacaaggtgtacgcctgcgaggtgacccaccagggcctgagcagtcctgtgac
 caagtcatttaatatagaggcagtggtga

Example nucleotide sequence encoding a foralumab light chain
 (SEQ ID NO: 126)

- 10 In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab may comprise
 from 5' to 3': a first signal sequence; a nucleotide encoding a teplizumab heavy chain (e.g. VH
 + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide
 encoding a teplizumab light chain, or a derivative thereof. In some embodiments, the
 nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal
 15 sequence; a nucleotide encoding a teplizumab light chain, or a derivative thereof; a linker
 sequence; a second signal sequence; and a nucleotide encoding a teplizumab heavy chain
 (e.g. VH + CH1), or a derivative thereof.

- In some embodiments, the nucleotide sequence encoding a teplizumab heavy chain (e.g. VH
 + CH1) comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at
 20 least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%,
 at least 99% identity, or 100% identity to SEQ ID NO: 127. In some embodiments, nucleotide
 sequence encoding a teplizumab heavy chain (e.g. VH + CH1) comprises or consists of SEQ
 ID NO: 127.

25 caggtgcagctggtgcagagcggcgggcggtggtgcagcctggcaggtctctgagactgtc
 ctgcaaggccagcgggtacaccttcacccggtacacatgcattgggtgcgccaggcaccag
 gaaaggggctggagtggatcggtacatcaaccctcaagaggggtacaccaactacaatcag
 aaggtgaaggaccggttcacatctccagagacaactccaagaataccgccttcctgcagat
 ggactccctccggccagaggataccggcggtgacttttgcgccagggtactatgatgatcact
 actgcctggattactggggccaggggacccctgtgacagtggtcaagcgccctctaccaaaggc
 30 ccctcagtggtccactggccccaaagcagcaagtctaccagcggcggcaccgcccgtctggg
 gtgcctggtgaaggactacttccccgaaccggtgacccgtgagctggaactccggtgccctga
 catctggggtgcacacattcccagccgtgctgcagctctagcggcctgtatagcctgagctct
 gtggtgactgtgccaagctcctccctggggacccagacctacatttgtaacgtgaaccataa
 accatctaacaccaaagtggacaagaagtgtag

- 35 Example nucleotide sequence encoding a teplizumab heavy chain
 (VH + CH1) (SEQ ID NO: 127)

- In some embodiments, the nucleotide sequence encoding a teplizumab light chain comprises
 or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least
 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity,
 40 or 100% identity to SEQ ID NO: 128. In some embodiments, nucleotide sequence encoding a
 teplizumab light chain comprises or consists of SEQ ID NO: 128.

gacatccagatgacccagtcctccagcctgagcgctctgtgggcgaccgggtgacaat
 tacatgttcagcctcatccagcgtgagctatatgaattggtaccagcagacccaggcaagg
 cccctaagagatggatctacgacaccagcaagctggcctctggcgtgcctagtcggttcagc
 5 gggtcgggctccggaaccgattacaccttcaccatctcatcactgcagcctgaggacatcg
 cacctactactgccagcagtggtcttctaaccattcactttcggccagggcaccaagctgc
 agatcacacgcaccgtggcggcccttctgtgttcattcttcccaccttccgatgaacagctg
 aagtcgggcacagccagcgtggtgtgcctgctgaacaacttctatcccaggaggccaaggt
 gcagtggaagtggataatgcactgcagagcggcaactcccaggagagcgtgaccgagcaag
 10 actctaaggactctacttattctctgtcatccaccctgaccctgagcaaggccgactacgag
 aagcacaagtgtacgcctgcgaagtgcacacaccaggggctgagcagcctgtgaccaagag
 cttcaatagaggagaatgctga

Example nucleotide sequence encoding a teplizumab light chain
 (SEQ ID NO: 128)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab may comprise
 15 from 5' to 3': a first signal sequence; a nucleotide encoding a visilizumab heavy chain (e.g. VH
 + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide
 encoding a visilizumab light chain, or a derivative thereof. In some embodiments, the
 nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal
 sequence; a nucleotide encoding a visilizumab light chain, or a derivative thereof; a linker
 20 sequence; a second signal sequence; and a nucleotide encoding a visilizumab heavy chain
 (e.g. VH + CH1), or a derivative thereof.

In some embodiments, the nucleotide sequence encoding a visilizumab heavy chain (e.g. VH
 + CH1) comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at
 least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%,
 25 at least 99% identity, or 100% identity to SEQ ID NO: 129. In some embodiments, the
 nucleotide sequence encoding a visilizumab heavy chain (e.g. VH + CH1) comprises or
 consists of SEQ ID NO: 129.

cagggtgcagctggtgcagagcggcgccgaagtgaagaagccaggagcctccgtgaaggtgtc
 ttgtaaagcctcaggttatactttcatctcatataccatgcactgggtgagacaggcccccg
 30 ggcagggccttgagtggatgggatacatataaccctcgctccggctacacccattataatcag
 aagctgaaggacaaggccaccctgaccgctgacaagagcgcctccactgcctatatggagct
 gagcagcctgcggtccgaagacaccgccgtgtactactgtgccaggtctgcctactacgact
 acgatgggtttgcctactggggccagggcacactggtgacagtgcagcgcgcagcaccaag
 ggccccagcgtgtttccactggccccctgcagcaggagcacctccgagagcaccgcccgtct
 35 gggatgcctcgtgaaggactatttccccgagcccgtagcagtgcagctggaactccggagcac
 tgacctccggcgtgcatacctttcccgccgtgctgcagagcagcggcctgtattccctgagc
 agcgtgggtgaccgtgcctagcagcaatttcgggacacagacatacacatgtaacgtggatca
 taagccttcaaacactaaggtggacaagacagtgtaa

Example nucleotide sequence encoding a visilizumab heavy chain
 40 (VH + CH1) (SEQ ID NO: 129)

In some embodiments, the nucleotide sequence encoding a visilizumab light chain comprises
 or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least

85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 130. In some embodiments, nucleotide sequence encoding a visilizumab light chain comprises or consists of SEQ ID NO: 130.

5 gatattcagatgacacagagccctagctccctgagcgccctccgtgggggacagagtgacaat
 cacatgcagcgcttcctcctcgtgtcttacatgaattggtaccagcagaagcccggaagg
 ctccaagaggctgatctacgacaccagcaaaactggcctctggcgtgccctctcgctttagc
 ggctccgggtccggcaccgacttcacactgaccatctcttctctgcaaccggaggatTTTgc
 cacttactactgtcagcagtggtcctccaacccccccactttcggcggaggcaccaaggtgg
 10 agatcaagcggacagtggtgctgccccagcgttttcatcttcccaccaagcgacgagcagctg
 aaaagcggcagcagctagcgtggtgtgcctgctgaataacttctacccccggaggccaaggt
 gcagtggaaggtggataacgccctgcagtcgggaacagccaggagtcagtgaccgagcagg
 actctaaggattccacctacagcctgagcagcaccctgaccctgtccaaagcagactacgag
 aaacacaaggtgtacgcctgcgaggtgacacaccagggcctgtcttcccccgtagccaagtc
 cttcaatcggggcgagtgtga
 15 Example nucleotide sequence encoding a visilizumab light chain
 (SEQ ID NO: 130)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a oteixizumab heavy chain (e.g. VH + CH1), or a derivative thereof; a linker sequence; a second signal sequence; and a
 20 nucleotide encoding a oteixizumab light chain, or a derivative thereof. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab may comprise from 5' to 3': a first signal sequence; a nucleotide encoding a oteixizumab light chain, or a derivative thereof; a linker sequence; a second signal sequence; and a nucleotide encoding a oteixizumab heavy chain (e.g. VH + CH1), or a derivative thereof.

25 In some embodiments, the nucleotide sequence encoding a oteixizumab heavy chain (e.g. VH + CH1) comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 131. In some embodiments, the
 30 nucleotide sequence encoding a oteixizumab heavy chain (e.g. VH + CH1) comprises or consists of SEQ ID NO: 131.

35 gaggtgcagctgctggagagcggagggggcctgggtgcagccaggtggctctctgaggtgtc
 atgcgctgccagtggttcaccttctccagctttcctatggcctgggtgcggcaggcccccg
 gcaagggctctggaatgggtgagcaccatcagccgagataattccaaaaacacactctacctgcagat
 agcgtgaagggcagattcaccatcagccgagataattccaaaaacacactctacctgcagat
 40 gaatagcctgagagctgaagatactgccgtgtactactgcgccaagttccggcagtagtccg
 gaggatttgattattggggccagggcaccctgggtgacagtgctcctcagccagcaccgaagggc
 ccagcgtgtttccctggccccagctctaagtccacctcaggcggcaccgcccctggg
 gtgcctgggtgaaggactatttccagaacctgtgacagtgagctggaattccggagctctca
 ccagcggcgtgcacacatttccctgccgtgctgcagagcagcggactgtatagtctgagttct
 gtggtgaccgtgccctcctcctccctggggcaccagacctatatctgtaacgtgaaccacaa
 acccagcaacaccaaagtggacaagaaggtgtga

Example nucleotide sequence encoding a otelixizumab heavy chain (VH + CH1) (SEQ ID NO: 131)

In some embodiments, the nucleotide sequence encoding a otelixizumab light chain comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 132. In some embodiments, nucleotide sequence encoding a otelixizumab light chain comprises or consists of SEQ ID NO: 132.

```

10      gacatccagctgacacagcctaatagtgtgtctacctctctggggagcaccgtgaagctgtc
      ctgcaccctgtctagcggcaacatcgaaaaacaactacgtgcactggtaccagctgtacgagg
      gcagaagcccaaccaccatgatttatgatgacgataagcggcctgatggcgtgcccagaccgg
15      ttcagcggcagcattgacagatcctccaacagtgcctttctgaccatccacaacgtggctat
      cgaggacgaggccatttacttttgccactcctatgtgagctctttcaatgtcttcggaggcg
      ggaccaagctgaccgtgctgagacagccaaaagccgccccttcctgaccctgttcccaccc
      tccagcgaggaactgcaggccaataagccacactggtgtgctgatctccgacttctatcc
20      cggagccgtgaccgtcgcttggaaggccgacagcagccccgtcaaagccggcgtggagacaa
      ccacccctccaaacagagcaataataagtatgcagccagcagctatctgtctctgacgccc
      gagcagtgggaagagccacagaagctattcctgcccaggtgacacacgagggcagcacagtga
      gaagaccgtggccccacagagtgtagctga

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Example nucleotide sequence encoding a otelixizumab light chain (SEQ ID NO: 132)

Example anti-CD3 Fab sequences

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino acid sequence comprising or consisting of from 5' to 3': an amino acid sequence having at least 70% identity to SEQ ID NO: 1, an amino acid sequence having at least 70% identity to SEQ ID NO: 76, an amino acid sequence having at least 70% sequence identity to any of SEQ ID NOs: 6-9, an amino acid sequence having at least 70% identity to SEQ ID NO: 1, and an amino acid sequence having at least 70% identity to SEQ ID NO: 77.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 133. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes the amino acid sequence of SEQ ID NO: 133.

```

35      MATGSRTSLLLAFLGLLCLPWLQEGSAQVQLVESGGGVVQPGRSRLRLSCAASGFKFSGYGMHW
      VRQAPGKGLEWVAVIWDGSKKYYVDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAR
      QMGYWHFDLWGRGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTISWN
      SGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKRRSG
      SGATNFSLLKQAGDVEENPGPMATGSRTSLLLAFLGLLCLPWLQEGSAEIVLTQSPATLSLSP
      GERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRTGIPARFSGSGSGTDFTLTISS
      LEPEDFAVYYCQQRSNWPPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNN

```

FYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNREGC

Example foralumab Fab amino acid sequence (SEQ ID NO: 133)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or
5 consists of from 5' to 3': a nucleotide sequence having at least 70% identity to any of SEQ ID
NOs: 2-5, a nucleotide sequence having at least 70% identity to SEQ ID NO: 125, a nucleotide
sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, a nucleotide
sequence having at least 70% identity to any of SEQ ID NOs: 2-5, and a nucleotide sequence
having at least 70% identity to SEQ ID NO: 126.

10 In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or
consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least
85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity,
or 100% identity to SEQ ID NO: 134. In some embodiments, the nucleotide sequence
encoding an anti-CD3 Fab comprises or consists of SEQ ID NO: 134.

15 atggctaccggctctaggacctcactgctgctggccttcggcctgctgtgcctgccctggct
gcaggagggcagcgcccaggctccagctgggtggagagcggcgggcgctgggtgcagcccggca
gaagcctgcggctgagctgtgccgccagcggattcaagtttagcggctacgggatgcactgg
gtcaggcaggctcctgaaaggactggagtgggtggcagtgatctggatatgatggctccaa
gaaatactacgtcgacagcgtgaaaggacgcttcaccatcagcagggataacagcaagaata
20 cactgtacctgcagatgaacagcctgagagcagaggatacagccgtgtactactgcgccagg
cagatgggctactggcacttcgacctatgggggaggggtacactgggtgacctgagctccgc
atccaccaaagggccctctgtgtccccctggccccctccagcaagtcacctctggaggaa
ccgctgccctgggctgtctggtgaaagattatttccagagcctgtcacctgtcttggaac
tccggcgctctgacatccggcggtgcacacattccccgcgctgctgcagagctctggcctcta
25 cagcctgtcttctgtggtgacagtgccagctccagcctggggaccagacctatatctgca
acgtgaaccacaagccatctaacaccaaagtggataaacgggtgaggaagaggaggtccggc
tccggcgccacaaacttttctctgtgaagcaggccggggacgtggaggagaacccgggcc
aatggccaccggttctagaaccagcctgctgctggccttggcctgctgtgcctgccttggc
tgcaggagggcagcgtgaaattgtgctgacacagctctccgccaccctgtccctgtcccc
30 ggcgagagggccacactgagctgtagggccagccagtcgctgagcagctatctggcctggta
ccagcagaaacccggacaggccccaagactgctgatttatgacgcctccaacagagctacgg
gcatccccgctcgtctttccggcagcgggaagcgggacagacttactctgaccattagctca
ctggaacccgaggacttcgctgtgtattattgtcagcaacgcagcaactggccccctctgac
cttcgggggggaacaaaagtggagattaaaagaaccgtggcgcctccttccgtgttcattct
35 ttcctccatccgatgagcagctgaagtccggtacagccagcgttgtgtgcctgctgaacaac
ttttatccccgggaggctaaagtgcagtggaagtggacaatgccctgcagagcggaaatag
ccaggagtcagtgaccgaacaggatagcaaggattccacctacagcctgagcagcacctga
cactgtctaaagccgactatgagaagcacaaggtgtatgcctgcgaggtgactcaccagggc
ctgagttctcccgtagcaagtcctttcaacaggggagaatgctga

40 Example foralumab Fab nucleotide sequence (SEQ ID NO: 134)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino
acid sequence comprising or consisting of from 5' to 3': an amino acid sequence having at
least 70% identity to SEQ ID NO: 1, an amino acid sequence having at least 70% identity to

SEQ ID NO: 87, an amino acid sequence having at least 70% sequence identity to any of SEQ ID NOs: 6-9, an amino acid sequence having at least 70% identity to SEQ ID NO: 1, and an amino acid sequence having at least 70% identity to SEQ ID NO: 88.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 135. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes the amino acid sequence of SEQ ID NO: 135.

```

10      MATGSRTSLLLAFLGLLCLPWLQEGSAQVQLVQSGGGVVQPGRLRLSCKASGYTFTRYTMHW
      VRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNKNTAFLQMDSLRPEDTGVYFCAR
      YYDDHYCLDYWGQGTPTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
      NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVRKRRS
      GSGATNFSLLKQAGDVEENPGPMATGSRTSLLLAFLGLLCLPWLQEGSADIQMTQSPSSLSAS
15      VGDRVITITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLAGVPSRFRSGSGSGTDYTFITIS
      LQPEDIATYYCQQWSSNPFTFGQGTKLQITRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNE
      YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGL
      SSPVTKSFNRGEC

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Example teplizumab Fab amino acid sequence (SEQ ID NO: 135)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of from 5' to 3': a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, a nucleotide sequence having at least 70% identity to SEQ ID NO: 127, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 128.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 136. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of SEQ ID NO: 136.

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30      atggctaccggctccagaacctccctgctgctggctttcggcctgctgtgtctgccttggt
      gcaggaaggctccgcccaggtgcagctggtgcagtcggaggaggagtggtgcagcccgga
      gatcccttcgcctgagctgcaaggcttctggctacacattcaccaggtacaccatgcactgg
      gtgagacaggcccccggaaaggcctggagtggttggtatattaacccagcaggggata
      caccaattacaatcagaaggtcaaggacaggttcaccatcagcagggacaacagtaagaata
35      cagcattcctgcagatggacagcctgagaccagaggatacaggcgtgtatTTTTTgcgccagg
      tattacgatgaccactactgtctggactactgggggcagggaacaccagtgaccgtgagctc
      cgccagcacaaggccctctgtgttccactggccccctccagcaagagcacctccggcg
      gaaccgcccgcctgggctgtctggtgaaggactactttcctgaacctgtgacagtcagctgg
      aactcaggcgccctgaccagcggagtgcataccttccccgccgtgctgcagagctctggcct
40      gtactccctgagcagcgtggtcacagtgcccagcagcagcctgggcacacagacttatact

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gcaacgtgaaccacaagcctagcaacactaaggctcgataaaaaggctcagaaagagaagatca
 ggcagcggcgctaccaacttcagcttgctgaagcaggcggcgacgtggaggagaaccccg
 cccaatggcaaccggctcaaggacttcctgctcctggctttcgggctgctgtgtctgcct
 ggctgcaggaggggagcgccgacatccagatgaccagctctccatcttcctgagcgccagc
 5 gtgggagatcgggtcacaatcacatgttctgccagctccagcgtgagctacatgaattggt
 ccagcagacccccggcaaggctcccaaaagggtggatttacgacacctcaaagctggcaagcg
 gagttccctctcggttctccggcagcggatccggaaccgactacaccttcacaatcagctcc
 ctgcagcctgaagacatcgctacatattactgccagcagtgaggcagcaaccctttcacctt
 cggccagggcacaagctgcagatcaccaggaccgtggctgcccctagcgtgttcaccttcc
 10 cccctcagatgagcagctcaagagcggcacagcctcagtggtgtgctcctcctgaacaatttc
 taccacagagaagcaaaggctgcagtggaagggtggacaatgcctgcagtcgggcaactctca
 ggagcgtgacagaacaggacagtaaaagactccacatatagcctgtctagcaccctgaccc
 tgtccaaggccgactacgagaagcacaagggtctacgcttgaggtgacacaccaagggtg
 tcttccctgtcaccaagtcatttaataagaggcgaatgctga

15 Example teplizumab Fab nucleotide sequence (SEQ ID NO: 136)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino acid sequence comprising or consisting of from 5' to 3': an amino acid sequence having at least 70% identity to SEQ ID NO: 1, an amino acid sequence having at least 70% identity to SEQ ID NO: 98, an amino acid sequence having at least 70% sequence identity to any of SEQ ID NOs: 6-9, an amino acid sequence having at least 70% identity to SEQ ID NO: 1, and an amino acid sequence having at least 70% identity to SEQ ID NO: 99.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 137. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes the amino acid sequence of SEQ ID NO: 137.

MATGSRTSLLLAFLGLCLPWLQEGSAQVQLVQSGAEVKKPGASVKVSKASGYTFISYTMHW
 VRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCAR
 SAYYDYDGFAYWGQGTLLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPTVS
 30 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSNFGTQTYTCNVDPKPSNTKVDKTVRKR
 SGSGATNFSLLKQAGDVEENPGPMATGSRTSLLLAFLGLCLPWLQEGSADIQMTQSPSSLSA
 SVGDRVTITCSASSSVSYMNWYQQKPKAPKRLIYDTSKLAGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQWSSNPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNN
 FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQG
 35 LSSPVTKSFNRGEC

Example visilizumab Fab amino acid sequence (SEQ ID NO: 137)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of from 5' to 3': a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, a nucleotide sequence having at least 70% identity to SEQ ID NO: 129, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 130.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 138. In some embodiments, the nucleotide sequence

5 encoding an anti-CD3 Fab comprises or consists of SEQ ID NO: 138.

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10  atggccaccggtctctcgacctccctgctgctggccttcggcctgctgtgtctgcccctggct
    ccaggaaggctccgcccaggtgcagctgggtgcagtctggggccgaagtgaagaaacccggcg
    ccagcgtaaggtgagctgcaaggcctctggctacaccttcacagctacaccatgcactgg
    gtgagacaggcccccgccagggaactggaatggatgggggtatatcaacccaaggtctgggta
15  caccactacaaccagaaactgaaagataaggccacgctgaccgcccagataagtctgccagca
    ccgctacatggagctgagtagcctgcggtcagaggataaccgcccgtgtactactgcgccaga
    tctgcctattacgactacgacgggttgcctactggggacaggggaactggtgaccgtgag
    ctctgcttctacgaaggggccatccgtgttcccactggccccctgcagccgcagcaccagcg
    agtccaccgcccgcctgggatgcctgggtgaaagactactttccagagcctgtgaccgtgtcc
20  tgggaattccggcgcccttaccagcggagtgcacacctttccagccgttctgcagtccagcgg
    cctgtactctctgtctagcgtggtagccgtgccagctccaattttggcaccagacctaca
    cctgcaacgtggaccacaagccatccaacactaaagtcgataagactgtgcgcaagagaaga
    agcggcagcggcgccaccaatttttccctgctgaagcaggctggagacgtcgaggagaaccc
    agggcctatggctaccggcagcggacttccctgctgctggcctttgggctgctgtgcctgc
25  cctggctccaggagggcagcgcgatataccagatgactcagtcctccatcctcactgtccgcc
    agcgtgggagatcgggtgactatcacatgtagcgcgaagcagcagcgtgagctatatgaactg
    gtatcagcagaagccaggaaaggcccaagaggtgatctatgacacctccaaactggcca
    gtggcgtgccttcacgtttctccgatctggctctgggaccgacttcaccctgacaatctcc
    agcctgcagccagaggatttcgctacttactactgccagcagtggaagctctaaccaccaac
30  cttcggcgcgccgacccaaggtggagatcaagagaacgctggccgccccatccgtgttcattt
    tccctccctctgacgagcagctgaagagcggcaccgcaagtgtggtgtgcctcctgaataat
    ttctaccctcgcgaagccaaggtgcagtgggaaggtggacaacgcctgcagtccggcaatag
    ccaggagtccgtgaccgaacaggacagcaaggactccacatactcctgagcagcacctga
    cactgagcaaggctgactacgagaaacacaaggtctatgcctgcgaggtgacccatcagggc
    ctgtcttcccctgtgacaaaaagctttaacagaggcgaatgttga

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Example visilizumab Fab nucleotide sequence (SEQ ID NO: 138)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino acid sequence comprising or consisting of from 5' to 3': an amino acid sequence having at least 70% identity to SEQ ID NO: 1, an amino acid sequence having at least 70% identity to

35 SEQ ID NO: 109, an amino acid sequence having at least 70% sequence identity to any of SEQ ID NOs: 6-9, an amino acid sequence having at least 70% identity to SEQ ID NO: 1, and an amino acid sequence having at least 70% identity to SEQ ID NO: 110.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at

40 least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 139. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab encodes the amino acid sequence of SEQ ID NO: 139.

5 MATGSRTSLLLAFLGLLCLPWLQEGSAEVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMW
VRQAPGKGLEWVSTISTSGGRYYRDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAK
FRQYSGGFDYWGGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVTVFPPSSSLGTQTYICNVNHKPSNTKVDKKVRKRRS
GSGATNFSLLKQAGDVEENPGPMATGSRTSLLLAFLGLLCLPWLQEGSADIQLTQPNVSTSL
GSTVKLSCTLSSGNIENNYVHWYQLYEGRSPTTMIYDDDKRPDGVPDFSGSIDRSSNSAFL
TIHNVAIEDEAIYFCHSYVSSFNVFGGGTKLTVLRQPKAAPSVTLFPPSSEELQANKATLVC
LISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVT
HEGSTVEKTVAPTECS

10 Example oteelixizumab Fab amino acid sequence (SEQ ID NO: 139)

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of from 5' to 3': a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, a nucleotide sequence having at least 70% identity to SEQ ID NO: 131, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, a nucleotide sequence having at least 70% identity to any of SEQ ID NOs: 2-5, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 132.

In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 140. In some embodiments, the nucleotide sequence encoding an anti-CD3 Fab comprises or consists of SEQ ID NO: 140.

25 atggccaccgggtctaggaccagcctgctgctggccttcggcctgctgtgtctgacctggct
gcaggagggctccgccgaggtgcagctgctggaatcaggaggcggcctgggtgcagcctggag
gcagcctgcgcctgtcatgcgcagcatctgggttcaccttctcctctttccctatggcctgg
gtgagacaggcccccggaagggctctggagtggtgtctaccatttctacatctggaggccg
gacctactacagagatagcgtgaagggccggttcaccatttctagagacaattccaagaata
ccctgtacctgcaaatgaatagcctgagagccgaagacactgccgtgtattactgcgctaag
ttccgcagctactccggcggttcgactactggtggggccaggccaccctagtgcagctgcctc
cgccagcacaaaaggccccctccgtgtttccactggccccctcttccaagtctacttcaggag
30 gcacagccgccccgtggctgctggtgaaagattacttccctgagcccgtagccgtgtcctgg
aactcaggagccctgaccagcggtgcacaccttcccagccgtgctgcagtcctctggcct
gtacagcctgtccagcgtggtgactgtgccaagctccagcctgggcacccagacctacatct
gcaatgtgaaccacaagcctagcaacacaaaggtggacaagaaagtgagaaaaagaaggtct
ggctccggcgcgaccaacttctccctgctgaaacaggccggggacgtggaggagaaccctgg
35 cccaatggccacaggctctagaaccagcctgctgctggctttcggcctgctgtgcctgacct
ggctgcaggaaggctccgccgatatccagcttaccagcccaatagcgtgtccacgtcactg
ggttccacagtcaagctgagttgtactctgagcagtggaatattgagaataactacgtgca
ctggtatcagctgtacgagggtaggagccctacaacctgatctatgacgacgacaagcgcc
cagacggcgctgcctgacagattctccggctcaatcgacagatccagtaactccgccttctg
40 acaattcacaacgtggctattgaggacgaggccatttacttttgccactcctacgtgagcag
cttcaatgtgttcggcgggcgactaagctgaccgttctgcgccagcctaagccgctccaa
gctgacctgttccccccagcagcgagggaactgcaggccaacaaagccacctgggtgtgc
ctgatcagcgatttctaccccgggccgtgaccgttgctggaaggccgacagcagccctgt
gaaggccggggtggagaccacaacccccctccaagcagagcaacaacaagtacgccgcaagct
45 cctacctgagcctgactcccgagcagtggaatctcataggtcttacagttgccaggttaca
cacgaaggagcagcagtggaaaaaacagtgggccccaccgaatgctcctga

Example otelixizumab Fab nucleotide sequence (SEQ ID NO: 140)

Polynucleotides

The present invention provides polynucleotides comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and/or (b) a nucleotide sequence encoding a CD3 inhibitor.

- 5 The present invention provides polynucleotides comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor. The present invention also provides a combination of: (a) a polynucleotide comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a polynucleotide comprising a nucleotide sequence encoding a TNF inhibitor.

- 10 The present invention also provides polynucleotides comprising a nucleotide sequence encoding the CD3 inhibitor of the invention.

Polynucleotides of the invention may comprise DNA or RNA, preferably DNA. They may be single-stranded or double-stranded. Polynucleotides such as DNA polynucleotides may be produced recombinantly, synthetically or by any means available to those of skill in the art.

- 15 They may also be cloned by standard techniques. The polynucleotides may be isolated polynucleotides.

- Longer polynucleotides will generally be produced using recombinant means, for example using polymerase chain reaction (PCR) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking the target sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture with an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned
- 20
- 25 into a suitable vector.

The polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the in vivo activity or lifespan of the polynucleotides.

Vectors

- The present invention provides vectors comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and/or (b) a nucleotide sequence encoding a CD3 inhibitor.
- 30

The present invention provides vectors comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor. The present invention also provides a combination of: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a TNF inhibitor.

- 5 The present invention also provides vectors comprising a nucleotide sequence encoding the CD3 inhibitor of the invention.

A vector is a tool that allows or facilitates the transfer of an entity from one environment to another. The four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes.

- 10 The vectors of the present invention may be capable of transducing ocular cells (e.g. retinal ganglion cells, RPE cells, photoreceptors, glial cells). In some embodiments, the vectors of the present invention are capable of specifically transducing ocular cells.

- In some embodiments, a vector of the present invention is capable of transducing retinal cells. In some embodiments, a vector of the present invention is capable of specifically transducing
15 retinal cells. The retina is the multi-layered membrane, which lines the inner posterior chamber of the eye and senses an image of the visual world which is communicated to the brain via the optic nerve. In order from the inside to the outside of the eye, the retina comprises the layers of the neurosensory retina and retinal pigment epithelium, with the choroid lying outside the retinal pigment epithelium.

- 20 In some embodiments, a vector of the present invention is capable of transducing retinal ganglion cells. In some embodiments, a vector of the present invention is capable of specifically transducing retinal ganglion cells. A retinal ganglion cell is a type of neuron located near the inner surface of the retina of the eye.

- Suitable vectors for transducing ocular cells include viral vectors such as parvovirus vectors
25 (e.g. AAV vectors), lentivirus vectors, adenovirus vectors and also non-viral delivery systems (see e.g. Rodrigues, G.A., et al., 2019. Pharmaceutical research, 36(2), pp.1-20).

- A vector of the present invention may be a viral vector. A viral vector of the present invention is preferably an adeno-associated viral (AAV), although it is contemplated that other viral vectors may be used. In some embodiments, the viral vector is any of a parvoviral vector, an
30 adenoviral vector, a herpes simplex viral vector, an anelloviral vector, a retroviral vector, or a lentiviral vector.

A vector of the present invention may be in the form of a viral vector particle. In some embodiments, the viral vector is any of a parvoviral vector particle, an adenoviral vector particle, a herpes simplex viral vector particle, an anelloviral vector particle, a retroviral vector particle, or a lentiviral vector particle. Preferably, a viral vector of the present invention is in the form of an AAV vector particle.

Methods of preparing and modifying viral vectors and viral vector particles, such as those derived from AAV, are known in the art. Suitable methods are described in Ayuso, E., et al., 2010. *Current gene therapy*, 10(6), pp.423-436, Merten, O.W., et al., 2016. *Molecular Therapy-Methods & Clinical Development*, 3, p.16017; and Nadeau, I. and Kamen, A., 2003. *Biotechnology advances*, 20(7-8), pp.475-489.

Parvovirus vectors

A vector of the present invention may be a parvovirus vector. A vector of the present invention may be in the form of a parvovirus vector particle.

Parvoviruses, and especially the adeno-associated virus (AAV), provide a versatile platform for the rational design of human gene-therapy vectors. Typically, parvoviruses are composed of a small, non-enveloped capsid containing a single-stranded DNA genome. Suitably, the parvovirus vector is from the *Parvovirinae* subfamily, which includes *Dependoparvovirus*, *Protoparvovirus*, and *Bocaparvovirus*. A vector of the present invention may be a hybrid gene therapy vector based on parvoviruses (see e.g. Fakhiri, J. and Grimm, D., 2021. *Molecular Therapy*, 29(12), pp.3359-3382).

In some embodiments, a vector of the present invention is a dependoparvovirus vector. In some embodiments, a vector of the present invention is in the form of a dependoparvovirus vector. Some dependoparvoviruses are also known as adeno-associated viruses because they cannot replicate productively in their host cell without the cell being co-infected by a helper virus such as an adenovirus.

In preferred embodiments, a vector of the present invention is an adeno-associated viral (AAV) vector. In preferred embodiments, a vector of the present invention is in the form of an AAV vector particle.

AAV genomes

The AAV vector or AAV vector particle may comprise an AAV genome or a fragment or derivative thereof. An AAV genome is a polynucleotide sequence, which may encode functions needed for production of an AAV particle. These functions include those operating in the

replication and packaging cycle of AAV in a host cell, including encapsidation of the AAV genome into an AAV particle. Naturally occurring AAVs are replication-deficient and rely on the provision of helper functions in trans for completion of a replication and packaging cycle. Accordingly, the AAV genome of the AAV vector of the invention is typically replication-deficient.

The AAV genome may be in single-stranded form, either positive or negative-sense, or alternatively in double-stranded form. The use of a double-stranded form allows bypass of the DNA replication step in the target cell and so can accelerate transgene expression.

AAVs occurring in nature may be classified according to various biological systems. The AAV genome may be from any naturally derived serotype, isolate or clade of AAV.

AAV may be referred to in terms of their serotype. A serotype corresponds to a variant subspecies of AAV which, owing to its profile of expression of capsid surface antigens, has a distinctive reactivity which can be used to distinguish it from other variant subspecies. Typically, an AAV vector particle having a particular AAV serotype does not efficiently cross-react with neutralising antibodies specific for any other AAV serotype. AAV serotypes include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 and AAV11. In some embodiments, the AAV vector of the present invention is an AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 serotype, or a variant thereof. In some embodiments, the AAV vector of the present invention is an AAV2 serotype, or a variant thereof.

The AAV genome may also comprise packaging genes, such as rep and/or cap genes which encode packaging functions for an AAV particle. The rep gene encodes one or more of the proteins Rep78, Rep68, Rep52 and Rep40 or variants thereof. The cap gene encodes one or more capsid proteins such as VP1, VP2 and VP3 or variants thereof. These proteins make up the capsid of an AAV particle, which determines the AAV serotype.

The AAV genome may be the full genome of a naturally occurring AAV. For example, a vector comprising a full AAV genome may be used to prepare an AAV vector or vector particle.

Preferably, the AAV genome is derivatised for the purpose of administration to patients. Such derivatisation is standard in the art and the invention encompasses the use of any known derivative of an AAV genome, and derivatives which could be generated by applying techniques known in the art. The AAV genome may be a derivative of any naturally occurring AAV. Suitably, the AAV genome is a derivative of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or AAV11. Suitably, the AAV genome is a derivative of AAV2.

Derivatives of an AAV genome include any truncated or modified forms of an AAV genome which allow for expression of a transgene from an AAV vector of the invention *in vivo*. Typically, it is possible to truncate the AAV genome significantly to include minimal viral sequence yet retain the above function. This is preferred for safety reasons to reduce the risk of recombination of the vector with wild-type virus, and also to avoid triggering a cellular immune response by the presence of viral gene proteins in the target cell.

- Typically, a derivative will include at least one inverted terminal repeat sequence (ITR), preferably more than one ITR, such as two ITRs or more. One or more of the ITRs may be derived from AAV genomes having different serotypes, or may be a chimeric or mutant ITR.
- A preferred mutant ITR is one having a deletion of a trs (terminal resolution site). This deletion allows for continued replication of the genome to generate a single-stranded genome which contains both coding and complementary sequences, i.e. a self-complementary AAV genome. This allows for bypass of DNA replication in the target cell, and so enables accelerated transgene expression.
- The AAV genome may comprise one or more ITR sequences from any naturally derived serotype, isolate or clade of AAV or a variant thereof. The AAV genome may comprise at least one, such as two, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or AAV11 ITRs, or variants thereof.

- Suitably, the AAV genome may comprise at least one, such as two, AAV2 ITRs, or variants thereof. In some embodiments, the AAV genome comprises an AAV2 5' ITR and/or an AAV2 3' ITR.

- In some embodiments, the AAV genome comprises a 5' ITR having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 141. In some embodiments, the AAV genome comprises a 5' ITR comprising or consisting of SEQ ID NO: 141.

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ctgctgctgctgctgctgctgagggccgcccgggcaaagcccgggcgctcgggcgacctttgg
tcgcccggcctcagtgagcgagcgagcgcgagagagggagtgccaactccatcactagggttcct
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Example AAV2 5' ITR (SEQ ID NO: 141)

- In some embodiments, the AAV genome comprises a 3' ITR having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 142. In some embodiments, the AAV genome comprises a 3' ITR comprising or consisting of SEQ ID NO: 142.

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aggaaccctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgagggc
gggcgaccaaaggtcgcccgaacggcggtttgcccggggcctcagtgagcgagcgagc
gcgcag

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Example AAV2 3' ITR (SEQ ID NO: 142)

- 5 The inclusion of one or more ITRs is preferred to aid concatamer formation of the AAV vector in the nucleus of a host cell, for example following the conversion of single-stranded vector DNA into double-stranded DNA by the action of host cell DNA polymerases. The formation of such episomal concatamers protects the AAV vector during the life of the host cell, thereby allowing for prolonged expression of the transgene in vivo.
- 10 The one or more ITRs may flank: (a) the nucleotide sequence encoding a TNF inhibitor; and/or (b) the nucleotide sequence encoding a CD3 inhibitor. In some embodiments, the AAV genome comprises (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor, flanked by one or more ITRs. In some embodiments, the AAV genome comprises (a) a nucleotide sequence encoding a TNF inhibitor; or (b) a
- 15 nucleotide sequence encoding a CD3 inhibitor, flanked by one or more ITRs.

- Suitably, the AAV genome may comprise one or more AAV2 ITR sequences flanking (a) the nucleotide sequence encoding a TNF inhibitor; and/or (b) the nucleotide sequence encoding a CD3 inhibitor. Suitably, the AAV genome may comprise two AAV2 ITR sequences flanking either side of (a) the nucleotide sequence encoding a TNF inhibitor; and/or (b) the nucleotide
- 20 sequence encoding a CD3 inhibitor. In some embodiments, the AAV genome comprises (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor, flanked by one or more AAV2 ITR sequences. In some embodiments, the AAV genome comprises two AAV2 ITR sequences flanking either side of (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor. In some
 - 25 embodiments, the AAV genome comprises (a) a nucleotide sequence encoding a TNF inhibitor; or (b) a nucleotide sequence encoding a CD3 inhibitor, flanked by one or more AAV2 ITRs. In some embodiments, the AAV genome comprises (a) two AAV2 ITR sequences flanking either side of a nucleotide sequence encoding a TNF inhibitor; or (b) two AAV2 ITR sequences flanking either side of a nucleotide sequence encoding a CD3 inhibitor.
 - 30 Suitably, ITR elements will be the only sequences retained from the native AAV genome in the derivative. A derivative will preferably not include the rep and/or cap genes of the native genome and any other sequences of the native genome. This is preferred for the reasons described above, and also to reduce the possibility of integration of the vector into the host cell genome. Additionally, reducing the size of the AAV genome allows for increased flexibility

in incorporating other sequence elements (such as regulatory elements) within the vector in addition to the transgene.

The following portions could therefore be removed in a derivative of the invention: one inverted terminal repeat (ITR) sequence, the replication (rep) and capsid (cap) genes. However, derivatives may additionally include one or more rep and/or cap genes or other viral sequences of an AAV genome. Naturally occurring AAV integrates with a high frequency at a specific site on human chromosome 19, and shows a negligible frequency of random integration, such that retention of an integrative capacity in the AAV vector may be tolerated in a therapeutic setting.

10 The invention additionally encompasses the provision of sequences of an AAV genome in a different order and configuration to that of a native AAV genome. The invention also encompasses the replacement of one or more AAV sequences or genes with sequences from another virus or with chimeric genes composed of sequences from more than one virus. Such chimeric genes may be composed of sequences from two or more related viral proteins of different viral species.

AAV capsid proteins

The AAV vector particle may be encapsidated by capsid proteins. The serotype may facilitate the transduction of ocular cells (e.g. retinal ganglion cells, RPE cells, photoreceptors, glial cells), for example specific transduction of ocular cells. The AAV vector particle may be an ocular tissue-specific vector particle. The AAV vector particle may be encapsidated by an ocular tissue-specific capsid. The AAV vector particle may comprise an ocular tissue-specific capsid protein.

In some embodiments, the AAV vector particle is a retinal-specific vector particle. In some embodiments, the AAV vector particle is encapsidated by a retinal-specific capsid. In some embodiments, the AAV vector particle comprises a retinal-specific capsid protein.

In some embodiments, the AAV vector particle is a retinal ganglion-specific vector particle. In some embodiments, the AAV vector particle is encapsidated by a retinal ganglion-specific capsid. In some embodiments, the AAV vector particle comprises a retinal ganglion-specific capsid protein.

30 Suitably, the AAV vector particles may be transcapsidated forms wherein an AAV genome or derivative having an ITR of one serotype is packaged in the capsid of a different serotype. The AAV vector particle also includes mosaic forms wherein a mixture of unmodified capsid proteins from two or more different serotypes makes up the viral capsid. The AAV vector

particle also includes chemically modified forms bearing ligands adsorbed to the capsid surface. For example, such ligands may include antibodies for targeting a particular cell surface receptor.

5 Where a derivative comprises capsid proteins i.e. VP1, VP2 and/or VP3, the derivative may be a chimeric, shuffled or capsid-modified derivative of one or more naturally occurring AAVs. In particular, the invention encompasses the provision of capsid protein sequences from different serotypes, clades, clones, or isolates of AAV within the same vector (i.e. a pseudotyped vector). The AAV vector may be in the form of a pseudotyped AAV vector particle.

10 Chimeric, shuffled or capsid-modified derivatives will be typically selected to provide one or more desired functionalities for the AAV vector. Thus, these derivatives may display increased efficiency of gene delivery, decreased immunogenicity (humoral or cellular), an altered tropism range and/or improved targeting of retinal cells compared to an AAV vector comprising a naturally occurring AAV genome. Increased efficiency of gene delivery may be effected by
15 improved receptor or co-receptor binding at the cell surface, improved internalisation, improved trafficking within the cell and into the nucleus, improved uncoating of the viral particle and improved conversion of a single-stranded genome to double-stranded form. Increased efficiency may also relate to an altered tropism range or targeting of retinal cells, such that the vector dose is not diluted by administration to tissues where it is not needed.

20 Chimeric capsid proteins include those generated by recombination between two or more capsid coding sequences of naturally occurring AAV serotypes. This may be performed for example by a marker rescue approach in which non-infectious capsid sequences of one serotype are co-transfected with capsid sequences of a different serotype, and directed selection is used to select for capsid sequences having desired properties. The capsid
25 sequences of the different serotypes can be altered by homologous recombination within the cell to produce novel chimeric capsid proteins.

For example, a directed evolution approach has been leveraged to generate novel AAV vectors that can more effectively cross biological barriers and target specific cell types. An example is the identification of the AAV.7m8 variant which, following intravitreal injection, is
30 capable of efficient gene delivery to all retina layers in both mice and primates. Similarly, SH10, an AAV6 variant, has increased tropism for glial cells following intravitreal delivery and has been shown to rescue retinal function in a rat model of RP (see e.g. Rodrigues, G.A., et al., 2019. Pharmaceutical research, 36(2), pp.1-20).

Chimeric capsid proteins also include those generated by engineering of capsid protein sequences to transfer specific capsid protein domains, surface loops or specific amino acid residues between two or more capsid proteins, for example between two or more capsid proteins of different serotypes.

- 5 Shuffled or chimeric capsid proteins may also be generated by DNA shuffling or by error-prone PCR. Hybrid AAV capsid genes can be created by randomly fragmenting the sequences of related AAV genes e.g. those encoding capsid proteins of multiple different serotypes and then subsequently reassembling the fragments in a self-priming polymerase reaction, which may also cause crossovers in regions of sequence homology. A library of hybrid AAV genes
10 created in this way by shuffling the capsid genes of several serotypes can be screened to identify viral clones having a desired functionality. Similarly, error prone PCR may be used to randomly mutate AAV capsid genes to create a diverse library of variants which may then be selected for a desired property.

- The sequences of the capsid genes may also be genetically modified to introduce specific
15 deletions, substitutions or insertions with respect to the native wild-type sequence. In particular, capsid genes may be modified by the insertion of a sequence of an unrelated protein or peptide within an open reading frame of a capsid coding sequence, or at the N- and/or C-terminus of a capsid coding sequence. The unrelated protein or peptide may advantageously be one which acts as a ligand for a particular cell type, thereby conferring
20 improved binding to a target cell or improving the specificity of targeting of the vector to a particular cell population. The unrelated protein may also be one which assists purification of the viral particle as part of the production process, i.e. an epitope or affinity tag. The site of insertion will typically be selected so as not to interfere with other functions of the viral particle e.g. internalisation, trafficking of the viral particle.

- 25 For example, AAV variants have been generated by site-directed mutagenesis of surface-exposed tyrosine residues, which prevents capsid phosphorylation and subsequent ubiquitination and proteasome-mediated degradation. AAV2, AAV8, and AAV9 carrying these mutations have been shown to have increased transduction efficiency both *in vitro* and *in vivo* (see e.g. Petrs-Silva, H., et al., 2009. Molecular therapy, 17(3), pp.463-471).

- 30 The capsid protein may be an artificial capsid protein. The term "artificial capsid" as used herein means that the capsid particle comprises an amino acid sequence which does not occur in nature or which comprises an amino acid sequence which has been engineered (e.g. modified) from a naturally occurring capsid amino acid sequence. In other words, the artificial capsid protein comprises a mutation or a variation in the amino acid sequence compared to

the sequence of the parent capsid from which it is derived where the artificial capsid amino acid sequence and the parent capsid amino acid sequences are aligned.

The capsid protein may comprise a mutation or modification relative to the wild type capsid protein which improves the ability to transduce ocular cells relative to an unmodified or wild type viral particle. Improved ability to transduce ocular cells may be measured for example by measuring the expression of a transgene, e.g. GFP, carried by the AAV vector particle, wherein expression of the transgene in ocular cells correlates with the ability of the AAV vector particle to transduce ocular cells.

Suitably, the AAV vector particle of the present invention is an AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 vector particle, or a variant thereof. AAV vector particles with these serotypes can transduce ocular cells.

The AAV vector particle of the present invention may comprise AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 capsid proteins, or variants thereof. Suitably, the AAV vector particle may comprise AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 capsid proteins VP1, VP2 and VP3, or variants thereof.

In one embodiment, the AAV vector particle comprises one or more AAV2 ITR sequences flanking (a) the nucleotide sequence encoding a TNF inhibitor; and/or (b) the nucleotide sequence encoding a CD3 inhibitor and AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 capsid proteins, or variants thereof. In one embodiment, the AAV vector particle comprises an AAV2 genome and AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9 capsid proteins, or variants thereof.

AAV2 vectors and variants thereof

In some embodiments, the AAV vector particle is an AAV2 vector particle, or a variant thereof. In some embodiments, the AAV vector particle comprises AAV2 capsid proteins, or variants thereof. Suitably, the AAV vector particle may comprise AAV2 capsid protein VP1, VP2 and VP3, or variants thereof.

AAV2 variants include AAV2.tYF, AAV2.7m8, R100, AAV2.GL, AAV2.NN, LSV1, R195-003, and dyno-86m. In some embodiments, the AAV vector particle is an AAV2 vector particle, an AAV2.tYF vector particle, an AAV2.7m8 vector particle, a R100 vector particle, an AAV2.GL vector particle, an AAV2.NN vector particle, an LSV1 vector particle, an R195-003 vector particle, or a dyno-86m vector particle. In some embodiments, the AAV vector particle comprises AAV2 capsid proteins, AAV2.tYF capsid proteins, AAV2.7m8 capsid proteins, R100 capsid proteins, AAV2.GL capsid proteins, AAV2.NN capsid proteins, LSV1 capsid proteins,

R195-003 capsid proteins, or dyno-86m capsid proteins. Suitably, the AAV vector particle may comprise AAV2 capsid protein VP1, VP2 and VP3, AAV2.tYF capsid protein VP1, VP2 and VP3, AAV2.7m8 capsid protein VP1, VP2 and VP3, R100 capsid protein VP1, VP2 and VP3, AAV2.GL capsid protein VP1, VP2 and VP3, AAV2.NN capsid protein VP1, VP2 and VP3,
 5 LSV1 capsid protein VP1, VP2 and VP3, R195-003 capsid protein VP1, VP2 and VP3, or dyno-86m capsid protein VP1, VP2 and VP3.

In some embodiments, the AAV vector particle is an AAV2 vector particle, an AAV2.tYF vector particle, an AAV2.7m8 vector particle, a R100 vector particle, an AAV2.GL vector particle, or an AAV2.NN vector particle. In some embodiments, the AAV vector particle comprises AAV2
 10 capsid proteins, AAV2.tYF capsid proteins, AAV2.7m8 capsid proteins, R100 capsid proteins, AAV2.GL capsid proteins, or AAV2.NN capsid proteins. Suitably, the AAV vector particle may comprise AAV2 capsid protein VP1, VP2 and VP3, AAV2.tYF capsid protein VP1, VP2 and VP3, AAV2.7m8 capsid protein VP1, VP2 and VP3, R100 capsid protein VP1, VP2 and VP3, AAV2.GL capsid protein VP1, VP2 and VP3, or AAV2.NN capsid protein VP1, VP2 and VP3.

15 In some embodiments, the AAV vector particle is an AAV2 vector particle. In some embodiments, the AAV vector particle comprises AAV2 capsid proteins. In some embodiments, the AAV vector particle comprises AAV2 capsid protein VP1, VP2 and VP3.

Suitably, an AAV2 VP1 capsid protein may comprise or consist of the amino acid sequence SEQ ID NO: 143, or a variant which is at least 90% identical to SEQ ID NO: 143. Suitably, the
 20 variant may be at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 143. Suitably, an AAV2 VP2 and VP3 capsid protein may be an N-terminal truncation of SEQ ID NO: 143, or an N-terminal truncation of a variant which is at least 90% identical, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 143.

25 MAADGYLPDWLEDTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFGNLDKG
 EPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEEFQERLKEDTSFGGNLGRAVFQAKKR
 VLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQ
 PLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWA
 30 LPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFSPRDWQRLINNNWGFRPKR
 LNFKL FNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMV
 PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQS LDRLM
 NPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNN
 NSEYSWTGATKYHLNGRDSL VNPGPAMASHKDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVM
 ITDEEEI RTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGP I WAK
 35 IPHTDGHFHPSPLMGGFGLKHPPQILIKNTVPANPSTTFSAAKFASFITQYSTGQVSVEI
 EWELQKENS KRWNPEIQYTSN YNKS VNVDFTVDTNGVYSEPRPIGTRYLTRNL

Example AAV2 VP1 capsid protein (SEQ ID NO: 143)

In some embodiments, the AAV vector particle is an AAV2.tYF vector particle. In some embodiments, the AAV vector particle comprises AAV2.tYF capsid proteins. In some embodiments, the AAV vector particle comprises AAV2.tYF capsid protein VP1, VP2 and VP3. Single phenylalanine (F) for tyrosine (Y) substitutions had increased the potency of AAV2 following intraocular injection (see e.g. Petrs-Silva, H., et al., 2009. Molecular therapy, 17(3), pp.463-471).

Suitably, an AAV2.tYF VP1 capsid protein may comprise or consist of the amino acid sequence SEQ ID NO: 144, or a variant which is at least 90% identical to SEQ ID NO: 144. Suitably, the variant may be at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 144. Suitably, an AAV2.tYF VP2 and VP3 capsid protein may be an N-terminal truncation of SEQ ID NO: 144, or an N-terminal truncation of a variant which is at least 90% identical, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 144.

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MAADGYLPDWLEDTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFGNLDKG
EPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQAKKR
VLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQ
PLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWA
LPTYNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFSRQDWQRLINNNWGFPRKR
LNFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMV
PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYHSQSGLDRLM
NPLIDQYLYFLSRTNTPSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNN
NSEFSWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVM
ITDEEEIIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPWAK
IPHTDGHFHPSPMLGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEI
EWELQKENS KRWNPEIQYTSNYNKSVNVDFTVD TNGVYSEPRPIGTRFLTRNL

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Example AAV2.tYF VP1 capsid protein (SEQ ID NO: 144)

In some embodiments, the AAV vector particle is an AAV2.7m8 vector particle. In some embodiments, the AAV vector particle comprises AAV2.7m8 capsid proteins. In some embodiments, the AAV vector particle comprises AAV2.7m8 capsid protein VP1, VP2 and VP3. AAV2.7m8 is an engineered capsid with a 10-amino acid insertion in adeno-associated virus (AAV) surface variable region VIII (VR-VIII) resulting in the ability to efficiently transduce retina cells following intravitreal administration (see e.g. Bennett, A., et al., 2020. Journal of structural biology, 209(2), p.107433).

Suitably, an AAV2.7m8 VP1 capsid protein may comprise or consist of the amino acid sequence SEQ ID NO: 145, or a variant which is at least 90% identical to SEQ ID NO: 145. Suitably, the variant may be at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 145. Suitably, an AAV2.7m8 VP2 and VP3 capsid protein may be an N-terminal truncation of SEQ ID NO: 145, or an N-terminal truncation of a variant which

is at least 90% identical, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 145.

5 MAADGYLPDWLEDTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFGNGLDKG
EPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEEFQERLKEDTSFGGNLGRAVFQAKKR
VLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQ
PLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWA
LPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFSRQWRLINNNWGFPRPKR
10 LNFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMV
PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHQSGLDRML
NPLIDQYLYLSRTNTPSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNN
NSEYSWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVM
ITDEEEIRTTPVATEQYGSVSTNLQRGN LALGETTRPAQAATADVNTQGVLPGMVWQDRD
VYLQGPWIWAKIPHTDGHFHPSPMLGGFGLKHPPPQILIKNTVPANPSTTFSAKFASFITQ
15 YSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRN
L

Example AAV2.7m8 VP1 capsid protein (SEQ ID NO: 145)

In some embodiments, the AAV vector particle is a R100 vector particle. In some
embodiments, the AAV vector particle comprises R100 capsid proteins. In some
embodiments, the AAV vector particle comprises R100 capsid protein VP1, VP2 and VP3.
20 R100 demonstrated superior transduction of human retinal cells compared to wild-type AAV
(see e.g. Kotterman, M., et al., 2021. bioRxiv 2021.06.24.449775).

Suitably, a R100 VP1 capsid protein may comprise or consist of the amino acid sequence
SEQ ID NO: 146, or a variant which is at least 90% identical to SEQ ID NO: 146. Suitably, the
variant may be at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical
25 to SEQ ID NO: 146. Suitably, a R100 and VP3 capsid protein may be an N-terminal truncation
of SEQ ID NO: 146, or an N-terminal truncation of a variant which is at least 90% identical, at
least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO:
146.

30 MAADGYLPDWLEDTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFGNGLDKG
EPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEEFQERLKEDTSFGGNLGRAVFQAKKR
VLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQ
PLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWA
LPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFSRQWRLINNNWGFPRPKR
35 LNFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMV
PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHQSGLDRML
NPLIDQYLYLSRTNTPSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNN
NSEYSWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVM
ITDEEEIRTTPVATEQYGSVSTNLQRGN LAI SDQTKHAQAATADVNTQGVLPGMVWQDRD
VYLQGPWIWAKIPHTDGHFHPSPMLGGFGLKHPPPQILIKNTVPANPSTTFSAKFASFITQ
40 YSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRN
L

Example R100 VP1 capsid protein (SEQ ID NO: 146)

In some embodiments, the AAV vector particle is an AAV2.GL vector particle. In some embodiments, the AAV vector particle comprises AAV2.GL capsid proteins. In some embodiments, the AAV vector particle comprises AAV2.GL capsid protein VP1, VP2 and VP3. In some embodiments, the AAV vector particle is an AAV2.NN vector particle. In some
 5 embodiments, the AAV vector particle comprises AAV2.NN capsid proteins. In some embodiments, the AAV vector particle comprises AAV2.NN capsid protein VP1, VP2 and VP3. AAV2.GL and AAV2.NN mediate widespread and high-level retinal transduction after intravitreal injection in mice, dogs and non-human primates (see e.g. Pavlou, M., et al., 2021. EMBO molecular medicine, 13(4), p.e13392).

- 10 Suitably, an AAV2.GL VP1 capsid protein may comprise or consist of the amino acid sequence SEQ ID NO: 147, or a variant which is at least 90% identical to SEQ ID NO: 147. Suitably, the variant may be at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 147. Suitably, an AAV2.GL VP2 and VP3 capsid protein may be an N-terminal truncation of SEQ ID NO: 147, or an N-terminal truncation of a variant which is at least 90%
 15 identical, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 147.

20 MAADGYLPDWLEDTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFGNGLDKG
 EPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKR
 VLEPLGLVEEPVKTPAGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQ
 PLGQPPAAPSGLTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWA
 LPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFSRQDWQRLINNNWGFRPKR
 LNFKLFNQVKEVTQNDGTTTIANNLSTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMV
 PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPPFHSSYAHSQSLDRLM
 25 NPLIDQYLYLSRTNTPSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNN
 NSEYSWTGATKYHLNGRDSLVPNGPAMASHKDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVM
 ITDEEEIRTTNPVATEQYGSVSTNLQRGN AAAGLSPPTRAARQAATADVNTQGVLPGMVWQD
 RDVYLQGPWAKIPHTDGHFHPSPMLGGGFLKHPPPPQILIKNTVPANPSTTFSAAKFASFI
 TQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYSVNVDFTVDTNGVYSEPRPIGTRYLT
 RNL

- 30 Example AAV2.GL VP1 capsid protein (SEQ ID NO: 147)

- Suitably, an AAV2.NN VP1 capsid protein may comprise or consist of the amino acid sequence SEQ ID NO: 148, or a variant which is at least 90% identical to SEQ ID NO: 148. Suitably, the variant may be at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 148. Suitably, an AAV2.NN VP2 and VP3 capsid protein may be
 35 an N-terminal truncation of SEQ ID NO: 148, or an N-terminal truncation of a variant which is at least 90% identical, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 148.

MAADGYLPDWLEDTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFGNGLDKG
 EPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKR

VLEPLGLVEEPPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDPQ
 PLGQPPAAPSGLTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWA
 LPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFSRQDWRLINNNWGFRPKR
 LNFKL FNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMV
 5 PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVFPFHSSYAHSQSLDRLM
 NPLIDQYLYLSRTNTPSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNN
 NSEYSWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVM
 ITDEEEIIRTNPVATEQYGSVSTNLQRGNAAANNPTPSRAARQAATADVNTQGVLPGMVWQD
 RDVYLQGPWAKIPHTDGHFHPSPMLGGFGLKHPPFQILIKNTVPANPSTTFSAKFASFI
 10 TQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLT
 RNL

Example AAV2.NN VP1 capsid protein (SEQ ID NO: 148)

In some embodiments, the AAV vector particle is a LSV1 vector particle. In some
 embodiments, the AAV vector particle comprises LSV1 capsid proteins. In some
 15 embodiments, the AAV vector particle comprises LSV1 capsid protein VP1, VP2 and VP3.
 Loop swap variant 1 (LSV1) transduces the retina and retinal pigment epithelium (RPE) from
 the vitreous and is based on AAV2.5T, a substitution from aa 571-579 (see e.g. Baker, C.K.,
 et al., 2022, Molecular Therapy, 30(4), p. 575).

Suitably, a LSV1 VP1 capsid protein may comprise or consist of the amino acid sequence
 20 SEQ ID NO: 149 or a variant which is at least 90% identical to SEQ ID NO: 149 Suitably, the
 variant may be at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical
 to SEQ ID NO: 149 Suitably, a LSV1 VP2 and VP3 capsid protein may be an N-terminal
 truncation of SEQ ID NO: 149 or an N-terminal truncation of a variant which is at least 90%
 identical, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to
 25 SEQ ID NO: 149

MAADGYLPDWLEDTLSEGI RQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKG
 EPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADA EFQERLKEDTSFGGNLGRAVFQAKKR
 VLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQLQIPAPPA
 30 SSLGADTMSAGGGGPLGDNNGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSSYNNHQY
 REIKSGSVDGSNANAYFGYSTPWGYFDNRFHSHWSPRDWRLINNYWGFRPRSLRVKIFNI
 QVKEVTVDSTTTIANNLSTVQVFTDDDYQLPYVVGNGTEGCLPAFPQPQVFTLPQYGYATL
 NRDNTENPTERSFFFCLEYFPSKMLRTGNNFEFTYNFEEVFPFHSSFPQSQNLFKLANPLVDQ
 YLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSATATNRM
 ELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQP
 35 VNRVAYNVGGQMLAHKFKSGDAPTTGTYNLQEI VPGSVWMERDVYLQGPWAKIPETGAHFH
 PSPAMGGFGLKHPPPMMLIKNTVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSK
 RWNPEIQYTNNYNDPQFVDFAPDSTGEYRTRPIGTRYLTRPL

Example LSV1 capsid protein (SEQ ID NO: 149)

In some embodiments, the AAV vector particle is a R195-003 vector particle. In some
 40 embodiments, the AAV vector particle comprises R195-003 capsid proteins. In some
 embodiments, the AAV vector particle comprises R195-003 capsid protein VP1, VP2 and VP3
 (see e.g. Human Gene Therapy Methods 2022; 33 (23-24): A27-A28).

In some embodiments, the AAV vector particle is a dyno-86m vector particle. In some embodiments, the AAV vector particle comprises dyno-86m capsid proteins. In some embodiments, the AAV vector particle comprises dyno-86m capsid protein VP1, VP2 and VP3 (see e.g. Molecular Therapy 2023; 31(4), S1, p.1284).

- 5 In some embodiments, the AAV vector particle comprises one or more AAV2 ITR sequences flanking (a) the nucleotide sequence encoding a TNF inhibitor; and/or (b) the nucleotide sequence encoding a CD3 inhibitor and AAV2 capsid proteins, or variants thereof. In some embodiments, the AAV vector particle comprises an AAV2 genome and AAV2 capsid proteins, or variants thereof.

10 *Other parvovirus vectors*

In some embodiments, a vector of the present invention is a protoparvovirus vector. In some embodiments, a vector of the present invention is in the form of a protoparvovirus vector. Protoparvoviruses have been studied extensively and utilized as vectors, including the minute virus of mice (MVM), the rat parvovirus H1, and the Lull virus. Human variants that have been
 15 found recently include bufavirus (BuV), tusavirus (TuV), and cutavirus (CuV) (see e.g. Fakhiri, J. and Grimm, D., 2021. Molecular Therapy, 29(12), pp.3359-3382).

- In some embodiments, a vector of the present invention is a bocaparvovirus vector. In some embodiments, a vector of the present invention is in the form of a bocaparvovirus vector. The use of Human bocavirus 1 (HBoV1) as a parvoviral vector for gene delivery has been
 20 described (see e.g. Shao, L., et al., 2021. Frontiers in Microbiology, 12, p.1463).

Other viral vectors

Retroviral and lentiviral vectors

A vector of the present invention may be a retroviral vector or a lentiviral vector. The vector of the present invention may be a retroviral vector particle or a lentiviral vector particle.

- 25 A retroviral vector may be derived from or may be derivable from any suitable retrovirus. A large number of different retroviruses have been identified. Examples include murine leukaemia virus (MLV), human T-cell leukaemia virus (HTLV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukaemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine
 30 sarcoma virus (Mo-MSV), Abelson murine leukaemia virus (A-MLV), avian myelocytomatosis virus-29 (MC29) and avian erythroblastosis virus (AEV).

Retroviruses may be broadly divided into two categories, “simple” and “complex”. Retroviruses may be even further divided into seven groups. Five of these groups represent retroviruses with oncogenic potential. The remaining two groups are the lentiviruses and the spumaviruses.

5 The basic structure of retrovirus and lentivirus genomes share many common features such as a 5' LTR and a 3' LTR. Between or within these are located a packaging signal to enable the genome to be packaged, a primer binding site, integration sites to enable integration into a host cell genome, and gag, pol and env genes encoding the packaging components – these are polypeptides required for the assembly of viral particles. Lentiviruses have additional features, such as rev and RRE sequences in HIV, which enable the efficient export of RNA
10 transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell.

In the provirus, these genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for proviral integration and transcription. LTRs also serve as enhancer-promoter sequences and can control the expression of the viral genes.

15 The LTRs themselves are identical sequences that can be divided into three elements: U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA. U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

20 In a defective retroviral vector genome gag, pol and env may be absent or not functional.

In a typical retroviral vector, at least part of one or more protein coding regions essential for replication may be removed from the virus. This makes the viral vector replication-defective. Portions of the viral genome may also be replaced by a library encoding candidate modulating moieties operably linked to a regulatory control region and a reporter moiety in the vector
25 genome in order to generate a vector comprising candidate modulating moieties which is capable of transducing a target host cell and/or integrating its genome into a host genome.

Lentivirus vectors are part of the larger group of retroviral vectors. In brief, lentiviruses can be divided into primate and non-primate groups. Examples of primate lentiviruses include but are not limited to human immunodeficiency virus (HIV), the causative agent of human acquired
30 immunodeficiency syndrome (AIDS); and simian immunodeficiency virus (SIV). Examples of non-primate lentiviruses include the prototype “slow virus” visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus

(EIAV), and the more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV).

The lentivirus family differs from retroviruses in that lentiviruses have the capability to infect both dividing and non-dividing cells. In contrast, other retroviruses, such as MLV, are unable to infect non-dividing or slowly dividing cells such as those that make up, for example, muscle, brain, lung and liver tissue.

A lentiviral vector, as used herein, is a vector which comprises at least one component part derivable from a lentivirus. Preferably, that component part is involved in the biological mechanisms by which the vector infects cells, expresses genes or is replicated.

The lentiviral vector may be a "primate" vector. The lentiviral vector may be a "non-primate" vector (i.e. derived from a virus which does not primarily infect primates, especially humans). Examples of non-primate lentiviruses may be any member of the family of lentiviridae which does not naturally infect a primate.

As examples of lentivirus-based vectors, HIV-1- and HIV-2-based vectors are described below.

The HIV-1 vector contains cis-acting elements that are also found in simple retroviruses. It has been shown that sequences that extend into the gag open reading frame are important for packaging of HIV-1. Therefore, HIV-1 vectors often contain the relevant portion of gag in which the translational initiation codon has been mutated. In addition, most HIV-1 vectors also contain a portion of the env gene that includes the RRE. Rev binds to RRE, which permits the transport of full-length or singly spliced mRNAs from the nucleus to the cytoplasm. In the absence of Rev and/or RRE, full-length HIV-1 RNAs accumulate in the nucleus. Alternatively, a constitutive transport element from certain simple retroviruses such as Mason-Pfizer monkey virus can be used to relieve the requirement for Rev and RRE. Efficient transcription from the HIV-1 LTR promoter requires the viral protein Tat.

Most HIV-2-based vectors are structurally very similar to HIV-1 vectors. Similar to HIV-1-based vectors, HIV-2 vectors also require RRE for efficient transport of the full-length or singly spliced viral RNAs.

Preferably, the viral vector used in the present invention has a minimal viral genome. By "minimal viral genome" it is to be understood that the viral vector has been manipulated so as to remove the non-essential elements and to retain the essential elements in order to provide the required functionality to infect, transduce and deliver a nucleotide sequence of interest to a target host cell. Further details of this strategy can be found in WO 1998/017815.

Preferably, the plasmid vector used to produce the viral genome within a host cell/packaging cell will have sufficient lentiviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle which is capable of infecting a target cell, but is incapable of independent replication to produce infectious viral particles within the final target cell. Preferably, the vector lacks a functional gag-pol and/or env gene and/or other genes essential for replication.

However, the plasmid vector used to produce the viral genome within a host cell/packaging cell will also include transcriptional regulatory control sequences operably linked to the lentiviral genome to direct transcription of the genome in a host cell/packaging cell. These regulatory sequences may be the natural sequences associated with the transcribed viral sequence (i.e. the 5' U3 region), or they may be a heterologous promoter, such as another viral promoter (e.g. the CMV promoter).

The vectors may be self-inactivating (SIN) vectors in which the viral enhancer and promoter sequences have been deleted. SIN vectors can be generated and transduce non-dividing cells in vivo with an efficacy similar to that of wild-type vectors. The transcriptional inactivation of the long terminal repeat (LTR) in the SIN provirus should prevent mobilisation by replication-competent virus. This should also enable the regulated expression of genes from internal promoters by eliminating any cis-acting effects of the LTR.

The vectors may be integration-defective. Integration defective lentiviral vectors (IDLVs) can be produced, for example, either by packaging the vector with catalytically inactive integrase (such as an HIV integrase bearing the D64V mutation in the catalytic site) or by modifying or deleting essential att sequences from the vector LTR, or by a combination of the above.

Adenoviral vectors

A vector of the present invention may be an adenoviral vector. A vector of the present invention may be an adenoviral vector particle.

The adenovirus is a double-stranded, linear DNA virus that does not go through an RNA intermediate. There are over 50 different human serotypes of adenovirus divided into 6 subgroups based on the genetic sequence homology. The natural targets of adenovirus are the respiratory and gastrointestinal epithelia, generally giving rise to only mild symptoms. Serotypes 2 and 5 (with 95% sequence homology) are most commonly used in adenoviral vector systems and are normally associated with upper respiratory tract infections in the young.

Adenoviruses have been used as vectors for gene therapy and for expression of heterologous genes. The large (36 kb) genome can accommodate up to 8 kb of foreign insert DNA and is able to replicate efficiently in complementing cell lines to produce very high titres of up to 10^{12} . Adenovirus is thus one of the best systems to study the expression of genes in primary non-replicative cells.

The expression of viral or foreign genes from the adenovirus genome does not require a replicating cell. Adenoviral vectors enter cells by receptor mediated endocytosis. Once inside the cell, adenovirus vectors rarely integrate into the host chromosome. Instead, they function episomally (independently from the host genome) as a linear genome in the host nucleus. Hence the use of recombinant adenovirus alleviates the problems associated with random integration into the host genome.

Herpes simplex viral vector

A vector of the present invention may be a herpes simplex viral vector. A vector of the present invention may be a herpes simplex viral vector particle.

Herpes simplex virus (HSV) is a neurotropic DNA virus with favorable properties as a gene delivery vector. HSV is highly infectious, so HSV vectors are efficient vehicles for the delivery of exogenous genetic material to cells. Viral replication is readily disrupted by null mutations in immediate early genes that in vitro can be complemented in trans, enabling straightforward production of high-titre pure preparations of non-pathogenic vector. The genome is large (152 Kb) and many of the viral genes are dispensable for replication in vitro, allowing their replacement with large or multiple transgenes. Latent infection with wild-type virus results in episomal viral persistence in sensory neuronal nuclei for the duration of the host lifetime. The vectors are non-pathogenic, unable to reactivate and persist long-term. The latency active promoter complex can be exploited in vector design to achieve long-term stable transgene expression in the nervous system. HSV vectors transduce a broad range of tissues because of the wide expression pattern of the cellular receptors recognized by the virus. Increasing understanding of the processes involved in cellular entry has allowed targeting the tropism of HSV vectors.

Vaccinia virus vectors

A vector of the present invention may be a vaccinia viral vector. A vector of the present invention may be a vaccinia viral vector particle.

Vaccinia virus is large enveloped virus that has an approximately 190 kb linear, double-stranded DNA genome. Vaccinia virus can accommodate up to approximately 25 kb of foreign

DNA, which also makes it useful for the delivery of large genes. A number of attenuated vaccinia virus strains are known in the art that are suitable for gene therapy applications, for example the MVA and NYVAC strains.

Anellovirus vectors

- 5 A vector of the present invention may be an anelloviral vector. A vector of the present invention may be an anelloviral vector particle.

Anelloviruses are small, single stranded circular DNA viruses. They are extremely diverse and have not been associated with any disease so far. Anelloviruses may infect the complete human population, and there is no evidence of disease association or of viral clearance from
10 infected individuals (see e.g. Venkataraman, T., et al., 2022. bioRxiv 2022.03.28.486145).

Non-viral delivery systems

In some embodiments, the vector is a non-viral vector. Suitable non-viral delivery systems will be known to the skilled person (see e.g. Zulliger, R., et al., 2015. Journal of Controlled Release, 219, pp.471-487; and Oliveira, A.V., et al., 2017. Materials Science and Engineering: C, 77, pp.1275-1289.).
15

In some embodiments, the vector is a plasmid. In some embodiments, the plasmid is modified to facilitate cellular and/or nuclear uptake. In some embodiments, the plasmid is comprised in a non-viral particle, e.g. a lipoplex particle or a polyplex particle.

Non-viral delivery systems include but are not limited to transfection methods. Here,
20 transfection includes a process using a non-viral vector to deliver a gene to a target cell. Typical transfection methods include electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection, liposomes, immunoliposomes, lipofectin, cationic agent-mediated transfection, cationic facial amphiphiles (CFAs), and combinations thereof.

25 **Promoters**

A polynucleotide or vector of the present invention may comprise a promoter, preferably an inflammation-inducible promoter.

Suitably, a promoter may be operably linked to (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a
30 CD3 inhibitor (e.g. anti-CD3 antibody fragment). In some embodiments, a promoter is operably linked to (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody

fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment). The term “operably linked” may mean that the components described are in a relationship permitting them to function in their intended manner.

A “promoter” may refer to a region of DNA that leads to initiation of transcription of a gene.

- 5 Promoters are typically located near the transcription start sites of genes, upstream on the DNA (towards the 5' region of the sense strand). Any suitable promoter may be used, the selection of which may be readily made by the skilled person.

- 10 A promoter typically comprises a “core” and a “proximal” region. The “core promoter region” may comprise promoter elements such as a transcription start site, RNA polymerase binding sites and general transcription factor binding sites (e.g. TATA box, B recognition element). The “proximal promoter region” may comprise primary regulatory elements and specific transcription factor binding sites which are required, for example, to facilitate effective and controllable transcription. The size and components of both the core and proximal promoter regions typically vary in a gene specific manner.

- 15 In some embodiments, the promoter is an ocular tissue-specific promoter. As used herein, an “ocular tissue-specific promoter” is a promoter which preferentially facilitates expression of a gene in ocular cells (e.g. photoreceptors, RPE cells, retinal ganglion cells etc.).

- In other embodiments, the promoter is a constitutive promoter. As used herein, a “constitutive promoter” is a promoter which is always active. Exemplary constitutive promoters include a
20 chicken beta-actin (CBA) promoter, or a variant or fragment thereof. The promoter may comprise or consist of a CAG promoter, or a variant or fragment thereof.

Inflammation-inducible promoters

A polynucleotide or vector of the present invention preferably comprises an inflammation-inducible promoter.

- 25 In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to an inflammation-inducible promoter. In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody
30 fragment) are operably linked to an inflammation-inducible promoter. The promoter may facilitate expression in response to inflammation.

The use of an inflammation-inducible promoter is advantageous because the TNF inhibitor and/or CD3 inhibitor may be expressed when inflammation occurs at a sub-clinical level and thereby prevent damage caused by flare-ups, before symptoms occur.

As used herein, an “inflammation-inducible promoter” may refer to a promoter which
 5 preferentially facilitates expression of an operably-linked transgene in response to inflammation. Inflammation may be characterised by, at a tissue level, redness, swelling, heat, pain and/or loss of tissue function (see e.g. Chen, L., et al., 2018. *Oncotarget*, 9(6), p.7204). Inflammation may be characterised by, at a cellular level, elevated levels of pro-inflammatory cytokines, activated immune cells, or acute phase proteins. Inflammation may be considered
 10 acute, i.e. an immediate response to harmful stimuli, or chronic, i.e. prolonged inflammation.

Suitably, an inflammation-inducible promoter may facilitate higher expression of an operably-linked transgene in response to higher levels of inflammation. Higher expression may be measured for example by measuring the expression of a transgene, e.g. green fluorescence protein (GFP), operably linked to the promoter, wherein expression of the transgene correlates
 15 with the ability of the promoter to facilitate expression of a gene. Levels of inflammation can be determined by suitable methods known in the art (see e.g. Menzel et al., 2021, *Antioxidants*, 10(3), p.414). For example, measurements of levels of markers of inflammation can be taken from appropriate media, such as body fluids. Appropriate body fluids may include blood, urine or vitreous humor.

20 Suitably, an inflammation-inducible promoter may facilitate higher expression of an operably-linked transgene in an ocular cell (e.g. photoreceptors, RPE cells, retinal ganglion cells etc.) in response to higher levels of inflammation in the ocular cell. The levels of inflammation in the eye may be determined by suitable non-invasive methods known in the art. For example, by grading ocular inflammation (see e.g. McNeil, R., 2016. *Eye news*, 22(5), pp.1-4-19) or by
 25 laser flare photometry (see e.g. Tugal-Tutkun, I. and Herbort, C.P., 2010. *International ophthalmology*, 30(5), pp.453-464).

The inflammation-inducible promoter may be (or may be derived from) a promoter associated with a gene with increased expression in response to inflammation. Suitably, the inflammation-inducible promoter may be (or may be derived from) a promoter associated with a gene with
 30 increased expression in ocular cells in response to inflammation (e.g. uveitis). For example, genes with increase expression in uveitis may include CCL16, IL15, CCL7, CYSLTR1, IL17, IL4, CCL8, IL15RA, CCR1, CDC25A, LAT, MEF2B, CSF3, MAP2K7, IL20, TYK2, MAF, COL1A1, IKBKG, MGC27165, IL10, CIITA, NFATC2IP, MEF2D, GSK3A, TH1L, IL3, IL10RB, SMAD9, C19orf10, IL13RA2, TGFB2, LEP, HAVCR2, SOCS5, IRF1, TXLNA, RFX2, FADD,

TGIF, CCR4, RFX1, MIF, IL10RA, CXCL5, LTA, IGFBP3, NFKBIB, CXCL13, IKBKE, MEF2A, LAG3, ICAM1, AMHR2, CCL15, and PDGFB (see e.g. Li, Z., et al., 2008. The Journal of Immunology, 181(7), pp.5147-5157). Methods to identify promoters associated with genes will be known to those of skill in the art.

- 5 In some embodiments, the inflammation inducible promoter comprises an IFN-beta minimal promoter, or a fragment or derivative thereof. In some embodiments, the inflammation-inducible promoter comprises a nucleotide sequence which is at least 70% identical to SEQ ID NO: 150 or a fragment thereof. In some embodiments, the inflammation-inducible promoter comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 150 or a fragment thereof. In some embodiments, the inflammation-inducible promoter comprises SEQ ID NO: 150.

15 `agcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactga
aagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaaatagca
caggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttg`

Example IFN-beta minimal promoter (SEQ ID NO: 150)

- The inflammation-inducible promoter may comprise one or more inflammation-associated transcription factor binding motif. An "inflammation-associated transcription factor binding motif" or "inflammation-associated transcription factor binding site" may refer to a nucleotide sequence that an inflammation-associated transcription factor binds. Exemplary inflammation-associated transcription factors include but are not limited to AP-1, NF-κB, IRFs, STATs and NFAT (see e.g. Smale ST. Cell. 2010 Mar 19;140(6):833-44., Platanitis E, Decker T. Front Immunol. 2018 Nov 13;9:2542., Pessler F, Dai L, Cron RQ, Schumacher HR. Autoimmun Rev. 2006 Feb;5(2):106-10.). Exemplary inflammation-associated transcription factor binding motifs may include, but are not limited to: AP-1 binding motifs, NF-κB binding motifs (κB sites), the interferon-stimulated response element (ISRE), the gamma-interferon-activated sequence (GAS), and NFAT binding motifs.

- In some embodiments, the inflammation-inducible promoter comprises at least one inflammation-associated transcription factor binding motif, which may be selected from an AP-1 transcription factor binding motif; a NF-κB transcription factor binding motif; an IRF transcription factor binding motif; a STAT transcription factor binding motif; and a NFAT transcription factor binding motif; or any combination thereof.

In some embodiments, the inflammation-inducible promoter comprises two or more inflammation-associated transcription factor binding motifs. In some embodiments, the

inflammation-inducible promoter comprises three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more inflammation-associated transcription factor binding motifs.

AP-1 binding motifs

- 5 In some embodiments, the inflammation-inducible promoter comprises one or more AP-1 binding motif.

Activator protein 1 (AP-1) is a transcription factor that regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, and bacterial and viral infections. An "AP-1 binding motif", also known as an "AP-1 transcription factor binding motif" or an "AP-1 promoter site", is a DNA sequence to which AP-1 transcription factors are able to bind (see e.g. Kim, H., et al., 1997. Biochemical Journal, 324(2), pp.547-553).

10

In some embodiments, the inflammation-inducible promoter comprises two or more AP-1 binding motifs. In some embodiments, the inflammation-inducible promoter comprises three or more AP-1 binding motifs. In some embodiments, the inflammation-inducible promoter comprises four or more AP-1 binding motifs. In some embodiments, the inflammation-inducible promoter comprises five or more AP-1 binding motifs. In some embodiments, the inflammation-inducible promoter comprises five AP-1 binding motifs.

15

Exemplary AP-1 binding motifs are shown as SEQ ID NO: 151 (where "s" is g or c and "m" is a or c), SEQ ID NO: 152, SEQ ID NO: 153, and SEQ ID NO: 154. Any other variant or derivative to which AP-1 binds may be used in the present invention.

20

In some embodiments, the inflammation-inducible promoter comprises the nucleotide sequence SEQ ID NO: 151.

tgastma

Example AP-1 binding consensus motif (SEQ ID NO: 151)

- 25 In some embodiments, the inflammation-inducible promoter comprises the nucleotide sequence SEQ ID NO: 152, or a derivative thereof having one or two nucleotide substitutions.

tgagtca

Example AP-1 binding motif 1 (SEQ ID NO: 152)

- In some embodiments, the inflammation-inducible promoter comprises the nucleotide sequence SEQ ID NO: 153, or a derivative thereof having one or two nucleotide substitutions.
- 30

tgactca

Example AP-1 binding motif 2 (SEQ ID NO: 153)

In some embodiments, the inflammation-inducible promoter comprises the nucleotide sequence SEQ ID NO: 154, or a derivative thereof having one or two nucleotide substitutions.

5 tgactaa

Example AP-1 binding motif 3 (SEQ ID NO: 154)

NF-kB binding motifs

In some embodiments, the inflammation-inducible promoter comprises one or more NF-kB binding motif.

- 10 Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) is a protein complex that controls transcription of DNA in response to stimuli such as stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. An “NF-kB binding motif”, also known as an “NF-kB transcription factor binding motif” or an “NF-kB promoter site”, is a DNA sequence to which NF-kB transcription factors are able to bind
- 15 (see e.g. Natoli, G., 2006. FEBS letters, 580(12), pp.2843-2849.).

- In some embodiments, the inflammation-inducible promoter comprises two or more NF-kB binding motifs. In some embodiments, the inflammation-inducible promoter comprises three or more NF-kB binding motifs. In some embodiments, the inflammation-inducible promoter comprises four or more NF-kB binding motifs. In some embodiments, the inflammation-inducible promoter comprises five or more NF-kB binding motifs. In some embodiments, the inflammation-inducible promoter comprises five NF-kB binding motifs.
- 20

Exemplary NF-kB binding motifs are shown as SEQ ID NO: 155 (where “r” is a purine, “y” is a pyrimidine and “n” is any nucleotide) and SEQ ID NO: 156. Any other variant or derivative to which NF-kB binds may be used in the present invention.

- 25 In some embodiments, the inflammation-inducible promoter comprises the nucleotide sequence SEQ ID NO: 155.

gggrnyyycc

Example NF-kB binding consensus motif (SEQ ID NO: 155)

In some embodiments, the inflammation-inducible promoter comprises the nucleotide sequence SEQ ID NO: 156, or a derivative thereof having one or two nucleotide substitutions.

ggggactttccact

Example NF- κ B binding motif (SEQ ID NO: 156)

5 *Other inflammation-associated transcription factor binding motifs*

In some embodiments, the inflammation-inducible promoter comprises one or more, two or more, three or more, four or more, or five or more interferon-stimulated response elements (ISREs). An ISRE (also known as an IRF transcription factor binding motif) is a DNA sequence to which IRF transcription factors are able to bind. All IRF family members possess an N-
10 terminal DNA-binding domain that recognises the ISRE, which may be characterized by the consensus sequence AANNGAAA (see e.g. Yanai, H., et al., 2012. Oncoimmunology, 1(8), pp.1376-1386).

In some embodiments, the inflammation-inducible promoter comprises one or more, two or more, three or more, four or more, or five or more gamma-interferon-activated sequences
15 (GASs). A GAS (also known as a "STAT transcription factor binding motif") is a DNA sequence to which STAT transcription factors are able to bind. Several members of the STAT protein family in particular STAT1, STAT2, STAT3, STAT4 and STAT6 act as transcription factors in modulating inflammatory responses. STAT transcription factors bind to similar sequences and may have the palindromic core motif TTCN₂₋₄GAA (see e.g. Ehret, G.B., et al., 2001. Journal
20 of Biological Chemistry, 276(9), pp.6675-6688).

In some embodiments, the inflammation-inducible promoter comprises one or more, two or more, three or more, four or more, or five or more NFAT binding motifs. A NFAT transcription factor binding motif is a DNA sequence to which NFAT transcription factors are able to bind. The NFAT family acts synergistically with AP-1 proteins on DNA elements which contain
25 adjacent NFAT and AP-1 binding sites, to regulate the expression of inducible genes. An NFAT binding motif may be characterized by the consensus sequence TGGAAA (see e.g. Macian, F., et al., 2001. Oncogene, 20(19), pp.2476-2489).

Example inflammation-inducible promoters

In some embodiments, the inflammation-inducible promoter comprises a combination of two
30 or more different inflammation-associated transcription factor binding motifs. For example, the inflammation-inducible promoter may comprise any combination of: one or more AP-1 binding motif, one or more NF- κ B binding motifs, one or more gamma-interferon-activated sequence

(GAS), one or more interferon-stimulated response element (ISRE), and one or more NFAT binding motif.

In some embodiments, the inflammation-inducible promoter comprises one or more AP-1 binding motifs and/or one or more NF- κ B binding motifs. In some embodiments, the inflammation-inducible promoter comprises two or more AP-1 binding motifs and/or two or more NF- κ B binding motifs. In some embodiments, the inflammation-inducible promoter comprises three or more AP-1 binding motifs and/or three or more NF- κ B binding motifs. In some embodiments, the inflammation-inducible promoter comprises four or more AP-1 binding motifs and/or four or more NF- κ B binding motifs. In some embodiments, the inflammation-inducible promoter comprises five or more AP-1 binding motifs and/or five or more NF- κ B binding motifs.

The inflammation-associated transcription factor binding motifs may be coupled. The term "coupled" may mean that the inflammation-associated transcription factor binding motifs are in a relationship permitting them to function in their intended manner (e.g. to bind inflammation-associated transcription factor). Suitably, the inflammation-associated transcription factor binding motifs may be linked by short nucleotide sequences or may be directly linked, or any combination thereof. Suitably, the inflammation-associated transcription factor binding motifs are linked by linker sequences of from 1 to about 20 nucleotides, or from 1 to about 10 nucleotides. Suitably, the inflammation-associated transcription factor binding motifs are directly linked (i.e. with no linker sequence in between).

Suitably, the inflammation-inducible promoter may comprise one or more inflammation-associated transcription factor binding sites and a minimal promoter (e.g. an IFN-beta minimal promoter). Suitably, the inflammation-inducible promoter may comprise a proximal promoter region comprising one or more inflammation-associated transcription factor binding sites. Suitably, the inflammation-inducible promoter may comprise a proximal promoter region comprising one or more inflammation-associated transcription factor binding sites and a core promoter region derived from a gene with selective expression in response to inflammation.

In some embodiments, the inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 157, or a fragment thereof. Suitably the inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 157 or a fragment thereof. In some embodiments, the inflammation-inducible promoter comprises or consists of the sequence SEQ ID NO: 157 or a fragment thereof.

5 tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
 gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
 gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
 aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaatagc
 acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttg

Example inflammation-inducible promoter (SEQ ID NO: 157)

Example inflammation-inducible promoter coupled to TNF inhibitor

In some embodiments, a nucleotide sequence encoding a TNF inhibitor operably linked to an inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 158 or a fragment thereof. Suitably, the nucleotide sequence encoding a TNF inhibitor operably linked to an inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 158 or a fragment thereof. In some embodiments, the nucleotide sequence encoding a TNF inhibitor operably linked to an inflammation-inducible promoter has a nucleotide sequence comprising or consisting of the sequence SEQ ID NO: 158 or a fragment thereof.

20 tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
 gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
 gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
 aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaatagc
 acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
 atcctgcagaagtgtgctgagtcactgggcaggtaagtatcaaggttacaagacaggttt
 aaggagaccaatagaaactgggcttgcgagacagagaagactccttgcgtttctgataggca
 25 cctattgggtcttactgacatccactttgcctttctctccacaggtgtccaggcggccgcat
 ggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttggctgc
 aagagggcagcgcgaggtgcaactggtggaaagcggcggcggcctggtccagcctggaagg
 tccctgagactgagctgtgccgacagcggttaccttcgacgactacgccatgcactgggt
 gcgccaggccctggcaaggcctggaatgggtctccgccatcacctggaatagcggccaca
 30 tcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaaagaactct
 ctgtatctgcaaatgaacagcctgcggtgaagatacagccgtgtactattgcgcaaaagt
 gagctacctctccaccgacagcctggactattggggacagggcaccctggtgaccgtgt
 ctagcgctccacaaaggcccttctgtgtttccactggctccaagctccaaaagcacatct
 ggaggaaccgctgccctgggtgcctggtaaggactacttccccgagcctgtgaccgtgag
 35 ctggaacagcggcgccctgacatctggtgttcataccttccctgccgttctgcaatcttctg
 gactctacagcctgtcttctgtggtgaccgtgccagcagcagccttggaaacacagacctac
 atctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcggag
 aagcgggaagcgggtgctaccaacttcagcctcctgaaacaggcggcgatgtggaggaaaacc
 ctggacctatggctaccggcagcagaaccagcctgctgctggcattcggccttctgtgcctg
 40 ccttggctgcaagagggtctgcccagatccagatgaccagagcccttccctcactgagcgc
 cagcgtgggcgacagagtactattacatgcagagccagccaaggcatccggaactacctgg
 cctggtatcagcagaagcccggcaaaagcccctaagctgctgatctacgccgacagcaccctg
 caaagcggcgtgcctagcagattcagcggctcaggctctggcactgatttcaccctgaccat
 45 ctcctctctgcaacctgaggacgtggccatactactgcccagagatacaacacagcccat
 acacctttggccagggcacaaaagtggaaatcaagagaaccgtggcggcctcccagtggttc
 atcttccccccagtgatgagcagctgaagtccggcacagcctctgtcgtgtgcctgctgaa

caacttctaccccagagaggccaaggtgcagtgggaaggtggataatgccttgcaaagcggca
 acagccaggagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagcaca
 ctgaccctgagcaaggccgactacgagaagcacaaggtgtacgcatgaggtgacccacca
 gggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgt

- 5 Example inflammation-inducible promoter and adalimumab Fab
 nucleotide sequence (SEQ ID NO: 158)

Example inflammation-inducible promoter coupled to CD3 inhibitor

- In some embodiments, a nucleotide sequence encoding a CD3 inhibitor operably linked to an
 inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at
 10 least 70% identical to SEQ ID NO: 159 or a fragment thereof. Suitably, the nucleotide
 sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter
 comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least
 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%
 identical to SEQ ID NO: 159 or a fragment thereof. In some embodiments, the nucleotide
 15 sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter has
 a nucleotide sequence comprising or consisting of the sequence SEQ ID NO: 159 or a
 fragment thereof.

- 20 tgagtcactgactcagtgcactgactcagtgcagtaaggaattctggggactttccactg
 gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
 gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
 aaaggagaaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaaatagc
 acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
 atcctgcagaagtgggtcgtgaggcactgggcaggtaagtatcaaggttacaagacaggttt
 aaggagaccaatagaaactgggcttgtcgagacagagaagactccttgctttctgataggca
 25 cctattgggtcttactgacatccactttgcctttctctccacagggtgtccaggcgccgcat
 ggctaccggctctaggaacctcactgctgctggccttcggcctgctgtgcctgccttggtgc
 aggagggcagcgccaggtccagctggtggagagcgggcggtgggtgcagcccgccaga
 agcctgcggctgagctgtgccgcagcggttcaagtttagcggtacgggatgcactgggt
 caggcaggctcctggaaaggactggagtggtggcagtgatctggtatgatggctccaaga
 30 aatactacgtcgacagcgtgaaaggacgcttcaccatcagcagggataacagcaagaataca
 ctgtacctgcagatgaacagcctgagagcagaggatacagccgtgtactactgcgccaggca
 gatgggctactggcacttcgacctatgggggaggggtacactggtgaccgtgagctccgcat
 ccaccaaagggccctctgtgttccccctggccccctccagcaagtccacctctggaggaacc
 gctgccctgggctgtctggtgaaagattattttccagagcctgtcaccgtgtcttggaactc
 35 cggcgctctgacatccggcggtgcacacattccccgcggtgctgcagagctctggcctctaca
 gcctgtcttctgtggtgacagtgccagctccagcctggggaccagacctatatctgcaac
 gtgaaccacaagccatctaaccacaaagtggataaacgggtgaggaagaggaggtccggctc
 cggcgccacaaacttttctctgctgaagcagggcggggacgtggaggagaacccccggccaa
 tggccaccggttctagaaccagcctgctgctggcctttggcctgctgtgcctgccttggtg
 40 caggagggcagcgtgaaattgtgctgacacagtcctccgccaccctgtccctgtccccgg
 cgagagggccacactgagctgtaggccagccagtcctgagcagctatctggcctggtacc
 agcagaaacccggacaggccccaagactgctgatttatgacgcctccaacagagctacgggc
 atccccgctcgcttttccggcagcggaagcgggacagacttactctgaccattagctcact
 ggaacccgaggacttcgctgtgtattattgtcagcaacgcagcaactggccccctctgacct
 45 tcggggggggaacaaaagtggagattaaaagaaccgtggccgctccttcctgtttcatcttt

5 cctccatccgatgagcagctgaagtccggtacagccagcgttggtgctgctgaacaactt
 ttatccccgggaggctaaagtgcagtggaaagtggacaatgccctgcagagcggaaatagcc
 aggagtcagtgaccgaacaggatagcaaggattccacctacagcctgagcagcacctgaca
 ctgtctaaagccgactatgagaagcacaagggtgtatgcctgcgaggtgactcaccagggcct
 gagttctcccgtgaccaagtctttcaacaggggagaatgctga

Example inflammation-inducible promoter and foralumab Fab
 nucleotide sequence (SEQ ID NO: 159)

In some embodiments, a nucleotide sequence encoding a CD3 inhibitor operably linked to an
 inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at
 10 least 70% identical to SEQ ID NO: 160 or a fragment thereof. Suitably, the nucleotide
 sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter
 comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least
 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%
 identical to SEQ ID NO: 160 or a fragment thereof. In some embodiments, the nucleotide
 15 sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter has
 a nucleotide sequence comprising or consisting of the sequence SEQ ID NO: 160 or a
 fragment thereof.

20 tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
 gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
 gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
 aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaatagc
 acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
 atcctgcagaagtggctcgtgaggcactgggcaggtaagtatcaaggttacaagacaggttt
 aaggagaccaatagaaaactgggcttgctgagacagagaagactcttgctttctgataggca
 25 cctatttggtcttactgacatccactttgcctttctctccacaggtgtccaggcgccgcat
 ggctaccggctccagaacctccctgctgctggctttcgccctgctgtgtctgccttggtgc
 aggaaggctccgcccagggtgcagctggtgcagtcgggaggaggagtggtgcagcccggcaga
 tcccttcgctgagctgcaaggcttctggctacacattcaccaggtacaccatgcactgggt
 gagacaggcccccgaaaggcctggagtggtggtatattaaccccagcaggggatata
 30 ccaattacaatcagaaggtcaaggacaggttcaccatcagcagggacaacagtaagaatata
 gcattcctgcagatggacagcctgagaccagaggatacaggcgtgtatTTTTGCGCCAGGTA
 ttacgatgaccactactgtctggactactgggggcagggaacaccagtgaccgtgagctccg
 ccagcaciaaagggccctctgtgttccactggccccctccagcaagagcacctccggcgga
 accgcccctgggctgtctggtgaaggactactttcctgaacctgtgacagtcagctggaa
 35 ctcaggcgccctgaccagcgagtgcataccttccccgcgctgctgcagagctctggcctgt
 actccctgagcagcgtggtcacagtgccagcagcagcctgggcacacagacttatactgc
 aacgtgaaccacaagcctagcaacactaagggtcgataaaaagggtcagaaagagaagatcagg
 cagcggcgctaccaacttcagcttgtctgaagcaggccggcgacgtggaggagaacccccggcc
 caatggcaaccggctcaaggacttccctgctcctggctttcgggctgctgtgtctgcctgg
 40 ctgcaggaggggagcgccgacatccagatgacctctccatcttccctgagcgccagcgt
 gggcatcgggtcacaatcacatgttctgccagctccagcgtgagctacatgaattggtacc
 agcagacccccggcaaggctccaaaagggtggatttacgacacctcaaagctggcaagcgga
 gttccctctcgttctccggcagcggtccggaaccgactacaccttcacaatcagctccct
 gcagcctgaagacatcgctacatattactgccagcagtgaggcagcaaccctttcaccttcg
 45 gccagggcacaaagctgcagatcaccaggaccgtggctgcccctagcgtgttcatcttcccc
 ccctcagatgagcagctcaagagcggcacagcctcagtggtgtgcctcctgaacaatttcta
 ccccagagaagcaagggtgcagtggaagggtggacaatgccctgcagtcgggcaactctcagg

agagcgtgacagaaacaggacagtaaaagactccacatatagcctgtctagcaccctgaccctg
 tccaaggccgactacgagaagcacaaggtctacgcttgcgaggtgacacaccaagggctgtc
 tccccctgtcaccaagtcatttaataagaggcgaatgctga

5 Example inflammation-inducible promoter and teplizumab Fab
 nucleotide sequence (SEQ ID NO: 160)

In some embodiments, a nucleotide sequence encoding a CD3 inhibitor operably linked to an
 inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at
 least 70% identical to SEQ ID NO: 161 or a fragment thereof. Suitably, the nucleotide
 sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter
 10 comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least
 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%
 identical to SEQ ID NO: 161 or a fragment thereof. In some embodiments, the nucleotide
 sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter has
 a nucleotide sequence comprising or consisting of the sequence SEQ ID NO: 161 or a
 15 fragment thereof.

tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
 gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
 gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
 20 aaagggagaaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaaatagc
 acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
 atcctgcagaagtgggtcgtgaggcactgggcaggttaagtatcaagggttacaagacaggttt
 aaggagaccaatagaaactgggcttgtcgagacagagaagactccttgcggtttctgataggca
 cctattgggtcttactgacatccactttgcctttctctccacaggtgtccaggcgccgcat
 25 gggccacggctctcgacctccctgctgctggccttcggcctgctgtgtctgcctggctcc
 aggaaggctccgcccaggtgcagctgggtgcagctctggggccgaagtgaagaaacccggcgcc
 agcgtaaggtgagctgcaaggcctctggctacaccttcacagctacaccatgcactgggt
 gagacaggcccccgccagggactggaatggatggggtatatcaacccaaggtctgggtaca
 cccactacaaccagaaactgaaagataaggccacgctgaccgcccagataagtctgccagcacc
 gcctacatggagctgagtagcctgcggtcagaggataccgcccgtgtactactgcgccagatc
 30 tgcctattacgactacgacgggtttgcctactggggacagggaaactggtgaccgtgagct
 ctgcttctacgaagggcccatccgtgttcccactggccccctgcagccgcagcaccagcgag
 tccaccgcccgcctgggatgcctggtgaaagactactttccagagcctgtgaccgtgtcctg
 gaattccggcgcccttaccagcggagtgcacacctttccagccgttctgcagtcagcggcc
 tgtactctctgtctagcgtggtgaccgtgcccagctccaattttggcaccagacctacacc
 35 tgcaacgtggaccacaagccatccaacactaaagtgcgataagactgtgcgcaagagaagaag
 cggcagcggcgccaccaatttttccctgctgaagcaggctggagacgtcgaggagaaccag
 ggccatggctaccggcagccggacttccctgctgctggcctttgggctgctgtgcctgcc
 tggctccaggagggcagcgccgatatccagatgactcagtcctccatcctcactgtccgccag
 cgtgggagatcgggtgactatcacatgtagcgcaagcagcagcgtgagctatatgaactggt
 40 atcagcagaagccaggaaaggccccaaaggcctgatctatgacacctccaaactggccagt
 ggcgtgccttcacgtttctccgcatctggctctgggaccgacttaccctgacaatctccag
 cctgcagccagaggatttcgtacttactactgccagcagtgagctctaaccacccaacct
 tcggcgggcgccaccaaggtggagatcaagagaaccgtggccgccccatccgtgttcattttc
 cctccctctgacgagcagctgaagagcggcaccgcaagtggtgtgcctcctgaataattt
 45 ctaccctcgcaagccaaggtgcagtggaaaggtggacaacgccttcagtcgggcaatagcc
 aggagtcctgacccaagcagcagcaaggactccacatactccctgagcagcaccctgaca

ctgagcaaggctgactacgagaaacacaaggtctatgcctgagaggtgacccatcagggcct
gtcttccccctgtgacaaaaagctttaacagaggcgaaatgttga

Example inflammation-inducible promoter and visilizumab Fab
nucleotide sequence (SEQ ID NO: 161)

- 5 In some embodiments, a nucleotide sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 162 or a fragment thereof. Suitably, the nucleotide sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 162 or a fragment thereof. In some embodiments, the nucleotide sequence encoding a CD3 inhibitor operably linked to an inflammation-inducible promoter has a nucleotide sequence comprising or consisting of the sequence SEQ ID NO: 162 or a fragment thereof.

15 tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaaatagc
acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
20 atcctgcagaagtgtgtcgtgaggcactgggcaggtaagtatcaaggttacaagacaggttt
aaggagaccaatagaaactgggcttgtcgagacagagaagactcttgcgtttctgataggca
cctattggctcttactgacatccactttgcctttctctccacaggtgtccaggcgccgcat
ggccaccgggtctaggaccagcctgctgctggccttcggcctgctgtgtctgcctgggtgc
25 agcctgcgcctgtcatgcgcagcatctgggttcaccttctcctctttccctatggcctgggt
gagacaggcccccggaagggctcggagtgggtgtctaccatttctacatctggaggccgga
cctactacagagatagcgtgaaggcggttcaccatttctagagacaattccaagaatacc
ctgtacctgcaaatgaatagcctgagagccgaagacactgccgtgtattactgcgctaagtt
ccgccagtactccggcggtatcgactactggggccaggccaccctagtgcagtgctcctccg
30 ccagcacaaaaggccccctcgtgtttccactggccccctcttccaagtctacttcaggaggc
acagccgccccctgggctgctgggtgaaagattacttccctgagcccgtgaccgtgtcctggaa
ctcaggagccccctgaccagcggtgcacaccttcccagccgtgctgcagtcctctggcctgt
acagcctgtccagcgtggtgactgtgccaagctccagcctgggcacccagacctacatctgc
aatgtgaaccacaagcctagcaacacaaaggtggacaagaaagtgagaaaaagaaggtctgg
35 ctccggcgcgaccaacttctccctgctgaaacaggccggggacgtggaggagaaccttgcc
caatggccacaggctctagaaccagcctgctgctggctttcggcctgctgtgcctgccctgg
ctgcaggaaggctccgccgatatccagcttaccagcccaatagcgtgtccacgtcactggg
ttccacagtcaagctgagttgtactctgagcagtggggaatattgagaataactacgtgcact
ggtatcagctgtacgaggttaggagccctacaaccatgatctatgacgacgacaagcgccca
40 gacggcgtgcctgacagattctccggctcaatcgacagatccagtaactccgccttctgac
aattcacaaactgtggtattgaggacgagggcatttacttttgccactcctacgtgagcagct
tcaatgtgttcggcgccgggactaagctgaccgttctgcccagcctaagccgctccaagc
gtgaccctgttcccccccagcagcgaggaaactgcaggccaacaaagccaccctgggtgtgct
gatcagcgatttctacccccgggcccgtgaccgttgctggaaggccgacagcagccctgtga
45 agggccgggggtggagaccacaacccccctccaagcagagcaacaacaagtacgcccgaagctcc
tacctgagcctgactcccgagcagtggaatctcataggtcttacagttgccaggttacaca
cgaaggggagcacagtggaacaaacagtgggccccccaccgaatgctcctga

Example inflammation-inducible promoter and orelizumab Fab
nucleotide sequence (SEQ ID NO: 162)

Other regulatory elements

5 A polynucleotide or vector of the present invention may comprise one or more further regulatory elements which may act pre- or post-transcriptionally.

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to one or more further regulatory elements which may act pre- or post-transcriptionally. In some embodiments, (a) the nucleotide sequence encoding
10 a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to one or more further regulatory elements which may act pre- or post-transcriptionally.

As used herein, a “regulatory element” may refer to any nucleotide sequence that facilitates expression of a polypeptide, e.g. acts to increase expression of a transcript or to enhance
15 mRNA stability. Suitable regulatory elements include for example enhancer elements, post-transcriptional regulatory elements, introns, polyadenylation sites, and Kozak sequences.

Enhancers

A polynucleotide or vector of the present invention may comprise an enhancer.

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to an enhancer. In some embodiments, (a) the
20 nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to an enhancer. The enhancer may facilitate expression of the TNF inhibitor and/or CD3
25 inhibitor in ocular cells (e.g. retinal ganglion cells, RPE cells, photoreceptors, glial cells).

An “enhancer” or “enhancer element” may refer to a region of DNA that can be bound by proteins (activators) to increase the likelihood that transcription of a particular gene will occur. Enhancers are cis-acting. They can be located up to 1 Mbp (1,000,000 bp) away from the gene, upstream or downstream from the start site.

30 The polynucleotide or vector of the present invention may comprise an ocular tissue-specific enhancer. Suitably, the enhancer may be operably linked to: (a) the nucleotide sequence

encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment). In some embodiments, the enhancer is operably linked to: (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment).

As used herein, a "tissue-specific enhancer" is an enhancer which preferentially facilitates expression of a gene in specific cells or tissues. Suitably, a tissue-specific enhancer may facilitate higher expression of a gene in specific cells-types as compared to other cell-types. Higher expression may be measured for example by measuring the expression of a transgene, e.g. green fluorescence protein (GFP), operably linked to the enhancer, wherein expression of the transgene correlates with the ability of the enhancer to facilitate expression of a gene. Suitable tissue-specific enhancers will be known to those of skill in the art. The enhancer may be a retinal-specific enhancer, preferably a retinal ganglion-specific enhancer. Suitably, the enhancer may be (or may be derived from) an enhancer associated with a gene with selective expression in human retinal cells. Methods to identify the enhancer regions associated with genes will be known to those of skill in the art.

Polyadenylation sequences

A polynucleotide or vector of the present invention may comprise a polyadenylation sequence. A polyadenylation sequence may be inserted after a nucleotide sequence to improve transgene expression.

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to a polyadenylation sequence. In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to a polyadenylation sequence.

A polyadenylation sequence typically comprises a polyadenylation signal, a polyadenylation site and a downstream element: the polyadenylation signal comprises the sequence motif recognised by the RNA cleavage complex; the polyadenylation site is the site of cleavage at which a poly-A tails is added to the mRNA; the downstream element is a GT-rich region which usually lies just downstream of the polyadenylation site, which is important for efficient processing.

Suitable polyadenylation sequences will be known to those of skill in the art (see e.g. Schambach, A., et al., 2007. *Molecular Therapy*, 15(6), pp.1167-1173; and Choi, J.H. et al., 2014. *Molecular brain*, 7(1), pp.1-10). Example polyadenylation sequences include the bovine growth hormone (bGH) polyadenylation sequence, the SV40 polyadenylation sequence, and the rabbit beta-globin polyadenylation sequence.

In some embodiments, the polyadenylation sequence comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 163 or a fragment thereof. Suitably, the polyadenylation sequence comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 163 or a fragment thereof.

In some embodiments, the polyadenylation sequence comprises or consists of the nucleotide sequence SEQ ID NO: 163 or a fragment thereof.

gcctcgactgtgccttctagttgccagccatctgttggtttgccctccccctgccttcctt
gaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcacgcatt
gtctgagtaggtgtcattctattctggggggtgggggtggggcaggacagcaagggggaggat
tggaagacaatagcaggcatgctgggga

Example bGH polyadenylation sequence (SEQ ID NO: 163)

Post-transcriptional regulatory elements

A polynucleotide or vector of the present invention may comprise a post-transcriptional regulatory element.

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to a post-transcriptional regulatory element. In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to a post-transcriptional regulatory element.

A polynucleotide or vector of the present invention may comprise a woodchuck hepatitis post-transcriptional regulatory element (WPRE).

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to a WPRE. In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide

sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to a WPRE.

Suitable WPRE sequences will be known to those of skill in the art (see e.g. Zufferey, R., et al., 1999. Journal of virology, 73(4), pp.2886-2892; and Zanta-Boussif, M.A. et al., 2009. Gene therapy, 16(5), pp.605-619). Suitably, the WPRE is a wild-type WPRE or is a mutant WPRE. For example, the WPRE may be mutated to abrogate translation of the woodchuck hepatitis virus X protein (WHX) e.g. by mutating the WHX ORF translation start codon.

In some embodiments, the WPRE comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 164 or a fragment thereof. Suitably, the WPRE comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 164 or a fragment thereof.

In some embodiments, the WPRE comprises or consists of the nucleotide sequence SEQ ID NO: 164 or a fragment thereof.

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15      aatcaacctctggattacaaaatttgtgaaagattgactgggtattcttaactatgttgctcc
      ttttacgctatgtggatacgtgctttaatgcctttgtatcatgctattgcttcccgatgg
      ctttcattttctcctccttgtataaatcctgggtgctgtctctttatgaggagtgtggccc
      gttgtcaggcaacgtggcgtggtgtgcaactgtgtttgctgacgcaacccccactggttgggg
20      cattgccaccacctgtcagctcctttccgggactttcgctttccccctccctattgccacgg
      cggaactcatcgccgctgccttgcccgtgctggacaggggctcggtgtgtgggcactgac
      aattccgtggtgtgtgcgggaaatcatcgctcctttccttggtgctcgccgtgtgtggcac
      ctggattctgcgcgggacgtccttctgtacgtcccttcggccctcaatccagcggaccttc
      cttcccgcggcctgctgccggtctgcggcctcttccgcgtcttcg

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Example WPRE sequence (SEQ ID NO: 164)

25 Introns

A polynucleotide or vector of the present invention may comprise an intron. An intron may be inserted between a promoter and a nucleotide sequence to increase expression.

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to an intron. In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to an intron.

Suitable introns will be known to those of skill in the art (see e.g. Powell, S.K., et al., 2015. Discovery medicine, 19(102), p.49) and may include an MVM intron, a F.IX truncated intron 1,

a chimeric β -globin/immunoglobulin heavy chain intron, a chimeric adenovirus/immunoglobulin intron, and a SV40 intron.

In some embodiments, the intron is an SV40 intron.

In some embodiments, the intron comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 165 or a fragment thereof. Suitably, the intron comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 165 or a fragment thereof.

In some embodiments, the intron comprises or consists of the nucleotide sequence SEQ ID NO: 165 or a fragment thereof.

```
gtaagtatcaagggttacaagacaggtttaaggagaccaatagaaactgggcttgtcgagaca
gagaagactcttgcgtttctgataggcacctattgggtcttactgacatccactttgcctttc
tctccacag
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Example SV40 intron (SEQ ID NO: 165)

15 Kozak sequences

A polynucleotide or vector of the present invention may comprise a Kozak sequence. A Kozak sequence may be inserted before the start codon to improve the initiation of translation.

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to a Kozak sequence. In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to a Kozak sequence.

Suitable Kozak sequences will be known to those of skill in the art (see e.g. Kozak, M., 1987. Nucleic acids research, 15(20), pp.8125-8148). A consensus Kozak sequence in vertebrates may have the sequence of SEQ ID NO: 166 or SEQ ID NO: 167.

Suitably, the Kozak sequence may comprise or consist of the nucleotide sequence of SEQ ID NO: 166 or 167, or variants thereof which have five or fewer deletions, substitutions or insertions. Suitably, the variants may have four or fewer, three or fewer, two or fewer, or one deletion(s), substitution(s) or insertion(s). Suitably, the variants may have three or fewer, two or fewer, or one deletion(s) and/or three or fewer, two or fewer, or one substitution(s). Suitably, the variants may have three or fewer, two or fewer, or one deletion(s) and/or three or fewer,

two or fewer, or one substitution(s). Suitably, the variants may have one deletion and/or one substitution. Suitably, the variants may have one deletion and one substitution.

gccrccatgg

Example consensus Kozak sequence 1 (SEQ ID NO: 166)

5

gccgccrccatgg

Example consensus Kozak sequence 2 (SEQ ID NO: 167)

Inflammation-inhibiting oligonucleotides

A polynucleotide or vector of the present invention may comprise a sequence encoding an inflammation-inhibiting oligonucleotide.

Suitably, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and/or (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) is operably linked to a sequence encoding an inflammation-inhibiting oligonucleotide. In some embodiments, (a) the nucleotide sequence encoding a TNF inhibitor (e.g. anti-TNF antibody fragment); and (b) the nucleotide sequence encoding a CD3 inhibitor (e.g. anti-CD3 antibody fragment) are operably linked to a sequence encoding an inflammation-inhibiting oligonucleotide.

The insertion of a sequence encoding an inflammation-inhibiting oligonucleotide into a vector may reduce innate immune and T cell responses and enhanced gene expression by “cloaking” the vector from inducing unwanted immune responses. Suitable sequences will be known to those of skill in the art (see e.g. Chan, Y.K., et al., 2021. Science translational medicine, 13(580), p.eabd3438).

The sequence encoding an inflammation-inhibiting oligonucleotide may antagonise TLR9 activation. In some embodiments, the inflammation-inhibiting oligonucleotide may be a TLR9-inhibiting oligonucleotide. In some embodiments, the sequence encoding an inflammation-inhibiting oligonucleotide includes one or more TLR9i sequences, for example one or more, two or more, three or more TLR9i sequences. Suitable TLR9i sequences are known in the art and a suitable TLR9i sequence is shown in SEQ ID NO: 168. In some embodiments, a TLR9i sequence comprises or consists of a nucleotide sequence which is at least 92%, at least 96% or 100% identical to SEQ ID NO: 168. The TLR9i sequences may be separated by any suitable linker (e.g. AAAAA linkers).

tttagggtagggtagggtaggg

Example TLR9i sequence (SEQ ID NO: 168)

In some embodiments, the sequence encoding an inflammation-inhibiting oligonucleotide comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 169 or a fragment thereof. Suitably, the sequence encoding an inflammation-inhibiting oligonucleotide comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 169 or a fragment thereof.

In some embodiments, the sequence encoding an inflammation-inhibiting oligonucleotide comprises or consists of the nucleotide sequence SEQ ID NO: 169 or a fragment thereof.

10 tttagggtttagggtttagggttagggaataatttagggtttagggtttagggtaatt
tagggtttagggtttagggttagggaataatgcagcggtaagttcccatccaggtttttgcag
cggtaagttcccatccaggtttttgcagcggtaagttcccatccaggttttt

Example io2 sequence (SEQ ID NO: 169)

Bicistronic or multicistronic polynucleotides or vectors

In one aspect, the present invention provides a polynucleotide or vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

In one aspect, the present invention provides an AAV vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor. AAV vector genome capacity permits up to ~5kb or cargo, enabling x2 transgenes (each ~1.5kb), promoter (~0.3kb) and regulatory elements such as WPRE (0.55kb) to be incorporated.

Bicistronic or multicistronic vectors

The polynucleotide or vector of the present invention may be a bicistronic polynucleotide or vector encoding a TNF inhibitor and a CD3 inhibitor. As used herein, a “bicistronic polynucleotide or vector” may refer to a polynucleotide or vector which expresses two separate genes from a single transcript.

The polynucleotide or vector of the present invention may be a multicistronic polynucleotide or vector encoding a TNF inhibitor and a CD3 inhibitor. As used herein, a “multicistronic polynucleotide or vector” may refer to a polynucleotide or vector which expresses two or more separate genes from a single transcript.

Typically, bicistronic or multicistronic polynucleotide or vectors contain IRES and/or self-cleaving 2A peptides to allow for co-expression of multiple genes from a single transcript (see

e.g. Liu, Z., et al., 2017. Scientific reports, 7(1), p.2193; and Renaud-Gabardos, E., et al., 2015. World journal of experimental medicine, 5(1), pp.11-20).

In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element.

In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence encoding a 2A self-cleaving peptide.

Suitable 2A self-cleaving peptides include T2A, P2A, E2A and F2A, or derivatives thereof. In some embodiments, the 2A self-cleaving peptide is a T2A self-cleaving peptide or derivative thereof.

In some embodiments, the 2A self-cleaving peptide has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 6-9. In some embodiments, the 2A self-cleaving peptide comprises or consists of any of SEQ ID NOs: 6-9.

In some embodiments, the 2A self-cleaving peptide has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 7. In some embodiments, the 2A self-cleaving peptide comprises or consists of SEQ ID NO: 7.

In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 170. In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence comprising or consisting of SEQ ID NO: 170.

gccaccaacttcagcctgctgaagcagggcaggggacgtggaggagaatcctggacca
Example T2A nucleotide sequence (SEQ ID NO: 170)

In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a cistronic linker sequence encoding a 2A self-cleaving peptide. The cistronic linker sequence may comprise any other suitable nucleotide sequences, for example nucleotide sequences which aid expression. Suitably, the linker sequence may comprise a furin site and/or a fusion protein linker sequence.

In some embodiments, the cistronic linker sequence comprises a furin site. Furin is a protease enzyme that may cleave at a conserved polybasic RNRR site. Suitably, the furin site encodes RKRR. In some embodiments, the cistronic linker sequence comprises fusion protein linker sequence. Fusion protein linker sequences may join two protein domains together. Suitably, the fusion protein linker sequence encodes SGSG. In some embodiments, the cistronic linker sequence encodes from 5' to 3': a furin site, a fusion protein linker sequence, and a 2A self-cleaving peptide.

In some embodiments, the cistronic linker sequence encodes an amino acid having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 171. In some embodiments, the cistronic linker sequence encodes an amino acid comprising or consisting of SEQ ID NO: 171.

RKRRSGSGEGRGSLTTCGDVEENPGP

Example cistronic linker peptide sequence (SEQ ID NO: 171)

In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 172. In some embodiments, the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence comprising or consisting of SEQ ID NO: 172.

cgaagcgagcaggagtggttctggggccaccaacttcagcctgctgaagcaggcaggggacgt
ggaggagaatcctggacca

Example cistronic linker sequence (SEQ ID NO: 172)

25 Example polynucleotides and vectors encoding TNF inhibitor and CD3 inhibitor

In the polynucleotide or vector, the nucleotide sequence encoding a TNF inhibitor; the nucleotide sequence encoding a CD3 inhibitor; and any further elements (e.g. promoter and/or other regulatory elements) may be arranged in any suitable orientation and/or configuration.

Suitably, the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor have the same orientation. In some embodiments, the nucleotide sequence encoding a TNF inhibitor is upstream from the nucleotide sequence encoding a CD3 inhibitor. In other embodiments, the nucleotide sequence encoding a CD3 inhibitor is upstream from the nucleotide sequence encoding a TNF inhibitor.

In preferred embodiments, the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are each comprised in the same expression cassette.

As used herein, an "expression cassette" may refer to a distinct component consisting of one
5 more genes and regulatory sequences to be expressed. Typically, an expression cassette is composed of one or more genes and the regulatory sequences controlling their expression. An expression cassette may comprise three components: a promoter sequence, an open reading frame, and a 3' untranslated region. In some embodiments, the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are each
10 comprised in the same open reading frame. In some embodiments, the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are each operably linked to the same regulatory elements.

In some embodiments, the polynucleotide or vector comprises only one promoter. In preferred
15 embodiments, the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are each operably linked to the same promoter.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a
20 CD3 inhibitor. In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a TNF inhibitor.

25 In other embodiments, the polynucleotide or vector comprises two promoters. In some embodiments, the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are operably linked to different promoters.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a TNF
30 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; a promoter; and the nucleotide sequence encoding a CD3 inhibitor. In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving

peptide, an enzymatically cleavable peptide motif, and/or an IRES element; a promoter; and the nucleotide sequence encoding a TNF inhibitor.

In preferred embodiments, the promoter is an inflammation-inducible promoter.

5 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a CD3 inhibitor. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; and the nucleotide sequence encoding a CD3 inhibitor.

15 In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a TNF inhibitor. In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; and the nucleotide sequence encoding a TNF inhibitor.

20 In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; an inflammation-inducible promoter; and the nucleotide sequence encoding a CD3 inhibitor. In some
25 embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; an inflammation-inducible promoter; and the nucleotide sequence encoding a CD3 inhibitor.

30 In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; an inflammation-inducible promoter; and the nucleotide sequence encoding a TNF inhibitor. In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or

consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; an inflammation-inducible promoter; and the nucleotide sequence encoding a TNF inhibitor.

5 In preferred embodiments, the polynucleotide or vector further comprises a polyadenylation sequence.

10 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; the nucleotide sequence encoding a CD3 inhibitor; and a polyadenylation sequence. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; the nucleotide sequence encoding a CD3 inhibitor; and a polyadenylation sequence.

15 In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; the nucleotide sequence encoding a TNF inhibitor; and a polyadenylation sequence. In other embodiments, 20 the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; the nucleotide sequence encoding a TNF inhibitor; and a polyadenylation sequence.

25 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; and a polyadenylation sequence. In some embodiments, the polynucleotide or vector comprises a nucleotide 30 sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; and a polyadenylation sequence.

In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; and a polyadenylation sequence. In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; and a polyadenylation sequence.

The polynucleotide or vector of the present invention may further comprise any other suitable elements, such as any other elements described herein or one or more spacer sequence. The spacer sequence(s) may comprise, for example, at least one (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10), at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten nucleotide bases. A spacer sequence may comprise a restriction site to enable the insertion of one or more further elements.

In some embodiments, the polynucleotide or vector further comprises one or more ITRs. In preferred embodiments, the vector is an AAV vector.

In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; the nucleotide sequence encoding a CD3 inhibitor; a polyadenylation sequence; and a 3' ITR. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; the nucleotide sequence encoding a CD3 inhibitor; a polyadenylation sequence; and a 3' ITR.

In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; the nucleotide sequence encoding a TNF inhibitor; a polyadenylation sequence; and a 3' ITR. In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-

cleaving peptide; the nucleotide sequence encoding a TNF inhibitor; a polyadenylation sequence; and a 3' ITR.

In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a polyadenylation sequence; and a 3' ITR. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a polyadenylation sequence; and a 3' ITR.

In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a polyadenylation sequence; and a 3' ITR. In other embodiments, the polynucleotide or vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a 5' ITR; an inflammation-inducible promoter; the nucleotide sequence encoding a CD3 inhibitor; a nucleotide sequence encoding a 2A self-cleaving peptide; an inflammation-inducible promoter; the nucleotide sequence encoding a TNF inhibitor; a polyadenylation sequence; and a 3' ITR.

Example polynucleotides and vectors encoding adalimumab Fab and foralumab Fab

In some embodiments, the polynucleotide or vector comprises: (a) a nucleotide sequence encoding an adalimumab Fab; and (b) a nucleotide sequence encoding a foralumab Fab.

In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide sequence encoding a foralumab Fab. In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding a foralumab Fab.

In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding a foralumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide sequence encoding an adalimumab Fab. In some embodiments, the polynucleotide or vector
5 comprises from 5' to 3': a nucleotide sequence encoding a foralumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding an adalimumab Fab.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof; an
10 adalimumab light chain, or a derivative thereof; a foralumab heavy chain (e.g. VH + CH1), or a derivative thereof; and a foralumab light chain, or a derivative thereof, wherein the chains are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an amino acid sequence having at least 70%, at least 75%, at least 80%, at least
15 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 24; an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 26; an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at
20 least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 78; and an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 80, wherein the amino acid sequences are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide having at least 70%, at least 75%,
25 at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 6-9.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: the amino acid sequence of SEQ ID NO: 24; the amino acid sequence of SEQ ID NO: 26; the amino acid sequence of SEQ ID NO: 78; and the amino acid sequence of SEQ ID
30 NO: 80, wherein the amino acid sequences are operably linked by nucleotide sequences encoding the 2A self-cleaving peptide of any of SEQ ID NOs: 6-9.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity,

or 100% identity to SEQ ID NO: 187. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding SEQ ID NO: 187.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 187. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding SEQ ID NO: 187.

10 MATGSRTSLLLLAFGLLCLPWLQEGSAEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHW
VRQAPGKGLEWVSAITWNSGHIDYADSVGEGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAK
VSYLSTASSLDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVRKR
RSGSGATNFSLLKQAGDVEENPGPMATGSRTSLLLLAFGLLCLPWLQEGSADIQMTQSPSSLS
ASVGDRTITCRASQGIIRNYLAWYQQKPKAPKLLIYAASLTQSGVPSRFSGSGSGTDFTLT
15 ISSLPEDVATYYCQRYNRPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGECRKRSGSGEGRGSLTCTGDEENPGPMATGSRTSLLLLAFGLLCLPWL
LQEGSAQVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWVAVIWDGS
KKYYVDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARQMGYWHFDLWGRGTLTVSS
20 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKRRSGSGATNFSLLKQAGDVEENPG
PMATGSRTSLLLLAFGLLCLPWLQEGSAEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAW
YQQKPGQAPRLLIYDASNRTGIPARFSGSGSGTDFTLTISLSEPEDFAVYYCQQRSNWPPPL
TFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSSTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

25 Example amino acid sequence encoding Adalimumab Fab and
foralumab Fab (SEQ ID NO: 187)

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 173 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 173 or a fragment thereof.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which is at least 70% identical to SEQ ID NO: 173 or a fragment thereof. Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 173 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 173 or a fragment thereof.

40 atggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttgct
gcaagagggcagcgccgaggtgcaactggtggaaagcggcgggcctggtccagcctggaa

5 ggtccctgagactgagctgtgccgccagcggtttaccttcgacgactacgccatgcactgg
 gtgccccaggccctggcaagggcctggaatgggtctccgccatcacctggaatagcggcc
 catcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaaagact
 ctctgtatctgcaaatgaacgcctgctgggtgaagatacagccgtgtactattgcgcaaaa
 10 gtgagctacctctccaccgccagcagcctggactattggggacagggcacccctggtgaccgt
 gtctagcgcctccacaaagggccttctgtgtttccactggctccaagctccaaaagcacat
 ctggaggaaccgctgccctgggtgcctgggttaaggactacttccccgagcctgtgaccgtg
 agctggaacagcggcgccctgacatctggtgttcataccttccctgccgttctgcaatcttc
 15 tggactctacagcctgtcttctgtggtgaccgtgccagcagcagccttggaaacagacac
 acatctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcgg
 agaagcgggaagcgggtgctaccaacttcagcctcctgaaacagggccggcgatgtggaggaaa
 ccctggacctatggctaccggcagcagaaccagcctgctgctggcattcggccttctgtgcc
 20 tgccttggctgcaagagggctctgccgacatccagatgaccagagcccttccctactgagc
 gccagcgtggggcagacagagtactattacatgcagagccagccaagcatccggaactacct
 ggcttggatcagcagaagcccgcaaaagccctaaagctgctgatctacgccgccagcacc
 25 tgcaagcggcgctgcctagcagattcagcggctcaggctctggcactgatttcaccctgacc
 atctcctctctgcaacctgaggacgtggccacatactactgccagagatacaacagagcccc
 atacacctttggccagggcacaaaagtggaaatcaagagaaccgtggccgctcccagtggt
 30 tcatcttccccccagtgatgagcagctgaagtcgggcacagcctctgtcgtgtgcctgctg
 aacaacttctaccccagagaggccaaggtgcagtggaaaggtggataatgccctgcaaagcgg
 caacagccaggagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagca
 cactgaccctgagcaaggccgactacgagaagcacaaggtgtacgcatgcgaggtgaccac
 35 cagggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgtcgaaagcgcaggag
 tggttctggggagggcaggggaagtctactaacatgcggggacgtggaggaaaatcccgcc
 caatggctaccggctctaggacctcactgctgctggccttcggcctgctgtgcctgcctgg
 ctgcaggagggcagcggccaggtccagctggtggagagcggcgggggcggtggtgcagcccg
 40 cagaagcctgcggctgagctgtgccgccagcggattcaagtttagcggctacgggatgact
 gggtcaggcaggctcctggaaaggactggagtgggtggcagtgatctggatgatggctcc
 aagaaatactacgtcgacagcgtgaaaggacgcttccaccatcagcagggataacagcaagaa
 45 tacactgtacctgcagatgaacagcctgagagcagaggatacagccgtgtactactgcgcca
 ggcagatgggctactggcacttcgacctatgggggaggggtacactgggtgaccgtgagctcc
 gcatccaccaaagggccctctgtgttccccctggccccctccagcaagtcacactctggagg
 aaccgctgccttgggtgtctgtgtgaaagattatttccagagcctgtcaccgtgtcttggga
 50 actccggcgctctgacatccggcgtgcacacattccccgcgctgctgcagacatctggcctc
 tacagcctgtcttctgtggtgacagtgccagctccagcctggggaccagacctatctg
 caacgtgaaccacaagccatctaaccaccaaagtggaataaacgggtgaggaaagagggtccg
 gctccggcgccacaaacttttctctgtgtagcaggccggggacgtggaggagaacccccggc
 ccaatggccaccggttctagaaccagcctgctgctggcctttggcctgctgtgcctgccttg
 gctgcaggagggcagcgtgaaattgtgctgacacagtctcccgccaccctgtccctgtccc
 40 cggcgagagggccacactgagctgtagggccagccagtcctgtgagcagctatctggcctgg
 taccagcagaaacccggacagccccaagactgctgatttatgacgcctccaacagagctac
 gggcatccccgctcgttttccggcagcgggaagcgggacagacttcaactctgaccattagct
 cactggaacccgaggacttcgctgtgtattattgtcagcaacgcagcaactggccccctctg
 45 accttcggggggggaacaaaagtggagattaaaagaaccgtggccgctccttccgtgttcat
 ctttcctccatccgatgagcagctgaagtccgggtacagccagcgttgtgtgcctgtgaaaca
 acttttatccccgggaggtataaagtgcagtggaaagtggacaatgcctgcagagcggaaat
 agccaggagtgcagtaccgaacaggatagcaaggattccacctacagcctgagcagcacct
 gacactgtctaaagccgactatgagaagcacaaggtgtatgctgaggtgactcaccagg
 gcctgagttctcccgtagaccaagtctttcaacaggggagaatgctga

50 Example polynucleotide encoding Adalimumab Fab and foralumab Fab
 (SEQ ID NO: 173)

In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a foralumab Fab are operably linked to an inflammation-

- inducible promoter. In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a foralumab Fab are operably linked to an inflammation-inducible promoter comprising or consisting of a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 157 or a fragment thereof. In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a foralumab Fab are operably linked to an inflammation-inducible promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 157 or a fragment thereof.
- 10 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 174 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 174 or a fragment thereof.
- 15 In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which is at least 70% identical to SEQ ID NO: 174 or a fragment thereof. Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 174 or a fragment thereof. In some
- 20 embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 174 or a fragment thereof.

25 tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
 gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
 gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
 aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaatagc
 acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
 atcctgcagaagtgtgtcgtgaggcactgggcaggtaagtatcaaggttacaagacaggttt
 aaggagaccaatagaaactgggcttgctcgagacagagaagactcttgcgtttctgataggca
 cctattggtcttactgacatccactttgcctttctctccacaggtgtccaggcggcggccat
 30 ggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttggtgc
 aagagggcagcgccgaggtgcaactggtggaagcggcggcggcctggtccagcctggaagg
 tccttgagactgagctgtgcccagcggcctttaccttcgacgactacgccatgactgggt
 gcgccaggcccctggcaaggcctggaatgggtctccgcatcacctggaatagcggccaca
 tcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaaagaactct
 35 ctgtatctgcaaatgaacagcctgcccggctgaagatacagccgtgtactattgcgccaaagt
 gagctacctctccaccgccagcagcctggactattggggacagggcaccctggtgaccgtgt
 ctagcgcctccacaaaggcccttctgtgtttccactggctccaagctccaaaagcacatct
 ggaggaaccgctgccttggtgctggttaaggactacttccccgagcctgtgaccgtgag
 ctggaacagcggcgccctgacatctggtgttcataccttccctgccgttctgcaatcttctg
 40 gactctacagcctgtcttctgtggtgaccgtgccagcagcagccttgaacacagacctac
 atctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcggag
 aagcgggaagcgggtgctaccaacttcagcctcctgaaacaggcggcgatgtggaggaaaacc

ctggacctatggctaccggcagcagaaccagcctgctgctggcattcggccttctgtgcctg
 ccttggctgcaagagggtctgcccacatccagatgaccagagcccttctcactgagcgc
 cagcgtgggacagagtgactattacatgcagagccagccaaggcatccggaactacctg
 cctggtatcagcagaagcccggcaaaagcccctaagctgctgatctacgccgagcaccctg
 5 caaagcggcgtgcctagcagattcagcggctcaggctctggcactgatttcaccctgaccat
 ctctctctgcaacctgaggacgtggccacatactactgccagagatacaacagagcccat
 acacctttggccagggcacaaaagtggaaatcaagagaaccgtggccgctcccagtggttc
 atcttccccccagtgatgagcagctgaagtccggcacagcctctgtcgtgtgcctgctgaa
 caacttctacccagagaggccaagggtgacgtggaagggtggataatgccctgcaaagcggca
 10 acagccaggagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagcaca
 ctgacctgagcaaggccgactacgagaagcacaagggtgtacgcatgcgaggtgaccacca
 gggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgctgaaaagcgcaggagt
 gttctggggagggcagggaagtctactaacatgcggggacgtggaggaaaatcccggcca
 atggctaccggctctaggacctcactgctgctggccttcggcctgctgtgcctgcctggct
 15 gcaggagggcagcggccaggtccagctggtggagagcggcgggggcggtggtgcagccggca
 gaagcctgcggctgagctgtgccgccagcgattcaagtttagcggctacgggatgactgg
 gtcaggcaggctcctgaaaggactggagtgggtggcagtgatctggtatgatggctccaa
 gaaatactacgtcgacagcgtgaaaggacgcttcaccatcagcagggataacagcaagaata
 cactgtacctgcagatgaacagcctgagagcagaggatacagccgtgtactactgcgccagg
 20 cagatgggctactggcacttcgacctatgggggaggggtacactgggtgacctgagctccgc
 atccaccaaaggccctctgtgttccccctggccccctccagcaagtccacctctggaggaa
 ccgctgcctgggctgtctgtgaaagattatttccagagcctgtcaccgtgtcttggaac
 tccggcgtctgacatccggcgtgcacacattccccgcgctgctgcagagctctggcctcta
 cagcctgtcttctgtggtgacagtgccagctccagcctggggacccagacctatatctgca
 25 acgtgaaccacaagccatctaaccaccaaagtggataaacgggtgaggaagaggaggtccggc
 tccggcgccacaaacttttctctgtgtaagcaggccggggacgtggaggagaacccccggccc
 aatggccaccggttctagaaccagcctgctgctggcctttggcctgctgtgcctgccttggc
 tgcaggagggcagcgtgaaattgtgctgacacagtctcccgccaccctgtccctgtcccc
 ggcgagagggccacactgagctgtagggccagccagtcctgtgagcagctatctggcctggt
 30 ccagcagaaacccggacaggccccaagactgctgatttatgacgcctccaacagagctacgg
 gcatccccgctcgttttccggcagcgggaagcgggacagacttcactctgaccattagctca
 ctggaacccgaggacttcgctgtgtattattgtcagcaacgcagcaactggccccctctgac
 ctctcggggggggaacaaaagtggagattaaaagaaccgtggccgctccttcctgttcatct
 35 ttctccatccgatgagcagctgaagtccgggtacagccagcgttgtgtgcctgctgaacaac
 ttttatccccgggaggctaaagtgcagtggaagtggacaatgccctgcagagcggaaatag
 ccaggagtcagtgaccgaacaggatagcaaggattccacctacagcctgagcagcacctga
 cactgtctaaagccgactatgagaagcacaagggtgtatgcctgcgaggtgactcaccagggc
 ctgagttctcccgtgaccaagtctttcaacaggggagaatgctga

40 Example polynucleotide encoding Adalimumab Fab and foralumab Fab
 operably linked to inflammation-inducible promoter (SEQ ID NO:
 174)

Example polynucleotides and vectors encoding adalimumab Fab and teplizumab Fab

In some embodiments, the polynucleotide or vector comprises: (a) a nucleotide sequence encoding an adalimumab Fab; and (b) a nucleotide sequence encoding a teplizumab Fab.

- 45 In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide sequence encoding a teplizumab Fab. In some embodiments, the polynucleotide or vector

comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding a teplizumab Fab.

5 In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding a teplizumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide sequence encoding an adalimumab Fab. In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding a teplizumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding an
10 adalimumab Fab.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof; an adalimumab light chain, or a derivative thereof; a teplizumab heavy chain (e.g. VH + CH1), or a derivative thereof; and a teplizumab light chain, or a derivative thereof, wherein the chains
15 are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 24; an amino acid sequence having at least 70%, at least
20 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 26; an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 89; and an amino acid sequence having at least 70%, at least 75%, at least 80%, at least
25 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 91, wherein the amino acid sequences are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 6-9.

30 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: the amino acid sequence of SEQ ID NO: 24; the amino acid sequence of SEQ ID NO: 26; the amino acid sequence of SEQ ID NO: 89; and the amino acid sequence of SEQ ID NO: 91, wherein the amino acid sequences are operably linked by nucleotide sequences encoding the 2A self-cleaving peptide of any of SEQ ID NOs: 6-9.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 188. In some embodiments, the polynucleotide or vector

5 comprises a nucleotide sequence encoding SEQ ID NO: 188.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 188. In some embodiments,

10 the polynucleotide or vector comprises a nucleotide sequence encoding SEQ ID NO: 188.

15 MATGSRTSLLLAFLGLLCLPWLQEGSAEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHW
VRQAPGKGLEWVSAITWNSGHIDYADSVGEGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAK
VSYLSTASSLDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVRKR
RSGSGATNFSLLKQAGDVEENPGPMATGSRTSLLLAFLGLLCLPWLQEGSADIQMTQSPSSLS
ASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASLTQSGVPSRFSGSGSGTDFTLT
ISSLPEDVATYYCQRYNRAPYTFGGQTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTH
20 QGLSSPVTKSFNRGECRKRSGSGEGRGSLTCDGVEENPGPMATGSRTSLLLAFLGLLCLPW
LQEGSAQVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKLEWIGYINPSRG
YTNYNQKVKDRFTISRDNKNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGTPTVVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVRKRSGSGATNFSLLKQAGDVEENP
25 GPMATGSRTSLLLAFLGLLCLPWLQEGSADIQMTQSPSSLSASVGDRVTITCSASSSVSYMNW
YQQTTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFITISSLPEDIATYYCQWSSNPFT
FGQGTKLQITRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Example amino acid sequence encoding Adalimumab Fab and
teplizumab Fab (SEQ ID NO: 188)

30 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 175 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 175 or a fragment thereof.

35 In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which is at least 70% identical to SEQ ID NO: 175 or a fragment thereof. Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 175 or a fragment thereof. In some

embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 175 or a fragment thereof.

atggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttggct
5 gcaagagggcagcgccgaggtgcaactgggtggaaagcggcgggcctgggtccagcctggaa
gggtccctgagactgagctgtgcccagcggcctttaccttcgacgactacgccatgactgg
gtgcccagggccctggcaagggcctggaatgggtctccgccatcacctggaatagcggcca
catcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaaagaact
ctctgtatctgcaaatgaacagcctgcgggctgaagatacagccgtgtactattgcgcaaaa
10 gtgagctacctctccaccggcagcctggactattggggacagggcacccctgggtgacctg
gtctagcgcctccacaaaggcccttctgtgtttccactggctccaagctccaaaagcacat
ctggaggaaccgctgcctgggctgctgggttaaggactacttccccgagcctgtgacctg
agctggaacagcggcgccctgacatctgggtgttcataccttccctgccgttctgcaatcttc
tggactctacagcctgtcttctgtgtgacctgcccagcagcagccttggaaacacagacct
15 acatctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcgg
agaagcggaaagcgggtgctaccaacttcagcctcctgaaacagggcggcgatgtggaggaaaa
ccctggacctatggctaccggcagcagaaccagcctgctgctggcattcggccttctgtgcc
tgccttggctgcaagagggctctgccgacatccagatgacccagagcccttccctactgagc
gccagcgtggggacagagtactattacatgcagagccagccaaggcatccggaactacct
ggcctgggtatcagcagaagcccggaagcccctaagctgctgatctacggccgacgacccc
20 atctcctctctgcaacctgaggacgtggccacatactactgccagagatacaacagagcccc
atacacctttggccagggcacaaaagtggaaatcaagagaaccgtggcgcctcccagtggt
tcacttccccccagtgatgagcagctgaagtccggcacagcctctgtcgtgtgcctgctg
aacaacttctaccccagagaggccaaggtgcagtggaaaggtggataatgccctgcaaagcgg
25 caacagccagagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagca
cactgacctgagcaaggccgactacgagaagcacaaggtgtacgcatgcgaggtgacctac
cagggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgctgaaagcgcaggag
tggttctggggagggcaggggaagtctactaacatgcggggacgtggaggaaaatcccgcc
caatggctaccggctccagaacctccctgctgctggctttcggcctgctgtgtctgccttg
30 ctgcaggaaggctccgcccaggtgcagctgggtgcagtcgggaggaggagtggtgcagcccg
cagatcccttcgcctgagctgcaaggcttctggctacacattcaccaggtacaccatgact
gggtgagacagggccccggaaagggcctggagtggtgattggctatattaaccccagcagggga
tacaccaattacaatcagaaggtcaaggacaggttcaccatcagcagggacaacagtaagaa
tacagcattcctgcagatggacagcctgagaccagaggatacaggcgtgtatTTTTGCGCCA
35 ggtattacgatgaccactactgtctggactactgggggcagggaacaccagtgacctgagc
tccgccagcacaaagggccctctgtgttccactggccccctccagcaagagcacctccgg
cggaaaccgcccgcctgggctgtctggtgaaggactacttccctgaacctgtgacagtcagct
ggaactcaggcgccctgaccagcggagtgcataccttccccgcccgtgctgcagagctctggc
ctgtactccctgagcagcgtggtcacagtgccagcagcagcctgggcacacagacttatat
40 ctgcaacgtgaaccacaagcctagcaacactaaggtcgataaaaaggctcagaaagagaagat
caggcagcggcgctaccaacttcagcttgcgtgaagcagggccggcgacgtggaggagaacccc
ggcccaatggcaaccggctcaaggacttccctgctcctggctttcgggctgctgtgtctgccc
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gcgtggcgcatcgggtcacaatcacatgttctgccagctccagcgtgagctacatgaattgg
45 taccagcagacccccggcaaggctcccaaaagggtggatttacgacacctcaaagctggcaag
cggagttccctctcggttctccggcagcggatccggaaccgactacaccttcacaatcagct
ccctgcagcctgaagacatcgctacatattactgccagcagtgaggcagcaaccctttcacc
ttcggccagggcacaaagctgcagatcaccaggaccgtggctgcccctagcgtgttcattct
ccccccctcagatgagcagctcaagagcggcacagcctcagtggtgtgcctcctgaacaatt
50 tctaccccagagaagcaaaggtgcagtggaaaggtggacaatgccctgcagtcgggcaactct
caggagagcgtgacagaacaggacagtaaaagactccacatatagcctgtctagcacctgac
cctgtccaaggccgactacgagaagcacaaggtctacgcttgcgaggtgacacaccaagggc
tgtcttccctgtcaccaagtcatttaatatagaggcgaatgctga

Example polynucleotide encoding Adalimumab Fab and teplizumab Fab (SEQ ID NO: 175)

In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a teplizumab Fab are operably linked to an inflammation-inducible promoter. In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a teplizumab Fab are operably linked to an inflammation-inducible promoter comprising or consisting of a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 157 or a fragment thereof. In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a teplizumab Fab are operably linked to an inflammation-inducible promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 157 or a fragment thereof.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 176 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 176 or a fragment thereof.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which is at least 70% identical to SEQ ID NO: 176 or a fragment thereof. Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 176 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 176 or a fragment thereof.

tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaaatagc
acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
atcctgcagaagtgtgctgagtcagtcagtcagtcagtcagtcagtcagtcagtcagtcag
aaggagaccaatagaaactggccttgctcgagacagagaagactcctgctgttctgataggca
cctattggtcttactgacatccactttgcctttctctccacaggtgtccaggcgccgcat
ggccacaggctctcgaccagcctgctgctggccttcggcctgctgtgctgctgctgctgctg
aagagggcagcgccgaggtgcaactggtggaaagcggcgccgctggtccagcctggaagg
tccctgagactgagctgtgcccagcgctttaccttcgacgactacgccatgcactgggt
gcccagggccctggcaaggcctggaatgggtctccgccatcacctggaatagcgccaca
tcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaagaactct
ctgtatctgcaaatgaacagcctgcccgtgaagatacagccgtgtactattgcgccaagt
gagctacctctccaccgccagcagcctggactattggggacagggcaccctggtgaccgtgt

5 ctagcgccctccacaaagggcccttctgtgtttccactggctccaagctccaaaagcacatct
 ggaggaaccgctgccctgggtgctgttaaggactacttccccgagcctgtgaccgtgag
 ctggaacagcggcgccctgacatctgtgttccataccttccctgccgttctgcaatcttctg
 gactctacagcctgtcttctgtgttgaccgtgccagcagcagccttggaacacagacctac
 atctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcggag
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 ccttggctgcaagagggctctgccgacatccagatgacccagagcccttccctcactgagcgc
 10 cagcgtggggcagacagagtactattacatgcagagccagccaaggcatccggaactacctgg
 cctgggtatcagcagaagcccggcaaagcccctaagctgctgatctacgcccgccagcaccttg
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 acacctttggccaggggcaciaaagtggaaatcaagagaaccgtggccgctcccagtggttct
 atcttccccccagtgatgagcagctgaagtccggcacagcctctgtcgtgtgacctgctgaa
 15 caacttctaccccagagaggccaaggtgcagtgggaaggtggataatgccctgcaaagcggca
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 ctgaccttgagcaaggccgactacgagaagcacaaggtgtacgcatgagaggtgacctacca
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 gttctggggagggcaggggaagtctactaacatgcccgggacgtggaggaaaatcccggccca
 20 atggctaccggctccagaacctccctgctgctggcttccggcctgctgtgtctgacctggct
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 gtgagacaggcccccgaaaggcctggagtggtgattggctatattaaccccagcaggggata
 caccaattacaatcagaaggtcaaggacaggttcaccatcagcagggaacagtaagaata
 25 cagcattcctgcagatggacagcctgagaccagaggatacaggcgtgtatTTTTTgcgccagg
 tattacgatgaccactactgtctggactactggggcaggggaacaccagtgacctgagctc
 cgccagcacaaagggcccctctgtgttccactggccccctccagcaagagcacctccggcg
 gaaccgcccgcctgggctgtctggtgaaggactactttcctgaacctgtgacagtcagctgg
 aactcaggcgccctgaccagcggagtgcataccttccccgcccgtgctgcagagctctggcct
 30 gtactccctgagcagcgtggtcacagtgcccagcagcagcctgggcacacagacttatatct
 gcaacgtgaaccacaagcctagcaacactaaggtcgataaaaaaggtcagaaagagaagatca
 ggcagcggcgctaccaacttcagcttgcagcagggccggcgacgtggaggagaacccccg
 cccaatggcaaccggctcaaggacttccctgctcctggcttccgggctgctgtgtctgcct
 35 ggctgcaggaggggagcgcgcgacatccagatgaccagctcctcatcttccctgagcggcagc
 gtgggcgatcgggtcacaatcacatgttctgccagctccagcgtgagctacatgaattggta
 ccagcagacccccggcaaggctcccaaaaggtggatttacgacacctcaaagctggcaagcg
 gagttccctctcggttctccggcagcggatccggaaccgactacaccttcacaatcagctcc
 ctgcagcctgaagacatcgctacatattactgccagcagtgaggcagcaaccctttcacctt
 40 cggccagggcacaaagctgcagatcaccaggaccgtggctgcccctagcgtgttcatcttcc
 cccctcagatgagcagctcaagagcggcacagcctcagtggtgtgcctcctgaacaatttc
 taccacagagaagcaaaggtgcagtggaaggtggacaatgccctgcagtcgggcaactctca
 ggagagcgtgacagaacaggacagtaaaagactccacatatagcctgtctagcacctgacct
 tgtccaaggccgactacgagaagcacaaggtctacgcttgccgaggtgacacaccaagggtg
 tcttccctgtcaccaagtcatttaatatagaggcgaatgctga

45 Example polynucleotide encoding Adalimumab Fab and teplizumab
 Fab operably linked to inflammation-inducible promoter (SEQ ID
 NO: 176)

Example polynucleotides and vectors encoding adalimumab Fab and visilizumab Fab

In some embodiments, the polynucleotide or vector comprises: (a) a nucleotide sequence
 50 encoding an adalimumab Fab; and (b) a nucleotide sequence encoding a visilizumab Fab.

In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide sequence encoding a visilizumab Fab. In some embodiments, the polynucleotide or vector
5 comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding a visilizumab Fab.

In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding a visilizumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide
10 sequence encoding an adalimumab Fab. In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding a visilizumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding an adalimumab Fab.

15 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof; an adalimumab light chain, or a derivative thereof; a visilizumab heavy chain (e.g. VH + CH1), or a derivative thereof; and a visilizumab light chain, or a derivative thereof, wherein the chains are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide.

20 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 24; an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at
25 least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 26; an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 100; and an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity,
30 or 100% identity to SEQ ID NO: 102, wherein the amino acid sequences are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 6-9.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: the amino acid sequence of SEQ ID NO: 24; the amino acid sequence of SEQ ID NO: 26; the amino acid sequence of SEQ ID NO: 100; and the amino acid sequence of SEQ ID NO: 102, wherein the amino acid sequences are operably linked by nucleotide sequences encoding the 2A self-cleaving peptide of any of SEQ ID NOs: 6-9.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 189. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding SEQ ID NO: 189.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 189. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding SEQ ID NO: 189.

MATGSR T S L L L A F G L L C L P W L Q E G S A E V Q L V E S G G G L V Q P G R S L R L S C A A S G F T F D D Y A M H W
V R Q A P G K G L E W V S A I T W N S G H I D Y A D S V E G R F T I S R D N A K N S L Y L Q M N S L R A E D T A V Y Y C A K
V S Y L S T A S S L D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V
S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S L G T Q T Y I C N V N H K P S N T K V D K K V R K R
R S G S G A T N F S L L K Q A G D V E E N P G P M A T G S R T S L L L A F G L L C L P W L Q E G S A D I Q M T Q S P S S L S
A S V G D R V T I T C R A S Q G I R N Y L A W Y Q Q K P G K A P K L L I Y A A S T L Q S G V P S R F S G S G S G T D F T L T
I S S L Q P E D V A T Y Y C Q R Y N R A P Y T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L
N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H
Q Q L S S P V T K S F N R G E C R K R S G S G E G R G S L L T C G D V E E N P G P M A T G S R T S L L L A F G L L C L P W
L Q E G S A Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F I S Y T M H W R Q A P G Q G L E W M G Y I N P R S G
Y T H Y N Q K L K D K A T L T A D K S A S T A Y M E L S S L R S E D T A V Y Y C A R S A Y Y D Y D G F A Y W G Q G T L V T V
S S A S T K G P S V F P L A P C S R S T S E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S
G L Y S L S S V V T V P S S N F G T Q T Y T C N V D H K P S N T K V D K T V R K R R S G S G A T N F S L L K Q A G D V E E N
P G P M A T G S R T S L L L A F G L L C L P W L Q E G S A D I Q M T Q S P S S L S A S V G D R V T I T C S A S S S V S Y M N
W Y Q Q K P G K A P K R L I Y D T S K L A S G V P S R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q W S S N P P
T F G G G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N
S Q E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q Q L S S P V T K S F N R G E C

Example amino acid sequence encoding Adalimumab Fab and visilizumab Fab (SEQ ID NO: 189)

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 177 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 177 or a fragment thereof.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which is at least 70% identical to SEQ ID NO: 177 or a fragment thereof. Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 177 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 177 or a fragment thereof.

10 atggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttggct
gcaagagggcagcgccgaggtgcaactggtggaaagcggcgggcctggtccagcctggaa
ggtccctgagactgagctgtgccgacagcggtttaccttcgacgactacgccatgcactgg
gtgcgccaggccccctggcaagggcctggaatgggtctccgccatcacctggaatagcggcca
catcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaagaact
ctctgtatctgcaaatgaacagcctgcgggctgaagatacagccgtgtactattgcgcaaa
15 gtgagctacctctccaccgacagcctggactattggggacagggcacccctggtagcctg
gtctagcgccctccaaaaggcccttctgtgtttccactggctccaagctccaaaagcacat
ctggaggaaccgctgccctgggctgctggttaaggactacttccccgagcctgtgaccgtg
agctggaacagcggcgccctgacatctggtgttcataccttccctgccgttctgcaatcttc
tggactctacagcctgtcttctgtggtgaccgtgccagcagcagccttggaacacagacct
acatctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcgg
20 agaagcggaagcgggtgctaccaacttcagcctcctgaaacaggcggcgatgtggaggaaaa
ccctggacctaaggctaccggcagcagaaccagcctgctgctggcatttcggccttctgtgcc
tgccttggctgcaagagggctctgccgacatccagatgacccagagcccttccctactgagc
gccagcgtgggacagagtgactattacatgcagagccagccaaggcatccggaactacct
ggcctgggtatcagcagaagcccgcaaaagccctaaagctgctgactacgcccagcagccc
25 tgcaagcggcgctgcctagcagattcagcggctcaggctctggcactgatttcaccctgacc
atctcctctctgcaacctgaggacgtggccacatactactgccagagatacaacagagcccc
atacacctttggccagggcacaaaagtggaaatcaagagaaccgtggccgctcccagtggt
tcatcttccccccagtgatgagcagctgaagtccggcacagcctctgtcgtgtgcctgctg
aacaacttctaccccagagaggccaaggtgcagtggaaggtggataatgccctgcaaagcgg
30 caacagccaggagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagca
cactgaccctgagcaaggccgactacgagaagcacaaggtgtacgcatgcgaggtgacccac
cagggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgctgaaagcgcaggag
tggttctggggagggcaggggaagtctactaacatgccccggagctggaggaaaatccccggc
caatggccaccggctctcgacctccctgctgctggccttcggcctgctgtgtctgcctgg
35 ctccaggaaggctccgcccaggtgcagctggtgcagctctggggccgaagtgaagaaaccgg
cgccagcgtaagggtgagctgcaaggcctctggtacaccttcatcagctacaccatgcact
gggtgagacaggcccccgccagggactggaatggatggggtatatcaaccaaggtctggg
tacaccactacaaccagaaactgaaagataaggccacgctgaccgcccagataagtctgccag
caccgcctacatggagctgagtagcctgcggtcagaggataaccgcccgtgtactactgcgcca
40 gatctgcctattacgactacgacgggtttgcctactggggacagggaaactggtgaccgtg
agctctgcttctacgaagggcccatccgtgttccactggccccctgcagccgcagcaccag
cgagtccaccgcccgcctgggatgcctggtgaaagactactttccagagcctgtgaccgtgt
cctggaattccggcgcccttaccagcggagtgacacacctttccagccgttctgcagtccagc
ggcctgtactctctgtctagcgtggtgaccgtgccagctccaattttggcaccagaccta
45 cacctgcaacgtggaccacaagccatccaacactaaagtcgataagactgtgcgcaagagaa
gaagcggcagcggcgccaccaattttccctgctgaagcaggctggagacgtcgaggagaac
ccaggccctatggctaccggcagccggaactccctgctgctggcctttggcgtgctgtgcct
gccctggctccaggaggcagcccgatatccagatgactcagtcctccactcactgtccg
ccagcgtgggagatcgggtgactatcacatgtagcgcaagcagcagcgtgagctatatgaac
50 tgggtatcagcagaagccaggaaaggcccaagaggctgatctatgacacctccaaactggc
cagtggcgtgccttcacgtttctccgatctggtctctgggaccgacttcaccctgacaatct

ccagcctgcagccagaggatttcgctacttactactgccagcagtgaggctctaaccaccca
accttcggcgcgccgacccaaggtggagatcaagagaaccgtggccgccccatccgtgttcat
tttcctccctctgacgagcagctgaagagcggcacccgaagtgtggtgtgcctcctgaata
atcttaccctcgcgaaagccaaggtgcagtggaaggtggacaacgccctgcagtcgggaat
agccaggagtcctgtaccgaacaggacagcaaggactccacatactccctgagcagcaccct
gacactgagcaaggctgactacgagaaacacaaggtctatgcctgagaggtgacccatcagg
gcctgtcttcccctgtgacaaaaagctttaacagaggcgaatgttga

Example polynucleotide encoding Adalimumab Fab and visilizumab
Fab (SEQ ID NO: 177)

- 10 In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a visilizumab Fab are operably linked to an inflammation-inducible promoter. In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a visilizumab Fab are operably linked to an inflammation-inducible promoter comprising or consisting of a nucleotide sequence which is
- 15 at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 157 or a fragment thereof. In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the nucleotide sequence encoding a visilizumab Fab are operably linked to an inflammation-inducible promoter comprising or consisting of the nucleotide sequence of SEQ ID NO: 157 or
- 20 a fragment thereof.

- In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 178 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide
- 25 sequence SEQ ID NO: 178 or a fragment thereof.

- In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which is at least 70% identical to SEQ ID NO: 178 or a fragment thereof. Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least
- 30 98%, or at least 99% identical to SEQ ID NO: 178 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 178 or a fragment thereof.

- tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg
aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaatagc
acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgag
atcctgcagaagttggtcgtgaggcactgggcaggtaagtatcaaggttacaagacaggttt
aaggagaccaatagaaaactgggcttgcgagacagagaagactcttgcgtttctgataggca
- 35

5 cctattggtcttactgacatccactttgcctttctctccacaggtgtccaggcggccgccaat
ggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtccttggtgc
aagagggcagcgcgaggtgcaactggtgaaagcggcggcggcctggtccagcctggaagg
tccctgagactgagctgtgccgcagcggctttaccttcgacgactacgccatgcactgggt
10 gcgccaggcccttggaagggcctggaatgggtctccgccatcacctggaatagcggccaca
tcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaagaactct
ctgtatctgcaaataaacagcctgcgggctgaagatacagccgtgtactattgcgccaaagt
gagctacctctccaccgcccagcagcctggactattggggacagggcaccctggtgaccgtgt
15 ctagcgcctccacaaagggcccttctgtgtttccactggctccaagctccaaaagcacatct
ggaggaaccgctgccttggtgctggttaaggactacttccccgagcctgtgaccgtgag
ctggaacagcggcgccctgacatctggtgttcataccttccctgcccgttctgcaatcttctg
gactctacagcctgtcttctgtggtgaccgtgcccagcagcagccttggaacacagacctac
atctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcggag
20 aagcggaaagcgggtgctaccaacttcagcctcctgaaacaggcggcgatgtggaggaaaacc
ctggacctatggctaccggcagcagaaccagcctgctgctggcatttcggccttctgtgctg
ccttggtgctgaagagggctctgcccacatccagatgaccagagcccttccctactgagcgc
cagcgtggggcagagagtgactattacatgcagagccagccaaggcatccggaactacctgg
cctggtatcagcagaagcccggcaaaagcccctaagctgctgatctacgccgccagcaccctg
25 caaagcggcgctgcctagcagattcagcggctcaggtctgtggcactgatttcaccctgacat
ctcctctctgcaacctgaggacgtggccacatactactgccagagatacaacagagcccat
acacctttggccaggggcacaaggtggaatcaagagaaccgtggccgctcccagtggttc
atcttccccccagtgatgagcagctgaagtcgggcacagcctctgtcgtgtgacctgtgaa
caacttctaccccagagagggcaaggtgcagtggaaggtggataatgccctgcaaagcggca
30 acagccaggagagcgtgacagagcagcagcaaggacagcacctacagcctctctagcaca
ctgacctgagcaaggccgactacgagaagcacaaggtgtacgcatgcgaggtgacctacca
gggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgctgaaagcgcaggagtgt
gttctggggagggcagggggaagtctactaacatgcggggacgtggaggaaaatcccggccca
atggccaccggctctcggacctccctgctgctggccttcggcctgctgtgtctgcctggct
35 ccaggaaggctccgcccaggtgcagctggtgcagctctggggccgaagtgaagaaaccggcg
ccagcgttaaggtgagctgcaaggcctctggctacaccttcacagctacaccatgcactgg
gtgagacaggcccccgccagggaactggaatggatgggtatatacaaccaaggtctgggtg
caccactacaaccagaaactgaaagataaggccacgctgaccgcccagataagtctgccagca
ccgcctacatggagctgagtagcctgcggtcagaggataccgcccgtgtactactgcgcaga
40 tctgcctattacgactacgacgggtttgctactggggacagggaacactggtagcctgag
ctctgcttctacgaagggccatccgtgttccactggccccctgcagccgcagcaccagcg
agtccaccgcccgcctgggatgcctggtgaaagactactttccagagcctgtgaccgtgtcc
tggaattccggcgcccttaccagcggagtgcacacctttccagccgttctgcagtccagcgg
cctgtactctctgtctagcgtggtgaccgtgccagctccaattttggcaccagacctaca
cctgcaacgtggaccacaagccatccaactaaagtcgataagactgtgacgagaaga
45 agcggcagcggcgccaccaattttccctgctgaagcaggctggagacgtcgaggagaacc
agggcctatggctaccggcagccggaacttccctgctgctggcctttgggctgctgtgctgc
cctggctccaggagggcagcgcgatataccagatgactcagtcctccatcctcactgtccgcc
agcgtgggagatcgggtgactatcacatgtagcgaagcagcagcgtgagctatatgaactg
gtatcagcagaagccaggaaaggcccaaagggtgatctatgacacctccaaactggcca
50 gtggcgtgccttcacgtttctccggtctggtctggtgggacgacttcacctgacaatctcc
agcctgcagccagaggatttcgctacttactactgccagcagtgagctctaaccaccaac
cttcggcgggcgccaccaaggtggagatcaagagaaccgtggcgccccatccgtgttcattt
tccctccctctgacgagcagctgaagagcggcaccgcaagtggtgtgctcctgaataat
ttctaccctcgcaagccaaggtgcagtggaaggtggacaacgccctgcagtccggcaatag
55 ccaggagtccgtgaccgaacaggacagcaaggactccacatactccctgagcagcaccctga
cactgagcaaggctgactacgagaaacacaaggtctatgcctgcgaggtgacctatcagggc
ctgtcttcccctgtgacaaaaagctttaacagaggcgaatgttga

Example polynucleotide encoding Adalimumab Fab and visilizumab
Fab operably linked to inflammation-inducible promoter (SEQ ID
NO: 178)

Example polynucleotides and vectors encoding adalimumab Fab and orelizumab Fab

In some embodiments, the polynucleotide or vector comprises: (a) a nucleotide sequence encoding an adalimumab Fab; and (b) a nucleotide sequence encoding an orelizumab Fab.

5 In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide sequence encoding an orelizumab Fab. In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding an adalimumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding an
10 orelizumab Fab.

In some embodiments, the polynucleotide or vector comprises from 5' to 3': a nucleotide sequence encoding an orelizumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and a nucleotide sequence encoding an adalimumab Fab. In some embodiments, the polynucleotide or vector
15 comprises from 5' to 3': a nucleotide sequence encoding an orelizumab Fab; a nucleotide sequence encoding a 2A self-cleaving peptide; and a nucleotide sequence encoding an adalimumab Fab.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an adalimumab heavy chain (e.g. VH + CH1), or a derivative thereof; an
20 adalimumab light chain, or a derivative thereof; an orelizumab heavy chain (e.g. VH + CH1), or a derivative thereof; and an orelizumab light chain, or a derivative thereof, wherein the chains are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: an amino acid sequence having at least 70%, at least 75%, at least 80%, at least
25 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 24; an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 26; an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at
30 least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 111; and an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 113, wherein the amino acid sequences are operably linked by nucleotide sequences encoding a 2A self-cleaving peptide having at least 70%, at least

75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to any of SEQ ID NOs: 6-9.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding: the amino acid sequence of SEQ ID NO: 24; the amino acid sequence of SEQ ID NO: 26; the amino acid sequence of SEQ ID NO: 111; and the amino acid sequence of SEQ ID NO: 113, wherein the amino acid sequences are operably linked by nucleotide sequences encoding the 2A self-cleaving peptide of any of SEQ ID NOs: 6-9.

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 190. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding SEQ ID NO: 190.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which encodes an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 190. In some embodiments, the polynucleotide or vector comprises a nucleotide sequence encoding SEQ ID NO: 190.

MATGSRTSLLLAFLGLLCLPWLQEGSAEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHW
VRQAPGKGLEWVSATWNSGHIDYADSVGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAK
VSYLSTASSLDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVRKR
RSGSGATNFSLKQAGDVEENPGPMATGSRTSLLLAFLGLLCLPWLQEGSADIQMTQSPSSLS
ASVGDRTITCRASQGI RNYLAWYQQKPGKAPKLLIYAASLTQSGVPSRFSGSGSGTDFLT
ISSLPEDVATYYCQRYNRAPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGECRKR RSGSGEGRGSLLTCDVEENPGPMATGSRTSLLLAFLGLLCLP
LQEGSAEVQLLES GGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKLEWVSTISTSGG
RTYYRDSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDYWGQGTLLTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVRKR RSGSGATNFSLKQAGDVEENP
GPMATGSRTSLLLAFLGLLCLPWLQEGSADIQLTQPNVSTSLGSTVKLSCTLSSGNIENNYV
HWYQLYEGRPSTMIYDDDKRPDGVDPDRFSGSIDRSSNSAFLTIHNVAIEDEAIYFCHSYVS
SFNVFGGKLTFLRQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSP
VKAGVETTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Example amino acid sequence encoding Adalimumab Fab and orelizumab Fab (SEQ ID NO: 190)

In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 179 or a fragment

thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 179 or a fragment thereof.

In one aspect, the present invention provides a polynucleotide or vector comprising a nucleotide sequence which is at least 70% identical to SEQ ID NO: 179 or a fragment thereof.

- 5 Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 179 or a fragment thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO: 179 or a fragment thereof.

10 atggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttggt
gcaagagggcagcgccgaggtgcaactgggtggaaagcggcgccgctgggtccagcctggaa
gggtccctgagactgagctgtgccgccagcggtttaccttcgacgactacgccatgcactgg
gtgcgccaggccctggcaagggcctggaatgggtctccgccatcacctggaatagcggcca
catcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaagaact
15 ctctgtatctgcaaatgaacagcctgcgggctgaagatacagccgtgtactattgcgcaaaa
gtgagctacctctccaccgccagcagcctggactattggggacagggcaccctggtgaccgt
gtctagcgccctccacaaagggcccttctgtgtttccactggctccaagctccaaaagcacat
ctggaggaaccgctgccttggtgctgctggttaaggactacttccccgagcctgtgaccgtg
agctggaacagcggcgccctgacatctggtgttcataccttccctgcccgttctgcaatcttc
20 tggactctacagcctgtcttctgtggtgaccgtgccagcagcagccttggaacacagacct
acatctgcaatgtgaaccacaagcctagcaacaccaaggtggacaagaaggtgagaaagcgg
agaagcggaaagcgggtgctaccaacttcagcctcctgaaacaggccggcgatgtggaggaaaa
ccctggacctatggctaccggcagcagaaccagcctgctgctggcattcggccttctgtgcc
tgccttggtgcaagagggctctgccgacatccagatgacccagagcccttccctactgagc
25 gccagcgtggggcagacagagtactattacatgcagagccagccaaggcatccggaactacct
ggcctggtatcagcagaagcccgcaaaagccctaaagctgctgatctacgccgccagcacc
tgcaaaagcggcgtgcctagcagattcagcggctcaggctctggcactgatttcaccctgacc
atctcctctctgcaacctgaggacgtggccacatactactgccagagatacaacagagcccc
atacacctttggccagggcacaaaagtggaaatcaagagaaccgtggccgctcccagtggt
30 tcatcttccccccagtgatgagcagctgaagtcgggcacagcctctgtcgtgtgcctgctg
aacaacttctaccccagagagccaaggtgcagtggaaaggtggataatgccctgcaaagcgg
caacagccagagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagca
cactgaccctgagcaagggcgcactacgagaagcacaaggtgtacgcatgcaggtgacccac
cagggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgctgaaagcgcaggag
35 tgggtctggggagggcaggggaagtctactaacatgcggggacgtggaggaaaatcccgcc
caatggccaccgggtctaggaccagcctgctgctggccttcggcctgctgtgtctgcctgg
ctgcaggagggctccgccgaggtgcagctgctggaatcaggaggcggcctgggtgcagcctgg
aggcagcctgcgcctgtcatgcgcagcatctgggttcaccttctcctctttccctatggcct
gggtgagacaggcccccggaagggctctggagtggtgtctaccatttctacatctggaggc
40 cggacctactacagagatagcgtgaagggccgggttcaccatttctagagacaattccaagaa
taccctgtacctgcaaatgaatagcctgagagccgaagacactgccgtgtattactgcgcta
agttccgccagtactccggcgattcgactactggggccagggcaccctagtgcagtgctcc
tccgccagcacaaaaggccctccgtgtttccactggccccctcttccaagtctacttcagg
aggcacagccgcccctgggtgctgctggtgaaagattacttccctgagcccgtgaccgtgtcct
45 ggaactcaggagccctgaccagcggcggtgcacacctccagccgtgctgcagtcctctggc
ctgtacagcctgtccagcgtggtgactgtgccaaagctccagcctgggcacccagacctacat
ctgcaatgtgaaccacaagcctagcaacacaaaggtggacaagaaagtgagaaaaagaaggt
ctggctccggcgaccaaacttctcctgctgaaacaggccggggacgtggaggagaaccct
ggcccaatggccacaggctctagaaccagcctgctgctggccttcggcctgctgtgcctgcc

5 ctggctgcaggaaggctccgccgatatccagcttaccagcccaatagcgtgtccacgtcac
 tgggttccacagtcaagctgagttgtactctgagcagtggaatattgagaataactacgtg
 cactggtatcagctgtacgagggtaggagccctacaaccatgatctatgacgacgacaagcg
 cccagacggcgtgcctgacagattctccggctcaatcgacagatccagtaactccgccttcc
 10 tgacaattcacaacgtggctattgaggacgaggccatttacttttgccactcctacgtgagc
 agcttcaatgtgttcggcgccgggactaagctgaccgttctgcgccagcctaagccgctcc
 aagcgtgaccctgttccccccagcagcgaggaactgcaggccaacaaagccaccctggtgt
 gcctgatcagcgatttctaccccggggcccgtgaccgttgccctggaaggccgacagcagccct
 gtgaaggccggggtggagaccacaacccccctccaagcagagcaacaacaagtacgccgcaag
 15 ctcttacctgagcctgactcccgagcagtggaatctcataggtcttacagttgccaggtta
 cacacgaaggaggacagtggaacacagtggtgccccaccgaatgctcctga

Example polynucleotide encoding Adalimumab Fab and orelizumab Fab (SEQ ID NO: 179)

- 15 In some embodiments, the nucleotide sequence encoding an adalimumab Fab and the
 nucleotide sequence encoding an orelizumab Fab are operably linked to an inflammation-
 inducible promoter. In some embodiments, the nucleotide sequence encoding an adalimumab
 Fab and the nucleotide sequence encoding an orelizumab Fab are operably linked to an
 inflammation-inducible promoter comprising or consisting of a nucleotide sequence which is
 at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least
 20 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 157 or a fragment
 thereof. In some embodiments, the nucleotide sequence encoding an adalimumab Fab and
 the nucleotide sequence encoding an orelizumab Fab are operably linked to an
 inflammation-inducible promoter comprising or consisting of the nucleotide sequence of SEQ
 ID NO: 157 or a fragment thereof.
- 25 In some embodiments, the polynucleotide or vector comprises a nucleotide sequence which
 is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least
 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 180 or a fragment
 thereof. In some embodiments, the polynucleotide or vector comprises the nucleotide
 sequence SEQ ID NO: 180 or a fragment thereof.
- 30 In one aspect, the present invention provides a polynucleotide or vector comprising a
 nucleotide sequence which is at least 70% identical to SEQ ID NO: 180 or a fragment thereof.
 Suitably, the polynucleotide or vector comprises a nucleotide sequence which is at least 75%,
 at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least
 98%, or at least 99% identical to SEQ ID NO: 180 or a fragment thereof. In some
 35 embodiments, the polynucleotide or vector comprises the nucleotide sequence SEQ ID NO:
 180 or a fragment thereof.

tgagtcactgactcagtgagtcactgactcagtgagtaaggaattctggggactttccactg
 gggactttccactggggactttccactggggactttccactggggactttccactcctgcag
 gagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacagaggaaaactg

aaagggagaactgaaagtgggaaattcctctgaggcagaaaggaccatcccttataaatagc
acaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgca
atcctgcagaagtgggtcgtgaggcactgggcaggtaagtatcaaggttacaagacaggttt
aaggagaccaatagaaactgggcttgcgagacagagaagactcttgcgtttctgataggca
5 cctattgggtcttactgacatccactttgcctttctctccacaggtgtccaggcggccgcat
ggccacaggctctcggaccagcctgctgctggccttcggcctgctgtgtctgccttggctgc
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tccttgagactgagctgtgccgccagcggtttaccttcgacgactacgccatgcactgggt
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tcgactacgccgatagcgtggaaggcagattcaccatcagccgggacaacgccaaagaactct
ctgtatctgcaaatgaacagcctgcgggctgaagatacagccgtgtactattgcgcaaaagt
gagctacctctccaccgccagcagcctggactattggggacagggcaccctgggtgacctgt
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15 ggaggaaccgctgcctgggtgctgtggttaaggactacttccccgagcctgtgacctgtgag
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aagcggaagcgggtgctaccaacttcagcctcctgaaacaggcggcgatgtggaggaaaacc
20 ctggacctatggctaccggcagcagaaccagcctgctgctggcattcggccttctgtgctg
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cagcgtggggcagacagagtactattacatgcagagccagccaaggcatccggaactacctgg
cctggtatcagcagaagcccgcaagcccctaagctgctgatctacgccgccagcaccctg
caaagcggcgtgcctagcagattcagcggctcaggtctctggcactgatttcaccctgacct
25 ctctctctgcaacctgaggactggccacatactactgccagagatacaacagagccccat
acacctttggccagggcacaaaagtggaaatcaagagaaccgtggcgctcccagtgtgttc
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caacttctaccccagagaggccaaggtgcagtggaaaggtggataatgccctgcaaagcggca
acagccaggagagcgtgacagagcaggacagcaaggacagcacctacagcctctctagcaca
30 ctgacctgagcaaggccgactacgagaagcacaaggtgtacgcatgcgaggtgacccacca
gggcctgagcagtcctgtgaccaagagcttcaaccggggcgagtgctgaaagcgcaggagt
gttctggggagggcaggggaagtctactaacatgcggggacgtggaggaaaatccccggcca
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gcaggagggctccgccgaggtgcagctgctggaatcaggaggcggcctgggtgcagcctggag
35 ggcagcctgcgcctgtcatgcgagcatctgggttcaccttctcctctttccctatggcctg
gtgagacaggcccccggaaggtctgagtggtgtctaccatttctacatctggaggccg
gacctactacagagatagcgtgaagggcgggttcaccatttctagagacaattccaagaata
ccctgtacctgcaaatgaatagcctgagagccgaagacactgccgtgtattactgcgctaag
ttccgccagtactccggcggattcgactactggggccaggggcaccctagtgcagtgctctc
40 cgccagcacaaaaggccccctccgtgtttccactggccccctcttccaagtctacttcaggag
gcacagccgccctgggctgctggtgaaagattacttccctgagcccgtgacctgtcctgg
aactcaggagccctgaccagcggcgtgcacaccttcccagccgtgctgcagtcctctggcct
gtacagcctgtccagcgtggtgactgtgccaagctccagcctgggcacccagacctacatct
gcaatgtgaaccacaagcctagcaacacaaaggtggacaagaaagtgagaaaaagaaggtct
45 ggctccggcgcgaccaacttctccctgctgaaacaggcgggggacgtggaggagaacctgg
cccaatggccacaggtctagaaaccagcctgctgctggcttccggcctgctgtgcctgacct
ggctgcaggaaggtccgccgatatacagcttaccagcccaatagcgtgtccacgtcactg
ggttccacagtcaagctgagttgtactctgagcagtgggaaatattgagaataactacgtgca
ctggtatcagctgtacgagggtaggagccctacaacctgatctatgacgacgacaagcgcc
cagacggcgtgacctgacagatttccggctcaatcgacagatccagtaactccgccttctg
50 acaattcacaacgtggctattgaggacgaggccatttacttttgccactcctacgtgagcag
cttcaatgtgttcggcgccgggactaagctgaccgttctgcgccagcctaagccgctccaa
gctgacacctgttccccccagcagcgagggaactgcaggccaacaaagccacctgggtgtgc
ctgatcagcgatttctaccccggggcccgtgacctgtgctggaaggccgacagcagcctgt
gaaggccgggggtggagaccacaacccccctccaagcagagcaacaacaagtacgccgcaagct
55 cctacctgagcctgactcccagcagtggaatctcataggtcttacagttgccaggttaca
cacgaaggagacagctggaaaaaacagtggtggccccaccgaatgctcctga

Example polynucleotide encoding Adalimumab Fab and orelizumab Fab operably linked to inflammation-inducible promoter (SEQ ID NO: 180)

Separate polynucleotides or vectors

5 In one aspect, the present invention provides a combination of: (a) a polynucleotide or vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a polynucleotide or vector comprising a nucleotide sequence encoding a CD3 inhibitor. The combination may be provided in any suitable form, for example in the form of a kit or composition.

10 In one aspect, the present invention provides a combination of: (a) an AAV vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) an AAV vector comprising a nucleotide sequence encoding a CD3 inhibitor. The combination may be provided in any suitable form, for example in the form of a kit or composition.

The polynucleotides or vectors may be identical in one or more aspects, except for the nucleotide sequence encoding a TNF inhibitor or CD3 inhibitor. In some embodiments, the polynucleotides or vectors are identical in all aspects, except for the nucleotide sequence encoding a TNF inhibitor or CD3 inhibitor. In other embodiments, the polynucleotides or vectors are different in one or more aspects, in addition to the nucleotide sequence encoding a TNF inhibitor or CD3 inhibitor

20 In some embodiments, both polynucleotides or vectors comprise a promoter (e.g. an inflammation-inducible promoter). In some embodiments, both polynucleotides or vectors comprise from 5' to 3': a promoter (e.g. an inflammation-inducible promoter); and a nucleotide sequence encoding a TNF inhibitor or a CD3 inhibitor.

25 In some embodiments, both polynucleotides or vectors comprise an inflammation-inducible promoter. In some embodiments, both polynucleotides or vectors comprise from 5' to 3': an inflammation-inducible promoter; and a nucleotide sequence encoding a TNF inhibitor or a CD3 inhibitor.

30 In preferred embodiments, both polynucleotides or vectors further comprise a polyadenylation sequence. In some embodiments, both polynucleotides or vectors comprise from 5' to 3': a promoter (e.g. an inflammation-inducible promoter); a nucleotide sequence encoding a TNF inhibitor or a CD3 inhibitor; and a polyadenylation sequence.

In some embodiments, both polynucleotides or vectors further comprise one or more ITRs. In preferred embodiments, both vectors are AAV vectors. In some embodiments, both AAV genomes comprise from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide

sequence encoding a TNF inhibitor or a CD3 inhibitor; and a 3' ITR. In some embodiments, both AAV genomes comprise from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a TNF inhibitor or a CD3 inhibitor; a polyadenylation sequence; and a 3' ITR. In some embodiments, both AAV genomes comprise from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a TNF inhibitor or a CD3 inhibitor; a WPRE; a polyadenylation sequence and a 3' ITR. In some embodiments, both AAV genomes comprise from 5' to 3': a 5' ITR; an inflammation inducible promoter; an intron; a nucleotide sequence encoding a TNF inhibitor or a CD3 inhibitor; a WPRE; a polyadenylation sequence; and a 3' ITR. In some embodiments, both AAV genomes comprise from 5' to 3': a 5' ITR; an inflammation inducible promoter; an intron; a nucleotide sequence encoding a TNF inhibitor or a CD3 inhibitor; a WPRE; a polyadenylation sequence; a sequence encoding an inflammation-inhibiting oligonucleotide; and a 3' ITR.

In preferred embodiments, both vectors are AAV vector particles. In some embodiments, both AAV vector particles are pseudotyped to confer ocular tissue tropism. In some embodiments, both AAV vector particles comprise AAV2 capsid proteins or AAV2 capsid variant proteins, optionally wherein the AAV2 capsid variant is selected from any of: AAV2.tYF, AAV2.7m8, R100, AAV2.GL and AAV2.NN.

Example polynucleotides or vectors encoding TNF inhibitor

A polynucleotide or vector encoding a TNF inhibitor may comprise from 5' to 3': a promoter (e.g. an inflammation-inducible promoter) and a nucleotide sequence encoding a TNF inhibitor.

The polynucleotide or vector may further comprise any other suitable elements, such as any other elements described herein or one or more spacer sequence. The spacer sequence(s) may comprise, for example, at least one (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10), at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten nucleotide bases. A spacer sequence may comprise a restriction site to enable the insertion of one or more further elements.

In preferred embodiments, the vector encoding a TNF inhibitor is an AAV vector. In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a TNF inhibitor; and a 3' ITR. In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a TNF inhibitor; a polyadenylation sequence; and a 3' ITR. In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a TNF inhibitor; a WPRE;

a polyadenylation sequence and a 3' ITR. In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; an intron; a nucleotide sequence encoding a TNF inhibitor; a WPRE; a polyadenylation sequence; and a 3' ITR. In some
 5 embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; an intron; a nucleotide sequence encoding a TNF inhibitor; a WPRE; a polyadenylation sequence; a sequence encoding an inflammation-inhibiting oligonucleotide; and a 3' ITR.

In some embodiments, an AAV genome encoding a TNF inhibitor comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 181 or a fragment thereof.
 10 Suitably, the AAV genome comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 181 or a fragment thereof. In some embodiments, the AAV genome comprises or consists of the nucleotide sequence SEQ ID NO: 181 or a fragment thereof.

15 ctgcgcgctcgctcgctcactgagggccgcccgggcaaagccgggctcgggcgacctttgg
 tcgcccggcctcagtgagcgagcgagcgcgagagagggagtggccaactccatcactaggg
 gttccttgtagttaatgattaacccgccatgctacttatctacgtagccatgctctaggaag
 atcggaattcgcccttaagctagctgagtcactgactcagtgagtcactgactcagtgagta
 aggaattctggggactttccactggggactttccactggggactttccactggggactttcc
 20 actggggactttccactcctgcaggagcttgaataaaatgaatattagaagctgttagaata
 agagaaaatgacagaggaaaactgaaagggagaactgaaagtgggaaattcctctgaggcag
 aaaggaccatcccttataaatagcacaggccatgaaggaagatcattctcactgcagccttt
 gacagcctttgacctcatcttcgagatcctgcagaagttggtcgtgaggcactgggcaggtaa
 gtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcttgtcgagacagaga
 25 agactcttgcgtttctgataggcacctattggtcttactgacatccactttgctttctctc
 cacaggtgtccaggcgccgcatggccacaggtctctcgaccagcctgctgctggccttcg
 gcctgctgtgtctgccttggctgcaagagggcgagcgccgaggtgcaactggtggaagcggc
 ggcgccctggtccagcctggaaggtccctgagactgagctgtgcccagcggtttacctt
 cgacgactacgccatgcactgggtgcgccaggccctggcaagggcctggaatgggtctccg
 30 ccatcacctggaatagcgccacatcgactacgccgatagcgtggaaggcagattcaccatc
 agccgggacaacgccaagaactctctgtatctgcaaatgaacagcctgcccgtgaagatac
 agccgtgtactattgcgcaaagtgagctacctctccaccgccagcagcctggactattggg
 gacagggcaccttggtgacctgtctagcgccctccacaaagggcccttctgtgtttccactg
 gctccaagctccaaaagcacatctggaggaaccgctgcccctgggctgctggttaaggacta
 35 cttccccgagcctgtgacctgagctggaacagcgcgccctgacatctggtgttcatacct
 tccttgccgttctgcaatcttctggactctacagcctgtcttctgtggtgacctgcccagc
 agcagccttggaaacacagacctacatctgcaatgtgaaccacaagcctagcaacacaaagt
 ggacaagaaggtgagaaagcggaagcggaagcggtgctaccaacttcagcctcctgaaac
 aggccggcgatgtggaggaaaaccctggacctatggctaccggcagcagaaccagcctgctg
 40 ctggcattcgcccttctgtgcctgccttggctgcaagagggctctgcccagatccagatgac
 ccagagcccttctcactgagcgccagcgtgggcgacagagtactattacatgcagagcca
 gccaaggcatccggaactacctggcctggtatcagcagaagcccggaagccctaaagctg
 ctgatctacgccgccagcaccctgcaaagcggtgcctagcagattcagcggtcaggctc
 tggcactgatttcaccctgacctctcctctctgcaacctgaggacgtggccacatactact
 45 gccagagatacaacagagccccatacacctttggccagggcacaaaagtggaaatcaagaga
 accgtggccgctcccagtggttcatcttccccccagtgatgagcagctgaagtcgggcac

agcctctgtcgtgtgcctgctgaacaacttctaccccagagaggccaaggtgcagtgggaagg
 tggataatgccttgcgaagcggaacagccaggagagcgtgacagagcaggacagcaaggac
 agcacctacagcctctctagcacactgacctgagcaaggccgactacgagaagcacaaggt
 gtacgcatgaggtgacccaccaggccctgagcagtcctgtgaccaagagcttcaaccggg
 5 gcgagtgttaccatacagatgttccagattacgcttgataagcttgatccaatcaacctct
 ggattacaaaatttgtgaaagattgactgggtattcttaactatgttgctccttttacgctat
 gtggatacgtgctttaatgcctttgtatcatgctattgcttcccgtatggcctttcattttc
 tcctccttgataaatcctggttgctgtctctttatgaggagttgtggcccgttgctcaggca
 acgtggcgtggtgtgactgtgtttgctgacgcaacccccactggttggggcattgccacca
 10 cctgtcagctcctttccgggactttcgctttccccctccctattgccacggcggaactcatc
 gccgctgaccttgcccgtgctggacaggggctcggtgttgggcactgacaattccgtggt
 gttgtcggggaaatcatcgtcctttccttggtgctcgctgctgctgttgccacctggattctgc
 gcgggacgtccttctgctacgtcccttcggccctcaatccagcggaccttccttcccgcggc
 ctgctgccggctctgcccctcttccgctcttcgagatctgctcgactgtgccttctagt
 15 tggcagccatctgttgtttgccctccccctgcttcccttgacctggaaggtgccactcc
 cactgtcctttcctaataaaaatgaggaaattgcatcgcatgtctgagtaggtgtcattcta
 ttctggggggtgggggtggggcaggacagcaagggggaggattgggaagacaatagcaggcat
 gctggggaggtacctttagggtagggtagggtagggaaaaattagggtagggtagg
 gttagggaaaaattagggtagggtagggtagggaaaaatgcagcggtaagttcccatc
 20 cagggtttttgcagcggtaagttcccatccagggtttttgcagcggtaagttcccatccagg
 tttttctcgagttaagggcgaattcccgcataaggatcttccctagagcatggctacgtagata
 agtagcatggcgggttaatcattaactacaaggaacccctagtgatggagttggccactccc
 tctctgcgcgtcgtcgtcactgagggccgggcgaccaaaggtcgcccgacgcccgggctt
 tgcccgggcccgtcagtgcagcgagcgagcgcgag

25 Example AAV vector encoding adalimumab Fab (SEQ ID NO: 181)

In some embodiments, an AAV genome encoding a TNF inhibitor comprises or consists of a
 nucleotide sequence which is at least 70% identical to SEQ ID NO: 182 or a fragment thereof.
 Suitably, the AAV genome comprises or consists of a nucleotide sequence which is at least
 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at
 30 least 98%, or at least AAV genome % identical to SEQ ID NO: 182 or a fragment thereof. In
 some embodiments, the vector comprises or consists of the nucleotide sequence SEQ ID NO:
 182 or a fragment thereof.

ctgcgcgtcgtcgtcgtcactgagggccgcccgggcaaagcccgggcgctcgggcgacctttgg
 35 tcgcccggcctcagtgcagcgagcgagcgcgagagaggagtggtggcaactccatcactaggg
 gttcctttagttaatgattaacccgcatgctacttatctacgtagccatgctctaggaag
 atcggaattcgcccttaagctagctgagtcactgactcagtgagtcactgactcagtgagta
 aggaattctggggactttccactggggactttccactggggactttccactggggactttcc
 actggggactttccactcctgcaggagcttgaataaaaatgaatattagaagctgttagaata
 agagaaaatgacagaggaaaactgaaagggagaactgaaagtgggaaattcctctgaggcag
 40 aaaggaccatcccttataaatagcacaggccatgaagggaagatcattctcactgcagccttt
 gacagcctttgcctcatcttgcatcctgcagaagttggtcgtgaggcactgggcaggtaa
 gtatcaagggttacaagacaggtttaaggagaccaatagaaaactgggcttgctcgagacagaga
 agactcttgcggttctgataggcacctattgggtcttactgacatccactttgcctttctctc
 cacagggtgtccaggcgccgcatggcaacaggtagtcgaaccagcctattactggccttcg
 45 gtctcctgtgtctgccttggttcaagagggtctgctgaggtgaaactggaggaaagtggg
 ggaggcctcggttcaaccaggcggtccatgaagctgtcgtgtgtggcatctggcttcatctt
 cagcaatcactggatgaactgggtcaggcaatctcctgagaaggggctagagtggtggcg
 agatccgctcaaaatcaatcaattccgccacacattatgcagagtcagtaaaaggcggttc
 accatttctagagatgacagcaaaagcgccgtgtacctccagatgaccgacctgcgaacaga

ggacactggggtctactactgctcccggaactactatgggtccacctatgactactggggcc
 aagggaccacattgacagtatcctcagcctccactaaaggctccttcagtgtttccgctggct
 cctcctccaaaagtacgtcaggcggcaccgctgctctgggctgtctggtgaaggattactt
 cctgaacctgtgactgtttcctggaacagtggagccttgacttcaggagtcacacatttc
 5 cggcagtgtctccagagcagtgggtctctattccctaagcagtgtagtgaccgtgccctctagc
 agcctcggaaaccagacatacatctgcaatgtcaatcacaagccaagcaatacaaaagtgga
 caagaaggtaggaagcggcgttccgggagcggggctacgaatttttcattgctcaagcaag
 cgggagatgtggaggagaacctggcccatggccacgggaagtgcgacttccttactactc
 10 gcctttggtcttctttgcttgccatggctccaggagggtagtgcagacattctcctgacca
 gtccctgtctatcttctgtctctccccggagagcgcgtctccttctcttgagagcttccc
 agtttggggcagcagcattcactgggtatcagcagagaacaaatggatcaccaaggcttttg
 atcaagtatgcttcagaaagcatgagtgggataccatccagggtttagtgggaagtggctctgg
 tactgacttcactctctctataaacacgggtggaaagcgaagatattgctgactattactgtc
 agcaaagccatagctggccatttacttttggatcagggaaccaacctggaagtcaagagaact
 15 gtggccgcgcttcggtttttattttccccccatctgatgaacagctgaagagcggtagacg
 cagtgtagtgtgctgtcaacaacttctaccctagagaagccaaggtgcagtggaaaggtcg
 acaatgcattacagagcgggaacagccaggaaagtgttactgagcaggatagcaaggacagc
 acctactctctgtctagcacactcactttgtctaaagcagattatgagaaacataaagtta
 tgctgtgaagttaccaccagggcctgagcagtcctcgtcaccaagtctttcaaccgcgggg
 20 agtgctaccatacagatgttccagattacgcttgataagccttgatccaatcaacctctgga
 ttacaaaatttgtgaaagattgactgggtattcttaactatgttgctccttttacgctatgtg
 gatacgtgctttaatgcctttgtatcatgctattgtctcccgatggcttccattttctcc
 tccttgataaatcctggtgtgtctctctttatgaggagttgtggcccggtgtcaggcaacg
 tggcgtggtgtgactgtgtttgctgacgcaacccccactgggtggggcattgccaccacct
 25 gtcagctcctttccgggactttcgctttccccctccctattgccacggcggaaactcatcgcc
 gcctgccttgcccgctgctggacaggggtcggtgtttgggactgacaattccgtgggtgtt
 gtcggggaaatcatcgctcctttccttggtgctgcgctgtgttgccacctggattctgcgcg
 ggacgtccttctgctacgtcccttcggccctcaatccagcggaccttccttcccgcggtg
 ctgcccgtctgcgccctctccgctcttcgagatctgcctcgactgtgccttctagtgtg
 30 cagccatctgttgtttgccccccccgtgccttccttgaccttgaaggtgccactccac
 tgtcctttccctaataaaatgaggaaattgcatcgcatgtctgagtaggtgtcattctattc
 tgggggggtgggtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgct
 ggggaggtacctttagggttagggtagggtaggggaaaaatttagggtagggtaggggt
 agggaaaaatttagggtagggtagggtaggggaaaaatgcagcggtaagttcccatccag
 35 gttttttgcagcggtaagtcccatccaggtttttttgcagcggtaagttcccatccaggttt
 ttctcgagttaagggcgaattcccgataaggatcttccctagagcatggctacgtagataagt
 agcatggcgggttaatcattaactacaaggaacccctagtgtgaggttgccactccctct
 ctgcgctcgtcgtcactgaggccgggcgaccaaagggtcgcccgacgcccgggctttgc
 ccgggcggcctcagtgcgagcgcgcgcag

40 Example AAV vector encoding infliximab Fab (SEQ ID NO: 182)

Example polynucleotides or vectors encoding CD3 inhibitor

A polynucleotide or vector encoding a CD3 inhibitor may comprise from 5' to 3': a promoter (e.g. an inflammation-inducible promoter) and a nucleotide sequence encoding a CD3 inhibitor.

45 The polynucleotide or vector may further comprise any other suitable elements, such as any other elements described herein or one or more spacer sequence. The spacer sequence(s) may comprise, for example, at least one (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10), at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at

least ten nucleotide bases. A spacer sequence may comprise a restriction site to enable the insertion of one or more further elements.

In preferred embodiments, the vector encoding a CD3 inhibitor is an AAV vector. In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a CD3 inhibitor; and a 3' ITR. In some
5 embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a CD3 inhibitor; a polyadenylation sequence; and a 3' ITR. In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; a nucleotide sequence encoding a CD3 inhibitor; a WPRE; a polyadenylation sequence and a 3' ITR. In some
10 embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; an intron; a nucleotide sequence encoding a CD3 inhibitor; a WPRE; a polyadenylation sequence; and a 3' ITR. In some embodiments, the AAV genome comprises from 5' to 3': a 5' ITR; an inflammation inducible promoter; an intron; a nucleotide sequence encoding a CD3 inhibitor; a WPRE; a
15 polyadenylation sequence; a sequence encoding an inflammation-inhibiting oligonucleotide; and a 3' ITR.

In some embodiments, an AAV genome encoding a CD3 inhibitor comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 183 or a fragment thereof. Suitably, the AAV genome comprises or consists of a nucleotide sequence which is at least
20 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 183 or a fragment thereof. In some embodiments, the AAV genome comprises or consists of the nucleotide sequence SEQ ID NO: 183 or a fragment thereof.

25 ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgctcgggcgacctttgg
tcgcccggcctcagtgagcgagcgagcgcgagagagggagtggccaactccatcactaggg
gttccttgtagttaatgattaaccggccatgctacttattctacgtagccatgctctaggaag
atcggaattcgcccttaagctagctgagtcactgactcagtgagtcactgactcagtgagta
aggaattctggggactttccactggggactttccactggggactttccactggggactttcc
actggggactttccactcctgcaggagcttgaataaaatgaatattagaagctgttagaata
30 agagaaaatgacagaggaaaactgaaagggagaactgaaagtgggaaattcctctgaggcag
aaaggaccatcccttataaatagcacaggccatgaagggaagatcattctcactgcagccttt
gacagcctttgcctcatcttgcatcctgcagaagttggctcgtagggcactgggcaggtaa
gtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcttgctgagacagaga
agactcttgcgcttctgataggcacctattggctcttactgacatccactttgcctttctctc
35 cacagggtgtccaggcgccgcccattggctaccggctctaggacctcactgctgctggccttcg
gcctgctgtgcctgccttggtgcaggagggcagcgcccagggtccagctggtggagagcggc
gggggctggtgcagcccggcagaagcctgcggtgagctgtgcccagcggttcaagtt
tagcggctacgggatgcactgggtcaggcaggctcctggaaaggactggagtgggtggcag
tgatctggtatgatggctccaagaaatactacgtcgacagcgtgaaaggacgcttcaccatc
40 agcagggataacagcaagaatacactgtacctgcagatgaacagcctgagagcagaggatac
agccgtgtactactgcgccaggcagatgggctactggcacttcgacctatgggggaggggta

5 cactggtgaccgtgagctccgcatccaccaaaagggccctctgtgttccccctggccccctcc
 agcaagtccacctctggaggaaccgctgccctgggctgtctggtgaaagattattttccaga
 gcctgtcacctgtcttggaaactccggcgtctgacatccggcgtgcacacattccccgccg
 tgctgcagagctctggcctctacagcctgtcttctgtggtgacagtgccagctccagcctg
 10 gggacccagacctatatctgcaacgtgaaccacaagccatctaaccacaaagtggataaacg
 ggtgaggaagaggaggtccggctccggcgccacaaacttttctctgtgtaagcaggccgggg
 acgtggaggagaaccccgcccaatggccaccgggttctagaaccagcctgctgctggccttt
 ggctgtgtgacctgacctggctgcaggagggcagcgtgaaattgtgctgacacagtctcc
 cgccacctgtccctgtcccccggcgagagggccacactgagctgtagggccagccagtcgg
 15 tgagcagctatctggcctggtaccagcagaaacccggacaggccccaagactgctgatttat
 gacgcctccaacagagctacgggcacccccgctcgttttccggcagcgggaagcgggacaga
 cttcactctgaccattagctcactggaacccgaggacttcgctgtgtattattgtcagcaac
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 gccgctccttccgtgttcatctttcctccatccgatgagcagctgaagtccggtacagccag
 20 cgttgtgtgacctgtgaacaacttttatccccgggagggttaaagtgcagtggaaagtggaca
 atgccctgcagagcggaaatagccaggagtgcagtaccgaacaggatagcaaggattccacc
 tacagcctgagcagcaccctgacactgtctaaagccgactatgagaagcacaagggtgatgc
 ctgcgaggtgactcaccagggcctgagttctcccgtagccaagtctttcaacagggggagaat
 gctgataagcttggatccaatcaacctctggattacaaaatttgtgaaagattgactggtat
 25 tcttaactatgttgtccttttacgctatgtggatacgtgctttaaagcctttgtatcatg
 ctattgtctcccgtagtggctttcatcttctcctccttgataaaatcctggttgcgtgtctct
 tatgaggagttgtggccggtgtcaggcaacgtggcgtggtgtgactgtgttgcgtgacgc
 aacccccactggttggggcattgccaccacctgtcagctcctttccgggactttcgtcttcc
 cctccctattgccacggcggaactcatcgccgctgccttgccgctgctggacaggggct
 30 cggctgttgggactgacaattccgtggtgttgcggggaaatcatcgtcctttccttggct
 gctcgctgtgttggcacctggattctgcgcgggacgtccttctgctacgtccttccggccc
 tcaatccagcggaccttccctcccgcgccctgctgcgggctctgcggcctcttccgctctt
 cgagatctgcctcgactgtgccttctagttgccagccatctgttgtttgccctcccccggtg
 ccttcccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgc
 35 atcgcatgtctgagtaggtgtcattctattctggggggtgggggtggggcaggacagcaagg
 gggaggattgggaagacaatagcaggcatgctggggaggtaccttaggggttaggggttaggg
 ttagggaaaaaatttaggggttaggggttaggggttagggaaaaaatttaggggttaggggt
 agggaaaaatgcagcggtaagttcccatccaggttttttgcagcggtaagttcccatccagg
 gatcttccctagagcatggctacgtagataagtagcatggcggttaatcattaactacaagg
 aacccttagtgatggagttggccactccctctctgcgcgctcgtcgtcactgaggccggg
 cgaccaaaggtcgcccgcgcccgggctttgcccgggcgccctcagtgagcgcgagcgcgcg
 cag

Example AAV vector encoding foralumab Fab (SEQ ID NO: 183)

- 40 In some embodiments, an AAV genome encoding a CD3 inhibitor comprises or consists of a
 nucleotide sequence which is at least 70% identical to SEQ ID NO: 184 or a fragment thereof.
 Suitably, the AAV genome comprises or consists of a nucleotide sequence which is at least
 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at
 least 98%, or at least AAV genome % identical to SEQ ID NO: 184 or a fragment thereof. In
 45 some embodiments, the vector comprises or consists of the nucleotide sequence SEQ ID NO:
 184 or a fragment thereof.

ctgcgcgctcgtcgtcactgaggccgcccgggcaaagcccgggcgctcgggcgacctttgg
 tcgcccggcctcagtgcgcgagcgcgcagagagggagtgccaactccatcactaggg
 gttcctttagttaatgattaacccgccatgctacttatctacgtagccatgctctaggaag

atcggaattcgcccttaagctagctgagtcactgactcagtgagtcactgactcagtgagta
aggaattctggggactttccactggggactttccactggggactttccactggggactttcc
actggggactttccactcctgcaggagcttgaataaaatgaatattagaagctgttagaata
agagaaaatgacagaggaaaactgaaagggagaactgaaagtgggaaattcctctgaggcag
5 aaaggaccatcccttataaatagcacaggccatgaaggaagatcattctcactgcagccttt
gacagcctttgcctcatcttgcatgacatcctgcagaagttggctcgtgaggcactgggcaggtaa
gtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcttgtcgagacagaga
agactcttgctgtttctgataggcacctattggcttactgacatccactttgcctttctctc
10 cacagggtgtccaggcggccgcatggctaccggctccagaacctcctgctgctggctttcg
gctgctgtgtctgccttggtgcaggaaggctccgcccagggtgcagctgggtgcagtcggga
ggaggagtgggtgcagcccgcatcccttcgctgagctgcaaggcttctggctacacatt
caccaggtacaccatgcaactgggtgagacaggcccccgaaaggcctggagtggttggct
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15 agcagggacaacagtaagaatacagcattcctgcagatggacagcctgagaccagaggatac
aggcgtgtatttttgcgccaggtattacgatgaccactactgtctggactactgggggcagg
gaacaccagtgaccgtgagctccgccagcacaaaggggccctctgtgttccactggcccc
tccagcaagagcacctccggcggaaccgcgcctgggctgtctggtgaaggactactttcc
tgaacctgtgacagtcagctggaactcaggcgccctgaccagcggagtgcataccttccccg
20 ccgtgctgcagagctctggcctgtactccctgagcagcgtgggtcacagtgcccagcagcagc
ctgggcacacagacttatactgcaacgtgaaccacaagcctagcaacactaaggtcgataa
aaaggtcagaaagagaagatcaggcagcggcgctaccaacttcagcttgtgaagcaggccg
gcgagctggaggagaaccccgcccaatggcaaccgggtcaaggacttccctgctcctgggt
ttcgggctgctgtgtctgcctgggtgcagggaggcgccgacatccagatgaccagtc
25 tccatcttccctgagcgcagcgtgggctgacgggtcacaatcacatgttctgccagctcca
gctgagctacatgaattgggtaccagcagaccccggaagggtcccaaaagggtggatttac
gacacctcaaagctggcaagcggagtccctctcggttctccggcagcggatccggaaccga
ctacaccttcacaatcagctccctgcagcctgaagacatcgctacataattactgccagcagt
ggagcagcaaccctttcaccttcggccagggcacaaagctgcagatcaccaggaccgtggct
30 gccctagcgtgttcatcttccccctcagatgagcagctcaagagcggcacagcctcagt
ggtgtgcctcctgaacaatttctaccccagagaagcaaagggtgcagtggaagggtggacaatg
cctgagctccggcaactctcaggagagcgtgacagaacaggacagtaaaagactccacatat
agcctgtctagcacctgaccctgtccaaggccgactacgagaagcacaagggtctacgcttg
cgagggtgacacaccaagggtgtcttccctgtcaccaagtcatttaatagaggcgaatgct
35 gataagcttgatccaatcaacctctggattacaaaatttgtaaaagattgacgttattct
taactatgttgctccttttacgctatgtggatacgtgctttaatgcctttgtatcatgcta
ttgcttcccgatggctttcattttctcctccttgataaaatcctgggtgctgtctctttat
gaggagtgtgtggccgtgtgcaggcaacgtggcgtgggtgtgactgtgtttgtgacgcaac
ccccactgggtggggcattgccaccacctgtcagctcctttccgggactttcgctttcccc
40 tccctattgccacggcggaactcatgcgcctgccttgcccgtgctggacaggggctcgg
ctgttgggcactgacaattccgtggtgtgtcggggaaatcatcgctcctttccttggtgct
cgctgtgttgccacctggattctgcgcgggacgtccttctgctacgtcccttcggccctca
atccagcggaccttccctcccgcgccctgctgcgggctctgcggcctcttccgctcttcga
gatctgcctcgactgtgccttctagttgccagccatctgttgtttgccccctcccccgctcct
45 tccctgacctggaagggtgacctcccactgtcctttcctaataaaatgaggaaattgcatc
gcattgtctgagtaggtgtcattctattctgtgggggtgggggtggggcaggacgaaggggg
aggattgggaagacaatagcaggcatgctggggagggtacctttagggttagggtagggtta
gggaaaaatttagggtagggtagggtagggtagggtagggtagggtagggtagggtaggg
gaaaaatgcagcggtaagttcccatccagggtttttgcagcggtaagttcccatccagggttt
50 tttgcagcggtaagttcccatccagggtttttctcgagttaagggcgaattcccataaggat
cttcttagagcatggctacgtagataagtagcatggcggttaatacattaactacaaggaac
ccctagtgtgagttggccactccctctctgcgcgtcgctcgctcactgaggccgggcga
ccaaagggtcgccgacgcccgggctttgcccggggcctcagtgagcagcagcgcgcag

Example AAV vector encoding teplizumab Fab (SEQ ID NO: 184)

In some embodiments, an AAV genome encoding a CD3 inhibitor comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 185 or a fragment thereof. Suitably, the AAV genome comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 185 or a fragment thereof. In some embodiments, the AAV genome comprises or consists of the nucleotide sequence SEQ ID NO: 185 or a fragment thereof.

```
10 ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgctcgggcgacctttgg
    tcgccccggcctcagtgagcgagcgagcgcgagagagggagtggccaactccatcactaggg
    gttccttgtagttaatgattaacccgccatgctacttatctacgtagccatgctctaggaag
    atcgggaattcgcccttaagctagctgagtcactgactcagtgagtcactgactcagtgagta
    aggaattctggggactttccactggggactttccactggggactttccactggggactttcc
    actggggactttccactcctgcaggagccttgaataaaatgaatattagaagctgttagaata
    agagaaaatgacagaggaaaactgaaaggggagaactgaaagtgggaaattcctctgaggcag
    15 aaaggaccatcccttataaatagcacaggccatgaaggaagatcattctcactgcagccttt
    gacagcctttgcctcatcttgcagatcctgcagaagttggctcgtagggcactgggcaggtaa
    gtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcttgtcgagacagaga
    agactcttgcgtttctgataggcacctattggctcttactgacatccactttgcctttctctc
    cacaggtgtccaggcgcccgcatggccaccggtctctcgacctccctgctgctggccttcg
    20 gcctgctgtgtctgccctggctccaggaaggctccgcccagggtgcagctggtgcagtctggg
    gccgaagtgaagaaaccggcgccagcggttaagggtgagctgcaaggcctctggctacacctt
    catcagctacaccatgcactgggtgagacaggcccccgccagggactggaatggatggggt
    atatcaaccaaggtctgggtacaccactacaaccagaaactgaaagataaggccacgctg
    accgcgataagtctgccagaccgcctacatggagctgagtagcctgcggtcagaggatac
    25 cgccgtgtactactgcgccagatctgcctattacgactacgacgggtttgcctactggggac
    agggaacactggtgaccgtgagctctgcttctacgaagggcccatccgtgttccactggcc
    ccctgcagccgcagcaccagcgagtcacccgcccctgggatgcctggtgaaagactactt
    tccagagcctgtgaccgtgtcctggaattccggcgcccttaccagcggagtgacacctttc
    cagccgttctgcagtcacagcggtgtactctctgtctagcgtggtgaccgtgccagctcc
    30 aattttggcaccagacctacacctgcaacgtggaccacaagccatccaacactaaagtcca
    taagactgtgcgaagagaagaagcgggcagcgggcgccaccaattttccctgctgaagcagg
    ctggagacgtcgaggagaaccagggcctatggctaccggcagccggacttccctgctgctg
    gcctttgggctgctgtgctgcccctggctccaggagggcagcgccgatatccagatgactca
    gtccccatcctcactgtccgccagcgtgggagatcggtgactatcacatgtagcgcaagca
    35 gcagcgtgagctatatgaactggtatcagcagaagccaggaaaggcccaagaggctgac
    tatgacacctccaaactggccagtggcgctgccttcacgtttctccggatctggtctggtgac
    cgacttcaccctgacaatctccagcctgcagccagaggatttcgctacttactactgccagc
    agtggagctctaaccaccaaacccttcggcgggcgccaccaaggtggagatcaagagaaccgtg
    gccgccccatccgtgttcattttccctccctctgacgagcagctgaagagcggcaccgcaag
    40 tgtggtgtgcctcctgaataatttctaccctcgcaagccaaggtgcagtgaaggtggaca
    acgcccctgcagtcggcaatagccaggagtcctgacccgaacaggacagcaaggactccaca
    tactccctgagcagcacctgacactgagcaaggctgactacgagaaaacacaaggtctatgc
    ctgagaggtgacctatcagggcctgtcttcccctgtgacaaaaagctttaacagaggcgaat
    gttgataagcttggatccaatcaacctctggattacaaaatttgtgaaagattgactggtat
    45 tcttaactatgttgctccttttacgctatgtggatacgtgctttaaagcctttgtatcatg
    ctattgcttccgctatggctttcattttctcctccttgataaaatcctggtgtgtctctt
    tataggagtttggtggccgttgtcaggcaacgtggcggtggtgtgactgtgttgctgacgc
    aacccccactggttgggcatgtgccaccacctgtcagctcctttccgggactttcgctttcc
    cctccctattgccagggcggaactcatcgccgctgccttggccgctgctggacaggggct
    50 cggctgttgggactgacaattccgtggtgttgcggggaaatcatcgtcctttccttggct
    gctcgccctgtgttgccacctggattctgcgcgggacgtccttctgctacgtcccttcggccc
```

5 tcaatccagcggaccttccttcccgcgccctgctgccggctctgcgccctcttccgcgtctt
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ccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaaatgaggaaattgc
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10 gggaggattgggaagacaatagcaggcatgctggggaggtacctttagggttagggttaggg
ttagggaaaaatttagggttagggttagggttagggaaaaatttagggttagggttagggtt
agggaaaaatgcagcggtaagttcccatccagggttttttgcagcggtaagttcccatccagg
tttttgcagcggtaagttcccatccagggttttttctcgagttaaggcggaattcccgataag
gatcttcctagagcatggctacgtagataagtagcatggcgggttaatcattaactacaagg
15 aacccttagtgatggagttggcactccctctctgcgcgctcgctcgctcactgaggccggg
cgaccaaaggtcgcccgacgcccgggctttgcccgggcgccctcagtgagcgagcgagcgcg
cag

Example AAV vector encoding visilizumab Fab (SEQ ID NO: 185)

15 In some embodiments, an AAV genome encoding a CD3 inhibitor comprises or consists of a
nucleotide sequence which is at least 70% identical to SEQ ID NO: 186 or a fragment thereof.
Suitably, the AAV genome comprises or consists of a nucleotide sequence which is at least
75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at
least 98%, or at least 99% identical to SEQ ID NO: 186 or a fragment thereof. In some
20 embodiments, the AAV genome comprises or consists of the nucleotide sequence SEQ ID
NO: 186 or a fragment thereof.

25 ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgctcgggcgacctttgg
tcgcccggcctcagtgagcgagcgagcgcgagagaggagtgcccaactccatcactaggg
gttcctttagttaatgattaacccgcatgctacttatctacgtagccatgctctaggaag
atcggaattcgcccttaagctagctgagtcactgactcagtgagtcactgactcagttagta
30 aggaattctggggactttccactggggactttccactggggactttccactggggactttcc
actggggactttccactcctgcaggagcttgaataaaatgaatattagaagctgttagaata
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aaaggaccatcccttataaatagcacaggccatgaagggaagatcattctcactgcagcctt
gacagcctttgctcatcttgcatatcctgcagaagtgggtcgtagggcactgggcaggtaa
35 gtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcttgctcgagacagaga
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35 ctctctttccctatggcctgggtgagacaggcccccggaagggtctggagtggtgtcta
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tctagagacaattccaagaataccctgtacctgcaaatgaatagcctgagagccgaagacac
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45 gaaagtgagaaaaagaaggtctggctccggcgcgaccaacttctcctgctgaaacaggccg
gggacgtggaggagaaccttgcccaatggccacaggctctagaaccagcctgctgctggct
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atattgagaataactacgtgcactgggtatcagctgtacgagggtaggagccctacaaccatg
atctatgacgacgacaagcgcccagacggcgctgctgacagattctccggctcaatcgacag

atccagtaactccgccttcctgacaattcacacgtggctattgaggacgaggccatttact
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 cgccagcctaagccgctccaagcgtgacctgttccccccagcagcaggaactgcaggc
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 5 ggaaggccgacagcagccctgtgaaggccgggtggagaccacaacccccctccaagcagagc
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 aatgctcctgataagcttggatccaatcaacctctggattacaaaatttgtgaaagattgac
 10 tggatattcttaactatgtgtccttttacgctatgtggatacgtgctttaatgccttgt
 atcatgctattgttcccgatggctttcattttctcctccttgataaatcctgggtgctg
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 15 ggggctcggtgttgggactgacaattccgtgggtgtgtcggggaaatcatcgctcctttcc
 ttggctgctcgctgtgttgcacctggattctgcgcgggacgtccttctgctacgtccctt
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 cccgtgccttcccttgacctggaagggtgccactcccactgtcctttcctaataaaatgagga
 aattgcatcgcatgtctgagtaggtgtcattctattctgggggtgggggtggggcaggaca
 20 gcaagggggaggattgggaagacaatagcaggcatgctggggaggtaccttagggtaggg
 ttagggtagggaaaaatttagggtagggtagggtagggtagggtagggtagggtaggg
 agggtagggaaaaatgcagcggtaagtcccatccagggttttttcgagcggtaagtccca
 tccagggttttttcgagcggtaagtcccatccagggttttttcgagcggtaagtccca
 gataaggatcttccctagagcatggctacgtagataagtagcatggcggttaataactaact
 25 acaaggaacccctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgag
 gccgggacgacaaaggtcgcccgacgccgggctttgccggggcgccctcagtgagcagcg
 agcgcgcag

Example AAV vector encoding otelixizumab Fab (SEQ ID NO: 186)

Variants, derivatives, analogues, and fragments

30 In addition to the specific amino acid sequences and nucleotide sequences described herein, the invention also encompasses variants, derivatives, and fragments thereof.

In the context of the invention, a “variant” of any given sequence is a sequence in which the
 specific sequence of residues (whether amino acid or nucleic acid residues) has been modified
 in such a manner that the amino acid or nucleotide sequence in question retains at least one
 35 or all of its endogenous functions. A variant sequence can be obtained by addition, deletion,
 substitution, modification, replacement and/or variation of at least one residue present in the
 naturally occurring amino acid or nucleotide sequence.

The term “derivative” as used herein in relation to amino acid sequences includes any
 substitution of, variation of, modification of, replacement of, deletion of and/or addition of one
 40 (or more) amino acid residues from or to the sequence, providing that the resultant amino acid
 sequence retains at least one or all of its endogenous functions.

Typically, amino acid substitutions may be made, for example from 1, 2 or 3, to 10 or 20 substitutions, provided that the modified amino acid sequence retains the required activity or ability. Amino acid substitutions may include the use of non-naturally occurring analogues.

Amino acid sequences used in the invention may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent amino acid sequence. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues as long as the endogenous function is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include asparagine, glutamine, serine, threonine and tyrosine.

Conservative substitutions may be made, for example according to the table below. Amino acids in the same block in the second column and in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R H
		F W Y
AROMATIC		

The effect of additions, deletions, substitutions, modifications, replacements and/or variations may be predicted using any suitable prediction tool e.g. SIFT (Vaser, R., et al., 2016. Nature protocols, 11(1), pp.1-9), PolyPhen-2 (Adzhubei, I., et al., 2013. Current protocols in human genetics, 76(1), pp.7-20), CADD (Rentzsch, P., et al., 2021. Genome medicine, 13(1), pp.1-12), REVEL (Ioannidis, N.M., et al., 2016. The American Journal of Human Genetics, 99(4), pp.877-885), MetaLR (Dong, C., et al., 2015. Human molecular genetics, 24(8), pp.2125-2137), and/or MutationAssessor (Reva, B., et al., 2011. Nucleic acids research, 39(17), pp.e118-e118) or based on clinical data e.g. ClinVar (Landrum, M.J., et al., 2016. Nucleic acids research, 44(D1), pp.D862-D868). Suitable additions, deletions, substitutions, modifications, replacements and/or variations may be considered tolerated, benign, and/or likely benign.

Typically, a variant may have a certain identity or similarity with the wild type amino acid sequence or the wild type nucleotide sequence.

In the present context, a variant sequence is taken to include an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85% or at least 90% identity or similarity, suitably at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity or similarity to the subject sequence. Although a variant can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express in terms of sequence identity.

In the present context, a variant sequence is taken to include a nucleotide sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85% or at least 90% identity or similarity, suitably at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity or similarity to the subject sequence. Although a variant can also be considered in terms of similarity, in the context of the present invention it is preferred to express it in terms of sequence identity.

Suitably, reference to a sequence which has a percent identity or similarity to any one of the SEQ ID NOs detailed herein refers to a sequence which has the stated percent identity or similarity over the entire length of the SEQ ID NO referred to.

Sequence identity or similarity comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percent identity or similarity between two or more sequences.

Percent identity or similarity may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid or nucleotide in one sequence is directly compared with the corresponding amino acid or nucleotide in the other sequence, one residue at a time. This is called an “ungapped” alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion in the amino acid or nucleotide sequence may cause the following residues or codons to be put out of alignment, thus potentially resulting in a large reduction in percent identity or similarity when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall identity or similarity score. This is achieved by inserting “gaps” in the sequence alignment to try to maximise local identity or similarity.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids or nucleotides, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example, when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum percent identity or similarity therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (see e.g. Devereux, J., et al., 1984. *Nucleic acids research*, 12(1), pp.387-395). Examples of other software that can perform sequence comparisons include, but are not limited to, the BLAST package (see e.g. Altschul, S.F., et al., 1990. *Journal of molecular biology*, 215(3), pp.403-410), BLAST 2 (see e.g. Tatusova, T.A. and Madden, T.L., 1999. *FEMS microbiology letters*, 174(2), pp.247-250), FASTA (see e.g. Pearson, W.R. and Lipman, D.J., 1988. *PNAS*, 85(8), pp.2444-2448.), EMBOSS Needle (Madeira, F., et al., 2019. *Nucleic acids research*, 47(W1), pp.W636-W641) and the GENWORKS suite of comparison tools. For some applications, it is preferred to use EMBOSS Needle.

Although the final percent identity or similarity can be measured, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix.

Once the software has produced an optimal alignment, it is possible to calculate percent sequence identity or similarity. The software typically does this as part of the sequence comparison and generates a numerical result. The percent sequence identity or similarity may be calculated as the number of identical or similar residues as a percentage of the total residues in the SEQ ID NO referred to.

"Fragments" are also variants and the term typically refers to a selected region of the amino acid or nucleotide sequence that is of interest either functionally or, for example, in an assay.

"Fragment" thus refers to an amino acid or nucleic acid sequence that is a portion of a full-length amino acid or nucleotide sequence.

Such variants, derivatives, and fragments may be prepared using standard recombinant DNA techniques such as site-directed mutagenesis. Where insertions are to be made, synthetic
5 DNA encoding the insertion together with 5' and 3' flanking regions corresponding to the naturally-occurring sequence either side of the insertion site may be made. The flanking regions will contain convenient restriction sites corresponding to sites in the naturally-occurring sequence so that the sequence may be cut with the appropriate enzyme(s) and the
10 synthetic DNA ligated into the cut. The DNA is then expressed in accordance with the invention to make the encoded amino acid sequence. These methods are only illustrative of the numerous standard techniques known in the art for manipulation of DNA sequences and other known techniques may also be used.

Vectors, Kits, and Systems

The present invention provides vectors (e.g. transfer vectors) encoding any of the viral
15 genomes described herein. The present invention also provides kit and systems for producing any of the vectors described herein.

Bicistronic or multicistronic vectors

In one aspect, the present invention provides a vector encoding a viral genome comprising:
(a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding
20 a CD3 inhibitor.

The vector may be a transfer vector, as described herein. For example, the vector may be a plasmid and/or the viral genome may be operably linked to a promoter (e.g. a viral promoter, such as a CMV promoter).

In one aspect, the present invention provides a kit or system for producing a vector (e.g. a
25 viral vector) comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

The kit or system may be a virus packaging kit or system or a virus production kit or system. As used herein, a "virus packaging kit or system" may comprise one or more components, and optionally instructions, for packaging the viral vector. As used herein, a "virus production kit or
30 system" may comprise one or more components, and optionally instructions, for producing a viral vector.

The kit or system may comprise a transfer vector encoding a viral genome comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor; and optionally one or more helper vectors. The kit or system may further comprise host cells (e.g. packaging cells or producer cells) and/or other reagents (e.g. transfection reagent, culture medium, etc.). The kit or system may further comprise any other suitable components, and optionally instructions for packaging and/or producing the viral vector.

Separate vectors

In one aspect, the present invention provides a combination comprising: (a) a vector encoding a viral genome encoding a TNF inhibitor; and (b) a vector encoding a viral genome encoding a CD3 inhibitor. The combination may be provided in the form of a kit, system, or composition.

The vectors may be transfer vectors, as described herein. For example, the vectors may be plasmids and/or the viral genomes may be operably linked to a promoter (e.g. a viral promoter, such as a CMV promoter).

In one aspect, the present invention provides a kit or system for producing: (a) a vector encoding a viral genome encoding a TNF inhibitor; and (b) a vector encoding a viral genome encoding a CD3 inhibitor. The kit or system may be a virus packaging kit or system or a virus production kit or system.

The kit or system may comprise: (a) a transfer vector encoding a viral genome encoding a TNF inhibitor; (b) a transfer vector encoding a viral genome encoding a CD3 inhibitor; and (c) optionally one or more helper vectors. The kit or system may further comprise host cells (e.g. packaging cells or producer cells) and/or other reagents (e.g. transfection reagent, culture medium, etc.). The kit or system may further comprise any other suitable components, and optionally instructions for packaging and/or producing viral vectors.

Cells

The present invention provides a cell comprising the CD3 inhibitor, polynucleotides or vectors of the present invention. The cell may be an isolated cell. Suitably, the cell is a mammalian cell, for example a human cell. The cell may be an isolated human cell.

In one aspect, the present invention provides a cell comprising a polynucleotide or vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

In one aspect, the present invention provides a cell comprising: (a) a polynucleotide or vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a polynucleotide or vector comprising a nucleotide sequence encoding a CD3 inhibitor.

5 Suitably, the cell may be a producer cell. The term “producer cell” includes a cell that produces viral particles, after transient transfection, stable transfection or vector transduction of all the elements necessary to produce the viral particles or any cell engineered to stably comprise the elements necessary to produce the viral particles. In some embodiments, the producer cell is an AAV producer cell. Suitable producer cells will be known to those of skill in the art (see e.g. Martin, J., et al. 2013. Human gene therapy methods, 24(4), pp.253-269) and may
10 include HEK293, COS-1, COS-7, CV-1, HeLa, CHO, and A549 cell lines. In some embodiments, the producer cell is a HEK293 cell, or a derivative thereof (e.g. a HEK293T cell).

Suitably, the cell may be a packaging cell. The term “packaging cell” includes a cell which contains some or all of the elements necessary for packaging a recombinant virus genome.
15 Typically, such packaging cells contain one or more vectors which are capable of expressing viral structural proteins (e.g. AAV *rep* and *cap* genes) and/or one or more genes encoding the viral structural proteins have been integrated into the genome of the packaging cell. Cells comprising only some of the elements required for the production of enveloped viral particles are useful as intermediate reagents in the generation of viral particle producer cell lines,
20 through subsequent steps of transient transfection, transduction or stable integration of each additional required element. These intermediate reagents are encompassed by the term “packaging cell”. In some embodiments, the packaging cell is an AAV packaging cell. Suitable packaging cells will be known to those of skill in the art (see e.g. Martin, J., et al. 2013. Human gene therapy methods, 24(4), pp.253-269).

25 **Pharmaceutical composition**

The present invention provides a pharmaceutical composition comprising the CD3 inhibitor, combination, polynucleotides, vectors or cells of the present invention. In preferred embodiments, the pharmaceutical composition comprises the vectors of the present invention in the form of viral vector particles.

30 In one aspect, the present invention provides a pharmaceutical composition comprising a polynucleotide or vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor. In preferred embodiments, the pharmaceutical composition comprises the vector in the form of a viral vector particle.

In one aspect, the present invention provides a pharmaceutical composition comprising: (a) a polynucleotide or vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a polynucleotide or vector comprising a nucleotide sequence encoding a CD3 inhibitor. In preferred embodiments, the pharmaceutical composition comprises the vectors in the form of a viral vector particles.

In one aspect, the present invention provides a pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

A pharmaceutical composition is a composition that comprises or consists of a therapeutically effective amount of a pharmaceutically active agent e.g. the vector. A pharmaceutical composition preferably includes a pharmaceutically acceptable carrier, diluent or excipient (including combinations thereof).

By "pharmaceutically acceptable" is included that the formulation is sterile and pyrogen free. The carrier, diluent, and/or excipient must be "acceptable" in the sense of being compatible with the vector and not deleterious to the recipients thereof. Typically, the carriers, diluents, and excipients will be saline or infusion media which will be sterile and pyrogen free, however, other acceptable carriers, diluents, and excipients may be used.

Acceptable carriers, diluents, and excipients for therapeutic use are well known in the pharmaceutical art. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as (or in addition to) the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s) or solubilising agent(s).

Examples of pharmaceutically acceptable carriers include, for example, water, salt solutions, alcohol, silicone, waxes, petroleum jelly, vegetable oils, polyethylene glycols, propylene glycol, liposomes, sugars, gelatin, lactose, amylose, magnesium stearate, talc, surfactants, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, and the like.

The pharmaceutical composition may be formulated for slow release, such as in microcapsules formed from biocompatible polymers or in liposomal carrier systems, according to methods known in the art (see e.g. Al-Qaysi, Z.K., et al., 2023. Expert Opinion on Drug Delivery, 20(7), pp.905-919). The pharmaceutical composition may be formulated for sustained release, for example as a depot injection or a long-acting injectable (LAI).

The pharmaceutical composition may be provided in the form of an implant, for example an ocular implant, such as an intraocular implant, for example an intravitreal implant (see e.g. Mohan, S. and Ratra, D., 2022. Intravitreal Implants, StatPearls).

5 The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions according to the present invention may be administered in a manner appropriate for treating and/or preventing the diseases described herein. Suitable administration routes will be known to the skilled person. The quantity and frequency of administration may be determined by the skilled person, for example depending by such factors as the condition of the subject, and the type and severity of the subject's disease. The pharmaceutical
10 composition may be formulated accordingly.

The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions according to the present invention may be administered locally to the eye. Suitably, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions according to the present invention are administered by subretinal injection, direct
15 retinal injection, subconjunctival injection, sub-Tenon's injection, periocular injection, suprachoroidal injection, or intravitreal injection. The pharmaceutical composition may be formulated accordingly.

In some embodiments, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions of the present invention are administered intraocularly. The term
20 "intraocular" may refer to the interior of the eye, thus intraocular administration may relate to the administration to the interior of the eye of a subject. In some embodiments, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions are administered to the eye of a subject by subretinal, direct retinal, suprachoroidal, or intravitreal injection. The skilled person will be familiar with and well able to carry out individual subretinal,
25 direct retinal, suprachoroidal, or intravitreal injections (see e.g. Hartman, R.R. and Kompella, U.B., 2018. Journal of Ocular Pharmacology and Therapeutics, 34(1-2), pp.141-153).

In some embodiments, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions are administered to the eye of a subject by subretinal, suprachoroidal, or intravitreal injection. In preferred embodiments, the CD3 inhibitor,
30 combination, polynucleotides, vectors, cells, or pharmaceutical compositions of the present invention are administered by intravitreal injection.

The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical composition may be administered as an ocular implant, such as an intraocular implant, for example an intravitreal implant.

The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical composition may be administered by the Port Delivery System, which is a refillable ocular implant (see e.g. Ranade, S.V., et al., 2022. Drug delivery, 29(1), pp.1326-1334).

5 The pharmaceutical compositions may comprise combinations, polynucleotides, vectors, cells, or pharmaceutical compositions of the invention in infusion media, for example sterile isotonic solution. The pharmaceutical composition may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

10 The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions may be administered in a single or in multiple doses. Suitably, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions may be administered in a single, one off dose. The pharmaceutical composition may be formulated accordingly.

15 The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions may be administered at varying doses (e.g. measured in viral genomes (vg) per mL). The physician in any event may determine the actual dosage which will be most suitable for any individual subject and the dosage may, for example, vary with the age, weight and response of the particular subject.

Suitably, a vector of the present invention is administered at a dose of at least about 10^{10} vg/mL, at least about 10^{11} vg/mL, at least about 10^{12} vg/mL, or at least about 5×10^{12} vg/mL.
20 Suitably, a vector of the present invention is administered at a dose of about 10^{13} vg/mL or less, about 10^{12} vg/mL or less, or about 10^{11} vg/mL or less. Suitably, a vector of the present invention is administered in a dose of from about 10^{10} to about 10^{13} vg/mL, or from about 10^{11} to about 10^{13} vg/mL. Suitably, a vector of the present invention is administered in a dose of from about 10^{10} to about 10^{12} vg/mL. Suitably, a vector of the present invention is administered
25 in a dose of from about 10^{11} to about 10^{13} vg/mL. Suitably, a vector of the present invention is administered in a dose of from about 10^{12} to about 10^{13} vg/mL. Suitably, a vector of the present invention is administered in a dose of from about 10^{12} to about 5×10^{12} vg/mL. The pharmaceutical composition may be formulated accordingly.

Suitably, a vector of the present invention is administered at a dose of at least about 10^9 vg/eye, at least about 2×10^9 vg/eye, at least about 5×10^9 vg/eye, at least about 10^{10} vg/eye, at least about 2×10^{10} vg/eye, at least about 5×10^{10} vg/eye, or at least about 10^{11} vg/eye. Suitably,
30 a vector of the present invention is administered at a dose of about 10^{13} vg/eye or less or about 5×10^{12} vg/eye or less. Suitably, a vector of the present invention is administered at a dose of from about 10^9 vg/eye to about 5×10^{12} vg/eye, from about 10^{10} vg/eye to about 5×10^{12}

vg/eye, from about 10^{10} vg/eye to about 10^{12} vg/eye, or from about 10^{10} vg/eye to about 5×10^{11} vg/eye.

Suitably, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions of the present invention may be administered in combination with one or more other therapeutic agents. The one or more other therapeutic agent may be administered separately, simultaneously or sequentially. The pharmaceutical composition may further comprise one or more other therapeutic agents. For example, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions of the present invention may be administered in combination with one or more immunosuppressant (e.g. antimetabolites, calcineurinic inhibitors, and alkylating agents).

The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions of the present invention may reduce the requirement for immunosuppressant therapy. In some embodiments, the CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical compositions of the present invention are administered in the absence of immunosuppressant therapy (i.e. the subject does not undergo an immunosuppressant therapy).

The invention further includes kits comprising the CD3 inhibitor, combination, polynucleotides, vectors, cells, and/or pharmaceutical compositions of the present invention. Preferably, said kits are for use in the methods and used as described herein, e.g., the therapeutic methods as described herein. Preferably, said kits comprise instructions for use of the kit components.

In one aspect, the present invention provides a kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor. In preferred embodiments, the kit comprises the vectors in the form of viral vector particles.

In one aspect, the present invention provides a kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

Methods for treating and/or preventing disease

The CD3 inhibitor, combination, polynucleotides, vectors, cells, or pharmaceutical composition of the present invention may be used in therapy.

In one aspect, the present invention provides the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention for use as a medicament.

5 In one aspect, the present invention provides use of the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention in the manufacture of a medicament.

In one aspect, the present invention provides a method of administering a therapeutically effective amount of the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention to a subject in need thereof.

10 The CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions may be administered to any subject in need thereof. The subject may be a mammal (e.g. a human). The CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention may be administered to a subject with or at risk of an inflammatory eye disease.

15 The combination of the present invention may be administered simultaneously, separately or sequentially. In some embodiments, the combination of the present invention is administered simultaneously.

The combination of the present invention may be administered in the same or a different form. In some embodiments, the combination of the present invention is administered in the same
20 form. In some embodiments, the combination of the present invention is administered in the form of a pharmaceutical composition comprising one or more vectors.

The combination of the present invention may be administered by the same or a different route of administration. In some embodiments, the combination of the present invention is administered by the same route of administration. In some embodiments, the combination of
25 the present invention is administered intraocularly.

Inflammatory eye diseases

The CD3 inhibitor, combination, polynucleotides, vectors, cells and pharmaceutical compositions according to the present invention may be used to prevent and/or treat inflammatory eye diseases.

In one aspect, the present invention provides the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention for use in preventing and/or treating an inflammatory eye disease.

5 In one aspect, the present invention provides use of the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention in the manufacture of a medicament for preventing and/or treating an inflammatory eye disease.

10 In one aspect, the present invention provides a method of preventing and/or treating an inflammatory eye disease, the method comprising administering a therapeutically effective amount of the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention to a subject in need thereof.

As used herein, an "inflammatory eye disease" may refer to any disorder associated with eye inflammation including uveitis, scleritis, keratitis, conjunctivitis, iritis, chorioretinitis, choroiditis, retinitis, and retinochoroiditis.

15 Following administration of the CD3 inhibitor, combination, polynucleotides, vectors, cells and pharmaceutical compositions according to the present invention, one or more symptoms of inflammatory eye disease may be prevented and/or treated in the subject. Any suitable method for determining the severity of inflammatory eye disease may be used (see e.g. McNeil, R., 2016. Eye news, 22(5), pp.1-4).

20 The CD3 inhibitor, combination, polynucleotides, vectors, cells and pharmaceutical compositions according to the present invention may prevent and/or reduce intraocular inflammation. Any suitable method may be used to determine intraocular inflammation. Suitable methods to quantify intraocular inflammation include laser flare photometry (see e.g. Tugal-Tutkun, I. and Herbort, C.P., 2010. International ophthalmology, 30(5), pp.453-464).

25 The CD3 inhibitor, combination, polynucleotides, vectors, cells and pharmaceutical compositions according to the present invention may reduce the inflammatory eye disease relapse rate. Any suitable method may be used to determine the relapse rate. For example, a recurrence (or flare) of uveitis is typically defined as an anterior chamber cells and/or vitreous haze grading of $\geq 2+$ using the SUN grading system (see e.g. McNeil, R., 2016. Eye news, 22(5), pp.1-4).

30

The CD3 inhibitor, combination, polynucleotides, vectors, cells and pharmaceutical compositions according to the present invention may prevent and/or reduce vision loss. Inflammatory eye diseases such as uveitis is a major cause of visual morbidity (see e.g.

Durrani, O.M., et al., 2004. British Journal of Ophthalmology, 88(9), pp.1159-1162). The CD3 inhibitor, combination, polynucleotides, vectors, cells and pharmaceutical compositions according to the present invention may maintain or improve visual acuity.

Uveitis

- 5 In preferred embodiments, the inflammatory eye disease is uveitis.

In one aspect, the present invention provides the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention for use in preventing and/or treating uveitis.

- 10 In one aspect, the present invention provides use of the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention in the manufacture of a medicament for preventing and/or treating uveitis.

- In one aspect, the present invention provides a method of preventing and/or treating uveitis, the method comprising administering a therapeutically effective amount of the CD3 inhibitor, combination, polynucleotides, vectors, cells and/or pharmaceutical compositions according to the present invention to a subject in need thereof.
- 15

- Uveitis refers to a group of intraocular inflammatory diseases of the uvea (i.e., the iris, ciliary body, and choroid) and adjacent structures, including the cornea, vitreous humor, retina, and optic nerve. Uveitis can be classified based on the primary anatomical site of inflammation (i.e., anterior, intermediate, and posterior) and/or etiologic origin, including infectious, non-infectious, or masquerade (see e.g. Rosenbaum, J.T., et al., 2019. Seminars in Arthritis and Rheumatism, 49(3), pp. 438-445; and Standardization of Uveitis Nomenclature (SUN) Working Group, 2005. American journal of ophthalmology, 140(3), pp.509-516).
- 20

- The uveitis may be anterior uveitis, intermediate uveitis, posterior uveitis, or panuveitis. The uveitis may be infectious, non-infectious, or masquerade. In preferred embodiments, the uveitis is non-infectious uveitis.
- 25

In some embodiments, the uveitis is selected from one or more of: sympathetic ophthalmia. Birdshot Chorioretinopathy, Sarcoid uveitis, Intermediate uveitis, Vogt Koyanaga Harada syndrome, JIA-associated uveitis, idiopathic retinal vasculitis, HLA-B27 associated non-anterior uveitis.

In some embodiments, the uveitis is sympathetic ophthalmia. Sympathetic ophthalmia is a rare, bilateral granulomatous uveitis that occurs after either surgical or accidental trauma to one eye (see e.g. Damico, F.M., et al., 2005. *Seminars in ophthalmology*, 20(3), pp. 191-197).

5 In some embodiments, the uveitis is Birdshot Chorioretinopathy. Birdshot retinochoroidopathy is a rare, chronic, bilateral, posterior uveitis (see e.g. Levinson, R.D., et al., 2006. *American journal of ophthalmology*, 141(1), pp.185-187).

10 In some embodiments, the uveitis is sarcoid uveitis. Sarcoid uveitis may also be known as sarcoid-related uveitis. Sarcoidosis is a disease that causes noncaseating granulomatous inflammation in one or more organs. The most common ocular manifestations are uveitis, dry eye and conjunctival nodules (see e.g. Jamilloux, Y., et al., 2014. *Autoimmunity reviews*, 13(8), pp.840-849).

In some embodiments, the uveitis is intermediate uveitis. Intermediate uveitis may refer to inflammation in the anterior vitreous, ciliary body and the peripheral retina (see e.g. Babu, B.M. and Rathinam, S.R., 2010. *Indian journal of ophthalmology*, 58(1), p.21).

15 In some embodiments, the uveitis is Vogt Koyanaga Harada syndrome. Vogt-Koyanagi-Harada syndrome is a bilateral, chronic, diffuse granulomatous panuveitis frequently associated with neurological, auditory, and integumentary manifestations (see e.g. Fang, W. and Yang, P., 2008. *Current eye research*, 33(7), pp.517-523).

20 In some embodiments, the uveitis is JIA-associated uveitis. JIA is the most common rheumatic disease of childhood, with JIA-associated uveitis its most common extra-articular manifestation (see e.g. Clarke, S.L., et al., 2016. *Pediatric Rheumatology*, 14(1), pp.1-11).

25 In some embodiments, the uveitis is idiopathic retinal vasculitis. Retinal vasculitis is a sight-threatening inflammatory eye condition that involves the retinal vessels. Based on the etiology, retinal vasculitis may be classified as either idiopathic or secondary to infection, neoplasia, or a systemic inflammatory disease (see e.g. Talat, L., et al., 2014. *Journal of ophthalmology*, 197675).

30 In some embodiments, the uveitis is HLA-B27 associated non-anterior uveitis. Human Leukocyte Antigen (HLA)-B27-associated uveitis is the most commonly diagnosed cause of acute anterior uveitis (see e.g. Loh, A.R. and Acharya, N.R., 2010. *American journal of ophthalmology*, 150(4), pp.534-542).

Bicistronic or multicistronic vectors

In one aspect, the present invention provides a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor for use as a medicament.

5 In one aspect, the present invention provides use of a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor in the manufacture of a medicament.

In one aspect, the present invention provides a method of administering a therapeutically effective amount of a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor to a subject in need thereof.

10 In one aspect, the present invention provides a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor for use in preventing and/or treating an inflammatory eye disease.

In one aspect, the present invention provides use of a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor
15 in the manufacture of a medicament for preventing and/or treating an inflammatory eye disease.

In one aspect, the present invention provides a method of preventing and/or treating an inflammatory eye disease, the method comprising administering a therapeutically effective amount of a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a
20 nucleotide sequence encoding a CD3 inhibitor to a subject in need thereof.

In one aspect, the present invention provides a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor for use in preventing and/or treating uveitis.

In one aspect, the present invention provides use of a vector comprising: (a) a nucleotide
25 sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor in the manufacture of a medicament for preventing and/or treating uveitis.

In one aspect, the present invention provides a method of preventing and/or treating uveitis, the method comprising administering a therapeutically effective amount of a vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide
30 sequence encoding a CD3 inhibitor to a subject in need thereof.

In some embodiments, the vector is an AAV vector. In some embodiments, the vector is an AAV vector particle. In some embodiments, the vector is an AAV vector particle comprising AAV2 capsid proteins or AAV2 capsid variant proteins.

5 In some embodiments, the vector is administered intraocularly. In some embodiments, the vector is administered via intravitreal, subretinal, direct retinal, subconjunctival, sub-Tenon's or suprachoroidal injection. In some embodiments, the vector is administered via intravitreal injection. In some embodiments, the vector is administered as a single dose.

10 In some embodiments, the vector is administered at a dose of at least about 10^{10} vg/mL, at least about 10^{11} vg/mL, at least about 10^{12} vg/mL, or at least about 5×10^{12} vg/mL. In some embodiments, the vector is administered at a dose of about 10^{13} vg/mL or less, about 10^{12} vg/mL or less, or about 10^{11} vg/mL or less. In some embodiments, the vector is administered in a dose of from about 10^{10} to about 10^{13} vg/mL, or from about 10^{11} to about 10^{13} vg/mL. In some embodiments, the vector is administered in a dose of from about 10^{10} to about 10^{12} vg/mL. In some embodiments, the vector is administered in a dose of from about 10^{11} to about
15 10^{13} vg/mL. In some embodiments, the vector is administered in a dose of from about 10^{12} to about 10^{13} vg/mL. In some embodiments, the vector is administered in a dose of from about 10^{12} to about 5×10^{12} vg/mL.

In some embodiments, the vector is administered at a dose of at least about 10^9 vg/eye, at least about 2×10^9 vg/eye, at least about 5×10^9 vg/eye, at least about 10^{10} vg/eye, at least about
20 2×10^{10} vg/eye, at least about 5×10^{10} vg/eye, or at least about 10^{11} vg/eye. In some embodiments, the vector is administered at a dose of about 10^{13} vg/eye or less or about 5×10^{12} vg/eye or less. In some embodiments, the vector is administered at a dose of from about 10^9 vg/eye to about 5×10^{12} vg/eye, from about 10^{10} vg/eye to about 5×10^{12} vg/eye, from about 10^{10} vg/eye to about 10^{12} vg/eye, or from about 10^{10} vg/eye to about 5×10^{11} vg/eye.

25 In some embodiments, the vector is administered in the form of a pharmaceutical composition. The pharmaceutical composition may be formulated according to the route of administration and/or dosage.

In some embodiments, the vector is administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.

30 Separate vectors

In one aspect, the present invention provides a product or kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a

nucleotide sequence encoding a CD3 inhibitor as a combined preparation for simultaneous, separate or sequential use in therapy.

In one aspect, the present invention provides use of a product or kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor in the manufacture of a combined preparation for simultaneous, separate or sequential use in therapy.

In one aspect, the present invention provides a method of administering a therapeutically effective amount of (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor, wherein the vectors are administered simultaneously, separately or sequentially.

In one aspect, the present invention provides a pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor for use as a medicament.

In one aspect, the present invention provides use of pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor in the manufacture of a medicament.

In one aspect, the present invention provides a method of administering a therapeutically effective amount of a pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

In one aspect, the present invention provides a product or kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor as a combined preparation for simultaneous, separate or sequential use in preventing and/or treating an inflammatory eye disease.

In one aspect, the present invention provides use of a product or kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor in the manufacture of a combined preparation for simultaneous, separate or sequential use in preventing and/or treating an inflammatory eye disease.

In one aspect, the present invention provides a method of preventing and/or treating an inflammatory eye disease, the method comprising administering a therapeutically effective

amount of (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor, wherein the vectors are administered simultaneously, separately or sequentially.

5 In one aspect, the present invention provides a pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor for use in preventing and/or treating an inflammatory eye disease.

10 In one aspect, the present invention provides use of pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor in the manufacture of a medicament for preventing and/or treating an inflammatory eye disease.

15 In one aspect, the present invention provides a method of preventing and/or treating an inflammatory eye disease, the method comprising administering a therapeutically effective amount of a pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

In some embodiments, the inflammatory eye disease is uveitis.

20 In some embodiments, the vectors are AAV vectors. In some embodiments, the vectors are AAV vector particles. In some embodiments, the vectors are AAV vector particles comprising AAV2 capsid proteins or AAV2 capsid variant proteins.

25 In some embodiments, the vectors are administered simultaneously, separately, or sequentially. In some embodiments, the vectors are administered simultaneously. In some embodiments, the vectors are administered intraocularly. In some embodiments, the vectors are administered via intravitreal, subretinal, direct retinal, subconjunctival, sub-Tenon's or suprachoroidal injection. In some embodiments, the vectors are administered via intravitreal injection. In some embodiments, the vectors are administered as a single dose.

30 In some embodiments, the vectors are administered at a dose of at least about 10^{10} vg/mL, at least about 10^{11} vg/mL, at least about 10^{12} vg/mL, or at least about 5×10^{12} vg/mL. In some embodiments, the vectors are administered at a dose of about 10^{13} vg/mL or less, about 10^{12} vg/mL or less, or about 10^{11} vg/mL or less. In some embodiments, the vectors are administered in a dose of from about 10^{10} to about 10^{13} vg/mL, or from about 10^{11} to about 10^{13} vg/mL. In some embodiments, the vectors are administered in a dose of from about 10^{10} to about 10^{12} vg/mL. In some embodiments, the vectors are administered in a dose of from about 10^{11} to

about 10^{13} vg/mL. In some embodiments, the vectors are administered in a dose of from about 10^{12} to about 10^{13} vg/mL. In some embodiments, the vectors are administered in a dose of from about 10^{12} to about 5×10^{12} vg/mL.

5 In some embodiments, the vectors are administered at a dose of at least about 10^9 vg/eye, at least about 2×10^9 vg/eye, at least about 5×10^9 vg/eye, at least about 10^{10} vg/eye, at least about 2×10^{10} vg/eye, at least about 5×10^{10} vg/eye, or at least about 10^{11} vg/eye. In some embodiments, the vectors are administered at a dose of about 10^{13} vg/eye or less or about 5×10^{12} vg/eye or less. In some embodiments, the vectors are administered at a dose of from about 10^9 vg/eye to about 5×10^{12} vg/eye, from about 10^{10} vg/eye to about 5×10^{12} vg/eye, from
10 about 10^{10} vg/eye to about 10^{12} vg/eye, or from about 10^{10} vg/eye to about 5×10^{11} vg/eye.

In some embodiments, the vectors are administered in the form of a pharmaceutical composition. The pharmaceutical composition may be formulated according to the route of administration and/or dosage.

15 In some embodiments, the vectors are administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.

Combination therapy

In one aspect, the present invention provides a product or kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same as a
20 combined preparation for simultaneous, separate or sequential use in therapy.

In one aspect, the present invention provides use of a product or kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same in the manufacture of a combined preparation for simultaneous, separate or sequential use in
25 therapy.

In one aspect, the present invention provides a method of administering a therapeutically effective amount of (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, wherein (a) and (b) are administered simultaneously,
30 separately or sequentially.

In one aspect, the present invention provides a pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and

(b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same for use as a medicament.

In one aspect, the present invention provides use of pharmaceutical composition comprising:

- (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same;
5 and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same in the manufacture of a medicament.

In one aspect, the present invention provides a method of administering a therapeutically effective amount of a pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor,
10 or a polynucleotide comprising a nucleotide sequence encoding the same.

In one aspect, the present invention provides a product or kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same as a combined preparation for simultaneous, separate or sequential use in preventing and/or
15 treating an inflammatory eye disease.

In one aspect, the present invention provides use of a product or kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same in the manufacture of a combined preparation for simultaneous, separate or sequential use in
20 preventing and/or treating an inflammatory eye disease.

In one aspect, the present invention provides a method of preventing and/or treating an inflammatory eye disease, the method comprising administering a therapeutically effective amount of (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence
25 encoding the same, wherein (a) and (b) are administered simultaneously, separately or sequentially.

In one aspect, the present invention provides a pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same
30 for use in preventing and/or treating an inflammatory eye disease.

In one aspect, the present invention provides use of pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the

same in the manufacture of a medicament for preventing and/or treating an inflammatory eye disease.

In one aspect, the present invention provides a method of preventing and/or treating an inflammatory eye disease, the method comprising administering a therapeutically effective amount of a pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

In some embodiments, the inflammatory eye disease is uveitis.

In some embodiments, (a) and (b) are administered simultaneously, separately, or sequentially. In some embodiments, (a) and (b) are administered simultaneously. In some embodiments, (a) and (b) are administered in the form of pharmaceutical compositions.

In some embodiments, (a) and (b) are administered intraocularly. In some embodiments, (a) and (b) are administered via intravitreal, subretinal, direct retinal, subconjunctival, sub-Tenon's or suprachoroidal injection. In some embodiments, (a) and (b) are administered via intravitreal injection. In some embodiments, (a) and (b) are administered as a single dose.

In some embodiments, (a) and (b) are administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.

EXAMPLES

The invention will now be further described by way of examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

Example 1 – Evaluation of monotherapy to suppress ocular inflammation

Local anti-TNF antibody fragment administration suppresses Experimental Autoimmune Uveoretinitis (EAU) *in vivo*

B10.RIII mice were immunized for Experimental Autoimmune Uveoretinitis (EAU) and eyes monitored using Topical Endoscopic Fundal Imaging (TEFI) from day 10 onward to select experimental mice displaying clinically evident disease. Groups of mice were injected via intravitreal route with 15 µg infliximab or vehicle control (EAU) on day 10. Eyes were enucleated (day 14), and retinal infiltrate characterized. Representative fundus images (**Figure 1A**), clinical disease scores (**Figure 1B**) and flow cytometric analysis of total CD45+ cell numbers from single eyes at day 14 (**Figure 1C**), demonstrate efficacy of intravitreal

Infliximab derived Fab molecules to suppress inflammation and retinal infiltrate in the B10.RIII EAU model. **P < 0.005; Data presented as means +/- SEM, representative of two independent experiments.

Therapeutic vector design

- 5 **Figure 2** is a schematic showing the vector organization of CMV.Infliximab Fab (constitutive CMV promoter) and AP1-NFkB.Infliximab Fab (inflammation-inducible promoter comprised of 5 repeated AP1 and NFkB binding sites). The heavy and light chains of Infliximab Fab are separated by a self-cleaving 2A peptide to generate the two separate peptides, which then form the Fab *in situ*. Following transduction of cells, AAV persists as episomal DNA in the
10 nucleus.

Evaluation of constitutive transgene expression *in vitro* and *in vivo*

- To evaluate constitutive Infliximab Fab transgene expression, HEK-293T (standard cell line for AAV development) or ARPE-19 (ocular cell line) cells were transduced with AAV2.CMV.Infliximab or AAV.CMV.NULL vectors [MOI 1E5vg/cell], and culture supernatants
15 assayed using a clinical IFX ELISA kit (**Figure 3A**). Detectable expression of Infliximab Fab from both cell types was evident by 72hrs (~30ng/ml). ****P < 0.0001. Data presented as means +/- SEM.

- To evaluate *in vivo* transgene expression, AAV2.CMV.Infliximab or AAV.CMV.NULL was administered by intravitreal (IVT) injection at 5E12 vg/ml into eyes of healthy B10.RIII mice.
20 At 4wks post-AAV, mice were killed, eyes dissected and supernatants (retina and vitreous) assayed using the clinical IFX ELISA kit (**Figure 3B**). Detectable expression of *in vivo* Infliximab Fab (~1.5ng/ml) was observed in eyes receiving the 5E12 vg/ml dose. *P<0.05. Data presented as means +/- SEM, with each data point representing a single eye.

These data demonstrate that AAV can mediate constitutive expression of an anti-TNF Fab.

- 25 Evaluation of inducible transgene expression *in vitro* and *in vivo* clinical model

- To demonstrate inducibility of the inflammation responsive promotor design, HEK-293T cells transduced with AAV2.AP1-NFkB.EGFP (reporter vector) or AAV2.AP1-NFkB.Infliximab (therapeutic vector) were stimulated with recIL-1b (2ng/mL). Activation leads to visible GFP expression at 24hs, increasing in intensity by 72hrs, with no GFP signal observed with
30 AAV2.AP1-NFkB.NULL (control vector) (**Figure 4A**). A higher GFP signal is observed with the constitutive AAV.CMV.EGFP vector. Stimulation results in a rapid induction of Infliximab Fab

expression (8hrs), accumulation reaching ~20ng/ml at 72hrs (**Figure 4B**). Images captured on EVOS FL, 10X magnification. ****P < 0.0001; Data presented as means +/- SEM.

To demonstrate *in vivo* inducibility of the inflammation responsive promotor design, AAV2.AP1-NFkB.EGFP (reporter) or AAV2.AP1-NFkB.NULL (control) at a 5E12 vg/ml dose was administered by intravitreal (IVT) injection in C57BL/6J mice. At 4wks post-AAV injection, mice were immunized to induce experimental autoimmune uveoretinitis (EAU), and imaged to monitor onset of ocular inflammation. At day 14 EAU, representative fundus and OCT images demonstrate clear clinical signs of disease (perivascular sheathing and vitreous infiltrate), and induction of GFP expression (**Figure 5A**). In mice that only received AAV (no EAU), no clinical signs of disease or expression of the GFP transgene are observed.

To demonstrate *in vivo* inducibility of the therapeutic transgene, groups of mice were injected with AAV2.AP1-NFkB.Infliximab or AAV2.AP1-NFkB.NULL in the contralateral eye, and EAU induced at 4wks post-AAV. At 3wks post-EAU, mice were killed, eyes dissected and supernatants (retina and vitreous) assayed using the clinical IFX ELISA kit (**Figure 5B**). Detectable expression of the Infliximab Fab is only observed in EAU eyes receiving the therapeutic vector and not the control. *P<0.05. Data presented as means +/- SEM, with each data point representing a single eye.

These data show inducibility of the inflammation responsive promotor design to express either a reporter (eGFP) or therapeutic (anti-TNF Fab) transgene *in vitro* and *in vivo*.

20 Evaluation of constitutive therapeutic transgene efficacy *in vivo*

To demonstrate efficacy of the constitutive therapeutic transgene, groups of mice were injected with AAV7m8.CMV.Infliximab or AAV7m8.CMV.NULL in the contralateral eye, followed by intravitreal administration of recombinant human TNF (rec_hTNF) at 4wks post-AAV. At 18hrs (peak of inflammatory response to rec_hTNF), representative fundus and OCT images demonstrate increased inflammation (vitreous infiltrate) in the control (NULL) vs infliximab eyes (**Figure 6A**). In mice that only received AAV, no clinical signs of disease are observed. At 18hrs mice were killed, eyes dissected prepared for flow cytometric analysis to determine absolute numbers of Ly6C+ monocytes (predominant infiltrate in this model) from single eyes. A significant reduction in number of monocytes was observed in eyes receiving the therapeutic vector and not the control (**Figure 6B**), this effect is further highlighted further with paired (contralateral eye) analysis (**Figure 6C**). **Wilcoxon signed rank test; *P<0.05 Wilcoxon matched pairs analysis.

These data show efficacy of the constitutive promotor design encoding a therapeutic (Infliximab Fab) transgene, to suppress human TNF mediated inflammation *in vivo*.

Constitutive expression of other anti-TNF biologics *in vitro* and *in vivo*

To evaluate constitutive expression of additional anti-TNF biologics, HEK-293T cells were transfected with the huTNFR1-hulgG plasmid, and culture supernatants assayed using an anti-human TNF antibody ELISA kit (**Figure 7A**). Detectable expression of huTNFR1-hulgG was evident by 48hrs. To evaluate AAV-mediated transgene expression, HEK-293T cells were transduced with AAV7m8.CMV.huTNFR1-hulgG or AAV7m8.CMV.NULL vectors [MOI 1E5vg/cell], and culture supernatants assayed using an anti-human TNF antibody ELISA kit (**Figure 7B**). Detectable expression of huTNFR1-IgG was evident by 72hrs (~30ng/ml).

To evaluate *in vivo* constitutive transgene expression of the huTNFR1-hulgG, AAV7m8.CMV.huTNFR1-hulgG or AAV7m8.CMV.NULL was administered by intravitreal (IVT) injection at range of doses [2E8 or 2E9 vg/eye] into the eyes of healthy C57BL/6J mice. At 4wks post-AAV, mice were killed, eyes dissected and supernatants (retina and vitreous) assayed using the anti-human TNF antibody ELISA kit (**Figure 7C**). Detectable expression *in vivo* of huTNFR1-hulgG at ~4ng/ml and 9ng/ml was observed in eyes receiving 2E8 and 2E9 vg/eye doses respectively.

These data provide additional evidence that gene therapy vectors can mediate constitutive expression of multiple clinically relevant anti-TNF biologics.

Bioactivity of constitutively expressed anti-TNF transgenes *in vitro*

To determine whether the transgene products are bioactive and functional, we evaluated AAV plasmids encoding Adalimumab Fab, huTNFR1-hulgG, and two modified constructs engineered to express a huTNFR1 ectodomain fused to mouse Fc (huTNFR1-msIgG) and a murine version (msTNFR1-msIgG). We also evaluated bioactivity of Infliximab Fab using the AAV2.CMV.Infliximab viral vector.

HEK-BLUE TNF reporter cells were first transfected with AAV.CMV.huTNFR1-hulgG, AAV.CMV.huTNFR1-msIgG, AAV.CMV.msTNFR1-msIgG, AAV.CMV.ADALIMUMAB plasmids or AAV2.CMV.Infliximab for 48hrs. Cells were stimulated with recombinant human TNF or mouse TNF (0.5ng/ml) for a further 24 hours and NFkB activation assessed. All the TNFR1 antibody-like plasmid constructs were bioactive, inhibiting huTNF- and msTNF-mediated activation in the reporter cell line compared to NULL or recTNF alone (**Figure 8A**). For the monoclonal Fab based anti-TNF biologics (both human specific), plasmid expression of

Adalimumab Fab (**Figure 8B**) or AAV-mediated Infliximab Fab expression (**Figure 8C**) both inhibit activation with huTNF.

These data demonstrate vectorized anti-TNF biologics, including huTNFRI-hulgG and Adalimumab are bioactive, and neutralize TNF mediated signalling and NFkB activation *in vitro*.

Evaluation of expression and bioactivity of an inducible anti-TNF transgene *in vitro* and *in vivo* clinical disease model

To further demonstrate inducibility of anti-TNF biologics via an inflammation responsive promotor, HEK-293T cells were transduced with AAV7m8.AP1-NFkB.huTNFRI-hulgG or AAV7m8.AP1-NFkB.NULL vectors [MOI 1E5vg/cell] and stimulated with IL-1b (2ng/ml). We selected this construct for further characterization as huTNFRI-hulgG can potentially neutralize both human and mouse TNF, facilitating efficacy testing in mice *in vivo*. Stimulation results in robust induction of huTNFRI-hulgG expression by 24hrs, accumulation reaching ~25ng/ml at 48hrs (**Figure 9A**).

To determine bioactivity of induced huTNFRI-hulgG protein, conditioned media (cell supernatant collected from the expression assay at 48hrs), was “spiked” with recombinant human or mouse TNF (final concentration 10ng/ml), and incubated with HEK-BLUE reporter cells for 24hrs. In response to both huTNF or mTNF stimulation, or with conditioned media from AAV7m8.AP1-NFkB.NULL, NFkB activation is robustly induced in the reporter cells. When supplemented with conditioned media from cells transduced with AAV7m8.AP1-NFkB.huTNFRI-hulgG and stimulated with IL-1b, NFkB activation in the reporter cells is completely suppressed, indicating potent bioactivity of the induced transgene (**Figure 9B**).

To demonstrate *in vivo* inducibility of the therapeutic transgene, B10.RIII mice were injected with AAV7m8.AP1-NFkB.huTNFRI-hulgG, and EAU induced at 4wks post-AAV. At day 19 post-EAU, when mild to moderate clinical signs of inflammation (not yet peak disease) were observed, mice were killed, eyes dissected and supernatants (retina and vitreous) assayed using the anti-human TNF antibody ELISA. We found that clinical disease drove detectable expression of the huTNFRI-hulgG in EAU eyes receiving the therapeutic vector and not the control (AAV only) (**Figure 10A**).

Using the recombinant human TNF model, we also evaluated inducibility of the huTNFRI-hulgG transgene in response to an acute inflammatory stimulus. B10.RIII mice were injected with AAV7m8.AP1-NFkB.huTNFRI-hulgG and AAV7m8.AP1-NFkB.NULL (contralateral eye control) at 2E9vg/eye. At 4wks post-AAV, mice received bilateral administration of

recombinant human TNF (rec_hTNF), 18hrs later were killed, eyes dissected and ocular supernatants (retina and vitreous) assayed for huTNFRI-hulgG expression. Acute activation elicits a significant increase in expression of the huTNFRI-hulgG compared to the control (**Figure 10B**).

- 5 These data show inducibility and sensitivity of the inflammation responsive promotor design to express a bioactive therapeutic (anti-TNF biologic) transgene *in vitro* and *in vivo*, using two different disease models, one with chronic low-grade inflammation and other with acute ocular inflammation.

Evaluation of inducible therapeutic transgene efficacy *in vivo*

- 10 To demonstrate efficacy of the inducible therapeutic transgene, B10.RIII mice were injected with AAV7m8.AP1-NFkB.huTNFRI-hulgG or AAV7m8.CMV.NULL [2E9 vg/eye] in contralateral eyes, and then immunized for EAU at 4wks post-AAV. At day 11, representative fundus and OCT images demonstrate increased inflammation (peri-vascular sheathing and vitreous infiltrate) in the eyes receiving the control NULL vector (**Figure 11A**). The
- 15 contralateral eyes of the same four animals, which received the inducible therapeutic vector, showed substantially reduced clinical inflammation, both peri-vascular sheathing and vitreous infiltrate. At this time-point mice were killed, eyes dissected prepared for flow cytometric analysis to determine absolute numbers of CD45+ (all leukocytes), CD3+ (lymphocytes), CD4+ (Th T cells) and CD11b+ (macrophages and monocytes) populations from single eyes
- 20 (**Figure 11B**). We observed a trend of reduction in immune cell infiltrates with AAV7m8.AP1-NFkB.huTNFRI-hulgG compared to AAV7m8.CMV.NULL.

These data show efficacy of inducible anti-TNF to locally suppress ocular inflammation in a mouse model of human uveitis *in vivo*.

Evaluation of constitutive and inducible expression of an anti-CD3 Fab transgene *in vitro*

- 25 To evaluate constitutive anti-CD3 Fab antibody expression, ARPE19 cells were transduced with AAV7m8.CMV.anti-CD3 Fab or AAV7m8.CMV.NULL vectors [MOI 1E5vg/cell]. Culture supernatants at 48hrs were collected and protein samples concentrated and prepared with non-reducing conditions for SDS-PAGE (4-20% gradient gel) analysis. Blots were probed with Anti-HA (Clone F7; sc-7392) primary monoclonal antibody, and secondary anti-mouse HRP
- 30 for ECL detection (**Figure 17A**). The SDS-PAGE shows HA-positive protein band (lanes 1-3), which corresponds to the expected light chain fragment of the anti-CD3 Fab (which includes the HA-tag sequence on the C-terminus). No band was detected in supernatants collected from AAV.CMV.NULL transfected cells (lanes 4-6).

To evaluate bioactivity of the anti-CD3 Fab transgene, conditioned media (CM; ARPE cell culture supernatant harvested from the expression assay at 48hrs), was preincubated with naïve mouse splenocytes for 1hr, before stimulation (TCR activation using CD3/CD28 antibodies). Cells were stained with CellTrace™ Violet, cultured for 72hrs to determine CD4+ T cell proliferation response by FACS (**Figure 17B**). Pre-incubation of splenocytes with anti-CD3 FAB antibody (2c11b; positive control) demonstrates suppressed proliferation, in a dose-dependent manner (0.005-5ug/ml). Compared to control (media only) or supernatant from AAV.NULL transfected ARPE-19 cells, conditioned media from AAV.CMV.anti-CD3 Fab also significantly reduces CD4+ proliferation comparable to 0.05ug/ml 2c11b control.

- 10 To demonstrate inducibility of anti-CD3 Fab via an inflammation responsive promotor, ARPE-19 cells were transduced with AAV7m8.AP1-NFkB.anti-CD3 Fab or AAV7m8.AP1-NFkB.NULL vectors [MOI 1E5vg/cell] and stimulated with IL-1b (2ng/ml). Conditioned media was collected at 0, 24 & 48hrs, and concentrated for SDS-PAGE (non-reducing conditions) (**Figure 18**). Stimulation results in robust induction of anti-CD3 Fab expression by 24hrs (lanes 4-6) and 48hrs (lanes 1-3), shown by HA-positive protein band, corresponding to the expected light chain fragment of the anti-CD3 Fab. No band is observed at 0hrs (lanes 7-9) or NULL (lanes 10-11).

- These data show successful expression (both constitutive and inducible) and bioactivity of an anti-CD3 Fab encoded as a transgene in both the AAV7m8.CMV.anti-CD3 Fab and AAV7m8.AP1-NFkB.anti-CD3 Fab vectors following transfection of an ocular cell type.

Evaluation of constitutive anti-CD3 Fab expression in vivo

- To demonstrate efficacy of the constitutive therapeutic transgene, C57BL/6J mice were injected in both eyes with AAV7m8.CMV.anti-CD3 Fab (n=7 eyes) or AAV7m8.CMV.NULL (n=3 eyes) [Vector dose: 4E9 vg/eye], and then immunized for Experimental Autoimmune Uveoretinitis (EAU) at 4wks post-AAV. At day 18, representative clinical images demonstrate inflammation (peri-vascular sheathing and vitreous infiltrate) in the eyes receiving the NULL vector; eyes receiving the therapeutic transgene show only mild clinical signs of disease (**Figure 19A**). At this time-point mice were killed, eyes dissected and prepared for flow cytometric analysis to determine absolute numbers of CD45+ (all leukocytes) and CD3+ (lymphocytes) populations from single eyes (**Figure 19B**). We observed a trend of reduction in immune cell infiltrate with AAV7m8.CMV.anti-CD3 Fab compared to AAV7m8.CMV.NULL (Figure 19B). AAV7m8.CMV.2c11b treatment promotes an exhausted T cell phenotype, shown by increased expression of CTLA-4 and TIM-3 on CD3+ cells (**Figure 19C**).

These data show efficacy of constitutive anti-CD3 Fab to locally suppress ocular inflammation and modulate the pathogenic phenotype of disease-causing T cells, in a model of human uveitis *in vivo*.

Methods

- 5 **Viral vectors:** Design, cloning and sequencing of plasmids performed in-house. Production, QC and quantification of ultra-pure AAV2 preparations performed by Vector Builder. Production, QC and quantification of ultra-pure AAV7m8 preparations performed by Vector BioLabs.

Name	Promotor	Transgene	Vector Type
AAV2.CMV.Infliximab	Constitutive	Infliximab Fab	Therapeutic
AAV2.CMV.NULL	Constitutive	Non-coding sequence	Control
AAV2.AP1-NFkB.Infliximab	Inducible	Infliximab Fab	Therapeutic
AAV2.AP1-NFkB.EGFP	Inducible	Enhanced GFP	Reporter
AAV2.AP1-NFkB.NULL	Inducible	Non-coding sequence	Control
AAV7m8.CMV.huTNFRI-hulgG	Constitutive	huTNFRI-hulgG	Therapeutic
AAV7m8.CMV.huTNFRI-msIgG	Constitutive	huTNFRI-msIgG	Therapeutic
AAV7m8.CMV.msTNFRI-msIgG	Constitutive	msTNFRI-msIgG	Therapeutic
AAV7m8.CMV.Adalimumab	Constitutive	Adalimumab Fab	Therapeutic
AAV7m8.CMV.NULL	Constitutive	Non-coding sequence	Control
AAV7m8.AP1-NFkB.huTNFRI-hulgG	Inducible	huTNFRI-hulgG	Therapeutic
AAV7m8.AP1-NFkB.huTNFRI-msIgG	Inducible	huTNFRI-msIgG	Therapeutic
AAV7m8.AP1-NFkB.msTNFRI-msIgG	Inducible	msTNFRI-msIgG	Therapeutic
AAV7m8.AP1-NFkB.Adalimumab	Inducible	Adalimumab Fab	Therapeutic
AAV7m8.AP1-NFkB.NULL	Inducible	Non-coding sequence	Control
AAV7m8.CMV.anti-CD3 Fab	Constitutive	Anti-CD3 Fab	Therapeutic
AAV7m8.AP1-NFkB.anti-CD3 Fab	Inducible	Anti-CD3 Fab	Therapeutic

- 10 **Cell lines:** HEK 293T or ARPE-19 cells cultured using standard complete medium. Plasmid transduction of cells performed using lipofectamine at concentration of 0.25ug/ml. AAV transduction of cells performed at range of Multiplicity of Infection (MOI; 1E4-1E5 vg/cell).

- Anti-TNF constructs:** Infliximab Fab was expressed via encoding the heavy and light chains of Infliximab Fab separated by a self-cleaving 2A peptide to generate the two separate peptides, which then form the Fab *in situ*. Adalimumab Fab was expressed similarly, but encoding the heavy and light chains of adalimumab Fab. For both Fabs, a Human Growth Hormone (HGH) signal peptide was added to the N-terminus of both heavy chain and light chain to allow secretion.
- 15

For antibody-like molecules, huTNFRI-hulgG was expressed by encoding the extracellular domain (N-terminus 211 amino acid residues, which include a native signal peptide) of human tumour necrosis factor receptor type 1 (TNFRI, also known as p55 receptor), followed by the Fc region of human IgG1. Similarly, huTNFRI-msIgG was expressed, but switching the Fc region of human IgG1 for that of mouse IgG1. Similarly, msTNFRI-msIgG was expressed, but additionally switching the extracellular domain (N-terminus 211 amino acid residues, which include a native signal peptide) of huTNFRI for the extracellular domain (N-terminus 212 amino acid residues, which include a native signal peptide) of msTNFRI. These soluble fusion proteins bind to and neutralize TNF.

- 10 **Infliximab Fab and huTNFRI-hulgG Characterization:** Levels of secreted Fab or IgG (cell culture or ex vivo retinal supernatants) assayed using a clinical IFX ELISA kit (R-Biopharm) or Anti-human TNF antibody ELISA kit (LS Bio; LS-F55832). Bioactivity of Infliximab Fab or huTNFRI-hulgG (i.e. the ability to neutralize human/mouse TNF) evaluated using TNF- α Reporter HEK 293 cells (Invivogen), which permits monitoring of NF- κ B pathway activation.
- 15 **Anti-CD3-FAB Characterization:** Levels of secreted IgG (cell culture or ex vivo retinal supernatants) assayed by SDS-PAGE using non-reducing conditions. Bioactivity of mouse specific anti-CD3 FAB (i.e. ability to inhibit T cell proliferation) evaluated using OT-II (OVA peptide specific TCR) splenocytes activated in vitro with peptide or CD3/CD28 beads. Proliferation of CD4⁺ T cells incubated with conditioned supernatants from transfected HEK-20 293T cells measured by FACS (CFSE dye incorporation).

Therapeutic intervention (AAV or biologics): Intravitreal injections performed under ketamine-based recovery anaesthesia, with an operating microscope using a 33G Hamilton syringe to inject up to 2 μ l volumes.

- 25 **Uveitis model:** Experimental Autoimmune Uveoretinitis (EAU) is an established preclinical model of human non-infectious Uveitis (Khalili, H., et al., Sci Rep, 2016. 6: p. 36905). Induced using standard immunizing protocol, with retinal peptides (RBP-3), with additional adjuvant (CFA and Pertussis Toxin) by appropriate systemic route (e.g. subcutaneous or intraperitoneal injection). EAU susceptibility is mouse strain dependent, with different RBP-3 epitopes eliciting acute, severe inflammation (B10.RIII) or persistent disease with reduced disease severity 30 (C57BL/6J). Initial therapeutic AAV2.Infliximab vector testing uses the B10.RIII strain, and subsequent therapeutic AAV7m8.huTNFRI-hulgG vector used both strains.

Recombinant human TNF model: To evaluate efficacy of therapeutic anti-TNF vectors to human protein, acute inflammation can be induced by intravitreal injection of recombinant

human TNF (20ng/eye). Susceptibility is not mouse strain dependent, with similar inflammatory kinetics in B10.RIII or C57BL/6J.

- Clinical assessment:** The Micron IV system permits repeated *in vivo* ocular assessment (Fundus, Fluorescence, OCT & ERG) of disease severity. *Ex vivo*, single retinas are processed for routine 15-colour flow cytometry (FACS) to immuno-phenotype and quantify CD45+ infiltrate cell populations.

Example 2 – Evaluation of combination therapy to suppress ocular inflammation

Vectorized constitutive expression of anti-TNF biologic (AAV.CMV.msTNFR1-msIgG) reduces inflammation Experimental Autoimmune Uveoretinitis (EAU) *in vivo*

- To further evaluate efficacy of vectorized anti-TNF biologics delivered locally to the eye, C57BL/6J mice injected with AAV7m8.CMV.msTNFR1-msIgG in one eye, and with AAV7m8.CMV.NULL in contralateral eye, were immunized for Experimental Autoimmune Uveoretinitis (EAU) at 4wks post-AAV. At day 18, clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) only in the eyes receiving the NULL vector; contralateral eyes receiving the therapeutic transgene appear normal with no clinical signs of disease (**Figure 12A**). FACS analysis of immune cell infiltrate from individual eyes (n=21 eyes/group combined from 2 independent experiments), demonstrates a significant reduction in CD45+ cells treated with AAV7m8.CMV.msTNFR1-msIgG compared to AAV7m8.CMV.NULL, confirmed through paired analysis which shows reduced lymphocyte (CD3+) and myeloid (CD11b+) cell counts. Wilcoxon matched pairs signed rank test. This demonstrated a significant difference [***p=0.0002, n=21, two tailed test], with 55% reduction in the average (mean) number of CD45+ cells (**Figure 12B**).

- These data show efficacy of a constitutive promotor design encoding anti-TNF therapeutic (msTNFR1-msIgG) transgene, to suppress ocular inflammation in a clinically relevant mouse model of human uveitis.

Local anti-CD3 Fab antibody administration suppresses EAU *in vivo*

- To identify another immune target, an anti-CD3-Fab was delivered locally to the eye. In contrast to blocking a cytokine like TNF, this approach directly targets T cells and can inhibit T cell proliferation, reduce CTL lysis or induce T cell depletion (see e.g. Khun & Weiner, 2016, Immunotherapy 8(8):889-906), which could be efficacious in uveitis.

Mice were immunized for Experimental Autoimmune Uveoretinitis (EAU) and clinical imaging used to select only mice displaying early mild signs of clinical disease at day 10. Mice received

a single intravitreal injection of 6ug Anti-CD3 Fab (Ref: BE001-1; Clone 145-2C11; BioxCell), with vehicle (PBS) administered to the contralateral eye. At day 14 (peak disease in the B10.RIII EAU model), mice were imaged and then killed, eyes dissected and immune cell infiltrate immunophenotyped using FACS. Representative clinical images (Fundus and OCT) demonstrate clinical inflammation in control (PBS) eyes compared to eyes receiving anti-CD3 Fab (**Figure 13A**), with substantially reduced CD45+ cell counts (**Figure 13B**) (One-way ANOVA, ** p=0.0021, n=6).

These data show efficacy of an anti-CD3-Fab, which targets disease-associated T cells in the eye though local delivery, to suppress ocular inflammation in the clinically relevant mouse EAU model of human uveitis.

Anti-CD3 Fab vector and Bicistronic vector design

To evaluate the efficacy of targeting both TNF and CD3 to suppress ocular inflammation, we developed separate AAV vectors and bicistronic AAV vectors encoding a TNF inhibitor and/or a CD3 inhibitor, under the control of either a constitutive or inducible promoter.

We developed constructs comprising a single therapeutic transgene, namely TNFRI-IgG (**Figure 14A**) or an Anti-CD3 Fab (**Figure 14B**), and either a constitutive promoter (CMV promoter) or an inflammation-inducible promoter (AP1-NFkB; inflammation responsive promoter comprised of x5 repeated AP1 & NFkB binding sites).

We also developed bicistronic constructs comprising both therapeutic transgenes (TNFRI-IgG and an Anti-CD3 Fab) and either a constitutive promoter (CMV promoter) or an inflammation-inducible promoter (AP1-NFkB; inflammation responsive promoter comprised of x5 repeated AP1 & NFkB binding sites) (**Figure 14C**). For the bicistronic constructs, the constitutive and inducible design allows encoding and expression of both therapeutic transgenes

Evaluation of constitutive anti-CD3 Fab transgene expression & bioactivity *in vitro*

To evaluate constitutive anti-CD3 Fab antibody expression, HEK-293T cells were transduced with AAV7m8.CMV.anti-CD3 Fab or AAV7m8.CMV.NULL vectors [MOI 1E5vg/cell]. Culture supernatants at 48hrs were collected and protein samples concentrated and prepared with reducing conditions for SDS-PAGE (4-20% gradient gel) analysis. Blots were probed with Anti-HA (Clone F7; sc-7392) primary monoclonal antibody, and secondary anti-mouse HRP for ECL detection (**Figure 15A**). The SDS-PAGE shows an HA-positive 22kD protein band (lanes 1-3), which corresponds to the expected light chain fragment of the anti-CD3 Fab (which includes the HA-tag sequence on the C-terminus). No band was detected in supernatants collected from AAV.CMV.NULL transfected cells (lanes 4-6).

To evaluate bioactivity of the anti-CD3 Fab transgene, conditioned media (CM; culture supernatant harvested from the expression assay at 48hrs), was preincubated with naïve mouse splenocytes for 1hr, before stimulation (TCR activation using CD3/CD28 antibodies). Cells were stained with CellTrace™ Violet, cultured for 72hrs to determine CD4+ T cell proliferation response by FACS (**Figure 15B**). Pre-incubation of splenocytes with anti-CD3 FAB antibody (2c11b; positive control) demonstrates suppressed proliferation, in a dose-dependent manner (0.01-1ug/ml). Compared to control (media only) or supernatant from AAV.NULL transfected HEK293T cells, CM from AAV.CMV.anti-CD3 FAB also significantly reduces CD4+ proliferation.

- 10 These data show successful expression and bioactivity of an anti-CD3 Fab encoded as a transgene in the AAV7m8.CMV.anti-CD3 FAB vector.

Evaluation of combined targeting therapy (AAV.CMV.msTNFRI-msIgG + AAV.CMV.anti-CD3 Fab) to suppress inflammation in EAU

- 15 To evaluate efficacy of combined therapeutic vectors, C57BL/6J mice were injected with AAV "MIX" containing AAV7m8.CMV.msTNFRI-msIgG [2E9 vg/eye] + AAV7m8.CMV.anti-CD3 FAB [2E9 vg/eye] in one eye, with AAV7m8.CMV.NULL [4E9 vg/eye] in contralateral eye, and were then immunized for EAU at 4wks post-AAV. To test if the combined treatment gives increased efficacy, in a separate group mice were injected with the AAV7m8.CMV.msTNFRI-msIgG [2E9 vg/eye] in one eye, with AAV7m8.CMV.NULL in contralateral eye. At day 18,
- 20 clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) only in the eyes receiving the NULL vector; contralateral eyes receiving either the combined "MIX" therapeutic transgenes or AAV.CMV.TNFRI vector only appear normal with no clinical signs of disease (**Figure 16A**). FACS analysis was then carried out for immune cell infiltrate from individual eyes of the AAV.TNFRI only and AAV "MIX" treatment groups.

- 25 Treatment with AAV7m8.CMV.msTNFRI-msIgG compared to AAV7m8.CMV.NULL, confirms overall reduction in total CD45+ cells from individual eyes receiving the single therapeutic transgene (**Figure 16B**). Wilcoxon matched pairs signed rank test demonstrates there is a significant difference [$**p=0.0039$, $n=9$, two tailed test], with 31% reduction in the average (mean) number of CD45+ cells/eye.

- 30 Treatment with combined AAV7m8.CMV.msTNFRI-msIgG + AAV7m8.CMV.anti-CD3 FAB (AAV "MIX") demonstrates significant reduction in CD45+ cells compared to AAV7m8.CMV.NULL, confirmed through paired analysis showing reduced lymphocyte (CD3+ & CD4+) and myeloid (CD11b+) populations (**Figure 16C**). Wilcoxon matched pairs signed

rank test demonstrates there is a significant difference [***p=0.0005, n=13, two tailed test], with 63% reduction in the average (mean) number of CD45+ cells.

These data show increased efficacy of combined administration of two therapeutic AAV vectors that constitutively express a TNF inhibitor and a CD3 inhibitor, to suppress ocular inflammation in the EAU mouse model of human uveitis.

Evaluation of constitutive expression of two transgenes (TNFRI-IgG and Anti-CD3 Fab) using a bi-cistronic construct *in vitro* - plasmids and vectors

To evaluate constitutive expression of both transgenes from the AAV.CMV.msTNFRI-IgG.anti-CD3 Fab (bi-cistronic) construct, ARPE-19 cells were transfected with AAV.CMV.anti-CD3 Fab, AAV.CMV.NULL or AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab plasmids (1ug/ml). Culture supernatants (conditioned media; CM) were collected at 72hrs, and protein samples concentrated for assays.

To demonstrate bioactivity of the expressed msTNFRI-IgG transgene, CM was “spiked” with recombinant mouse TNF (final concentration 0.1ng/ml), before incubation with HEK-BLUE reporter cells for 24hrs, and NFkB activation assessment. CM from AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab (Bi-cistronic plasmid) inhibited mTNF-mediated activation in the reporter cell line compared to AAV.CMV.NULL or recTNF alone. One-way ANOVA; ****p<0.0001; Data presented as means +/- SEM (**Figure 20A**).

To demonstrate expression of the anti-CD3 Fab transgene, CM from AAV.CMV.anti-CD3 Fab, AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab or AAV.CMV.NULL prepared with non-reducing conditions for SDS-PAGE (4-20% gradient gel) analysis. Blots were probed with Anti-HA (Clone F7; sc-7392) primary monoclonal antibody, and secondary anti-mouse HRP for ECL detection. SDS-PAGE image shows HA-positive protein band (light chain fragment of the anti-CD3 Fab) present in the AAV.CMV.anti-CD3 Fab and AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab samples. No band was detected in supernatants collected from AAV.CMV.NULL. This demonstrates expression of the anti-CD3 Fab transgene from the bi-cistronic plasmid (**Figure 20B**).

To evaluate constitutive expression of both transgenes from the AAV.CMV.msTNFRI-IgG.anti-CD3 Fab (bi-cistronic) construct, HEK293T or ARPE-19 cells were transduced with AAV7m8.CMV.msTNFRI-IgG, AAV7m8.CMV.anti-CD3 Fab, AAV7m8.CMV.NULL or AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab vectors [MOI 1E5vg/cell], and incubated for 48hrs to generate conditioned media (CM).

To demonstrate bioactivity of the expressed msTNFRI-IgG transgene, CM was “spiked” with recombinant mouse TNF (final concentration 0.1ng/ml), before incubation with HEK-BLUE reporter cells for 24hrs, and NFkB activity assessed (**Figure 21A**). CM from AAV7m8.CMV.msTNFRI-msIgG and AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab transfected HEK293T or ARPE-19 inhibited mTNF-mediated activation in the reporter cell line compared to AAV7m8.CMV.NULL. One-way ANOVA; ****p<0.0001; Data presented as means +/- SEM.

To demonstrate expression of the anti-CD3 Fab transgene, CM from HEK293T transduced with AAV7m8.CMV.anti-CD3 Fab, AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab or AAV7m8.CMV.NULL prepared with non-reducing conditions for SDS-PAGE (4-20% gradient gel) analysis. Blots were probed with Anti-HA (Clone F7; sc-7392) primary monoclonal antibody, and secondary anti-mouse HRP for ECL detection (**Figure 21B**). SDS-PAGE image shows HA-positive 50kD protein band (heavy chain fragment of the anti-CD3 Fab) present in the AAV7m8.CMV.anti-CD3 Fab and AAV7m8.CMV.msTNFRI-msIgG.anti-CD3 Fab samples only. No band was detected in supernatants collected from AAV7m8.CMV.NULL.

These data show successful expression and bioactivity of two therapeutic transgenes using a constitutive promotor in the bi-cistronic plasmid and vector.

Evaluation of combined targeting therapy (AAV.CMV.msTNFRI-msIgG + AAV.CMV.anti-CD3 Fab) to maintain reduced inflammation in EAU

To evaluate the long-term efficacy of combined therapeutic vectors, C57BL/6J mice were injected with AAV “MIX” containing AAV7m8.CMV.msTNFRI-msIgG [2E9 vg/eye] + AAV7m8.CMV.anti-CD3 Fab [2E9 vg/eye] in one eye, with AAV7m8.CMV.NULL [4E9 vg/eye] in contralateral eye, and were then immunized for EAU at 4wks post-AAV. At day 28 EAU (post-peak inflammation), clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) in eyes receiving the NULL vector, with contralateral eyes receiving the combined “MIX” therapeutic transgenes showing mild clinical signs of disease (**Figure 22A**). FACS analysis was then carried out for immune cell infiltrate from individual eyes AAV “MIX” vs NULL eyes, shows 34% reduction in the average (mean) number of CD45+ cells [**p=0.005, n=7, paired t test] (**Figure 22B**).

These data provide evidence showing maintained efficacy of a combined targeting therapy approach to suppress disease in the EAU mouse model of human uveitis.

Evaluation of bi-cistronic vector (AAV.CMV.msTNFRI-msIgG.anti-CD3 Fab) to suppress peak inflammation in EAU

To evaluate efficacy of the bi-cistronic vector to suppress inflammation, C57BL/6J mice injected with AAV7m8.CMV.msTNFR1- μ sgG.anti-CD3 Fab in one eye, and with AAV7m8.CMV.NULL in contralateral eye, were immunized for Experimental Autoimmune Uveoretinitis (EAU) at 4wks post-AAV. At day 18 (peak disease), clinical imaging demonstrates inflammation (peri-vascular sheathing and vitreous infiltrate) only in the eyes receiving the NULL vector, with contralateral eyes injected with the bicistronic AAV expressing therapeutic transgenes appearing normal with no clinical signs of disease (**Figure 23A**). FACS analysis of immune cell infiltrate from individual eyes (n=8 eyes/group), demonstrates a significant reduction in CD45⁺ cells treated with AAV7m8.CMV.msTNFR1- μ sgG.anti-CD3 Fab compared to AAV7m8.CMV.NULL, confirmed through paired analysis which shows reduced lymphocyte (CD3⁺) and myeloid (CD11b⁺) cell counts [* p=0.05; **p=0.005, n=8, two tailed test] (**Figure 23B**).

These data provide evidence supporting efficacy of AAV.CMV.mTNFR1-IgG.2c11b (Bicistronic vector) to inhibit immune cell infiltrate and inflammation in the EAU mouse model of human uveitis.

Methods

Viral vectors: Design, cloning and sequencing of plasmids performed in-house. Plasmid maps for constitutive (AAV.CMV.mTNFR1-IgG.anti-CD3 Fab) and inducible (AAV.AP1-NFkB.mTNFR1-IgG.anti-CD3 Fab) bi-cistronic constructs are provided in **Figure 24**. Production, QC and quantification of ultra-pure AAV7m8 preparations performed by Vector BioLabs.

Name	Promotor	Transgene(s)	Vector Type
AAV7m8.CMV.msTNFR1- μ sgG	Constitutive	msTNFR1- μ sgG	Therapeutic
AAV7m8.CMV.ms anti-CD3 FAB	Constitutive	ms_anti-CD3 FAB	Therapeutic
AAV7m8.CMV.NULL	Constitutive	Non-coding sequence	Control
AAV7m8.AP1-NFkB.msTNFR1- μ sgG	Inducible	msTNFR1- μ sgG	Therapeutic
AAV7m8.AP1-NFkB.ms anti-CD3 FAB	Inducible	ms_anti-CD3 FAB	Therapeutic
AAV7m8.CMV.NULL	Inducible	Non-coding sequence	Control
AAV7m8.CMV.bicistronic	Constitutive	msTNFR1- μ sgG + ms_anti-CD3 FAB	Therapeutic
AAV7m8.AP1-NFkB.bicistronic	Inducible	msTNFR1- μ sgG + ms_anti-CD3 FAB	Control

Cell lines: HEK 293T cultured using standard complete medium. Plasmid transduction of cells performed using lipofectamine at concentration of 0.25ug/ml. AAV transduction of cells performed at range of Multiplicity of Infection (MOI; 1E4-1E5 vg/cell).

Anti-TNF constructs: A transgene encoding msTNFRI-msIgG was constructed by encoding the extracellular domain (N-terminus 212 amino acid residues, which include a native signal peptide) of msTNFRI followed by the Fc region of mouse IgG1.

5 **Anti-CD3 FAB constructs:** A transgene encoding an anti-CD3 Fab was constructed by encoding the Fab domain of 145-2C11 along with an N-terminus human growth factor (HGH) signal peptide. 145-2C11 is an Armenian Hamster IgG monoclonal antibody raised against mouse CD3e and has been shown to block cytotoxic T cell-mediated cell lysis and deplete T cells. A furin site and P2A peptide separated the heavy chain and light chain coding regions, allowing the heavy chain and light chain polypeptides to be expressed separately and to form
10 the Fab.

Bicistronic constructs: To encode both TNFRI-hulgG and anti-CD3 Fab in a single vector, the two transgenes were separated by a furin site and T2A peptide.

msTNFRI-msIgG Characterization: Levels of secreted IgG (cell culture or ex vivo retinal supernatants) assayed using an anti-mouse TNF antibody ELISA kit (LS Bio; LS-F55832).
15 Bioactivity of msTNFRI-msIgG (i.e. the ability to neutralize mouse TNF) evaluated using TNF- α Reporter HEK 293 cells (Invivogen), which permits monitoring of NF- κ B pathway activation.

Anti-CD3-FAB Characterization: Levels of secreted IgG (cell culture or ex vivo retinal supernatants) assayed by SDS-PAGE using non-reducing conditions. Bioactivity of mouse specific anti-CD3 FAB (i.e. ability to inhibit T cell proliferation) evaluated using OT-II (OVA
20 peptide specific TCR) splenocytes activated in vitro with peptide or CD3/CD28 beads. Proliferation of CD4⁺ T cells incubated with conditioned supernatants from transfected HEK-293T cells measured by FACS (CFSE dye incorporation).

Therapeutic intervention (AAV or biologics): Intravitreal injections performed under ketamine-based recovery anaesthesia, with an operating microscope using a 33G Hamilton
25 syringe to inject up to 2 μ l volumes.

Uveitis model: Experimental Autoimmune Uveoretinitis (EAU) is an established preclinical model of human non-infectious Uveitis (Khalili, H., et al., Sci Rep, 2016. 6: p. 36905). Induced using standard immunizing protocol, with retinal peptides (RBP-3), with additional adjuvant (CFA and Pertussis Toxin) by appropriate systemic route (e.g. subcutaneous or intraperitoneal
30 injection). EAU induced in C57BL/6J mice generates a persistent chronic disease phenotype, used for in vivo evaluation of therapeutic AAV7m8 vector efficacy.

Clinical assessment: The Micron IV system permits repeated *in vivo* ocular assessment (Fundus, Fluorescence, OCT & ERG) of disease severity. *Ex vivo*, single retinas are processed for routine 15-colour flow cytometry (FACS) to immuno-phenotype and quantify CD45+ infiltrate cell populations.

- 5 The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, biochemistry, molecular biology, microbiology and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example: Skoog, D.A., et al. (2013) Fundamentals of Analytical Chemistry, 9th edition, Cengage learning; Walker J.M. (2009) The Protein Protocols Handbook, 3rd edition, Springer Nature; Green, M.R. and Sambrook, J. (2012) Molecular Cloning: A Laboratory Manual, 4th Edition, Cold Spring Harbor Laboratory Press; Ausubel, F.M., et al. (2003) Current Protocols in Molecular Biology, John Wiley & Sons; Hill, A. J. (2013) DNA Sequencing Protocols, Humana Press; Nielsen, B.S. and Jones, J. (2021) In Situ Hybridization Protocols, Springer US; Herdewijn, P. (2010) Oligonucleotide Synthesis: Methods and Applications, Humana Press; and Luo, Y. (2019) CRISPR Gene Editing: Methods and Protocols, Springer New York. Each of these general texts is herein incorporated by reference.

EMBODIMENTS

- Various preferred features and embodiments of the present invention will now be described with reference to the following numbered paragraphs (paras).

Combinations, vectors, kits, products, and pharmaceutical compositions for preventing or treating an inflammatory eye disease

1. A vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.
- 25 2. The vector according to any preceding para, wherein the nucleotide sequence encoding a TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element.
3. The vector according to any preceding para, wherein the nucleotide sequence encoding a
- 30 TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide sequence encoding a 2A self-cleaving peptide.

4. The vector according to any preceding para, wherein the nucleotide sequence encoding a TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are each operably linked to the same promoter.
5. The vector according to any preceding para, wherein the vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a TNF inhibitor; a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a CD3 inhibitor.
6. The vector according to any of paras 1-5, wherein the vector comprises a nucleotide sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence encoding a CD3 inhibitor; a 2A self-cleaving peptide, an enzymatically cleavable peptide motif, and/or an IRES element; and the nucleotide sequence encoding a TNF inhibitor.
7. A kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
8. A pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
9. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the TNF inhibitor is any of adalimumab or a fragment thereof, infliximab or a fragment thereof, golimumab or a fragment thereof, or certolizumab or a fragment thereof.
10. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the TNF inhibitor is adalimumab or a fragment thereof, or infliximab or a fragment thereof, preferably wherein the TNF inhibitor is adalimumab or a fragment thereof.
11. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the TNF inhibitor is an antigen binding fragment (Fab) of adalimumab.
12. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the TNF inhibitor is an anti-TNF antibody or fragment thereof comprising one or more CDR regions selected from SEQ ID NOs: 16 to 21 or derivatives thereof comprising one amino acid substitution, preferably wherein the TNF inhibitor is an anti-TNF antibody or fragment thereof comprising CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 16, 17, 18, 19, 20 and 21 respectively or derivatives thereof comprising one amino acid substitution.

13. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the TNF inhibitor is an anti-TNF antibody or fragment thereof comprising a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 22 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 23.

14. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the CD3 inhibitor is any of foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, otelixizumab or a fragment thereof, muromonab-CD3 or a fragment thereof, T3/4.A or a fragment thereof, YTH12.5 or a fragment thereof, or HuM291 or a fragment thereof.

15. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the CD3 inhibitor is foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, or otelixizumab or a fragment thereof.

16. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the CD3 inhibitor is an antigen binding fragment (Fab) of foralumab, teplizumab, visilizumab, or otelixizumab.

17. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the CD3 inhibitor is an anti-CD3 antibody or fragment thereof comprising one or more CDR regions selected from: (a) SEQ ID NOs: 70 to 75 or derivatives thereof comprising one amino acid substitution, (b) SEQ ID NOs: 81 to 86 or derivatives thereof comprising one amino acid substitution, (c) SEQ ID NOs: 92 to 97 or derivatives thereof comprising one amino acid substitution, and (d) SEQ ID NOs: 103 to 108 or derivatives thereof comprising one amino acid substitution, preferably wherein the CD3 inhibitor is an anti-CD3 antibody or fragment thereof comprising (a) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 70, 71, 72, 73, 74 and 75 respectively or derivatives thereof comprising one amino acid substitution; (b) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 81, 82, 83, 84, 85 and 86 respectively or derivatives thereof comprising one amino acid substitution; (c) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 92, 93, 94, 95, 96 and 97 respectively or derivatives thereof comprising one amino acid substitution; or (d) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 103, 104, 105, 106, 107 and 108 respectively or derivatives thereof comprising one amino acid substitution.

18. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the CD3 inhibitor is an anti-CD3 antibody or fragment thereof comprising: (a) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 76 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110.

19. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the nucleotide sequence encoding a TNF inhibitor is operably linked to an inflammation-inducible promoter and/or the nucleotide sequence encoding a CD3 inhibitor is operably linked to an inflammation-inducible promoter.

20. The vector, kit, or pharmaceutical composition according to para 19, wherein the inflammation-inducible promoter comprises one or more inflammation-inducible transcription factor binding motif selected from: an AP-1 transcription factor binding motif; a NF- κ B transcription factor binding motif; an IRF transcription factor binding motif; a STAT transcription factor binding motif; and a NFAT transcription factor binding motif or any combination thereof.

21. The vector, kit, or pharmaceutical composition according to para 19 or 20, wherein the inflammation-inducible promoter comprises one or more AP-1 binding motif and/or one or more NF- κ B binding motif.

22. The vector, kit, or pharmaceutical composition according to any of paras 19-21, wherein the inflammation-inducible promoter comprises two or more AP-1 binding motifs and/or two or more NF- κ B binding motifs, three or more AP-1 binding motifs and/or three or more NF- κ B binding motifs, four or more AP-1 binding motifs and/or four or more NF- κ B binding motifs, or five or more AP-1 binding motifs and/or five or more NF- κ B binding motifs

23. The vector, kit, or pharmaceutical composition according to any of paras 19-22, wherein the inflammation-inducible promoter comprises at least one AP-1 binding motif coupled to at least one NF- κ B binding motif.

24. The vector, kit, or pharmaceutical composition according to any of paras 19-23, wherein the inflammation-inducible promoter comprises five AP-1 binding motifs coupled to five NF-κB binding motifs.
25. The vector, kit, or pharmaceutical composition according to any of paras 20-24, wherein
5 the AP-1 binding motif comprises or consists of SEQ ID NO: 151, or wherein the AP-1 binding motif comprises or consists of any of SEQ ID NOs: 152-154 or derivatives thereof comprising one nucleotide substitution.
26. The vector, kit, or pharmaceutical composition according to any of paras 20-25, wherein
10 the NF-κB binding motif comprises or consists of SEQ ID NO: 155, or wherein the NF-κB binding motif comprises or consists of SEQ ID NO: 156 or a derivative thereof comprising two or fewer nucleotide substitutions.
27. The vector, kit, or pharmaceutical composition according to any of paras 19-26, wherein the inflammation-inducible promoter comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 157.
- 15 28. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the vector(s) further comprise a polyadenylation sequence.
29. The vector, kit, or pharmaceutical composition according to para 28, wherein the polyadenylation sequence is selected from any of: a bovine growth hormone (bGH) polyadenylation sequence, a SV40 polyadenylation sequence, and a rabbit beta-globin
20 polyadenylation sequence.
30. The vector, kit, or pharmaceutical composition according to para 28 or 29, wherein the polyadenylation sequence comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 163.
31. The vector, kit, or pharmaceutical composition according to any preceding para, wherein
25 the vector(s) further comprise a woodchuck hepatitis post-transcriptional regulatory element (WPRE).
32. The vector, kit, or pharmaceutical composition according to para 31, wherein the WPRE comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 164.

33. The vector, kit, or pharmaceutical composition according to any preceding para, wherein the vector(s) further comprise an intron, optionally wherein the intron is selected from a beta-globin intron or a SV40 intron.
34. The vector, kit, or pharmaceutical composition according to para 33, wherein the intron
5 comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 165.
35. The vector, kit, or pharmaceutical composition according to any previous para, wherein the vector(s) are a viral vector, optionally wherein the vector(s) are any of a parvoviral vector, an adenoviral vector, a herpes simplex viral vector, an anelloviral vector, a retroviral vector or
10 a lentiviral vector.
36. The vector, kit, or pharmaceutical composition according to any previous para, wherein the vector(s) are an adeno-associated virus (AAV) vector.
37. The vector, kit, or pharmaceutical composition according to any previous para, wherein the vector(s) are an AAV vector particle.
- 15 38. The vector, kit, or pharmaceutical composition according to para 37, wherein the AAV vector particle is pseudotyped to confer ocular tissue tropism.
39. The vector, kit, or pharmaceutical composition according to para 37 or 38, wherein the AAV vector particle comprises AAV2 capsid proteins or AAV2 capsid variant proteins, optionally wherein the AAV2 capsid variant is selected from any of: AAV2.tYF, AAV2.7m8,
20 R100, AAV2.GL and AAV2.NN.
40. The vector, kit, or pharmaceutical composition according to any previous para, wherein the vector(s) comprise one or more inverted terminal repeats (ITRs).
41. An isolated cell comprising: (i) the vector according to any of paras 1-6 or 9-40; and/or (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector
25 comprising a nucleotide sequence encoding a CD3 inhibitor.
42. A kit for the production of: (i) the vector of any one of paras 1-6 or 9-40; and/or (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
43. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent
30 or excipient in combination with: (i) the vector according to any one of paras 1-6 or 9-40; (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector

comprising a nucleotide sequence encoding a CD3 inhibitor; and/or (iii) the isolated cell according to para 41.

44. A vector according to any one of paras 1-6 or 9-40, an isolated cell according to para 41, and/or a pharmaceutical composition according to para 43, for use as a medicament.

5 45. Use of a vector according to any one of paras 1-6 or 9-40, an isolated cell according to para 41, or a pharmaceutical composition according to para 43, for the manufacture of a medicament.

46. A method comprising administering a vector according to any one of paras 1-6 or 9-40, an isolated cell according to para 41, or a pharmaceutical composition according to para 43, to a
10 subject in need thereof.

47. A vector for use in preventing or treating an inflammatory eye disease, wherein the vector comprises: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

48. Use of a vector in the manufacture of a medicament for preventing or treating an
15 inflammatory eye disease, wherein the vector comprises: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

49. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a vector to a subject in need thereof, wherein the vector comprises:
20 (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

50. A product comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor, as a combined preparation for simultaneous, separate or sequential use in preventing or treating an inflammatory eye disease.

25 51. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering to a subject in need thereof: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

52. A vector according to any one of paras 1-6 or 9-40, or a pharmaceutical composition
30 according to para 43, for use in preventing or treating an inflammatory eye disease.

53. Use of a vector according to any one of paras 1-6 or 9-40, or a pharmaceutical composition according to para 43, for the manufacture of a medicament for preventing or treating an inflammatory eye disease.
54. A method of preventing or treating an inflammatory eye disease comprising administering
5 a vector according to any one of paras 1-6 or 7-40, or a pharmaceutical composition according to para 43, to a subject in need thereof.
55. The vector or pharmaceutical composition for use according to para 47 or 52, the use according to para 48 or 53, the method according to para 49, 51 or 54, or the product for use according to para 50, wherein the inflammatory eye disease is uveitis.
- 10 56. The vector or pharmaceutical composition for use according to any of paras 47, 52 or 55, the use according to any of paras 48, 53 or 55, the method according to any of paras 49, 51 or 54-55, or the product for use according to para 50 or 55, wherein the vector(s) or pharmaceutical composition are administered intraocularly.
- 15 57. The vector or pharmaceutical composition for use according to any of paras 47, 52, or 55-56, the use according to any of paras 48, 53 or 55-56, the method according to any of paras 49, 51 or 54-56, or the product for use according to any of paras 50 or 55-56, wherein the vector(s) or pharmaceutical composition are administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection.
- 20 58. The vector or pharmaceutical composition for use according to any of paras 47, 52, or 55-57, the use according to any of paras 48, 53 or 55-57, the method according to any of paras 49, 51 or 54-57, or the product for use according to any of paras 50 or 55-57, wherein the vector(s) or pharmaceutical composition are administered via intravitreal injection.
- 25 59. The vector or pharmaceutical composition for use according to any of paras 47, 52, or 55-58, the use according to any of paras 48, 53 or 55-58, the method according to any of paras 49, 51 or 54-58, or the product for use according to any of paras 50 or 55-58, wherein the vector(s) or pharmaceutical composition are administered as a single dose.
- 30 60. The vector or pharmaceutical composition for use according to any of paras 47, 52, or 55-59, the use according to any of paras 48, 53 or 55-59, the method according to any of paras 49, 51 or 54-59, or the product for use according to any of paras 50 or 55-59, wherein the vector(s) are administered at a dose of at least about $1E10$ vg/mL, at least about $1E11$ vg/mL, at least about $1E12$ vg/mL, or at least about $5E12$ vg/mL.

61. The vector or pharmaceutical composition for use according to any of paras 47, 52, or 55-60, the use according to any of paras 48, 53 or 55-60, the method according to any of paras 49, 51 or 54-60, or the product for use according to any of paras 50 or 55-60, wherein the vector(s) are administered at a dose of at least about 1E9 vg/eye, at least about 1E10 vg/eye,
5 or at least about 1E11 vg/eye, preferably wherein the vector(s) are administered at a dose of about 1E9 vg/eye to about 5E12 vg/eye.
62. The vector or pharmaceutical composition for use according to any of paras 47, 52, or 55-61, the use according to any of paras 48, 53 or 55-61, the method according to any of paras 49, 51 or 54-61, or the product for use according to any of paras 50 or 55-61, wherein the
10 vector(s) or pharmaceutical composition are administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.
63. A kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.
- 15 64. A pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.
65. A product comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a
20 nucleotide sequence encoding the same, as a combined preparation for simultaneous, separate or sequential use in therapy.
66. A product comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a
25 nucleotide sequence encoding the same, as a combined preparation for simultaneous, separate or sequential use in preventing or treating an inflammatory eye disease.
67. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering to a subject in need thereof: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a
polynucleotide comprising a nucleotide sequence encoding the same.
- 30 68. The product for use according to para 66 or the method according to para 67, wherein the inflammatory eye disease is uveitis.

69. The product for use according to para 66 or 68 or the method according to para 67 or 68, wherein (a) the TNF inhibitor or polynucleotide is administered intraocularly; and/or (b) the CD3 inhibitor or polynucleotide is administered intraocularly.

5 70. The product for use according to any of paras 66 or 68-69 or the method according to any of paras 67-69, wherein (a) the TNF inhibitor or polynucleotide is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection; and/or (b) the CD3 inhibitor or polynucleotide is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection.

10 71. The product for use according to any of paras 66 or 68-70 or the method according to any of paras 67-70, wherein (a) the TNF inhibitor or polynucleotide is administered via intravitreal injection; and/or (b) the CD3 inhibitor or polynucleotide is administered via intravitreal injection.

15 72. The product for use according to any of paras 66 or 68-71 or the method according to any of paras 67-71, wherein (a) the TNF inhibitor or polynucleotide is administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis; and/or (b) the CD3 inhibitor or polynucleotide is administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.

20 73. The product for use according to any of paras 66 or 68-72 or the method according to any of paras 67-72, wherein (a) the TNF inhibitor or polynucleotide is administered in the form of a vector comprising a nucleotide sequence encoding a TNF inhibitor; and/or (b) the CD3 inhibitor or polynucleotide is administered in the form of a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

25 74. The product for use according to any of paras 66 or 68-73 or the method according to any of paras 67-73, wherein (a) the TNF inhibitor or polynucleotide is administered in the form of a pharmaceutical composition comprising the TNF inhibitor or polynucleotide; and/or (b) the CD3 inhibitor or polynucleotide is administered in the form of a pharmaceutical composition comprising the CD3 inhibitor or polynucleotide.

CD3 inhibitors, polynucleotides, cells, and pharmaceutical compositions for preventing or treating an inflammatory eye disease

30 1. A vector comprising a nucleotide sequence encoding a CD3 inhibitor, wherein the nucleotide sequence encoding the CD3 inhibitor is operably linked to an inflammation-inducible promoter.

2. The vector according to para 1, wherein the CD3 inhibitor is an anti-CD3 antibody or fragment thereof.
3. The vector according to para 1 or 2, wherein the CD3 inhibitor is an antibody fragment.
4. The vector according to any preceding para, wherein the CD3 inhibitor is an antigen-binding
5 fragment (Fab), a fragment antibody (F(ab')₂), a single chain antibody (scFv), or a single-domain antibody (sdAb).
5. The vector according to any preceding para, wherein the CD3 inhibitor is an antigen-binding fragment (Fab).
6. The vector according to any preceding para, wherein the CD3 inhibitor thereof is any of
10 foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, otelexizumab or a fragment thereof, muromonab-CD3 or a fragment thereof, T3/4.A or a fragment thereof, YTH12.5 or a fragment thereof, or HuM291 or a fragment thereof.
7. The vector according to any preceding para, wherein the CD3 inhibitor is foralumab or a fragment thereof, teplizumab or a fragment thereof, visilizumab or a fragment thereof, or
15 otelexizumab or a fragment thereof.
8. The vector according to any preceding para, wherein the CD3 inhibitor is an antigen binding fragment (Fab) of foralumab, teplizumab, visilizumab, or otelexizumab.
9. The vector according to any preceding para, wherein the CD3 inhibitor is an anti-CD3 antibody or fragment thereof comprising one or more CDR regions selected from: (a) SEQ ID
20 NOs: 70 to 75 or derivatives thereof comprising one amino acid substitution, (b) SEQ ID NOs: 81 to 86 or derivatives thereof comprising one amino acid substitution, (c) SEQ ID NOs: 92 to 97 or derivatives thereof comprising one amino acid substitution, and (d) SEQ ID NOs: 103 to 108 or derivatives thereof comprising one amino acid substitution.
10. The vector according to any preceding para, wherein the CD3 inhibitor is an anti-CD3
25 antibody or fragment thereof comprising: (a) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 70, 71, 72, 73, 74 and 75 respectively, or derivatives thereof comprising one amino acid substitution; (b) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 81, 82, 83, 84, 85 and 86 respectively, or derivatives thereof comprising one amino acid
30 substitution; (c) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 92, 93, 94, 95, 96 and 97 respectively, or derivatives thereof comprising one amino acid substitution; or (d) CDR regions HCDR1, HCDR2, HCDR3,

LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 103, 104, 105, 106, 107 and 108 respectively, or derivatives thereof comprising one amino acid substitution.

11. The vector according to any preceding para, wherein the CD3 inhibitor comprises: (a) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 76 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110.

12. The vector according to para 11, wherein: (a) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 125 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 126; (b) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 127 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 128; (c) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 129 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 130; or (d) the heavy chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 131 and/or the light chain is encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 132.

13. The vector according to para 12, wherein the nucleotide sequence encoding the heavy chain and the nucleotide sequence encoding the light chain are connected via a linker sequence.

14. The vector according to para 13, wherein the linker sequence encodes a 2A self-cleaving peptide, and/or an enzymatically cleavable peptide motif.

15. The vector according to para 13 or 14, wherein the linker sequence encodes a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9.

16. The vector according to any of paras 12 to 15, wherein the nucleotide sequence encoding the heavy chain and/or the nucleotide sequence encoding the light chain is operably linked to a signal sequence, optionally wherein the signal sequence encodes a signal peptide selected from any of: a Human Growth Hormone (HGH) signal peptide, an interleukin-2 (IL-2) signal

peptide, a CD5 signal peptide, an immunoglobulin Kappa light chain signal peptide, a trypsinogen signal peptide, a serum albumin signal peptide, and a prolactin signal peptide.

17. The vector according to any preceding para, wherein the nucleotide sequence encoding a CD3 inhibitor encodes: (a) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 76, a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87, a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98, a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109, a 2A self-cleaving peptide having at least 70% sequence identity to any of SEQ ID NOs: 6-9, and a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110.

18. The vector according to any preceding para, wherein the nucleotide sequence encoding a CD3 inhibitor comprises or consists of: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 125, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 126; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 127, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 128; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 129, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 130; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 131, a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 10 or 11, and a nucleotide sequence having at least 70% identity to SEQ ID NO: 132.

19. The vector according to any preceding para, wherein the nucleotide sequence encoding a CD3 inhibitor comprises or consists of: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 134; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 136; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 138; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 140.

20. The vector according to any preceding para, wherein the inflammation-inducible promoter comprises one or more inflammation-inducible transcription factor binding motif selected from: an AP-1 transcription factor binding motif; a NF-κB transcription factor binding motif; an IRF transcription factor binding motif; a STAT transcription factor binding motif; and a NFAT transcription factor binding motif or any combination thereof.
21. The vector according to any preceding para, wherein the inflammation-inducible promoter comprises one or more AP-1 binding motif and/or one or more NF-κB binding motif.
22. The vector according to any preceding para, wherein the inflammation-inducible promoter comprises two or more AP-1 binding motifs and/or two or more NF-κB binding motifs, three or more AP-1 binding motifs and/or three or more NF-κB binding motifs, four or more AP-1 binding motifs and/or four or more NF-κB binding motifs, or five or more AP-1 binding motifs and/or five or more NF-κB binding motifs
23. The vector according to any preceding para, wherein the inflammation-inducible promoter comprises at least one AP-1 binding motif coupled to at least one NF-κB binding motif.
24. The vector according to any preceding para, wherein the inflammation-inducible promoter comprises five AP-1 binding motifs coupled to five NF-κB binding motifs.
25. The vector according to any of paras 20-24, wherein the AP-1 binding motif comprises or consists of SEQ ID NO: 151, or wherein the AP-1 binding motif comprises or consists of any of SEQ ID NOs: 152-154 or derivatives thereof comprising one nucleotide substitution.
26. The vector according to any of paras 20-25, wherein the NF-κB binding motif comprises or consists of SEQ ID NO: 155, or wherein the NF-κB binding motif comprises or consists of SEQ ID NO: 156 or a derivative thereof comprising two or fewer nucleotide substitutions.
27. The vector according to any preceding para, wherein the inflammation-inducible promoter comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 157.
28. The vector according to any preceding para, wherein the vector comprises: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 159; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 160; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 161; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 162.

29. The vector according to any preceding para, wherein the nucleotide sequence encoding a CD3 inhibitor is operably linked to a polyadenylation sequence, optionally wherein the polyadenylation sequence is selected from any of: a bovine growth hormone (bGH) polyadenylation sequence, a SV40 polyadenylation sequence, and a rabbit beta-globin polyadenylation sequence.
30. The vector according to para 29, wherein the polyadenylation sequence comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 163.
31. The vector according to any preceding para, wherein the nucleotide sequence encoding a CD3 inhibitor is operably linked to a woodchuck hepatitis post-transcriptional regulatory element (WPRE).
32. The vector according to para 31, wherein the WPRE comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 164.
33. The vector according to any preceding para, wherein the nucleotide sequence encoding a CD3 inhibitor is operably linked to an intron, optionally wherein the intron is selected from a beta-globin intron or a SV40 intron.
34. The vector according to para 33, wherein the intron comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 165.
35. The vector according to any preceding para, wherein the vector is a viral vector.
36. The vector according to any preceding para, wherein the vector is any of a parvoviral vector, an adenoviral vector, a herpes simplex viral vector, an anelloviral vector, a retroviral vector or a lentiviral vector, preferably wherein the vector is an adeno-associated virus (AAV) vector.
37. The vector according to any preceding para, wherein the vector is an AAV vector particle.
38. The vector according to para 37, wherein the AAV vector particle is pseudotyped to confer ocular tissue tropism.
39. The vector according to para 37 or 38, wherein the AAV vector particle comprises AAV2 capsid proteins or AAV2 capsid variant proteins, optionally wherein the AAV2 capsid variant is selected from any of: AAV2.tYF, AAV2.7m8, R100, AAV2.GL and AAV2.NN.
40. The vector according to any preceding para, wherein the vector comprises one or more inverted terminal repeats (ITRs).

41. The vector according to any preceding para, wherein the vector comprises: (a) a nucleotide sequence having at least 70% identity to SEQ ID NO: 183; (b) a nucleotide sequence having at least 70% identity to SEQ ID NO: 184; (c) a nucleotide sequence having at least 70% identity to SEQ ID NO: 185; or (d) a nucleotide sequence having at least 70% identity to SEQ ID NO: 186.
42. A vector comprising a nucleotide sequence having at least 70% identity to SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, or SEQ ID NO: 186.
43. An isolated cell comprising the vector according to any of paras 1-42.
44. A kit for the production of the vector of any one of paras 1-42.
45. A pharmaceutical composition comprising the vector according to any one of paras 1 to 42 or the isolated cell according to para 43, in combination with a pharmaceutically acceptable carrier, diluent or excipient.
46. A vector according to any one of paras 1 to 42, an isolated cell according to para 43, and/or a pharmaceutical composition according to para 45, for use as a medicament.
47. Use of a vector according to any one of paras 1 to 42, an isolated cell according to para 43, or a pharmaceutical composition according to para 45, for the manufacture of a medicament.
48. A method comprising administering a vector according to any one of paras 1 to 42, an isolated cell according to para 43, or a pharmaceutical composition according to para 45, to a subject in need thereof.
49. A vector for use in preventing or treating an inflammatory eye disease, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.
50. Use of a vector in the manufacture of a medicament for preventing or treating an inflammatory eye disease, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.
51. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a vector to a subject in need thereof, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.
52. A vector according to any one of paras 1 to 42, or a pharmaceutical composition according to para 45, for use in preventing or treating an inflammatory eye disease.

53. Use of a vector according to any one of paras 1 to 42, or a pharmaceutical composition according to para 45, for the manufacture of a medicament for preventing or treating an inflammatory eye disease.
54. A method of preventing or treating an inflammatory eye disease comprising administering
5 a vector according to any one of paras 1 to 42, or a pharmaceutical composition according to para 45, to a subject in need thereof.
55. The vector or pharmaceutical composition for use according to para 49 or 52, the use according to para 50 or 53, or the method according to para 51 or 54, wherein the inflammatory eye disease is uveitis.
- 10 56. The vector or pharmaceutical composition for use according to any of paras 49, 52 or 55, the use according to any of paras 50, 53 or 55, or the method according to any of paras 51 or 54-55, wherein the vector or pharmaceutical composition is administered intraocularly.
57. The vector or pharmaceutical composition for use according to any of paras 49, 52, or 55-56, the use according to any of paras 50, 53 or 55-56, or the method according to any of paras
15 51 or 54-56, wherein the vector or pharmaceutical composition is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection.
58. The vector or pharmaceutical composition for use according to any of paras 49, 52, or 55-57, the use according to any of paras 50, 53 or 55-57, or the method according to any of paras 51 or 54-57, wherein the vector or pharmaceutical composition is administered via intravitreal
20 injection.
59. The vector or pharmaceutical composition for use according to any of paras 49, 52, or 55-58, the use according to any of paras 50, 53 or 55-58, or the method according to any of paras 51 or 54-58, wherein the vector or pharmaceutical composition is administered as a single dose.
- 25 60. The vector or pharmaceutical composition for use according to any of paras 49, 52, or 55-59, the use according to any of paras 50, 53 or 55-59, or the method according to any of paras 51 or 54-59, wherein the vector is administered at a dose of at least about 1E10 vg/mL, at least about 1E11 vg/mL, at least about 1E12 vg/mL, or at least about 5E12 vg/mL.
61. The vector or pharmaceutical composition for use according to any of paras 49, 52, or 55-
30 60, the use according to any of paras 50, 53 or 55-60, or the method according to any of paras 51 or 54-60, wherein the vector is administered at a dose of at least about 1E9 vg/eye, at least

about 1E10 vg/eye, or at least about 1E11 vg/eye, preferably wherein the vector is administered at a dose of about 1E9 vg/eye to about 5E12 vg/eye.

62. The vector or pharmaceutical composition for use according to any of paras 49, 52, or 55-61, the use according to any of paras 50, 53 or 55-61, or the method according to any of paras 51 or 54-61, wherein the vector or pharmaceutical composition is administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.

63. A CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, for use in preventing or treating an inflammatory eye disease, wherein the CD3 inhibitor or polynucleotide is administered intraocularly.

64. Use of a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, in the manufacture of a medicament for preventing or treating an inflammatory eye disease, wherein the CD3 inhibitor or polynucleotide is administered intraocularly.

65. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, to a subject in need thereof, wherein the CD3 inhibitor or polynucleotide is administered intraocularly.

66. The CD3 inhibitor or polynucleotide for use according to para 63, the use according to para 64, or the method according to para 65, wherein the inflammatory eye disease is uveitis.

67. The CD3 inhibitor or polynucleotide for use according to para 63 or 66, the use according to para 64 or 66, or the method according to para 65 or 66, wherein the CD3 inhibitor or polynucleotide is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection.

68. The CD3 inhibitor or polynucleotide for use according to any of paras 63 or 66-67, the use according to any of paras 64 or 66-67, or the method according to any of paras 65-67, wherein the CD3 inhibitor or polynucleotide is administered via intravitreal injection.

69. The CD3 inhibitor or polynucleotide for use according to any of paras 63 or 66-68, the use according to any of paras 64 or 66-68, or the method according to any of paras 65-68, wherein the CD3 inhibitor or polynucleotide is administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.

70. The CD3 inhibitor or polynucleotide for use according to any of paras 63 or 66-69, the use according to any of paras 64 or 66-69, or the method according to any of paras 65-69, wherein the CD3 inhibitor or polynucleotide is administered in the form of a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
- 5 71. The CD3 inhibitor or polynucleotide for use according to any of paras 63 or 66-70, the use according to any of paras 64 or 66-70, or the method according to any of paras 65-70, wherein the CD3 inhibitor or polynucleotide is administered in the form of a pharmaceutical composition comprising the CD3 inhibitor or polynucleotide.

CLAIMS

1. A vector comprising: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.
2. The vector according to any preceding claim, wherein the nucleotide sequence encoding a
5 TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide
sequence encoding a 2A self-cleaving peptide, an enzymatically cleavable peptide motif,
and/or an IRES element.
3. The vector according to any preceding claim, wherein the nucleotide sequence encoding a
10 TNF inhibitor is linked to the nucleotide sequence encoding a CD3 inhibitor by a nucleotide
sequence encoding a 2A self-cleaving peptide.
4. The vector according to any preceding claim, wherein the nucleotide sequence encoding a
TNF inhibitor and the nucleotide sequence encoding a CD3 inhibitor are each operably linked
to the same promoter.
5. The vector according to any preceding claim, wherein the vector comprises a nucleotide
15 sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence
encoding a TNF inhibitor; a 2A self-cleaving peptide, an enzymatically cleavable peptide motif,
and/or an IRES element; and the nucleotide sequence encoding a CD3 inhibitor.
6. The vector according to any of claims 1-5, wherein the vector comprises a nucleotide
20 sequence comprising or consisting of from 5' to 3': a promoter; the nucleotide sequence
encoding a CD3 inhibitor; a 2A self-cleaving peptide, an enzymatically cleavable peptide motif,
and/or an IRES element; and the nucleotide sequence encoding a TNF inhibitor.
7. A kit comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor;
and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
8. A pharmaceutical composition comprising: (a) a vector comprising a nucleotide sequence
25 encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3
inhibitor.
9. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein
the TNF inhibitor is any of adalimumab or a fragment thereof, infliximab or a fragment thereof,
golimumab or a fragment thereof, or certolizumab or a fragment thereof.

10. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the TNF inhibitor is adalimumab or a fragment thereof, or infliximab or a fragment thereof, preferably wherein the TNF inhibitor is adalimumab or a fragment thereof.
11. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein
5 the TNF inhibitor is an antigen binding fragment (Fab) of adalimumab.
12. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the TNF inhibitor is an anti-TNF antibody or fragment thereof comprising one or more CDR regions selected from SEQ ID NOs: 16 to 21 or derivatives thereof comprising one amino acid substitution, preferably wherein the TNF inhibitor is an anti-TNF antibody or fragment thereof
10 comprising CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 16, 17, 18, 19, 20 and 21 respectively or derivatives thereof comprising one amino acid substitution.
13. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the TNF inhibitor is an anti-TNF antibody or fragment thereof comprising a heavy chain
15 comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 22 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 23.
14. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the CD3 inhibitor is any of foralumab or a fragment thereof, teplizumab or a fragment thereof,
20 visilizumab or a fragment thereof, otelixizumab or a fragment thereof, muromonab-CD3 or a fragment thereof, T3/4.A or a fragment thereof, YTH12.5 or a fragment thereof, or HuM291 or a fragment thereof.
15. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the CD3 inhibitor is foralumab or a fragment thereof, teplizumab or a fragment thereof,
25 visilizumab or a fragment thereof, or otelixizumab or a fragment thereof.
16. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the CD3 inhibitor is an antigen binding fragment (Fab) of foralumab, teplizumab, visilizumab, or otelixizumab.
17. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein
30 the CD3 inhibitor is an anti-CD3 antibody or fragment thereof comprising one or more CDR regions selected from: (a) SEQ ID NOs: 70 to 75 or derivatives thereof comprising one amino acid substitution, (b) SEQ ID NOs: 81 to 86 or derivatives thereof comprising one amino acid substitution, (c) SEQ ID NOs: 92 to 97 or derivatives thereof comprising one amino acid

substitution, and (d) SEQ ID NOs: 103 to 108 or derivatives thereof comprising one amino acid substitution, preferably wherein the CD3 inhibitor is an anti-CD3 antibody or fragment thereof comprising (a) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 70, 71, 72, 73, 74 and 75 respectively or derivatives thereof comprising one amino acid substitution; (b) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 81, 82, 83, 84, 85 and 86 respectively or derivatives thereof comprising one amino acid substitution; (c) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 92, 93, 94, 95, 96 and 97 respectively or derivatives thereof comprising one amino acid substitution; or (d) CDR regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 comprising or consisting of SEQ ID NOs: 103, 104, 105, 106, 107 and 108 respectively or derivatives thereof comprising one amino acid substitution.

18. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the CD3 inhibitor is an anti-CD3 antibody or fragment thereof comprising: (a) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 76 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 77; (b) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 87 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 88; (c) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 98 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 99; or (d) a heavy chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 109 and/or a light chain comprising or consisting of a sequence with at least 70% identity to SEQ ID NO: 110.

19. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the nucleotide sequence encoding a TNF inhibitor is operably linked to an inflammation-inducible promoter and/or the nucleotide sequence encoding a CD3 inhibitor is operably linked to an inflammation-inducible promoter.

20. The vector, kit, or pharmaceutical composition according to claim 19, wherein the inflammation-inducible promoter comprises one or more inflammation-inducible transcription factor binding motif selected from: an AP-1 transcription factor binding motif; a NF- κ B transcription factor binding motif; an IRF transcription factor binding motif; a STAT transcription factor binding motif; and a NFAT transcription factor binding motif or any combination thereof.

21. The vector, kit, or pharmaceutical composition according to claim 19 or 20, wherein the inflammation-inducible promoter comprises one or more AP-1 binding motif and/or one or more NF- κ B binding motif.
22. The vector, kit, or pharmaceutical composition according to any of claims 19-21, wherein
5 the inflammation-inducible promoter comprises two or more AP-1 binding motifs and/or two or more NF- κ B binding motifs, three or more AP-1 binding motifs and/or three or more NF- κ B binding motifs, four or more AP-1 binding motifs and/or four or more NF- κ B binding motifs, or five or more AP-1 binding motifs and/or five or more NF- κ B binding motifs
23. The vector, kit, or pharmaceutical composition according to any of claims 19-22, wherein
10 the inflammation-inducible promoter comprises at least one AP-1 binding motif coupled to at least one NF- κ B binding motif.
24. The vector, kit, or pharmaceutical composition according to any of claims 19-23, wherein the inflammation-inducible promoter comprises five AP-1 binding motifs coupled to five NF- κ B binding motifs.
- 15 25. The vector, kit, or pharmaceutical composition according to any of claims 20-24, wherein the AP-1 binding motif comprises or consists of SEQ ID NO: 151, or wherein the AP-1 binding motif comprises or consists of any of SEQ ID NOs: 152-154 or derivatives thereof comprising one nucleotide substitution.
26. The vector, kit, or pharmaceutical composition according to any of claims 20-25, wherein
20 the NF- κ B binding motif comprises or consists of SEQ ID NO: 155, or wherein the NF- κ B binding motif comprises or consists of SEQ ID NO: 156 or a derivative thereof comprising two or fewer nucleotide substitutions.
27. The vector, kit, or pharmaceutical composition according to any of claims 19-26, wherein
25 the inflammation-inducible promoter comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 157.
28. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the vector(s) further comprise a polyadenylation sequence.
29. The vector, kit, or pharmaceutical composition according to claim 28, wherein the polyadenylation sequence is selected from any of: a bovine growth hormone (bGH)
30 polyadenylation sequence, a SV40 polyadenylation sequence, and a rabbit beta-globin polyadenylation sequence.

30. The vector, kit, or pharmaceutical composition according to claim 28 or 29, wherein the polyadenylation sequence comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 163.
31. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein
5 the vector(s) further comprise a woodchuck hepatitis post-transcriptional regulatory element (WPRE).
32. The vector, kit, or pharmaceutical composition according to claim 31, wherein the WPRE comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO: 164.
- 10 33. The vector, kit, or pharmaceutical composition according to any preceding claim, wherein the vector(s) further comprise an intron, optionally wherein the intron is selected from a beta-globin intron or a SV40 intron.
34. The vector, kit, or pharmaceutical composition according to claim 33, wherein the intron comprises or consists of a nucleotide sequence having at least 70% identity to SEQ ID NO:
15 165.
35. The vector, kit, or pharmaceutical composition according to any previous claim, wherein the vector(s) are a viral vector, optionally wherein the vector(s) are any of a parvoviral vector, an adenoviral vector, a herpes simplex viral vector, an anelloviral vector, a retroviral vector or a lentiviral vector.
- 20 36. The vector, kit, or pharmaceutical composition according to any previous claim, wherein the vector(s) are an adeno-associated virus (AAV) vector.
37. The vector, kit, or pharmaceutical composition according to any previous claim, wherein the vector(s) are an AAV vector particle.
38. The vector, kit, or pharmaceutical composition according to claim 37, wherein the AAV
25 vector particle is pseudotyped to confer ocular tissue tropism.
39. The vector, kit, or pharmaceutical composition according to claim 37 or 38, wherein the AAV vector particle comprises AAV2 capsid proteins or AAV2 capsid variant proteins, optionally wherein the AAV2 capsid variant is selected from any of: AAV2.tYF, AAV2.7m8, R100, AAV2.GL and AAV2.NN.
- 30 40. The vector, kit, or pharmaceutical composition according to any previous claim, wherein the vector(s) comprise one or more inverted terminal repeats (ITRs).

41. An isolated cell comprising:
- (i) the vector according to any of claims 1-6 or 9-40; and/or
 - (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
- 5 42. A kit for the production of:
- (i) the vector of any one of claims 1-6 or 9-40; and/or
 - (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
- 10 43. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent or excipient in combination with:
- (i) the vector according to any one of claims 1-6 or 9-40;
 - (ii) (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor; and/or
 - (iii) the isolated cell according to claim 41.
- 15 44. A vector according to any one of claims 1-6 or 9-40, an isolated cell according to claim 41, and/or a pharmaceutical composition according to claim 43, for use as a medicament.
45. Use of a vector according to any one of claims 1-6 or 9-40, an isolated cell according to claim 41, or a pharmaceutical composition according to claim 43, for the manufacture of a medicament.
- 20 46. A method comprising administering a vector according to any one of claims 1-6 or 9-40, an isolated cell according to claim 41, or a pharmaceutical composition according to claim 43, to a subject in need thereof.
- 25 47. A vector for use in preventing or treating an inflammatory eye disease, wherein the vector comprises: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.
48. Use of a vector in the manufacture of a medicament for preventing or treating an inflammatory eye disease, wherein the vector comprises: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.

49. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a vector to a subject in need thereof, wherein the vector comprises: (a) a nucleotide sequence encoding a TNF inhibitor; and (b) a nucleotide sequence encoding a CD3 inhibitor.
- 5 50. A product comprising: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor, as a combined preparation for simultaneous, separate or sequential use in preventing or treating an inflammatory eye disease.
- 10 51. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering to a subject in need thereof: (a) a vector comprising a nucleotide sequence encoding a TNF inhibitor; and (b) a vector comprising a nucleotide sequence encoding a CD3 inhibitor.
52. A vector according to any one of claims 1-6 or 9-40, or a pharmaceutical composition according to claim 43, for use in preventing or treating an inflammatory eye disease.
- 15 53. Use of a vector according to any one of claims 1-6 or 9-40, or a pharmaceutical composition according to claim 43, for the manufacture of a medicament for preventing or treating an inflammatory eye disease.
54. A method of preventing or treating an inflammatory eye disease comprising administering a vector according to any one of claims 1-6 or 7-40, or a pharmaceutical composition
20 according to claim 43, to a subject in need thereof.
55. The vector or pharmaceutical composition for use according to claim 47 or 52, the use according to claim 48 or 53, the method according to claim 49, 51 or 54, or the product for use according to claim 50, wherein the inflammatory eye disease is uveitis.
- 25 56. The vector or pharmaceutical composition for use according to any of claims 47, 52 or 55, the use according to any of claims 48, 53 or 55, the method according to any of claims 49, 51 or 54-55, or the product for use according to claim 50 or 55, wherein the vector(s) or pharmaceutical composition are administered intraocularly.
- 30 57. The vector or pharmaceutical composition for use according to any of claims 47, 52, or 55-56, the use according to any of claims 48, 53 or 55-56, the method according to any of claims 49, 51 or 54-56, or the product for use according to any of claims 50 or 55-56, wherein the vector(s) or pharmaceutical composition are administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection.

58. The vector or pharmaceutical composition for use according to any of claims 47, 52, or 55-57, the use according to any of claims 48, 53 or 55-57, the method according to any of claims 49, 51 or 54-57, or the product for use according to any of claims 50 or 55-57, wherein the vector(s) or pharmaceutical composition are administered via intravitreal injection.
- 5 59. The vector or pharmaceutical composition for use according to any of claims 47, 52, or 55-58, the use according to any of claims 48, 53 or 55-58, the method according to any of claims 49, 51 or 54-58, or the product for use according to any of claims 50 or 55-58, wherein the vector(s) or pharmaceutical composition are administered as a single dose.
60. The vector or pharmaceutical composition for use according to any of claims 47, 52, or
10 55-59, the use according to any of claims 48, 53 or 55-59, the method according to any of claims 49, 51 or 54-59, or the product for use according to any of claims 50 or 55-59, wherein the vector(s) are administered at a dose of at least about 1E10 vg/mL, at least about 1E11 vg/mL, at least about 1E12 vg/mL, or at least about 5E12 vg/mL.
61. The vector or pharmaceutical composition for use according to any of claims 47, 52, or
15 55-60, the use according to any of claims 48, 53 or 55-60, the method according to any of claims 49, 51 or 54-60, or the product for use according to any of claims 50 or 55-60, wherein the vector(s) are administered at a dose of at least about 1E9 vg/eye, at least about 1E10 vg/eye, or at least about 1E11 vg/eye, preferably wherein the vector(s) are administered at a dose of about 1E9 vg/eye to about 5E12 vg/eye.
- 20 62. The vector or pharmaceutical composition for use according to any of claims 47, 52, or 55-61, the use according to any of claims 48, 53 or 55-61, the method according to any of claims 49, 51 or 54-61, or the product for use according to any of claims 50 or 55-61, wherein the vector(s) or pharmaceutical composition are administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.
- 25 63. A kit comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.
64. A pharmaceutical composition comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a
30 polynucleotide comprising a nucleotide sequence encoding the same.
65. A product comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a

nucleotide sequence encoding the same, as a combined preparation for simultaneous, separate or sequential use in therapy.

66. A product comprising: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, as a combined preparation for simultaneous, separate or sequential use in preventing or treating an inflammatory eye disease.

67. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering to a subject in need thereof: (a) a TNF inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same; and (b) a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same.

68. The product for use according to claim 66 or the method according to claim 67, wherein the inflammatory eye disease is uveitis.

69. The product for use according to claim 66 or 68 or the method according to claim 67 or 68, wherein (a) the TNF inhibitor or polynucleotide is administered intraocularly; and/or (b) the CD3 inhibitor or polynucleotide is administered intraocularly.

70. The product for use according to any of claims 66 or 68-69 or the method according to any of claims 67-69, wherein (a) the TNF inhibitor or polynucleotide is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection; and/or (b) the CD3 inhibitor or polynucleotide is administered via intravitreal, subretinal, subconjunctival, sub-Tenon's or suprachoroidal injection.

71. The product for use according to any of claims 66 or 68-70 or the method according to any of claims 67-70, wherein (a) the TNF inhibitor or polynucleotide is administered via intravitreal injection; and/or (b) the CD3 inhibitor or polynucleotide is administered via intravitreal injection.

72. The product for use according to any of claims 66 or 68-71 or the method according to any of claims 67-71, wherein (a) the TNF inhibitor or polynucleotide is administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis; and/or (b) the CD3 inhibitor or polynucleotide is administered in response to relapse of an inflammatory eye disease, preferably wherein the inflammatory eye disease is uveitis.

73. The product for use according to any of claims 66 or 68-72 or the method according to any of claims 67-72, wherein (a) the TNF inhibitor or polynucleotide is administered in the form of a vector comprising a nucleotide sequence encoding a TNF inhibitor; and/or (b) the CD3

inhibitor or polynucleotide is administered in the form of a vector comprising a nucleotide sequence encoding a CD3 inhibitor.

74. The product for use according to any of claims 66 or 68-73 or the method according to any of claims 67-73, wherein (a) the TNF inhibitor or polynucleotide is administered in the form of a pharmaceutical composition comprising the TNF inhibitor or polynucleotide; and/or (b) the CD3 inhibitor or polynucleotide is administered in the form of a pharmaceutical composition comprising the CD3 inhibitor or polynucleotide.

75. A vector comprising a nucleotide sequence encoding a CD3 inhibitor, wherein the nucleotide sequence encoding the CD3 inhibitor is operably linked to an inflammation-inducible promoter.

76. A vector comprising a nucleotide sequence having at least 70% identity to SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, or SEQ ID NO: 186.

77. An isolated cell comprising the vector according to claim 75 or 76.

78. A kit for the production of the vector of claim 75 or 76.

79. A pharmaceutical composition comprising the vector according to claim 75 or 76 or the isolated cell according to claim 77, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

80. A vector according to claim 75 or 76, an isolated cell according to claim 77, and/or a pharmaceutical composition according to claim 79, for use as a medicament.

81. Use of a vector according to claim 75 or 76, an isolated cell according to claim 77, or a pharmaceutical composition according to claim 79, for the manufacture of a medicament.

82. A method comprising administering a vector according to claim 75 or 76, an isolated cell according to claim 77, or a pharmaceutical composition according to claim 79, to a subject in need thereof.

83. A vector for use in preventing or treating an inflammatory eye disease, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.

84. Use of a vector in the manufacture of a medicament for preventing or treating an inflammatory eye disease, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.

85. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a vector to a subject in need thereof, wherein the vector comprises a nucleotide sequence encoding a CD3 inhibitor.
86. A vector according to claim 75 or 76, or a pharmaceutical composition according to claim 5 79, for use in preventing or treating an inflammatory eye disease.
87. Use of a vector according to claim 75 or 76, or a pharmaceutical composition according to claim 79, for the manufacture of a medicament for preventing or treating an inflammatory eye disease.
88. A method of preventing or treating an inflammatory eye disease comprising administering 10 a vector according to claim 75 or 76, or a pharmaceutical composition according to claim 79, to a subject in need thereof.
89. A CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, for use in preventing or treating an inflammatory eye disease, wherein the CD3 inhibitor or polynucleotide is administered intraocularly.
- 15 90. Use of a CD3 inhibitor, or a polynucleotide comprising a nucleotide sequence encoding the same, in the manufacture of a medicament for preventing or treating an inflammatory eye disease, wherein the CD3 inhibitor or polynucleotide is administered intraocularly.
91. A method for preventing or treating an inflammatory eye disease, wherein the method comprises administering a CD3 inhibitor, or a polynucleotide comprising a nucleotide 20 sequence encoding the same, to a subject in need thereof, wherein the CD3 inhibitor or polynucleotide is administered intraocularly.