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(54) **BIFUNCTIONAL MOLECULE FORMED BY FUSION OF PD1 ANTIBODY AND INTERLEUKIN 2**

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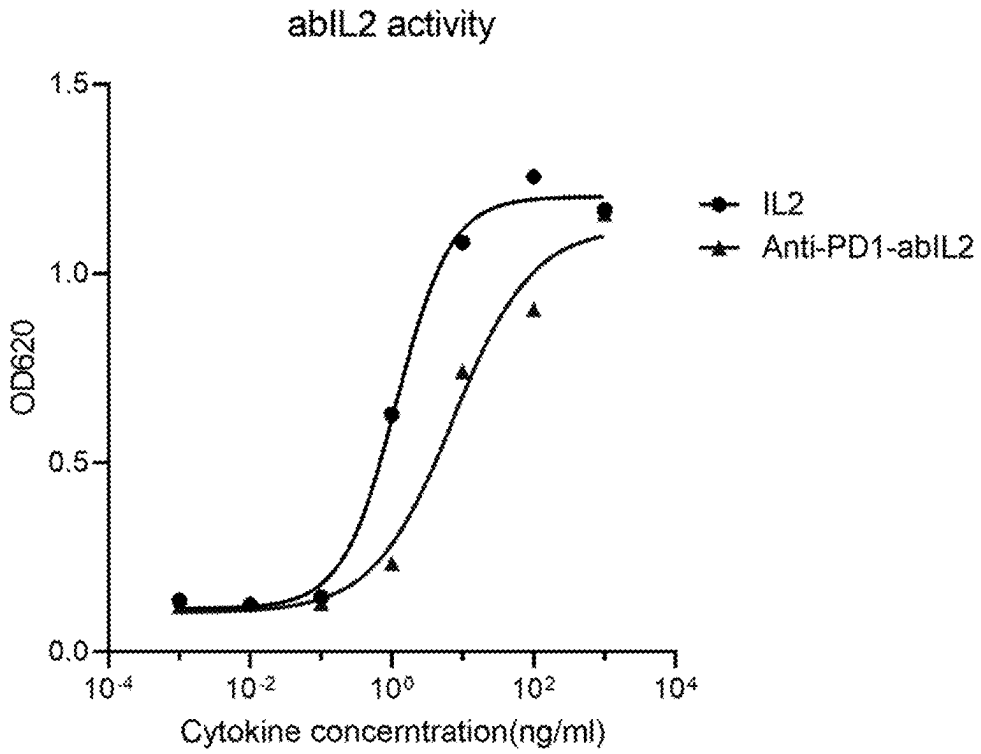
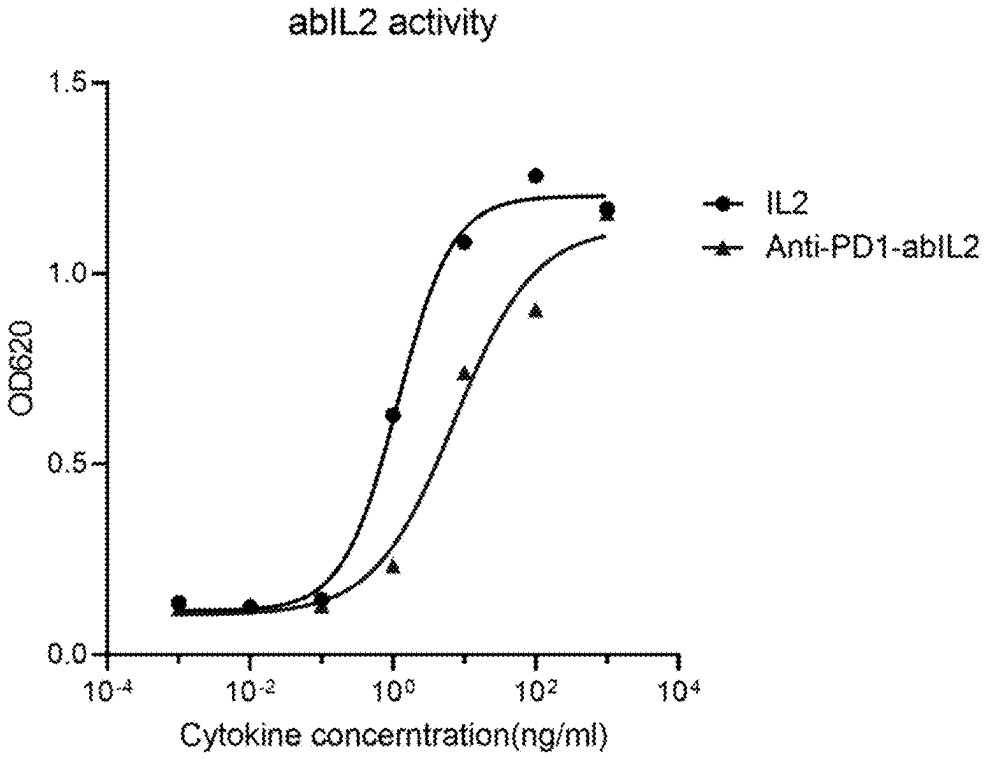
(57) **ABSTRACT**

The present invention relates to a bifunctional molecule formed by fusion of a PD1 antibody and interleukin 2, comprising: a heterodimer composed of a first monomer and a second monomer as follows: (1) a first monomer, formed by linking interleukin 2 (IL2) to an immunoglobulin Fc single chain; and (2) a second monomer, formed by linking an Fab/ScFv of an anti-T cell surface molecule antibody to an Fc single chain. The bifunctional molecule may also be a homodimer comprising a monomer comprising: (1) an interleukin 2 functional block; and (2) a monomer of an antibody or a monomer formed by linking an Fab/ScFv of an antibody to an Fc single chain, the antibody being an anti-T cell surface molecule antibody. The present invention further relates to use of the bifunctional molecule in the manufacture of anti-tumor medicament.

Specification includes a Sequence Listing.



	IL2	Anti-PD1-abIL2
EC50	1.166	7.264



	IL2	Anti-PD1-ablL2
EC50	1.166	7.264

FIG. 1

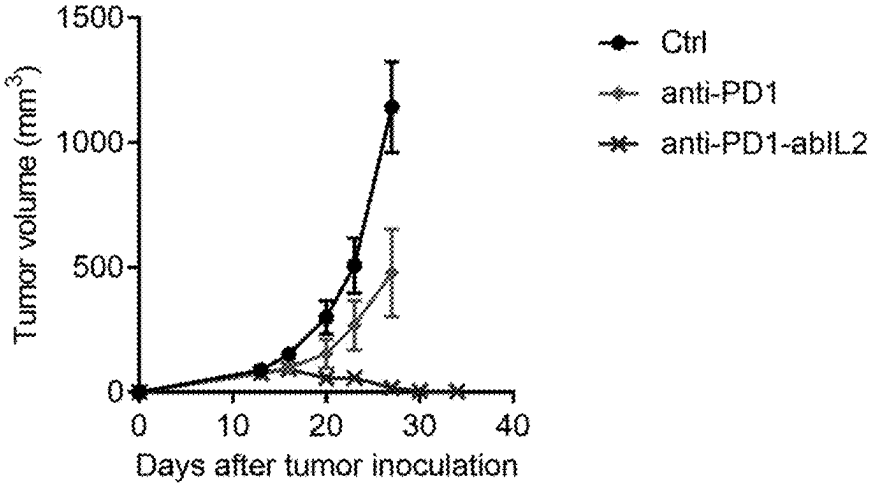


FIG. 2

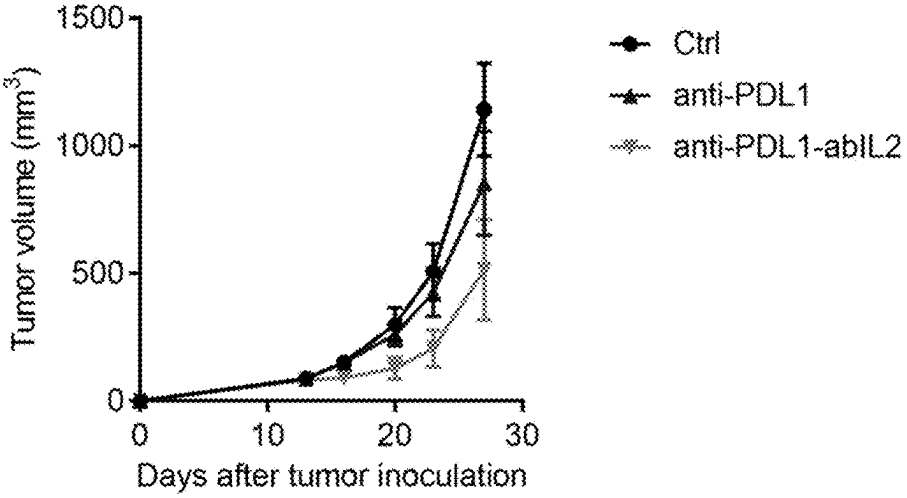

1500
1000
500
0
Tumor volume (mm3)
0
10
20
30
Days after tumor inoculation
Ctrl
anti-PDL1
anti-PDL1-ablL2

FIG. 3

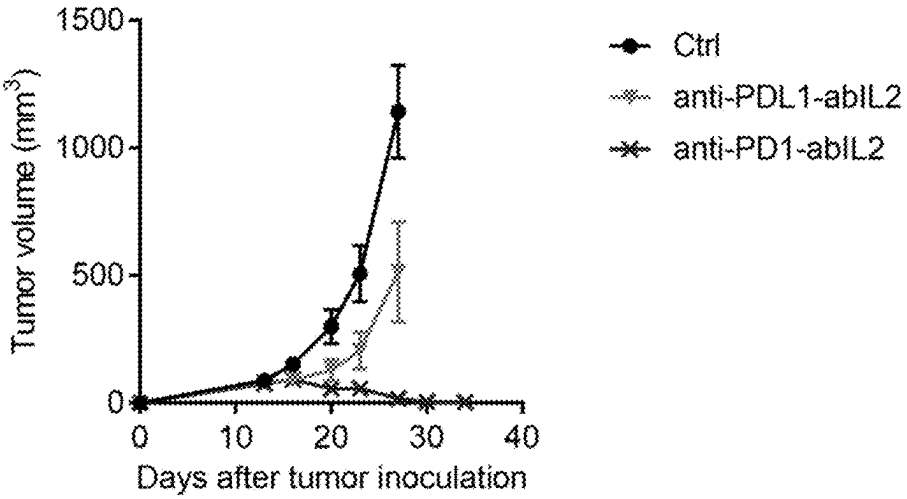

1500
1000
500
0
Tumor volume (mm3)
0
10
20
30
40
Days after tumor inoculation
Ctrl
anti-PDL1-ablL2
anti-PD1-ablL2

FIG. 4

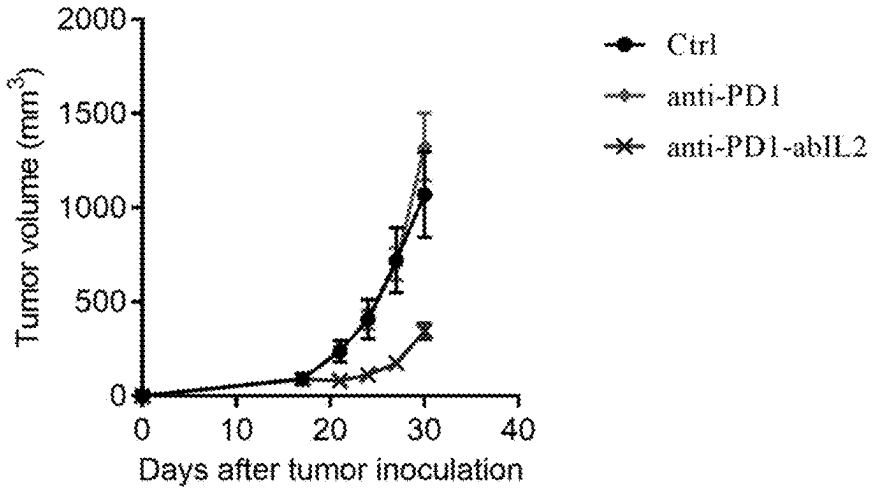

2000
1500
1000
500
0
Tumor volume (mm3)
0
10
20
30
40
Days after tumor inoculation
Ctrl
anti-PD1
anti-PD1-abIL2

FIG. 5

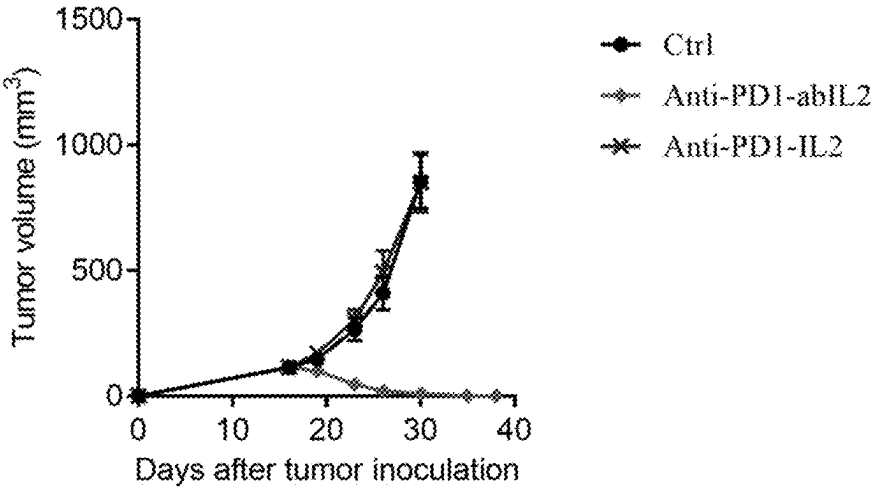

1500
1000
500
0
Tumor volume (mm3)
0
10
20
30
40
Days after tumor inoculation
Ctrl
Anti-PD1-abIL2
Anti-PD1-IL2

FIG. 6

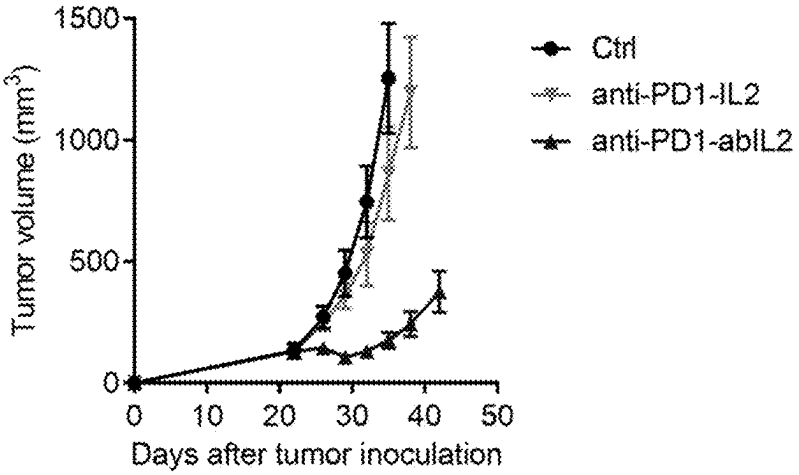

1500
1000
500
0
Tumor volume (mm3)
0 10 20 30 40 50
Days after tumor inoculation
Ctrl
anti-PD1-IL2
anti-PD1-ablL2

FIG. 7

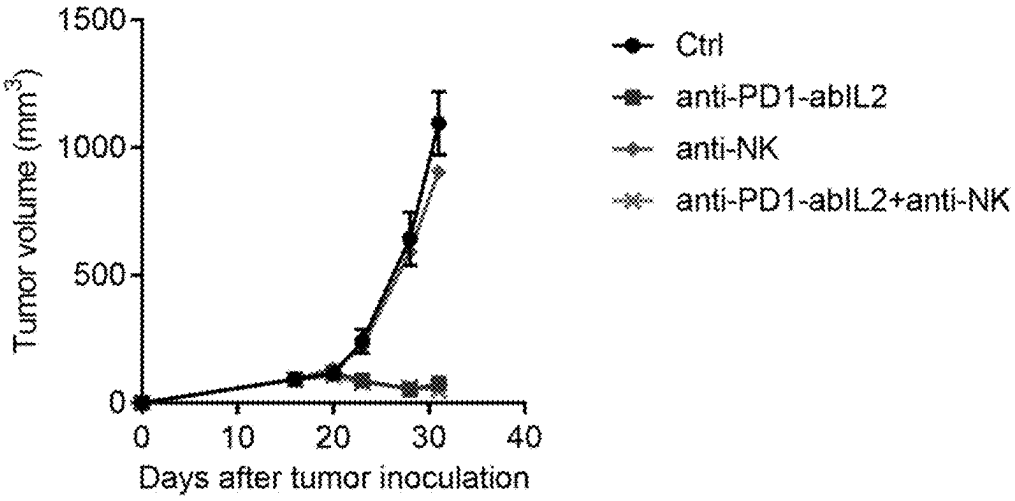

1500
1000
500
0
Tumor volume (mm3)
0 10 20 30 40
Days after tumor inoculation
Ctrl
anti-PD1-ablL2
anti-NK
anti-PD1-ablL2+anti-NK

FIG. 8

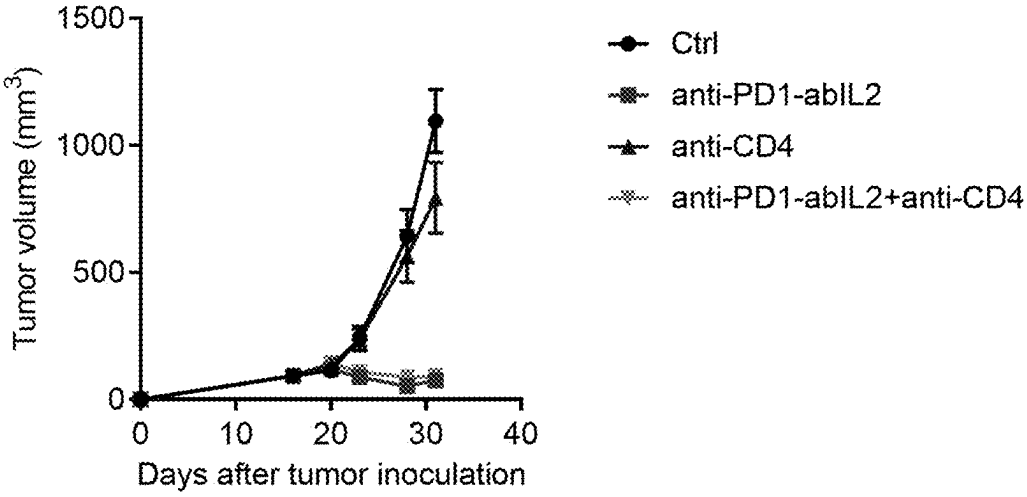

1500
1000
500
0
Tumor volume (mm3)
0
10
20
30
40
Days after tumor inoculation
Ctrl
anti-PD1-ablL2
anti-CD4
anti-PD1-ablL2+anti-CD4

FIG. 9

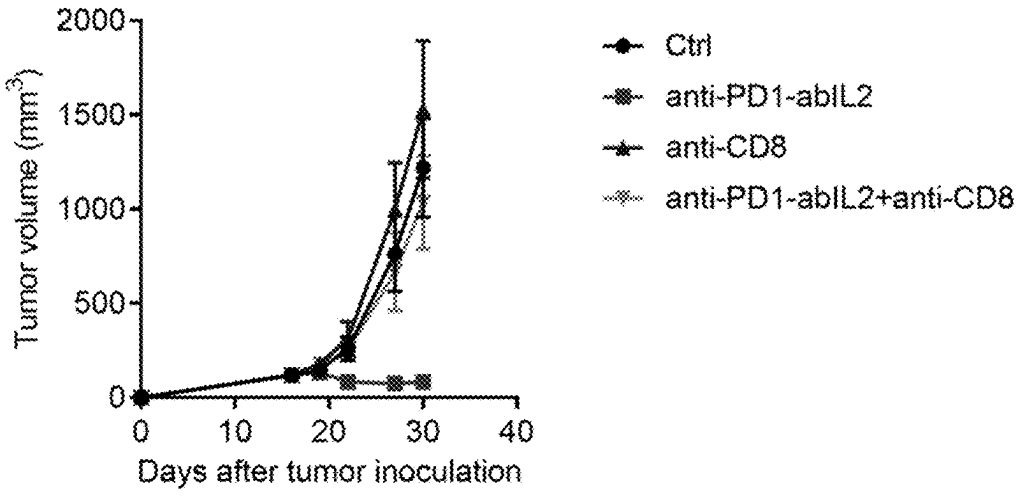

2000
1500
1000
500
0
Tumor volume (mm3)
0
10
20
30
40
Days after tumor inoculation
Ctrl
anti-PD1-ablL2
anti-CD8
anti-PD1-ablL2+anti-CD8

FIG. 10

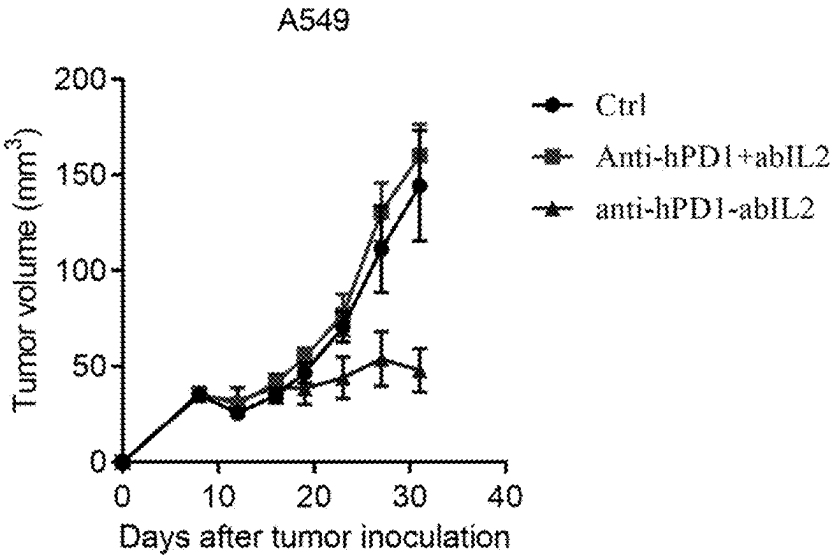

A549
Tumor volume (mm3)
200
150
100
50
0
0
10
20
30
40
Days after tumor inoculation
Ctrl
Anti-hPD1+abIL2
anti-hPD1-abIL2

FIG. 11

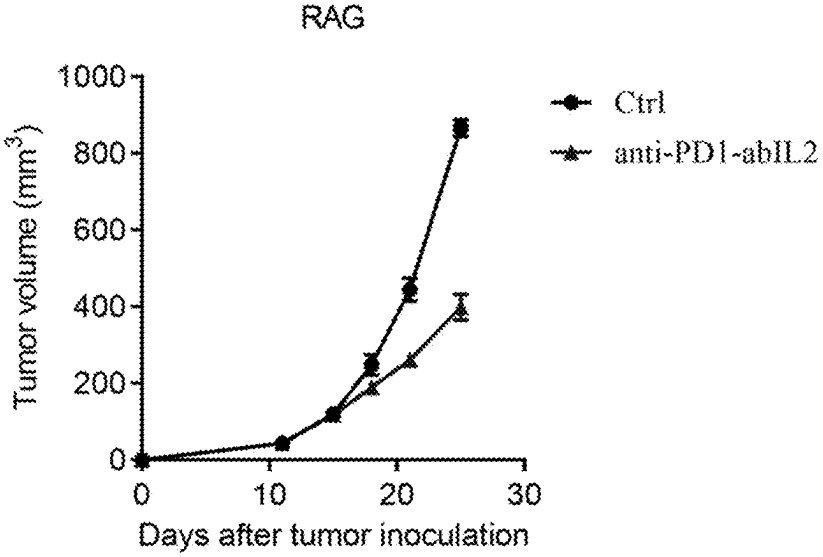

RAG
Tumor volume (mm3)
1000
800
600
400
200
0
0
10
20
30
Days after tumor inoculation
Ctrl
anti-PD1-abIL2

FIG. 12

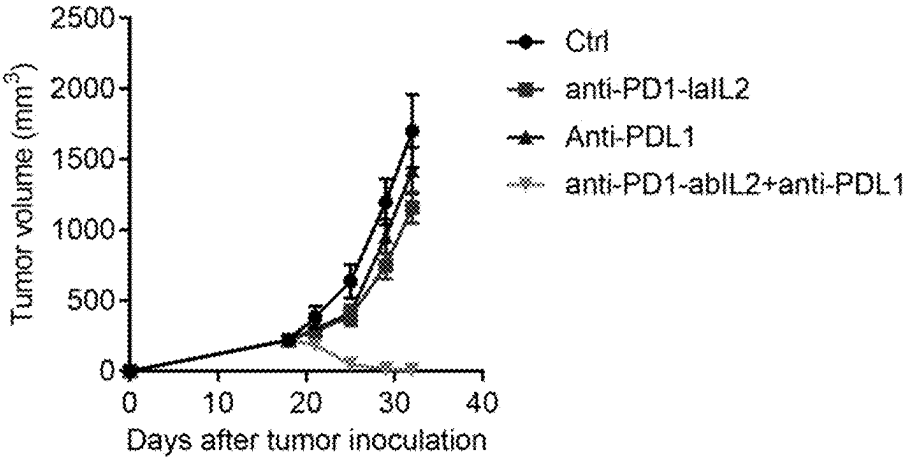

2500
2000
1500
1000
500
0
Tumor volume (mm3)
0
10
20
30
40
Days after tumor inoculation
Ctrl
anti-PD1-laIL2
Anti-PDL1
anti-PD1-abIL2+anti-PDL1

FIG. 13

BIFUNCTIONAL MOLECULE FORMED BY FUSION OF PD1 ANTIBODY AND INTERLEUKIN 2

FIELD OF THE INVENTION

[0001] The invention belongs to the field of biomedical technology, and specifically relates to a bifunctional molecule formed by fusion of PD1 antibody and interleukin 2.

BACKGROUND OF THE INVENTION

[0002] IL-2 cytokine is a potent growth factor of T cells. It exerts its activity by binding with the IL-2 receptor (IL-2R) on the surface of T and NK cells, leading to its own phosphorylation through a JAK/STAT5-dependent pathway, and finally triggering the activation and proliferation of the corresponding cells (1). The exact mechanism by which IL-2 exerts its durable complete response (CR) remains controversial (2). The stimulating effect of IL-2 has been validated in multiple pathways required for the successful generation of adaptive and CTL-mediated anti-tumor responses (3). Following the recognition of antigens in MHC by T cell receptors (TCRs) and the binding of the co-stimulatory molecule CD28 to B7, IL-2 is considered as a third essential signal required for T cell clonal expansion and effector function (1, 3). Similarly, the function of CD8+ CTL is also heavily dependent on IL-2, as shown by their reduced effect or cytotoxic function in IL-2- or IL-2R-deficient mice (4-6). IL-2 increases the transport of CTL to extralymphatic sites of infection or tumors (1, 7). IL-2 is produced by Th1 cells in response to the activation of dendritic cells (DCs), which leads to the activation and proliferation of CD8 (3). A unique mechanism of IL-2 anti-tumor activity may be mediated by activating natural killer (NK) cells (8).

[0003] The efficacy of IL-2 in inducing durable complete response (CR) and partial response (PR) in patients with clear cell renal cell carcinoma (RCC) (9-11) was clinically validated in multiple phase II and phase III trials. In contrast, molecular targeted therapy failed to induce CR or cure cancer. Response rate (RR) (600,000-800,000 IU/kg q8h×14 as tolerated) of IL-2 therapy reported in multiple phase III trials ranged from 20% to 23.2%, while CR ranged from 7% to 9% (9-11). Among patients who achieved CR, the efficacy was durable in most patients, with a median survival of more than 10 years (1, 15, 16). Alternative regimens and reduced doses of IL-2 were further tested, but did not show any improvement in efficacy (10-12).

[0004] Tumor microenvironment (TME) usually limits the efficacy of immunotherapy by increasing the production of regulatory T cells (Tregs) and/or decreasing T cell growth factors or their signaling. A major challenge is to provide sufficient cytokines to reactivate Cytotoxic T cells (CTLs) or inhibit Tregs. IL-2 is a "T cell growth factor", a pleiotropic cytokine that is produced upon antigen activation and plays a key role in immune response. IL-2 is a potent inducer of cytotoxic T cells and NK cells. Therefore, it is a sought-after treatment for various cancers. However, there are two main obstacles to the use of IL-2 in anti-cancer immunotherapy. Some T cells, such as Tregs, express heterotrimeric high-affinity receptors composed of CD25 (IL-2Rα), CD122 (IL-2Rβ) and CD132 (a common cytokine receptor γ chain) subunits. In contrast, immature CD8 T cells, CD4/CD8 memory T cells and NK cells express dimeric receptors (lacking the CD25 subunit) with low affinity (13, 14). When immature CD8 T cells are activated, they up-regulate the expression of CD25 (15). Therefore, Tregs can better compete with effector T cells for the use of IL-2. At present, IL-2 immunotherapy requires high-dose administration and multiple injections. In addition to the preferential expansion of Tregs, high dose of IL-2 may lead to vascular leakage syndrome, which may result in increased vascular permeability, hypotension, pulmonary edema, hepatocyte damage and renal failure (16-18).

[0005] Two fundamentally important strategies can be used to improve the use of IL-2 in immunotherapy: 1) how to remain active in tumor tissues while limiting systemic side effects; 2) how to preferentially activate effector T cells while limiting stimulation of Tregs. For the first issue, many research groups hope to reduce the toxicity of IL-2 by using antibody-based IL-2 delivery (19-24). For the second issue, some researchers have constructed IL-2 mutants which preferentially reduce their binding to CD25, resulting in better expansion of non-Treg population (25-27). Christopher Garcia's team constructed the IL-2 superkine (also called super-2) to eliminate the functional requirement of IL-2 for CD25 expression and increase the binding affinity for IL-2Rβ. Compared to IL-2, the IL-2 superkine induced super expansion of cytotoxic T cells, leading to improved antitumor responses in vivo, and elicited proportionally less expansion of T regulatory cells thereby reducing systemic side effects (26).

[0006] Whether a targeted IL-2 approach can improve the efficacy has been controversial. A recent study showed that F8-IL-2, an immune cytokine composed of F8 antibody fused to human IL-2, had a strong and improved inhibition of lymphoma progression compared to targeted IL-2 (21). Another recent study suggested that antigen specificity may not be important to the efficacy and biodistribution of immune cytokines (28, 29). They found that immunocytokine antigen specificity and Fcγ receptor interactions did not seem necessary for therapeutic efficacy or biodistribution patterns because immunocytokines with irrelevant specificity and/or inactive mutant Fc domains behaved similarly to tumor-specific IL-2. They speculated that the biodistribution of IL-2 is mainly related to innate immune cells expressing IL-2R. We believe that the difference is related to the tumor model, the targeting ability of antibodies and the affinity between IL-2 and IL-2 receptors. Therefore, it is necessary to evaluate the targeting effect.

[0007] Here, we designed a bifunctional molecule (IL-2 linked to an antibody against the T cell surface antigen PD1) to target tumor infiltrating lymphocytes (TILs), because TILs express more T cell surface antigen PD1 than other cells. To reduce the binding of IL-2 to Tregs, we chose an IL-2 mutant (abIL2) with greatly reduced binding to IL-2a and IL-2B. We linked abIL2 to antibody against the T cell surface antigen PD1 (anti-PD1-abIL2) to increase its affinity for CD8+ T cells in tumors. Anti-PD1-abIL2 showed improved intratumoral T cell binding and potent anti-tumor effect. The PDL1 treatment resistance can also be overcome by using such bifunctional molecules.

[0008] Therefore, the anti-PD1-abIL2 bifunctional molecule is a novel and promising tumor treatment in clinic.

SUMMARY OF THE INVENTION

[0009] The present invention firstly relates to a bifunctional molecule, which is a heterodimer, wherein the heterodimer comprises: (1) a first monomer of the heterodimer,

formed by linking interleukin 2 (IL2) to an immunoglobulin Fc single chain; (2) a second monomer of the heterodimer, formed by linking a Fab or ScFv of an anti-T cell surface molecule antibody to an immunoglobulin Fc single chain; the first monomer and the second monomer are linked through dimerization of the Fc single chain to form the heterodimer; the T cell surface molecule includes but is not limited to PD1, TIM-3, LAG-3, OX40, 4-1BB, ICOS and GITR; the immunoglobulin Fc single chain is a natural immunoglobulin Fc single chain or an immunoglobulin Fc single chain in which ADCC effect is knocked out by gene mutation; preferably, the immunoglobulin Fc single chain is a natural immunoglobulin Fc single chain or an immunoglobulin Fc single chain in which ADCC effect is knocked out by gene mutation; more preferably, the immunoglobulin Fc single chain is a human IgG Fc single chain.

[0010] The T cell surface molecule is PD1, and the anti-T cell surface molecule antibody is an anti-PD1 antibody (aPD1); in the second monomer, the Fab of the antibody is a Fab of a humanized antibody or a Fab of a fully human antibody; the ScFv of the antibody is a ScFv of a humanized antibody or a ScFv of a fully human antibody; more preferably, the second monomer is: a monomer of an anti-T cell surface molecule antibody, comprising a light chain and a heavy chain; preferably, the antibody is a humanized antibody or a fully human antibody.

[0011] More preferably, the heterodimer comprises: (1) a first monomer, comprising sequentially from the N-terminus: 1) a wild-type IL-2 protein having a sequence as shown in SEQ ID NO.1 or a mutant thereof comprising any one or any combination of mutations of R38L, F42A, D20K, R38A, F42K and K43E; 2) an essential linker structure (G4S linker sequence), preferably, having a sequence as shown in SEQ ID NO.6; 3) an IgG Fc single chain having a sequence as shown in SEQ ID NO.2, or a No-ADCC mutated IgG Fc having a sequence as shown in SEQ ID NO.3, or a knob mutant Fc having a sequence as shown in SEQ ID NO.4, or a hole mutant Fc having a sequence as shown in SEQ ID NO.5; (2) a second monomer, comprising: 1) an anti-PD1 antibody Fab region consisting of anti-PD1 antibody light chain VL-KCL having a sequence as shown in SEQ ID NO.7 and anti-PD1 antibody heavy chain VH&CH1 having a sequence as shown in SEQ ID NO.8; or 2) an anti-PD1 single-chain antibody (ScFv) having a sequence as shown in SEQ ID NO.9; and 3) an IgG Fc single chain having a sequence as shown in SEQ ID NO.2, or a No-ADCC mutated IgG Fc having a sequence as shown in SEQ ID NO.3, or a knob mutant Fc having a sequence as shown in SEQ ID NO.4, or a hole mutant Fc having a sequence as shown in SEQ ID NO.5; more preferably, the heterodimer comprises: a first monomer, which is a polypeptide having a sequence as shown in SEQ ID NO.10 (abIL2-Fc); a second monomer, which is: (1) a second monomer consisting of an anti-PD1 antibody VH-CH1-Fc (knob) having a sequence as shown in SEQ ID NO.11 and an anti-PD1 antibody light chain VL-KCL having a sequence as shown in SEQ ID NO.7; or (2) a polypeptide having a sequence as shown in SEQ ID NO.12 (aPD1ScFv-Fc(knob)).

[0012] The present invention also relates to a bifunctional molecule, which is a homodimer, wherein, a monomer of the homodimer is: a monomer formed by linking a molecule of interleukin 2 (IL2) to a molecule of anti-PD1 antibody Fab by any means, or, a monomer formed by linking a molecule of interleukin 2 (IL2) to a molecule of anti-PD1 single chain antibody (ScFv) by any means.

[0013] Preferably, the monomer of the homodimer comprises sequentially from the N-terminus: (1) a wild-type IL-2 protein having a sequence as shown in SEQ ID NO.1 or a mutant thereof comprising any one or any combination of mutations of R38L, F42A, D20K, R38A, F42K and K43E; (2) an essential linker structure (G4S linker sequence), preferably, having a sequence as shown in SEQ ID NO.6; (3) a Fab or ScFv of an anti-PD1 antibody; the Fab is a Fab of a humanized antibody or a Fab of a fully human antibody, and the ScFv is a ScFv of a humanized antibody or a ScFv of a fully human antibody; (4) an antibody Fc; the antibody Fc is a fully human wild-type Fc or a No-ADCC mutant Fc.

[0014] More preferably, the monomer of the homodimer has a sequence as shown in: (1) SEQ ID NO.18 (aPD1-abIL2: VL-VH(ScFv)-Fc-abIL2); (2) SEQ ID NO.19 (abIL2-aPD1: abIL2-VL-VH(ScFv)-Fc).

[0015] The present invention also relates to another bifunctional molecule, which is a bifunctional molecule comprising an anti-PD1 antibody (K) and abIL2, and the bifunctional molecule is a heterodimer; the heterodimer comprises: (1) a first monomer, comprising sequentially from the N-terminus: 1) a wild-type IL-2 protein having a sequence as shown in SEQ ID NO.1 or a mutant thereof comprising any one or any combination of mutations of R38L, F42A, D20K, R38A, F42K and K43E; 2) an essential linker structure (G4S linker sequence), preferably, having a sequence as shown in SEQ ID NO.6; 3) an IgG Fc single chain having a sequence as shown in SEQ ID NO.2, or a No-ADCC mutated IgG Fc having a sequence as shown in SEQ ID NO.3, or a knob mutant Fc having a sequence as shown in SEQ ID NO.4, or a hole mutant Fc having a sequence as shown in SEQ ID NO.5; (2) a second monomer, comprising: 1) an antibody Fab region consisting of anti-PD1 antibody (K) light chain VL-KCL having a sequence as shown in SEQ ID NO.13 and anti-PD1 antibody (K) heavy chain VH&CH1 having a sequence as shown in SEQ ID NO.14; or 2) an anti-PD1 single-chain antibody (K) (ScFv) having a sequence as shown in SEQ ID NO.15; and 3) an IgG Fc single chain having a sequence as shown in SEQ ID NO.2, or a No-ADCC mutated IgG Fc having a sequence as shown in SEQ ID NO.3, or a knob mutant Fc having a sequence as shown in SEQ ID NO.4, or a hole mutant Fc having a sequence as shown in SEQ ID NO.5; more preferably, the heterodimer comprises: a first monomer, which is a polypeptide having a sequence as shown in SEQ ID NO.10 (abIL2-Fc); a second monomer, which is: (1) a second monomer consisting of a polypeptide having a sequence as shown in SEQ ID NO.16 (aPD1(K)VH-CH1-Fc (knob)) and an anti-PD1 antibody (K) light chain having a sequence as shown in SEQ ID NO.13; or (2) a polypeptide having a sequence as shown in SEQ ID NO.17 or SEQ ID NO.22 (aPD1(K) ScFv-Fc (knob)).

[0016] The present invention also relates to a bifunctional molecule, which is a homodimer, wherein, a monomer of the homodimer is: a monomer formed by linking a molecule of interleukin 2 (IL2) to a molecule of anti-PD1 antibody (K) Fab by any means, or, a monomer formed by linking a molecule of interleukin 2 (IL2) to a molecule of anti-PD1 single-chain antibody (K) (ScFv) by any means.

[0017] Preferably, the monomer of the homodimer comprises sequentially from the N-terminus: (1) a wild-type IL-2 protein having a sequence as shown in SEQ ID NO.1 or a

mutant thereof comprising any one or any combination of mutations of R38L, F42A, D20K, R38A, F42K and K43E; (2) an essential linker structure (G4S linker sequence), preferably, having a sequence as shown in SEQ ID NO.6; (3) a Fab or ScFv of an anti-PD1 antibody (K); the Fab is a Fab of a humanized antibody or a Fab of a fully human antibody, and the ScFv is a ScFv of a humanized antibody or a ScFv of a fully human antibody; (4) an antibody Fc; the antibody Fc is a fully human wild-type Fc or a No-ADCC mutant Fc.

[0018] More preferably, the monomer of the homodimer has a sequence as shown in: (1) SEQ ID NO.20 (aPD1(K)-abIL2: VL-VH(ScFv)-Fc-abIL2), (2) SEQ ID NO.21 (IL2-a PD1(K): IL2-VL-VH(ScFv)-Fc).

[0019] The present invention also relates to use of the bifunctional molecules: (1) in the manufacture of an anti-tumor medicament; (2) in the manufacture of an anti-tumor medicament used in combination with an immune checkpoint inhibitor; (3) in the manufacture of an anti-tumor medicament that overcomes resistance to an immune checkpoint inhibitor; (4) in the manufacture of an anti-tumor medicament used in combination with a T cell adoptive transfer; (5) in the manufacture of an anti-tumor medicament that overcomes non-response to a T cell adoptive transfer.

[0020] Preferably, the immune checkpoint inhibitor is a PDL1 antibody;

[0021] Preferably, the T cell is an anti-tumor T cell; more preferably, the T cell is an anti-tumor CAR T cell or a structural analog thereof.

[0022] Beneficial effects of the invention: (1) The present invention provides a corresponding solution to the existing bottleneck of clinical application of IL-2. Specifically, the abIL2/aPD1-hIgG1 antibody reduces the binding of IL2 to intratumoral Tregs while retaining the activation of IL2 on effector cells, thereby overcoming the adverse effect of Treg expansion caused by the use of IL2. These results provide a new idea for the clinical use of IL2; (2) It is of great significance to overcome the resistance to immune checkpoint blockade therapy and the non-response to T cell adoptive transfer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. **1**. Anti-PD1-abIL2 has lower in vitro activity.

[0024] FIG. **2**. Anti-PD1-abIL2 can effectively control the growth of A20 tumor.

[0025] FIG. **3**. Anti-PDL1-abIL2 cannot effectively control the growth of A20 tumor.

[0026] FIG. **4**. The anti-tumor effect of anti-PD1-abIL2 is significantly better than that of anti-PDL1-abIL2.

[0027] FIG. **5**. Anti-PD1-abIL2 can effectively control the growth of MC38 tumors.

[0028] FIG. **6**. In A20 tumors, the anti-tumor effect of anti-PD1-abIL2 is significantly better than that of anti-PD1-IL2.

[0029] FIG. **7**. In MC38 tumors, the anti-tumor effect of anti-PD1-abIL2 is significantly better than that of anti-PD1-IL2.

[0030] FIG. **8**. The anti-tumor effect of anti-PD1-abIL2 does not depend on CD4 T cells.

[0031] FIG. **9**. The anti-tumor effect of anti-PD1-abIL2 does not depend on NK cells.

[0032] FIG. **10**. The anti-tumor effect of anti-PD1-abIL2 depends on CD8 T cells.

[0033] FIG. **11**. Anti-hPD1-abIL2 can better control human tumors.

[0034] FIG. **12**. Anti-PD1-abIL2 can act synergistically with T cell transplantation in tumor control.

[0035] FIG. **13**. Anti-PD1-abIL2 can act synergistically with anti-PDL1 in tumor control.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Experimental Materials

1. Strains and Plasmids

[0036] Strains: Top10 *E. coli* and DH5a *E. coli* competent cells (Beijing TransGen Biotech Co., Ltd.)

[0037] Plasmid: pEE6.4-IgGκ-hIgG1, comprising the signal peptide of mouse IgGκ and the Fc sequence of human IgG1, was used for the expression of antibodies. pEE6.4-IgGκ-hlgG1-Fc-hole and pEE6.4-IgGκ-hlgG1-Fc-knob were used for the expression of heterodimer proteins. pEE6.4-PD1 VH-CH1-Fc-knob and pEE6.4-PD1 VL-CL were used for the expression of the antibody portion of heterodimer proteins; pEE6.4-abIL2-Fc-hole was used for the expression of the abIL2 portion of heterodimer proteins.

2. Laboratory Animals

[0038] Wild-type C57BL/6, BALB/c and BALB/c-Rag mice were purchased from Beijing Vital River Laboratory Animal Center. Unless otherwise specified, female mice aged 8-10 weeks were used in all experiments. Mice were raised in a specific pathogen-free (SPF) barrier environment. Animal feeding and experimental operations complied with the relevant regulations of the Animal Management Committee of the Institute of Biophysics, Chinese Academy of Sciences.

3. Cell Lines

[0039] MC38 cell line, a colorectal cancer cell line derived from a C57 mouse, was cultured in DMEM complete medium (containing 10% inactivated fetal bovine serum, 2 mmol/l L-glutamine, 0.1 mmol/l non-essential amino acids, 100U penicillin and 100 μg/ml streptomycin).

[0040] A20 cell line, a B cell lymphoma cell line derived from a BALB/c mouse, was cultured in RPMI1640 complete medium (containing 10% inactivated fetal bovine serum, 2 mmol/L L-glutamine, 0.1 mmol/L non-essential amino acid, 100U penicillin and 100 μg/ml streptomycin).

[0041] FreeStyle™ 293F cell line (Invitrogen), a suspension cell derived from HEK293 cell line, was cultured in SMM293-TII or CD OptiCHO™ medium and mainly used for transient transfection to express bifunctional molecules.

[0042] CTLL-2 cell lin, a murine T cell line, was used to detect the biological activity of IL2 and cultured in RPMI1640 complete medium (containing 10% inactivated fetal bovine serum, 2 mmol/L L-glutamine, 0.1 mmol/L non-essential amino acid, 100U penicillin, 100 μg/ml streptomycin, and 100 IU/ml recombinant IL2).

Design and Synthesis of Genes and Primers

[0043] The human wild-type IL2 gene sequences are shown in SEQ ID NO.1. The primers used in the experiment were designed by DNAMAN software and synthesized by Invitrogen.

[0044] The proteins used in the following examples are all heterodimers, specifically: 1. aPD1-abIL2 consists of the

following first monomer and second monomer: (1) a polypeptide formed by fusing a Fc fragment to an IL2 containing mutations of R38L, F42A, D20K, R38A, F42K and K43E, having a sequence as shown in SEQ ID NO.10, (2) a second monomer of aPD1 having a sequence as shown in SEQ ID NO.22; 2. aPDL1-abIL2 consists of the following first monomer and second monomer: (1) a polypeptide formed by fusing a Fc fragment to an IL2 containing mutations of R38L, F42A, D20K, R38A, F42K and K43E, having a sequence as shown in SEQ ID NO.10, (2) a second monomer of aPDL1 having a sequence as shown in SEQ ID NO.23; 3. ahPD1-abIL2 consists of the following first monomer and second monomer: (1) a polypeptide formed by fusing a Fc fragment to an IL2 containing mutations of R38L, F42A, D20K, R38A, F42K and K43E, having a sequence as shown in SEQ ID NO.10, (2) a second monomer of aPD1 having a sequence as shown in SEQ ID NO.17; 4. aPDL1 antibody is commercialized atezolizumab consisting of: a first monomer: a polypeptide formed by fusing a Fc fragment to an IL2 containing mutations of R38L, F42A, D20K, R38A, F42K and K43E; a polypeptide having a sequence as shown in SEQ ID NO.10 (abIL2-Fc) when involving a murine mode; a second monomer: an scFv-Fc of a PD1 antibody; when involving a murine mode, the polypeptide having a sequence as shown in SEQ ID NO.10 (abIL2-Fc).

Tumor Inoculation and Treatment in Mice

(1) Tumor Inoculation and Measurement:

Tumor Model Establishment

[0045] 5×10^5-7.5×10^5 MC38 and MC38-EGFR5 single cells were suspended in 100 μl PBS, and then inoculated subcutaneously on the back of C57BL/6 mice; 2×10^6 A20 single cells were suspended in 100 μl PBS, and then inoculated subcutaneously on the back of BALB/c mice.

[0046] When a re-challenge experiment of the same tumor cells was performed on mice with tumor regression, the number of tumor cells inoculated was 5 times that of the initial tumor modeling, and the inoculation site was subcutaneous on the other side of the back of the mice. The tumor size was measured twice a week by using a vernier caliper to measure the long diameter (a), short diameter (b) and height (c) of the tumor. The tumor volume of mice=a×b×c/2.

(2) Treatment:

[0047] Antibodies or bifunctional molecules were injected intraperitoneally. Intratumoral administration was also used in some experiments. The specific dosage will be described in specific experiments.

In Vivo Cell Deletion in Mice

[0048] (1) Depletion of CD4+T cells and CD8+T cells: 200 μg GK1.5 or TIB210 antibody were injected intraperitoneally to deplete CD4+ T cells and CD8+ T cells on the day before IL2 or IL2 bifunctional molecular therapy, then injected every 3 days, and the number of injections was adjusted according to the treatment cycle. Depletion efficiency was detected by flow cytometry.

[0049] (2) Depletion of NK cells: 20 μl of NK cell-depleting antibody were injected intraperitoneally to deplete NK cells on the day before IL2 or IL2 bifunctional molecular therapy. Depletion efficiency was detected by flow cytometry.

Example 1. aPD1-abIL2 had a Lower Ability to Activate Receptors, Thereby Avoiding Peripheral Side Effects

[0050] To reduce the toxicity of IL2, the binding ability of IL2 to IL2Rα and IL2Rβ was reduced. The design was then validated by in vitro CTLL2 proliferation experiments. CTLL2 cells were added with different concentrations of IL-2 or aPD1-abIL2, cultured for 72h and detected for the proliferation levels under different concentrations of IL2 or aPD1-abIL2 by CCK8 kit. The results showed that aPD1-abIL2 had a lower ability to expand CTLL2 cells (FIG. **1**), indicating that the constructed antibody reduced IL2 activity.

Example 2. aPD1-abIL2 Bispecific Antibody had a Better Anti-Tumor Activity

1. aPD1-abIL2 Bispecific Antibody had a Significantly Improved Therapeutic Effect Compared with aPD1 Antibody Alone

[0051] BALB/c mice were subcutaneously inoculated with 2×10^6 A20 tumor cells, and intraperitoneally injected with 10 μg aPD1 antibody or 20 μg aPD1-abIL2 antibody protein on Day 14 (D14) after tumor inoculation, respectively. Tumor size was measured twice a week. The results showed that aPD1-abIL2 bispecific antibody had a better therapeutic effect compared with aPD1 antibody alone (FIG. **2**).

2. aPDL1-abIL2 Bispecific Antibody Failed to Significantly Improve the Therapeutic Effect

[0052] BALB/c mice were subcutaneously inoculated with 2×10^6 A20 tumor cells, and intraperitoneally injected with 10 μg aPDL1 antibody or 20 μg aPDL1-abIL2 antibody protein on D14 after tumor inoculation, respectively. Tumor size was measured twice a week. The results showed that aPD1-abIL2 bispecific antibody did not have a better therapeutic effect compared with aPD1 antibody alone (FIG. **3**).

3. aPD1-abIL2 Bispecific Antibody had a Significantly Improved Therapeutic Effect Compared with aPDL1-abIL2 Bispecific Antibody

[0053] BALB/c mice were subcutaneously inoculated with 2×10^6 A20 tumor cells, and intraperitoneally injected with 20 μg aPD1-abIL2 antibody or 20 μg aPDL1-abIL2 antibody protein on D14 after tumor inoculation, respectively. Tumor size was measured twice a week. The results showed that the aPD1-abIL2 bispecific antibody had a better therapeutic effect compared with aPDL1-abIL2 antibody alone (FIG. **4**).

4. aPD1-abIL2 Bispecific Antibody had a Significantly Improved Therapeutic Effect Compared with aPD1 Antibody Alone in MC38 Colorectal Cancer Tumors

[0054] C57BL/6 mice were subcutaneously inoculated with 5×10^5 MC38 tumor cells, and intraperitoneally injected with 10 μg aPD1 antibody or 20 μg aPD1-abIL2 antibody protein on Day 18 (D18) after tumor inoculation, respectively. Tumor size was measured twice a week. The results showed that aPD1-abIL2 bispecific antibody had a better therapeutic effect compared with aPD1 antibody alone (FIG. **5**).

5. aPD1-abIL2 Bispecific Antibody had a Significantly Improved Therapeutic Effect Compared with aPDL1-abIL2 Bispecific Antibody

[0055] BALB/c mice were subcutaneously inoculated with 2×10^6 A20 tumor cells, and intraperitoneally injected with 20 μg aPD1-IL2 antibody or 20 μg aPDL1-abIL2 antibody protein on D14 after tumor inoculation, respectively. Tumor size was measured twice a week. The results showed that aPD1-abIL2 bispecific antibody had a better therapeutic effect compared with aPDL1-IL2 antibody alone (FIG. **6**).

[0056] C57BL/6 mice were subcutaneously inoculated with 5×10^5 MC38 tumor cells, and intraperitoneally injected with 20 μg aPD1-IL2 antibody or 20 μg aPD1-abIL2 antibody protein on D18 after tumor inoculation, respectively. Tumor size was measured twice a week. The results showed that aPD1-abIL2 bispecific antibody had a better therapeutic effect compared with aPD1-IL2 antibody alone (FIG. **7**).

Example 3. aPD1-abIL2 was Capable of Activating CD8 T Cells

1. The Therapeutic Effect of aPD1-abIL2 Bispecific Antibody Did not Depend on NK Cells

[0057] Since CD25 (IL2 receptor a) and PD1 were mainly expressed on activated effector T cells and NK cells, deletion experiments of different cell populations were performed separately to determine which population of immune cells the treatment of aPD1-abIL2 antibody mainly depended on.

[0058] BALB/c mice were subcutaneously inoculated with 2×10^6 A20 tumor cells, intraperitoneally injected with 20 μg aPD1-abIL2 bifunctional molecule on day 17, and intraperitoneally injected with 20 μl of NK cell-depleting antibody one day before the treatment every 4 days for a total of 3 injections.

[0059] In the experiment, the aPD1-abIL2 antibody still had a therapeutic effect after the deletion of NK cells, indicating that NK cells were not the main effector cells for the therapeutic effect of the antibody (FIG. **8**).

2. The Therapeutic Effect of aPD1-abIL2 Antibody Depended on CD8 T Cells

[0060] The role of T cells in antibody therapy was further verified.

[0061] BALB/c mice were subcutaneously inoculated with 2×10^6 A20 tumor cells and intraperitoneally injected with 20 μg aPD1-abIL2 protein on day 17 after tumor inoculation, along with the intraperitoneal injection of 200 μg of CD4 T cell depleting antibody (clone number: GK1.5, prepared in our laboratory), 200 μg of CD8 T cell depleting antibody (clone number: TIB210, prepared in our laboratory) or both for a total of 3 injections.

[0062] Depletion experiments of CD4 T and CD8 T cells showed that the therapeutic effect of antibody therapy was not significantly reduced after depleting CD4 T cells, but significantly reduced after depleting CD8 T cells; and completely disappeared after depleting both CD4 T and CD8 T cells. It indicated that the therapeutic effect of aPD1-abIL2 antibody depended on T cells, and mainly CD8 T cells (FIG. **9** and FIG. **10**).

Example 4. aPD1-abIL2 Bispecific Antibody had a Better Anti-Tumor Activity in Humanized Mice

[0063] Humanized mice were subcutaneously inoculated with 2×10^6 A549 tumor cells, and intraperitoneally injected with 10 μg ahPD1 antibody plus 10 μg abIL2, or 20 μg ahPD1-abIL2 antibody protein on D9 after tumor inoculation, respectively. The tumor size was measured twice a week. The results showed that ahPD1-abIL2 bispecific antibody had a better therapeutic effect compared with the combined antibody (FIG. **11**).

Example 5. aPD1-abIL2 was Capable of Overcoming Non-Response to T Cell Adoptive Transfer Therapy

[0064] Rag knockout mice were subcutaneously inoculated with 2×10^6 A20-HA tumor cells, adoptively transferred with 2×10^6 OTI cells 12 days later, and intraperitoneally injected with 20 μg aPD1-abIL2. The tumor size was measured twice a week. The results showed that aPD1-abIL2 bispecific antibody had a better therapeutic effect compared with the T cell adoptive transfer therapy alone (FIG. **12**).

Example 6. aPD1-abIL2 was Capable of Overcoming Non-Response to PDL1 Antibody Therapy

[0065] When the tumor of A20 tumor-bearing mice was below 150 mm^3, aPD1-abIL2 antibody therapy alone had a good elimination effect. However, when the tumor was larger, antibody therapy alone could only control tumor growth but not completely eliminate it. To improve the therapeutic effect, bispecific antibodies and immune checkpoint inhibitory antibodies were combined to see whether the therapeutic effect can be improved.

[0066] Specific protocol: BALB/c mice were subcutaneously inoculated with 2×10^6 A20 tumor cells, and intraperitoneally injected with 50 μg aPDL1 antibody or/and 20 μg aPDL1-abIL2 antibody protein on D20 after tumor inoculation, respectively. Tumor size was measured twice a week. The results showed that the combination therapy had a better therapeutic effect compared with aPDL1 antibody or aPD1-abIL2 bispecific antibody alone (FIG. **13**).

[0067] Finally, it should be noted that the above examples are only used to help those skilled in the art to understand the essence of the present invention, and are not intended to limit the protection scope of the present invention.

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REFERENCE TO A "SEQUENCE LISTING" SUBMITTED AS AN XML FILE

[0097] The material in the XML file, named "TN-OF240953SP-US-Sequence-Listing", created Mar. 7, 2025, file size of 45,056 bytes, is hereby incorporated by reference.

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THRTHRPHEM ETCYSGLUTY RALAASPGLU THRALATHRI LEVALGLUPH ELEUASNARG  360
TRPILETHRP HECYSGLNSE RILEILESER THRLEUTHRG LYGLYGLYGL YSERASPLYS  420
THRHISTHRC YSPROPROCY SPROALAPRO GLULEULEUG LYGLYPROSE RVALPHELEU  480
PHEPROPROL YSPROLYSAS PTHRLEUMET ILESERARGT HRPROGLUVA LTHRCYSVAL  540
VALVALASPV ALSERHISGL UASPPROGLU VALLYSPHEA SNTRPTYRVA LASPGLYVAL  600
GLUVALHISA SNALALYSTH RLYSPROARG GLUGLUGLNT YRASNSERTH RTYRARGVAL  660
VALSERVALL EUTHRVALLE UHISGLNASP TRPLEUASNG LYLYSGLUTY RLYSCYSLYS  720
VALSERASNL YSALALEUPR OALAPROILE GLULYSTHRI LESERLYSAL ALYSGLYGLN  780
PROARGGLUP ROGLNVALCY STHRLEUPRO PROSERARGA SPGLULEUTH RLYSASNGLN  840
VALSERLEUS ERCYSALAVA LLYSGLYPHE TYRPROSERA SPILEALAVA LGLUTRPGLU  900
SERASNGLYG LNPROGLUAS NASNTYRLYS THRTHRPROP ROVALLEUAS PSERASPGLY  960
SERPHELYSL EUVALSERLY SLEUTHRVAL ASPLYSSERA RGTRPGLNGL NGLYASNVAL  1020
PHESERCYSS ERVALMETHI SGLUALALEU HISASNHIST YRTHRGLNLY SSERLEUSER  1080
LEUSERPROG LYLYS                                                   1095

SEQ ID NO: 11           moltype = AA  length = 1329
FEATURE                 Location/Qualifiers
source                  1..1329
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 11
GLNVALGLNL EUVALGLUSE RGLYGLYGLY VALVALGLNP ROGLYARGSE RLEUARGLEU  60
ASPCYSLYSA LASERGLYIL ETHRPHESER ASNSERGLYM ETHISTRPVA LARGGLNALA  120
PROGLYLYSG LYLEUGLUTR PVALALAVAL ILETRPTYRA SPGLYSERLY SARGTYRTYR  180
ALAASPSERV ALLYSGLYAR GPHETHRILE SERARGASPA SNSERLYSAS NTHRLEUPHE  240
LEUGLNMETA SNSERLEUAR GALAGLUASP THRALAVALT YRTYRCYSAL ATHRASNASP  300
ASPTYRTRPG LYGLNGLYTH RLEUVALTHR VALSERSERA LASERTHRLY SGLYPROSER  360
VALPHEPROL EUALAPROSE RSERLYSSER THRSERGLYG LYTHRALAAL ALEUGLYCYS  420
LEUVALLYSA SPTYRPHEPR OGLUPROVAL THRVALSERT RPASNSERGL YALALEUTHR  480
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SERGLYVALH ISTHRPHEPR OALAVALLEU GLNSERSERG LYLEUTYRSE RLEUSERSER  540
VALVALTHRV ALPROSERSE RSERLEUGLY THRGLNTHRT YRILECYSAS NVALASNHIS  600
LYSPROSERA SNTHRLYSVA LASPLYSARG VALGLUPROL YSSERCYSAS PLYSTHRHIS  660
THRCYSPROP ROCYSPROAL APROGLULEU LEUGLYGLYP ROSERVALPH ELEUPHEPRO  720
PROLYSPROL YSASPTHRLE UMETILESER ARGTHRPROG LUVALTHRCY SVALVALVAL  780
ASPVALSERH ISGLUASPPR OGLUVALLYS PHEASNTRPT YRVALASPGL YVALGLUVAL  840
HISASNALAL YSTHRLYSPR OARGGLUGLU GLNTYRASNS ERTHRTYRAR GVALVALSER  900
VALLEUTHRV ALLEUHISGL NASPTRPLEU ASNGLYLYSG LUTYRLYSCY SLYSVALSER  960
ASNLYSALAL EUPROALAPR OILEGLULYS THRILESERL YSALALYSGL YGLNPROARG 1020
GLUPROGLNV ALTYRTHRLE UPROPROCYS ARGASPGLUL EUTHRLYSAS NGLNVALSER 1080
LEUTRPCYSL EUVALLYSGL YPHETYRPRO SERASPILEA LAVALGLUTR PGLUSERASN 1140
GLYGLNPROG LUASNASNTY RLYSTHRTHR PROPROVALL EUASPSERAS PGLYSERPHE 1200
PHELEUTYRS ERALALEUTH RVALASPLYS SERARGTRPG LNGLNGLYAS NVALPHESER 1260
CYSSERVALM ETHISGLUAL ALEUHISASN HISTYRTHRG LNLYSSERLE USERLEUSER 1320
PROGLYLYS                                                         1329

SEQ ID NO: 12          moltype = AA  length = 1386
FEATURE                Location/Qualifiers
source                 1..1386
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 12
GLUILEVALL EUTHRGLNSE RPROALATHR LEUSERLEUS ERPROGLYGL UARGALATHR  60
LEUSERCYSA RGALASERGL NSERVALSER SERTYRLEUA LATRPTYRGL NGLNLYSPRO  120
GLYGLNALAP ROARGLEULE UILETYRASP ALASERASNA RGALATHRGL YILEPROALA  180
ARGPHESERG LYSERGLYSE RGLYTHRASP PHETHRLEUT HRILESERSE RLEUGLUPRO  240
GLUASPPHEA LAVALTYRTY RCYSGLNGLN SERSERASNT RPPROARGTH RPHEGLYGLN  300
GLYTHRLYSV ALGLUILELY SGLYGLYGLY GLYSERGLYG LYGLYGLYSE RGLYGLYGLY  360
GLYSERGLNV ALGLNLEUVA LGLUSERGLY GLYGLYVALV ALGLNPROGL YARGSERLEU  420
ARGLEUASPC YSLYSALASE RGLYILETHR PHESERASNS ERGLYMETHI STRPVALARG  480
GLNALAPROG LYLYSGLYLE UGLUTRPVAL ALAVALILET RPTYRASPGL YSERLYSARG  540
TYRTYRALAA SPSERVALLY SGLYARGPHE THRILESERA RGASPASNSE RLYSASNTHR  600
LEUPHELEUG LNMETASNSE RLEUARGALA GLUASPTHRA LAVALTYRTY RCYSALATHR  660
ASNASPASPT YRTRPGLYGL NGLYTHRLEU VALTHRVALS ERSERASPLY STHRHISTHR  720
CYSPROPROC YSPROALAPR OGLULEULEU GLYGLYPROS ERVALPHELE UPHEPROPRO  780
LYSPROLYSA SPTHRLEUME TILESERARG THRPROGLUV ALTHRCYSVA LVALVALASP  840
VALSERHISG LUASPPROGL UVALLYSPHE ASNTRPTYRV ALASPGLYVA LGLUVALHIS  900
ASNALALYST HRLYSPROAR GGLUGLUGLN TYRASNSERT HRTYRARGVA LVALSERVAL  960
LEUTHRVALL EUHISGLNAS PTRPLEUASN GLYLYSGLUT YRLYSCYSLY SVALSERASN 1020
LYSALALEUP ROALAPROIL EGLULYSTHR ILESERLYSA LALYSGLYGL NPROARGGLU 1080
PROGLNVALT YRTHRLEUPR OPROCYSARG ASPGLULEUT HRLYSASNGL NVALSERLEU 1140
TRPCYSLEUV ALLYSGLYPH ETYRPROSER ASPILEALAV ALGLUTRPGL USERASNGLY 1200
GLNPROGLUA SNASNTYRLY STHRTHRPRO PROVALLEUA SPSERASPGL YSERPHEPHE 1260
LEUTYRSERA LALEUTHRVA LASPLYSSER ARGTRPGLNG LNGLYASNVA LPHESERCYS 1320
SERVALMETH ISGLUALALE UHISASNHIS TYRTHRGLNL YSSERLEUSE RLEUSERPRO 1380
GLYLYS                                                            1386

SEQ ID NO: 13          moltype = AA  length = 218
FEATURE                Location/Qualifiers
source                 1..218
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 13
EIVLTQSPAT LSLSPGERAT LSCRASKGVS TSGYSYLHWY QQKPGQAPRL LIYLASYLES  60
GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQHSRDLPL TFGGGTKVEI KRTVAAPSVF  120
IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS  180
STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC                          218

SEQ ID NO: 14          moltype = AA  length = 669
FEATURE                Location/Qualifiers
source                 1..669
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 14
GLNVALGLNL EUVALGLNSE RGLYVALGLU VALLYSLYSP ROGLYALASE RVALLYSVAL  60
SERCYSLYSA LASERGLYTY RTHRPHETHR ASNTYRTYRM ETTYRTRPVA LARGGLNALA  120
PROGLYGLNG LYLEUGLUTR PMETGLYGLY ILEASNPROS ERASNGLYGL YTHRASNPHE  180
ASNGLULYSP HELYSASNAR GVALTHRLEU THRTHRASPS ERSERTHRTH RTHRALATYR  240
METGLULEUL YSSERLEUGL NPHEASPASP THRALAVALT YRTYRCYSAL AARGARGASP  300
TYRARGPHEA SPMETGLYPH EASPTYRTRP GLYGLNGLYT HRTHRVALTH RVALSERSER  360
ALASERTHRL YSGLYPROSE RVALPHEPRO LEUALAPROS ERSERLYSSE RTHRSERGLY  420
GLYTHRALAA LALEUGLYCY SLEUVALLYS ASPTYRPHEP ROGLUPROVA LTHRVALSER  480
TRPASNSERG LYALALEUTH RSERGLYVAL HISTHRPHEP ROALAVALLE UGLNSERSER  540
GLYLEUTYRS ERLEUSERSE RVALVALTHR VALPROSERS ERSERLEUGL YTHRGLNTHR  600
TYRILECYSA SNVALASNHI SLYSPROSER ASNTHRLYSV ALASPLYSAR GVALGLUPRO  660
LYSSERCYS                                                          669
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SEQ ID NO: 15 moltype = AA length = 738
FEATURE Location/Qualifiers
source 1..738
mol_type = protein
organism = synthetic construct
SEQUENCE: 15
GLUILEVALL EUTHRGLNSE RPROALATHR LEUSERLEUS ERPROGLYGL UARGALATHR 60
LEUSERCYSA RGALASERLY SGLYVALSER THRSERGLYT YRSERTYRLE UHISTRPTYR 120
GLNGLNLYSP ROGLYGLNAL APROARGLEU LEUILETYRL EUALASERTY RLEUGLUSER 180
GLYVALPROA LAARGPHESE RGLYSERGLY SERGLYTHRA SPPHETHRLE UTHRILESER 240
SERLEUGLUP ROGLUASPPH EALAVALTYR TYRCYSGLNH ISSERARGAS PLEUPROLEU 300
THRPHEGLYG LYGLYTHRLY SVALGLUILE LYSGLYGLYG LYGLYSERGL YGLYGLYGLY 360
SERGLYGLYG LYGLYSERGL NVALGLNLEU VALGLNSERG LYVALGLUVA LLYSLYSPRO 420
GLYALASERV ALLYSVALSE RCYSLYSALA SERGLYTYRT HRPHETHRAS NTYRTYRMET 480
TYRTRPVALA RGGLNALAPR OGLYGLNGLY LEUGLUTRPM ETGLYGLYIL EASNPROSER 540
ASNGLYGLYT HRASNPHEAS NGLULYSPHE LYSASNARGV ALTHRLEUTH RTHRASPSER 600
SERTHRTHRT HRALATYRME TGLULEULYS SERLEUGLNP HEASPASPTH RALAVALTYR 660
TYRCYSALAA RGARGASPTY RARGPHEASP METGLYPHEA SPTYRTRPGL YGLNGLYTHR 720
THRVALTHRV ALSERSER 738

SEQ ID NO: 16 moltype = AA length = 1350
FEATURE Location/Qualifiers
source 1..1350
mol_type = protein
organism = synthetic construct
SEQUENCE: 16
GLNVALGLNL EUVALGLNSE RGLYVALGLU VALLYSLYSP ROGLYALASE RVALLYSVAL 60
SERCYSLYSA LASERGLYTY RTHRPHETHR ASNTYRTYRM ETTYRTRPVA LARGGLNALA 120
PROGLYGLNG LYLEUGLUTR PMETGLYGLY ILEASNPROS ERASNGLYGL YTHRASNPHE 180
ASNGLULYSP HELYSASNAR GVALTHRLEU THRTHRASPS ERSERTHRTH RTHRALATYR 240
METGLULEUL YSSERLEUGL NPHEASPASP THRALAVALT YRTYRCYSAL AARGARGASP 300
TYRARGPHEA SPMETGLYPH EASPTYRTRP GLYGLNGLYT HRTHRVALTH RVALSERSER 360
ALASERTHRL YSGLYPROSE RVALPHEPRO LEUALAPROS ERSERLYSSE RTHRSERGLY 420
GLYTHRALAA LALEUGLYCY SLEUVALLYS ASPTYRPHEP ROGLUPROVA LTHRVALSER 480
TRPASNSERG LYALALEUTH RSERGLYVAL HISTHRPHEP ROALAVALLE UGLNSERSER 540
GLYLEUTYRS ERLEUSERSE RVALVALTHR VALPROSERS ERSERLEUGL YTHRGLNTHR 600
TYRILECYSA SNVALASNHI SLYSPROSER ASNTHRLYSV ALASPLYSAR GVALGLUPRO 660
LYSSERCYSA SPLYSTHRHI STHRCYSPRO PROCYSPROA LAPROGLULE ULEUGLYGLY 720
PROSERVALP HELEUPHEPR OPROLYSPRO LYSASPTHRL EUMETILESE RARGTHRPRO 780
GLUVALTHRC YSVALVALVA LASPVALSER HISGLUASPP ROGLUVALLY SPHEASNTRP 840
TYRVALASPG LYVALGLUVA LHISASNALA LYSTHRLYSP ROARGGLUGL UGLNTYRASN 900
SERTHRTYRA RGVALVALSE RVALLEUTHR VALLEUHISG LNASPTRPLE UASNGLYLYS 960
GLUTYRLYSC YSLYSVALSE RASNLYSALA LEUPROALAP ROILEGLULY STHRILESER 1020
LYSALALYSG LYGLNPROAR GGLUPROGLN VALTYRTHRL EUPROPROCY SARGASPGLU 1080
LEUTHRLYSA SNGLNVALSE RLEUTRPCYS LEUVALLYSG LYPHETYRPR OSERASPILE 1140
ALAVALGLUT RPGLUSERAS NGLYGLNPRO GLUASNASNT YRLYSTHRTH RPROPROVAL 1200
LEUASPSERA SPGLYSERPH EPHELEUTYR SERALALEUT HRVALASPLY SSERARGTRP 1260
GLNGLNGLYA SNVALPHESE RCYSSERVAL METHISGLUA LALEUHISAS NHISTYRTHR 1320
GLNLYSSERL EUSERLEUSE RPROGLYLYS 1350

SEQ ID NO: 17 moltype = AA length = 1419
FEATURE Location/Qualifiers
source 1..1419
mol_type = protein
organism = synthetic construct
SEQUENCE: 17
GLUILEVALL EUTHRGLNSE RPROALATHR LEUSERLEUS ERPROGLYGL UARGALATHR 60
LEUSERCYSA RGALASERLY SGLYVALSER THRSERGLYT YRSERTYRLE UHISTRPTYR 120
GLNGLNLYSP ROGLYGLNAL APROARGLEU LEUILETYRL EUALASERTY RLEUGLUSER 180
GLYVALPROA LAARGPHESE RGLYSERGLY SERGLYTHRA SPPHETHRLE UTHRILESER 240
SERLEUGLUP ROGLUASPPH EALAVALTYR TYRCYSGLNH ISSERARGAS PLEUPROLEU 300
THRPHEGLYG LYGLYTHRLY SVALGLUILE LYSGLYGLYG LYGLYSERGL YGLYGLYGLY 360
SERGLYGLYG LYGLYSERGL NVALGLNLEU VALGLNSERG LYVALGLUVA LLYSLYSPRO 420
GLYALASERV ALLYSVALSE RCYSLYSALA SERGLYTYRT HRPHETHRAS NTYRTYRMET 480
TYRTRPVALA RGGLNALAPR OGLYGLNGLY LEUGLUTRPM ETGLYGLYIL EASNPROSER 540
ASNGLYGLYT HRASNPHEAS NGLULYSPHE LYSASNARGV ALTHRLEUTH RTHRASPSER 600
SERTHRTHRT HRALATYRME TGLULEULYS SERLEUGLNP HEASPASPTH RALAVALTYR 660
TYRCYSALAA RGARGASPTY RARGPHEASP METGLYPHEA SPTYRTRPGL YGLNGLYTHR 720
THRVALTHRV ALSERSERAS PLYSTHRHIS THRCYSPROP ROCYSPROAL APROGLULEU 780
LEUGLYGLYP ROSERVALPH ELEUPHEPRO PROLYSPROL YSASPTHRLE UMETILESER 840
ARGTHRPROG LUVALTHRCY SVALVALVAL ASPVALSERH ISGLUASPPR OGLUVALLYS 900
PHEASNTRPT YRVALASPGL YVALGLUVAL HISASNALAL YSTHRLYSPR OARGGLUGLU 960
GLNTYRASNS ERTHRTYRAR GVALVALSER VALLEUTHRV ALLEUHISGL NASPTRPLEU 1020
ASNGLYLYSG LUTYRLYSCY SLYSVALSER ASNLYSALAL EUPROALAPR OILEGLULYS 1080
THRILESERL YSALALYSGL YGLNPROARG GLUPROGLNV ALTYRTHRLE UPROPROCYS 1140

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ARGASPGLUL EUTHRLYSAS NGLNVALSER LEUTRPCYSL EUVALLYSGL YPHETYRPRO  1200
SERASPILEA LAVALGLUTR PGLUSERASN GLYGLNPROG LUASNASNTY RLYSTHRTHR  1260
PROPROVALL EUASPSERAS PGLYSERPHE PHELEUTYRS ERALALEUTH RVALASPLYS  1320
SERARGTRPG LNGLNGLYAS NVALPHESER CYSSERVALM ETHISGLUAL ALEUHISASN  1380
HISTYRTHRG LNLYSSERLE USERLEUSER PROGLYLYS                         1419

SEQ ID NO: 18          moltype = AA  length = 1800
FEATURE                Location/Qualifiers
source                 1..1800
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 18
GLUILEVALL EUTHRGLNSE RPROALATHR LEUSERLEUS ERPROGLYGL UARGALATHR  60
LEUSERCYSA RGALASERGL NSERVALSER SERTYRLEUA LATRPTYRGL NGLNLYSPRO  120
GLYGLNALAP ROARGLEULE UILETYRASP ALASERASNA RGALATHRGL YILEPROALA  180
ARGPHESERG LYSERGLYSE RGLYTHRASP PHETHRLEUT HRILESERSE RLEUGLUPRO  240
GLUASPPHEA LAVALTYRTY RCYSGLNGLN SERSERASNT RPPROARGTH RPHEGLYGLN  300
GLYTHRLYSV ALGLUILELY SGLYGLYGLY GLYSERGLYG LYGLYGLYSE RGLYGLYGLY  360
GLYSERGLNV ALGLNLEUVA LGLUSERGLY GLYGLYVALV ALGLNPROGL YARGSERLEU  420
ARGLEUASPC YSLYSALASE RGLYILETHR PHESERASNS ERGLYMETHI STRPVALARG  480
GLNALAPROG LYLYSGLYLE UGLUTRPVAL ALAVALILET RPTYRASPGL YSERLYSARG  540
TYRTYRALAA SPSERVALLY SGLYARGPHE THRILESERA RGASPASNSE RLYSASNTHR  600
LEUPHELEUG LNMETASNSE RLEUARGALA GLUASPTHRA LAVALTYRTY RCYSALATHR  660
ASNASPASPT YRTRPGLYGL NGLYTHRLEU VALTHRVALS ERSERASPLY STHRHISTHR  720
CYSPROPROC YSPROALAPR OGLULEULEU GLYGLYPROS ERVALPHELE UPHEPROPRO  780
LYSPROLYSA SPTHRLEUME TILESERARG THRPROGLUV ALTHRCYSVA LVALVALASP  840
VALSERHISG LUASPPROGL UVALLYSPHE ASNTRPTYRV ALASPGLYVA LGLUVALHIS  900
ASNALALYST HRLYSPROAR GGLUGLUGLN TYRASNSERT HRTYRARGVA LVALSERVAL  960
LEUTHRVALL EUHISGLNAS PTRPLEUASN GLYLYSGLUT YRLYSCYSLY SVALSERASN  1020
LYSALALEUP ROALAPROIL EGLULYSTHR ILESERLYSA LALYSGLYGL NPROARGGLU  1080
PROGLNVALT YRTHRLEUPR OPROSERARG ASPGLULEUT HRLYSASNGL NVALSERLEU  1140
THRCYSLEUV ALLYSGLYPH ETYRPROSER ASPILEALAV ALGLUTRPGL USERASNGLY  1200
GLNPROGLUA SNASNTYRLY STHRTHRPRO PROVALLEUA SPSERASPGL YSERPHEPHE  1260
LEUTYRSERL YSLEUTHRVA LASPLYSSER ARGTRPGLNG LNGLYASNVA LPHESERCYS  1320
SERVALMETH ISGLUALALE UHISASNHIS TYRTHRGLNL YSSERLEUSE RLEUSERPRO  1380
GLYLYSGLYG LYGLYGLYSE RALAPROTHR SERSERSERT HRLYSLYSTH RGLNLEUGLN  1440
LEUGLUHISL EULEULEUAS PLEUGLNMET ILELEUASNG LYILEASNAS NTYRLYSASN  1500
PROLYSLEUT HRLEUMETLE UTHRALALYS PHETYRMETP ROLYSLYSAL ATHRGLULEU  1560
LYSHISLEUG LNCYSLEUGL UGLUGLULEU LYSPROLEUG LUGLUVALLE UASNLEUALA  1620
GLNSERLYSA SNPHEHISLE UARGPROARG ASPLEUILES ERASNILEAS NVALILEVAL  1680
LEUGLULEUL YSGLYSERGL UTHRTHRPHE METCYSGLUT YRALAASPGL UTHRALATHR  1740
ILEVALGLUP HELEUASNAR GTRPILETHR PHECYSGLNS ERILEILESE RTHRLEUTHR  1800

SEQ ID NO: 19          moltype = AA  length = 1800
FEATURE                Location/Qualifiers
source                 1..1800
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 19
ALAPROTHRS ERSERSERTH RLYSLYSTHR GLNLEUGLNL EUGLUHISLE ULEULEUASP  60
LEUGLNMETI LELEUASNGL YILEASNASN TYRLYSASNP ROLYSLEUTH RLEUMETLEU  120
THRALALYSP HETYRMETPR OLYSLYSALA THRGLULEUL YSHISLEUGL NCYSLEUGLU  180
GLUGLULEUL YSPROLEUGL UGLUVALLEU ASNLEUALAG LNSERLYSAS NPHEHISLEU  240
ARGPROARGA SPLEUILESE RASNILEASN VALILEVALL EUGLULEULY SGLYSERGLU  300
THRTHRPHEM ETCYSGLUTY RALAASPGLU THRALATHRI LEVALGLUPH ELEUASNARG  360
TRPILETHRP HECYSGLNSE RILEILESER THRLEUTHRG LYGLYGLYGL YSERGLUILE  420
VALLEUTHRG LNSERPROAL ATHRLEUSER LEUSERPROG LYGLUARGAL ATHRLEUSER  480
CYSARGALAS ERGLNSERVA LSERSERTYR LEUALATRPT YRGLNGLNLY SPROGLYGLN  540
ALAPROARGL EULEUILETY RASPALASER ASNARGALAT HRGLYILEPR OALAARGPHE  600
SERGLYSERG LYSERGLYTH RASPPHETHR LEUTHRILES ERSERLEUGL UPROGLUASP  660
PHEALAVALT YRTYRCYSGL NGLNSERSER ASNTRPPROA RGTHRPHEGL YGLNGLYTHR  720
LYSVALGLUI LELYSGLYGL YGLYGLYSER GLYGLYGLYG LYSERGLYGL YGLYGLYSER  780
GLNVALGLNL EUVALGLUSE RGLYGLYGLY VALVALGLNP ROGLYARGSE RLEUARGLEU  840
ASPCYSLYSA LASERGLYIL ETHRPHESER ASNSERGLYM ETHISTRPVA LARGGLNALA  900
PROGLYLYSG LYLEUGLUTR PVALALAVAL ILETRPTYRA SPGLYSERLY SARGTYRTYR  960
ALAASPSERV ALLYSGLYAR GPHETHRILE SERARGASPA SNSERLYSAS NTHRLEUPHE  1020
LEUGLNMETA SNSERLEUAR GALAGLUASP THRALAVALT YRTYRCYSAL ATHRASNASP  1080
ASPTYRTRPG LYGLNGLYTH RLEUVALTHR VALSERSERA SPLYSTHRHI STHRCYSPRO  1140
PROCYSPROA LAPROGLULE ULEUGLYGLY PROSERVALP HELEUPHEPR OPROLYSPRO  1200
LYSASPTHRL EUMETILESE RARGTHRPRO GLUVALTHRC YSVALVALVA LASPVALSER  1260
HISGLUASPP ROGLUVALLY SPHEASNTRP TYRVALASPG LYVALGLUVA LHISASNALA  1320
LYSTHRLYSP ROARGGLUGL UGLNTYRASN SERTHRTYRA RGVALVALSE RVALLEUTHR  1380
VALLEUHISG LNASPTRPLE UASNGLYLYS GLUTYRLYSC YSLYSVALSE RASNLYSALA  1440
LEUPROALAP ROILEGLULY STHRILESER LYSALALYSG LYGLNPROAR GGLUPROGLN  1500
VALTYRTHRL EUPROPROSE RARGASPGLU LEUTHRLYSA SNGLNVALSE RLEUTHRCYS  1560
LEUVALLYSG LYPHETYRPR OSERASPILE ALAVALGLUT RPGLUSERAS NGLYGLNPRO  1620
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GLUASNASNT YRLYSTHRTH RPROPROVAL LEUASPSERA SPGLYSERPH EPHELEUTYR  1680
SERLYSLEUT HRVALASPLY SSERARGTRP GLNGLNGLYA SNVALPHESE RCYSSERVAL  1740
METHISGLUA LALEUHISAS NHISTYRTHR GLNLYSSERL EUSERLEUSE RPROGLYLYS  1800

SEQ ID NO: 20          moltype = AA  length = 1833
FEATURE                Location/Qualifiers
source                 1..1833
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 20
GLUILEVALL EUTHRGLNSE RPROALATHR LEUSERLEUS ERPROGLYGL UARGALATHR  60
LEUSERCYSA RGALASERLY SGLYVALSER THRSERGLYT YRSERTYRLE UHISTRPTYR  120
GLNGLNLYSP ROGLYGLNAL APROARGLEU LEUILETYRL EUALASERTY RLEUGLUSER  180
GLYVALPROA LAARGPHESE RGLYSERGLY SERGLYTHRA SPPHETHRLE UTHRILESER  240
SERLEUGLUP ROGLUASPPH EALAVALTYR TYRCYSGLNH ISSERARGAS PLEUPROLEU  300
THRPHEGLYG LYGLYTHRLY SVALGLUILE LYSGLYGLYG LYGLYSERGL YGLYGLYGLY  360
SERGLYGLYG LYGLYSERGL NVALGLNLEU VALGLNSERG LYVALGLUVA LLYSLYSPRO  420
GLYALASERV ALLYSVALSE RCYSLYSALA SERGLYTYRT HRPHETHRAS NTYRTYRMET  480
TYRTRPVALA RGGLNALAPR OGLYGLNGLY LEUGLUTRPM ETGLYGLYIL EASNPROSER  540
ASNGLYGLYT HRASNPHEAS NGLULYSPHE LYSASNARGV ALTHRLEUTH RTHRASPSER  600
SERTHRTHRT HRALATYRME TGLULEULYS SERLEUGLNP HEASPASPTH RALAVALTYR  660
TYRCYSALAA RGARGASPTY RARGPHEASP METGLYPHEA SPTYRTRPGL YGLNGLYTHR  720
THRVALTHRV ALSERSERAS PLYSTHRHIS THRCYSPROP ROCYSPROAL APROGLULEU  780
LEUGLYGLYP ROSERVALPH ELEUPHEPRO PROLYSPROL YSASPTHRLE UMETILESER  840
ARGTHRPROG LUVALTHRCY SVALVALVAL ASPVALSERH ISGLUASPPR OGLUVALLYS  900
PHEASNTRPT YRVALASPGL YVALGLUVAL HISASNALAL YSTHRLYSPR OARGGLUGLU  960
GLNTYRASNS ERTHRTYRAR GVALVALSER VALLEUTHRV ALLEUHISGL NASPTRPLEU  1020
ASNGLYLYSG LUTYRLYSCY SLYSVALSER ASNLYSALAL EUPROALAPR OILEGLULYS  1080
THRILESERL YSALALYSGL YGLNPROARG GLUPROGLNV ALTYRTHRLE UPROPROSER  1140
ARGASPGLUL EUTHRLYSAS NGLNVALSER LEUTHRCYSL EUVALLYSGL YPHETYRPRO  1200
SERASPILEA LAVALGLUTR PGLUSERASN GLYGLNPROG LUASNASNTY RLYSTHRTHR  1260
PROPROVALL EUASPSERAS PGLYSERPHE PHELEUTYRS ERLYSLEUTH RVALASPLYS  1320
SERARGTRPG LNGLNGLYAS NVALPHESER CYSSERVALM ETHISGLUAL ALEUHISASN  1380
HISTYRTHRG LNLYSSERLE USERLEUSER PROGLYLYSG LYGLYGLYGL YSERALAPRO  1440
THRSERSERS ERTHRLYSLY STHRGLNLEU GLNLEUGLUH ISLEULEULE UASPLEUGLN  1500
METILELEUA SNGLYILEAS NASNTYRLYS ASNPROLYSL EUTHRLEUME TLEUTHRALA  1560
LYSPHETYRM ETPROLYSLY SALATHRGLU LEULYSHISL EUGLNCYSLE UGLUGLUGLU  1620
LEULYSPROL EUGLUGLUVA LLEUASNLEU ALAGLNSERL YSASNPHEHI SLEUARGPRO  1680
ARGASPLEUI LESERASNIL EASNVALILE VALLEUGLUL EULYSGLYSE RGLUTHRTHR  1740
PHEMETCYSG LUTYRALAAS PGLUTHRALA THRILEVALG LUPHELEUAS NARGTRPILE  1800
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THRALALYSP HETYRMETPR OLYSLYSALA THRGLULEUL YSHISLEUGL NCYSLEUGLU  180
GLUGLULEUL YSPROLEUGL UGLUVALLEU ASNLEUALAG LNSERLYSAS NPHEHISLEU  240
ARGPROARGA SPLEUILESE RASNILEASN VALILEVALL EUGLULEULY SGLYSERGLU  300
THRTHRPHEM ETCYSGLUTY RALAASPGLU THRALATHRI LEVALGLUPH ELEUASNARG  360
TRPILETHRP HECYSGLNSE RILEILESER THRLEUTHRG LYGLYGLYGL YSERGLUILE  420
VALLEUTHRG LNSERPROAL ATHRLEUSER LEUSERPROG LYGLUARGAL ATHRLEUSER  480
CYSARGALAS ERLYSGLYVA LSERTHRSER GLYTYRSERT YRLEUHISTR PTYRGLNGLN  540
LYSPROGLYG LNALAPROAR GLEULEUILE TYRLEUALAS ERTYRLEUGL USERGLYVAL  600
PROALAARGP HESERGLYSE RGLYSERGLY THRASPPHET HRLEUTHRIL ESERSERLEU  660
GLUPROGLUA SPPHEALAVA LTYRTYRCYS GLNHISSERA RGASPLEUPR OLEUTHRPHE  720
GLYGLYGLYT HRLYSVALGL UILELYSGLY GLYGLYGLYS ERGLYGLYGL YGLYSERGLY  780
GLYGLYGLYS ERGLNVALGL NLEUVALGLN SERGLYVALG LUVALLYSLY SPROGLYALA  840
SERVALLYSV ALSERCYSLY SALASERGLY TYRTHRPHET HRASNTYRTY RMETTYRTRP  900
VALARGGLNA LAPROGLYGL NGLYLEUGLU TRPMETGLYG LYILEASNPR OSERASNGLY  960
GLYTHRASNP HEASNGLULY SPHELYSASN ARGVALTHRL EUTHRTHRAS PSERSERTHR  1020
THRTHRALAT YRMETGLULE ULYSSERLEU GLNPHEASPA SPTHRALAVA LTYRTYRCYS  1080
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THRVALSERS ERASPLYSTH RHISTHRCYS PROPROCYSP ROALAPROGL ULEULEUGLY  1200
GLYPROSERV ALPHELEUPH EPROPROLYS PROLYSASPT HRLEUMETIL ESERARGTHR  1260
PROGLUVALT HRCYSVALVA LVALASPVAL SERHISGLUA SPPROGLUVA LLYSPHEASN  1320
TRPTYRVALA SPGLYVALGL UVALHISASN ALALYSTHRL YSPROARGGL UGLUGLNTYR  1380
ASNSERTHRT YRARGVALVA LSERVALLEU THRVALLEUH ISGLNASPTR PLEUASNGLY  1440
LYSGLUTYRL YSCYSLYSVA LSERASNLYS ALALEUPROA LAPROILEGL ULYSTHRILE  1500
SERLYSALAL YSGLYGLNPR OARGGLUPRO GLNVALTYRT HRLEUPROPR OSERARGASP  1560
GLULEUTHRL YSASNGLNVA LSERLEUTHR CYSLEUVALL YSGLYPHETY RPROSERASP  1620
ILEALAVALG LUTRPGLUSE RASNGLYGLN PROGLUASNA SNTYRLYSTH RTHRPROPRO  1680
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VALLEUASPS ERASPGLYSE RPHEPHELEU TYRSERLYSL EUTHRVALAS PLYSSERARG  1740
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THRGLNLYSS ERLEUSERLE USERPROGLY LYS                               1833

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source                 1..1419
                       mol_type = protein
                       organism = synthetic construct
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GLNTHRILEL EUGLNVALIL ETYRASPASP ASNLYSARGP ROSERGLYIL EPROGLUARG  180
ILESERGLYS ERSERSERGL YTHRTHRALA THRLEUTHRI LEARGASPVA LARGALAGLU  240
ASPGLUGLYA SPTYRTYRCY SPHESERGLY TYRVALASPS ERASPSERLY SLEUTYRVAL  300
PHEGLYSERG LYTHRGLNLE UTHRVALLEU GLYGLYGLYG LYSERGLYGL YGLYGLYSER  360
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GLYSERLEUL YSLEUSERCY SVALALASER GLYPHETHRP HESERASPTY RPHEMETSER  480
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TYRASNTYRA LATHRTYRTY RSERGLYSER VALLYSGLYA RGPHETHRIL ESERARGASP  600
ASPSERARGS ERMETVALTY RLEUGLNMET ASNASNLEUA RGTHRGLUAS PTHRALATHR  660
TYRTYRCYST HRARGASPGL YSERGLYTYR PROSERLEUA SPPHETRPGL YGLNGLYTHR  720
GLNVALTHRV ALSERSERAS PLYSTHRHIS THRCYSPROP ROCYSPROAL APROGLULEU  780
LEUGLYGLYP ROSERVALPH ELEUPHEPRO PROLYSPROL YSASPTHRLE UMETILESER  840
ARGTHRPROG LUVALTHRCY SVALVALVAL ASPVALSERH ISGLUASPPR OGLUVALLYS  900
PHEASNTRPT YRVALASPGL YVALGLUVAL HISASNALAL YSTHRLYSPR OARGGLUGLU  960
GLNTYRASNS ERTHRTYRAR GVALVALSER VALLEUTHRV ALLEUHISGL NASPTRPLEU  1020
ASNGLYLYSG LUTYRLYSCY SLYSVALSER ASNLYSALAL EUPROALAPR OILEGLULYS  1080
THRILESERL YSALALYSGL YGLNPROARG GLUPROGLNV ALTYRTHRLE UPROPROCYS  1140
ARGASPGLUL EUTHRLYSAS NGLNVALSER LEUTRPCYSL EUVALLYSGL YPHETYRPRO  1200
SERASPILEA LAVALGLUTR PGLUSERASN GLYGLNPROG LUASNASNTY RLYSTHRTHR  1260
PROPROVALL EUASPSERAS PGLYSERPHE PHELEUTYRS ERALALEUTH RVALASPLYS  1320
SERARGTRPG LNGLNGLYAS NVALPHESER CYSSERVALM ETHISGLUAL ALEUHISASN  1380
HISTYRTHRG LNLYSSERLE USERLEUSER PROGLYLYS                         1419

SEQ ID NO: 23          moltype = AA  length = 1401
FEATURE                Location/Qualifiers
source                 1..1401
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GLYLYSALAP ROLYSLEULE UILETYRSER ALASERPHEL EUTYRSERGL YVALPROSER  180
ARGPHESERG LYSERGLYSE RGLYTHRASP PHETHRLEUT HRILESERSE RLEUGLNPRO  240
GLUASPPHEA LATHRTYRTY RCYSGLNGLN TYRLEUTYRH ISPROALATH RPHEGLYGLN  300
GLYTHRLYSV ALGLUILELY SGLYGLYGLY GLYSERGLYG LYGLYGLYSE RGLYGLYGLY  360
GLYSERGLUV ALGLNLEUVA LGLUSERGLY GLYGLYLEUV ALGLNPROGL YGLYSERLEU  420
ARGLEUSERC YSALAALASE RGLYPHETHR PHESERASPS ERTRPILEHI STRPVALARG  480
GLNALAPROG LYLYSGLYLE UGLUTRPVAL ALATRPILES ERPROTYRGL YGLYSERTHR  540
TYRTYRALAA SPSERVALLY SGLYARGPHE THRILESERA LAASPTHRSE RLYSASNTHR  600
ALATYRLEUG LNMETASNSE RLEUARGALA GLUASPTHRA LAVALTYRTY RCYSALAARG  660
ARGHISTRPP ROGLYGLYPH EASPTYRTRP GLYGLNGLYT HRLEUVALTH RVALSERALA  720
ASPLYSTHRH ISTHRCYSPR OPROCYSPRO ALAPROGLUL EULEUGLYGL YPROSERVAL  780
PHELEUPHEP ROPROLYSPR OLYSASPTHR LEUMETILES ERARGTHRPR OGLUVALTHR  840
CYSVALVALV ALASPVALSE RHISGLUASP PROGLUVALL YSPHEASNTR PTYRVALASP  900
GLYVALGLUV ALHISASNAL ALYSTHRLYS PROARGGLUG LUGLNTYRAS NSERTHRTYR  960
ARGVALVALS ERVALLEUTH RVALLEUHIS GLNASPTRPL EUASNGLYLY SGLUTYRLYS  1020
CYSLYSVALS ERASNLYSAL ALEUPROALA PROILEGLUL YSTHRILESE RLYSALALYS  1080
GLYGLNPROA RGGLUPROGL NVALTYRTHR LEUPROPROC YSARGASPGL ULEUTHRLYS  1140
ASNGLNVALS ERLEUTRPCY SLEUVALLYS GLYPHETYRP ROSERASPIL EALAVALGLU  1200
TRPGLUSERA SNGLYGLNPR OGLUASNASN TYRLYSTHRT HRPROPROVA LLEUASPSER  1260
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LEUSERLEUS ERPROGLYLY S                                            1401
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1. A bifunctional molecule, which is a heterodimer, wherein,

the heterodimer comprises:

(1) a first monomer, formed by linking interleukin 2 (IL2) to an immunoglobulin Fc single chain;

(2) a second monomer, formed by linking a Fab or ScFv of an anti-T cell surface molecule antibody to an immunoglobulin Fc single chain;

the first monomer and the second monomer are linked through dimerization of the Fc single chain to form the heterodimer;

the T cell surface molecule includes but is not limited to PD1, TIM-3, LAG-3, OX40, 4-1BB, ICOS and GITR.

2. The bifunctional molecule according to claim 1, wherein the immunoglobulin Fc single chain is a natural

immunoglobulin Fc single chain or an immunoglobulin Fc single chain in which ADCC effect is knocked out by gene mutation; preferably, the immunoglobulin Fc single chain is a human IgG Fc single chain.

3. The bifunctional molecule according to claim **1**, wherein, in the second monomer,

the Fab of the antibody is a Fab of a humanized antibody or a Fab of a fully human antibody;

the ScFv of the antibody is a ScFv of a humanized antibody or a ScFv of a fully human antibody;

preferably, the second monomer is:

a monomer of an anti-T cell surface molecule antibody, comprising a light chain and a heavy chain;

more preferably, the antibody is a monomer of a humanized anti-T cell surface molecule antibody or a fully human anti-T cell surface molecule antibody, comprising a light chain and a heavy chain.

4. The bifunctional molecule according to claim **1**, wherein,

the T cell surface molecule is PD1,

the anti-T cell surface molecule antibody is an anti-PD1 antibody (aPD1).

5. The bifunctional molecule according to claim **1**, wherein,

the heterodimer comprises:

(1) a first monomer, comprising sequentially from the N-terminus:

1) a wild-type IL-2 protein having a sequence as shown in SEQ ID NO.1 or a mutant thereof comprising any one or any combination of mutations of R38L, F42A, D20K, R38A, F42K and K43E;

2) an essential linker structure (G4S linker sequence), preferably, having a sequence as shown in SEQ ID NO.6;

3) an IgG Fc single chain having a sequence as shown in SEQ ID NO.2, or an ADCC-knockout mutated IgG Fc having a sequence as shown in SEQ ID NO.3, or a knob mutant Fc having a sequence as shown in SEQ ID NO.4, or a hole mutant Fc having a sequence as shown in SEQ ID NO.5;

(2) a second monomer, comprising:

1) an anti-PD1 antibody Fab region consisting of anti-PD1 antibody light chain VL-KCL having a sequence as shown in SEQ ID NO.7 and anti-PD1 antibody heavy chain VH&CH1 having a sequence as shown in SEQ ID NO.8; or an anti-PD1 single-chain antibody (ScFv) having a sequence as shown in SEQ ID NO.9; and,

2) an IgG Fc single chain having a sequence as shown in SEQ ID NO.2, or an ADCC-knockout mutated IgG Fc having a sequence as shown in SEQ ID NO.3, or a knob mutant Fc having a sequence as shown in SEQ ID NO.4, or a hole mutant Fc having a sequence as shown in SEQ ID NO.5.

6. The bifunctional molecule according to claim **1**, wherein,

the heterodimer comprises:

a first monomer, which is a polypeptide having a sequence as shown in SEQ ID NO.10 (abIL2-Fc);

a second monomer, which is:

(1) a Fab construct of an anti-PD1 antibody consisting of an anti-PD1 antibody light chain VL-KCL having a sequence as shown in SEQ ID NO.7 and an anti-PD1 antibody VH-CH1-Fc (knob) having a sequence as shown in SEQ ID NO.11; or

(2) a polypeptide having a sequence as shown in SEQ ID NO.12 (aPD1ScFv-Fc(knob)).

7. The bifunctional molecule according to claim **5**, wherein,

the second monomer comprises:

1) an antibody Fab construct consisting of anti-PD1 antibody (K) light chain having a sequence as shown in SEQ ID NO.13 and anti-PD1 antibody (K) heavy chain VH&CH1 having a sequence as shown in SEQ ID NO.14; or

2) an anti-PD1 single-chain antibody (K) (ScFv) having a sequence as shown in SEQ ID NO.15; and

3) an IgG Fc single chain having a sequence as shown in SEQ ID NO.2, or a No-ADCC mutated IgG Fc having a sequence as shown in SEQ ID NO.3, or a knob mutant Fc having a sequence as shown in SEQ ID NO.4, or a hole mutant Fc having a sequence as shown in SEQ ID NO.5.

8. The bifunctional molecule according to claim **6**, wherein,

the second monomer is:

(1) a Fab construct of an anti-PD1 antibody consisting of a polypeptide having a sequence as shown in SEQ ID NO.16 (aPD1(K)VH-CH1-Fc (knob)) and a variable region of an anti-PD1 antibody light chain having a sequence as shown in SEQ ID NO.13; or

(2) a polypeptide having a sequence as shown in SEQ ID NO.17 or SEQ ID NO.22 (aPD1(K) ScFv-Fc (knob)).

9-11. (canceled)

12. Use of the bifunctional molecule according to claim **1**:

(1) in treatment of tumors;

(2) in treatment of tumors in combination with immune checkpoint inhibitors;

(3) in treatment of tumors which are resistant to immune checkpoint inhibitors;

(4) in treating tumors in combination with T-cell adoptive transfer; or

(5) in treatment of tumors that are not responsive to T-cell adoptive transfer.

13. The use according to claim **12**, wherein, the immune checkpoint inhibitor is an anti-PDL1 antagonist; preferably, the anti-PDL1 antagonist is an anti-PDL1 antibody.

14. The use according to claim **12**, wherein, the T cell is an anti-tumor T cell; more preferably, the T cell is an anti-tumor CAR T cell or a structural analog thereof.

15. A medicament or a pharmaceutical composition comprising the bifunctional molecule of claim **1**.

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