CLAIMS

What we claim is:

1. A guidance and navigation control (GNC) protein, comprising,

a binding domain for a T cell activating receptor, wherein the T cell activating receptor comprises CD3 and the binding domain is configured to activate a T cell through the binding with CD3,

a binding domain for a tumor associated antigen (TAA), wherein the tumor associated antigen comprises EGFRvIII, CD19 or ROR1,

a binding domain for an immune checkpoint receptor, wherein the immune checkpoint receptor comprises PD-L1 or PD-1 and the binding domain is configured to block PD-L1 and PD-1 binding interaction,

a binding domain for a T cell co-stimulating receptor, wherein the T cell co-stimulating receptor comprises 4-1BB and the binding domain is configured to stimulate a T cell through the binding with 4-1BB, and

an IgG Fc domain,

wherein the binding domain for the tumor associated antigen is not adjacent to the binding domain for the T cell co-stimulating receptor,

wherein the IgG Fc domain intermediates the binding domains for the T cell activating receptor and tumor associated antigen, and the binding domains for the immune checkpoint receptor and T cell co-stimulating receptor.

2. The GNC protein of claim 1, wherein the binding domain for the T cell activating receptor is adjacent to the binding domain for the tumor associated antigen (TAA).

3. The GNC protein of claim 1 or 2, wherein the binding domain for the tumor associated antigen has a binding affinity to ROR1 IgD domain, ROR1 Frizzled domain or ROR1 Kringle domain.

4. The GNC protein of any one of claims 1 to 3, wherein the tumor associated antigen is a receptor on a lung cancer cell, a liver cancer cell, a breast cancer cell, a colorectal cancer cell, an anal cancer cell, a pancreatic cancer cell, a gallbladder cancer cell, a bile duct cancer cell, a head and neck cancer cell, a nasopharyngeal cancer cell, a skin cancer cell, a melanoma cell, an ovarian cancer cell, a prostate cancer cell, a urethral cancer cell, a non-small lung cell cancer cell, a small cell lung cancer cell, a brain tumour cell, a glioma cell, a neuroblastoma cell, an esophageal cancer cell, a gastric cancer cell, a kidney cancer cell, a bladder cancer cell, a cervical cancer cell, an endometrial cancer cell, a thyroid cancer cell, an eye cancer cell, a sarcoma cell, a bone cancer cell, a leukemia cell, a myeloma cell, or a lymphoma cell, or a combination thereof.

5. The GNC protein of any one of claims 1 to 4, wherein the protein is a tetra-specific antibody.

6. The GNC protein of claim 5, wherein the binding domain for the T cell activating receptor comprising CD3 is linked to the binding domain for the tumor associated (TAA) antigen through a peptide linker to form a CD3-TAA pair, wherein the peptide linker has a length not exceeding 100 amino acids.

7. The GNC protein of claim 6, wherein the peptide linker has a length not exceeding 20 amino acids.

8. The GNC protein of claim 6, wherein the peptide linker has a length from about 2 amino acids to about 10 amino acids.

9. The GNC protein of any one of claims 6 to 8, wherein the IgG Fc domain intermediates the CD3-TAA pair and the binding domain for the immune checkpoint receptor.

10. The GNC protein of any one of claims 1 to 9, wherein:

i) a) the binding domain for the T cell co-stimulating receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 18 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 20 according to Kabat numbering,

b) the binding domain for the immune checkpoint receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 10 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 12 according to Kabat numbering,

c) the binding domain for the tumor associated antigen comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 30 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 32 according to Kabat numbering, and

d) the binding domain for the T cell activating receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 2 according to Kabat numbering and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 4 according to Kabat numbering;

or

ii) a) the binding domain for the T cell activating receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 2 according to Kabat numbering, and a light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 4 according to Kabat numbering,

b) the binding domain for the tumor associated antigen comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 64 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 66 according to Kabat numbering,

c) the binding domain for the immune checkpoint receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 60 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 62 according to Kabat numbering, and

d) the binding domain for the T cell co-stimulating receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 18 according to Kabat numbering and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 20 according to Kabat numbering;

or

iii) a) the binding domain for the T cell activating receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 2 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 4 according to Kabat numbering,

b) the binding domain for the tumor associated antigen comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 68 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 70 according to Kabat numbering,

c) the binding domain for the immune checkpoint receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 60 according to Kabat numbering, and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 62 according to Kabat numbering, and

d) the binding domain for the T cell co-stimulating receptor comprises heavy chain complementarity determining regions 1-3 (HCDR1-3) as set forth in HCDR1-3 of SEQ ID NO. 14 according to Kabat numbering and light chain complementarity determining regions (LCDR1-3) as set forth in LCDR1-3 of SEQ ID NO. 16 according to Kabat numbering.

11. The GNC protein of claim 10, comprising:

i) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 50 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 52;

ii) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 80 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 82;

iii) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 84 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 86;

iv) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 88 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 90;

v) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 92 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 94;

vi) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 96 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 98;

vii) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 100 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 102;

viii) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 104 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 106; or

ix) a heavy chain comprising an amino acid sequence having at least 98% sequence identity to 108 and a light chain comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO. 110.

12. The GNC protein of any one of claims 6 to 11 having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal,

a binding domain for CD3,

a binding domain for EGFRvIII,

an IgG Fc domain,

a binding domain for PD-L1, and

the binding domain for 4-1BB.

13. The GNC protein of claim 12, comprising a heavy chain amino acid sequence as set forth in SEQ ID NO. 80 and a light chain amino acid sequence as set forth in SEQ ID NO. 82.

14. The GNC protein of any one of claims 6 to 11 having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal,

a binding domain for 4-1BB,

a binding domain for PD-L1,

an IgG Fc domain,

a binding domain for ROR1, and

a binding domain for CD3.

15. The GNC protein of claim 14, comprising a heavy chain amino acid sequence as set forth in SEQ ID NO. 88 and a light chain amino acid sequence as set forth in SEQ ID NO. 90.

16. The GNC protein of any one of claims 6 to 11 having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal,

a binding domain for CD3,

a binding domain for CD19,

an IgG Fc domain,

a binding domain for PD-L1, and

a binding domain for 4-1BB.

17. The GNC protein of claim 16, comprising a heavy chain amino acid sequence as set forth in SEQ ID NO. 104 and a light chain amino acid sequence as set forth in SEQ ID NO. 106.

18. A nucleic acid, encoding the GNC protein of any one of claims 1 to 17.

19. The nucleic acid of claim 18 having a nucleotide sequence as set forth in:

i) SEQ ID NO. 49 and SEQ ID NO. 51;

ii) SEQ ID NO. 79 and SEQ ID NO. 81;

iii) SEQ ID NO. 83 and SEQ ID NO. 85;

iv) SEQ ID NO. 87 and SEQ ID NO. 89;

v) SEQ ID NO. 91 and SEQ ID NO. 93;

vi) SEQ ID NO. 95 and SEQ ID NO. 97;

vii) SEQ ID NO. 99 and SEQ ID NO. 101;

viii) SEQ ID NO. 103 and SEQ ID NO. 105; or

ix) SEQ ID NO. 107 and SEQ ID NO. 109.

20. An isolated cytotoxic cell, comprising:

a T cell having a T cell activating receptor and a T cell co-stimulating receptor, and

the GNC protein of any one of claims 1 to 17 bound to the T cell through interaction with the T cell activating receptor, the T cell co-stimulating receptor, or a combination thereof.

21. An isolated cancer cell, comprising:

a cancer cell having a tumor associated antigen, and

the GNC protein of any one of claims 1 to 17 bound to the cancer cell through the interaction with the tumor associated antigen.

22. A biological complex, comprising,

an isolated T cell having a T cell activating receptor and a T cell co-stimulating receptor,

an isolated cancer cell having a tumor associated antigen, and

the GNC protein of any one of claims 1 to 17, wherein the GNC protein is bound to the T cell through the interaction with the T cell activating receptor, the T cell co-stimulating receptor, or a combination thereof, and wherein the GNC protein is bound to the cancer cell through the interaction with the tumor associated antigen.

23. A pharmaceutical composition, comprising

the GNC protein of any one of claims 1 to 17, or the cytotoxic cell of claim 20, or a combination thereof, and

a pharmaceutically acceptable carrier.

24. Use of a GNC protein of any one of claims 1 to 17, and/or a cytotoxic cell of claim 20 in the manufacture of a medicament for treating a subject having a cancer, wherein the GNC protein is capable of binding to the cancer cell through the interaction with the tumor associated antigen and/or PD-L1.