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HLA-B57 open conformers

Abstract

The invention relates to a HLA-B57 open conformer or a HLA-B57 Fc fusion protein for use in the treatment or prevention of cancer. The Fc open conformer comprises or consists of a first and a second monomer, and each monomer comprises a HLA-B57 chain. The Fc fusion protein further comprises a protein stabilizing polypeptide sequence and optionally an amino acid linker. Further aspects of the invention provide combination medicaments comprising the HLA-B57 Fc open conformer and immune checkpoint inhibitors and/or checkpoint agonist agents. Furthermore, the invention relates to the use of HLA-B57 open conformer as an immunomodulator, particularly in diseases where modulation of diverse immune cell components (e.g. cytotoxic CD8+ T cells, Tregs) is a therapeutic strategy, e.g. infectious diseases.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This is a continuation the U.S. patent application Ser. No. 16/083,508 filed Sep. 9, 2018, which is the US National Stage of International Patent Application No. PCT/EP2017/055373 filed Mar. 7, 2017, which in turn claims the benefit of European Patent Application No. 16159099.7 filed Mar. 8, 2016. The contents of the foregoing applications are incorporated by reference herein in their entirety.

FIELD

(1) The present invention relates to the use of HLA-B57 open conformers, particularly for use in

the prophylaxis or treatment of cancer, and for use as an immunomodulator.

BACKGROUND

(2) Human leukocyte antigens (HLA) belong to the classical major histocompatibility complex (MHC) protein family. The HLA complex helps the immune system distinguish the body's own proteins from proteins made by foreign invaders such as viruses and bacteria. Humans have three main classical MHC class I genes, known as HLA-A, HLA-B, and HLA-C. Classical HLA genes have many possible variations, allowing each person's immune system to react to a wide range of foreign invaders. Some HLA genes have hundreds of identified versions (alleles), each of which is given a particular number (such as HLA-B57). Closely related alleles are categorized together; for example, at least 82 very similar alleles are subtypes of HLA-B57. These subtypes are designated as HLA-B*5701 to HLA-B*5782, and the closely related HLA-B*5801.

(3) Classical MHC-I molecules (designated HLA-I in humans) are trimeric structures comprising a membrane-bound heavy chain with three extracellular domains (α1, α2 and α3) that associates non-covalently with β2-microglobulin (β3 m) and a small peptide. HLA I heavy chains may exist in a form not associated to β2-microglobulin or peptide. These forms are referred to as open conformers.

(4) As all other HLA molecules, HLA-B57's principle function is to present cell-derived peptides to CD8.sup.+ cytotoxic T lymphocytes (CTLs), as part of the adaptive immune response. Under normal physiological conditions, HLA-B57 molecules form heterotrimeric complexes that consist of B57 heavy chains, β2-microglobulin, and peptides which are derived from self-proteins, viruses or bacteria. In this respect, HLA-B57 resembles all other class I HLA alleles. However, HLA-molecules may also be present in cells as free-heavy chains lacking β2m-microglobulin and peptide, and can be referred to as HLA-B57 open conformers (Arosa et al. Open conformers: the hidden face of MHC-I molecules, Trends in Immunology 2007 March; 28(3):115-23).

(5) Cancer is a group of diseases characterized by abnormal cells of the body undergoing uncontrolled and destructive growth. Cancer cells can spread around the body and metastasize to form tumors; this growth pattern is called malignant. Cancer can be treated by surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and immunotherapy. The choice of therapy depends on the type of cancer, the stage of the cancer (how much it has spread), age, health status, and additional personal characteristics. There is no single treatment for cancer, and patients often receive a combination of therapies and palliative care.

(6) Cancer immunotherapy refers to a diverse set of therapeutic strategies designed to induce the patient's own immune system to fight the tumor, and is based on the insight that the progression of cancer, which involves the accumulation of diverse mutations, is monitored by the immune system. Immunotherapies either stimulate the activities of specific cell components of the immune system or counteract signals produced by cancer cells that suppress immune responses (Mahoney et al., Nat Rev Drug Discov. 2015 August; 14(8):561-84).

(7) Different type of immune cells are involved in the immune response against cancer. Within this pool of white blood cells (immune contexture), the most notorious cells are: T-cells (cytotoxic CD8+ T-cells, T helper CD4+ cells—Th1, Th2, and Th17 phenotype), regulatory T cells (Tregs), Macrophages (M1 type-pro-inflammatory and M2 type-pro-tumoral), myeloid derived suppressor cells (MDSCs), natural killer cells (NK cells), and dendritic cells (DCs). These immune cells can be located in the center of the tumor, in the invasive margin or in the adjacent tertiary lymphoid structures (Fridman et al., Nat. Rev. Cancer. 2012, April: 12, 298-306).

(8) The density and composition of the immune microenvironment is heterogeneous among patients and tumors. It is now well established that in general the tumor infiltration with M2-phenotype macrophages and myeloid derived suppressor cells (MDSCs) promotes tumor progression, whereas infiltration of cytotoxic CD8+ T-cells, Th1 phenotype cells and M1 type macrophages are often associated with good clinical outcome, and good response to immunotherapy. The clinical impact of other lymphoid and myeloid cell populations is less

consistent and seems dependent on the tumor type and stage. The presence of Th17, and NK cells, and the absence/reduction of Treg cells in tumor infiltrates is correlated with good outcome in some cancer indications (Giraldo et al., Current Opinion in Immunology 2014, 27:8-15). A general overview of the balance between leukocyte infiltrates and clinical outcome is reviewed in FIG. **1**. (Becht et al. Current Opinion in Immunology. 2016, 39:17-13).

(9) Overall, modulating the immune contexture of tumors favoring the infiltration of M1 type macrophages, cytotoxic CD8 T-cells, and Th1 cells, and/or reducing the infiltration of MDSCs and M2 type macrophages is an enormous therapeutic avenue to treat cancer that is explored here with the use of B57.sub.2-Fc proteins in diverse cancer indications.

SUMMARY

(10) The present invention provides HLA-B57 open conformers.

(11) According to one aspect, the invention provides HLA-B57 open conformers for use as a medicament.

(12) According to an alternative aspect, the invention provides HLA-B57 open conformers for use in prevention or treatment of cancer, or as an immunomodulator.

(13) According to another aspect of the invention, an isolated HLA-B57 open conformer protein is provided, particularly as a medicament, more particularly for use in the treatment or prevention of cancer, or as an immunomodulator.

(14) According to another aspect of the invention an isolated HLA-B57 open conformer protein is provided as an immunomodulatory agent or for use as negative modulator of regulatory T cells (Tregs), for use in human diseases where Tregs impair the development of protective immunity, such as cancer and infectious diseases (von Boehmer et al. ibid.).

(15) In certain embodiments, the HLA-B57 open conformer comprises two identical HLA-B57 polypeptide chains. In certain embodiments, the HLA-B57 open conformer comprises two different HLA-B57 polypeptide chains.

(16) According to an alternative of this first aspect of the invention, an HLA-B57 open conformer is provided for use in the treatment or prevention of cancer, or for use as an immunomodulatory agent to treat infectious diseases, particularly for use in prevention or therapy human immunodeficiency virus (HIV), hepatitis A, B, C, virus (HAV HBV, HCV respectively), influenza virus, Respiratory Syncytial Virus (RSV), measles virus, herpes viruses and/or yellow fever virus. The open conformer according to this aspect is a fusion protein that exists as a dimer of two monomers, and each monomer independently of the other monomer comprises an HLA-B57 chain, and a polypeptide domain known to metabolically stabilize a polypeptide in vivo. One example of such stabilizing domain is an Fc (crystallisable fragment) domain of an immunoglobulin, particularly the Fc polypeptide domain of a gamma immunoglobulin. The HLA-B57 chain and the stabilizing domain may optionally be joined by an amino acid linker. An open conformer fusion protein comprising the HLA-B57 chain and an immunoglobulin Fc fragment is henceforth termed HLA-B57 Fc open conformer or B57.sub.2-Fc herein.

(17) The presence of the Fc domain in the fusion protein facilitates increasing the solubility, stability, avidity, half-life, and from a technological point of view, cost-effective production and purification in mammalian systems (protein A or G purification).

(18) In certain embodiments, the HLA-B57 open conformer homodimer additionally comprises a peptide epitope fragment.

(19) According to a second aspect of the invention an HLA-B57 open conformer monomer (i.e., the HLA-B57 unattached to a second HLA-B57 heavy chain polypeptide, and not bound by β2-microglobulin) is provided for use in the treatment or prevention of cancer, or for use as an immunomodulatory agent. In certain embodiments of this aspect, the HLA-B57 monomer additionally comprises a peptide epitope fragment.

(20) This aspect can be summarized in the following items: Item 1: An isolated single HLA-B57 heavy chain polypeptide monomer essentially free of associated β2-microglobulin for use as a

medicament, particularly for use in the treatment or prevention of cancer, or for use as an immunomodulatory agent. Item 2: The isolated single HLA-B57 heavy chain polypeptide monomer for use in the treatment or prevention of cancer or as an immunomodulatory agent according to item 1, wherein the monomer additionally comprises a peptide epitope fragment. Item 3: The isolated single HLA-B57 heavy chain polypeptide monomer for use in the treatment or prevention of cancer or as an immunomodulatory agent according to items 1 or 2, wherein the HLA-B57 chain only consists of the HLA-B57 alpha 1, 2 and 3 domains. Item 4: The isolated single HLA-B57 heavy chain polypeptide monomer for use in the treatment or prevention of cancer or as an immunomodulatory agent according to any one of the preceding items, wherein the HLA-B57 chain comprises the transmembrane domain and does not comprise the intracellular domain (cytoplasmic tail). Item 5: The isolated single HLA-B57 heavy chain polypeptide monomer for use in the treatment or prevention of cancer or as an immunomodulatory agent according to any one of the preceding items, wherein the HLA-B57 chain has ≥70%, ≥80%, ≥85%, ≥90%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97% or ≥98%, or 100%, sequence identity compared to any one of the sequences provided in table 1. Item 6: A combination medicament comprising a. an isolated single HLA-B57 heavy chain polypeptide monomer as specified in any one of items 1 to 5, and b. a checkpoint inhibitory agent, particularly a checkpoint inhibitory antibody, and/or a checkpoint agonist agent, particularly a checkpoint agonist antibody. Item 7: The combination medicament according to item 6, wherein said checkpoint inhibitory agent is selected from an inhibitor of CTLA4 interaction with CD80 or CD86, and an inhibitor of the interaction of PD-1 with its ligand PD-L1, particularly an antibody against any one of CTLA4, CD80, CD86, PD-1, PD-L1, more particularly a monoclonal antibody against human CTLA4, PD-1, or PD-L1, and/or wherein said checkpoint agonist agent is selected from an agonist antibody or ligand to 4-1 BB and/or 4-1 BBL (CD137L, Uniprot P41273).

Description

BRIEF DESCRIPTION OF THE FIGURES

(1) FIG. **1** Schematic representation depicting three different classifications of tumors based on their immune cell infiltrates. The ‘immunogenic tumors’ are characterized by abundant Cytotoxic T-Lymphocyte (CTL) infiltration, M1 type macrophages, the presence of Tertiary Lymphoid Structures (TLS) and low/moderate vascularization while associated with the longest patient's survival. The ‘immune neglected’ tumors are characterized by the lack of infiltration by immune cells, low/moderate vascularization and intermediate prognosis. Finally, the ‘inflammatory tumors’ are characterized by abundant CTL in the absence of TLS, conspicuous infiltration with M2 macrophages, severe vascularization and poor prognosis (Becht et al. Current Opinion in Immunology. 2016, 39:17-13).

(2) FIG. **2** shows that B57.sub.2-Fc blocks mouse CD4.sup.+ T cell conversion into iTreg. Incubation of B57.sub.2-Fc in a dose dependent manner with naïve CD4.sup.+ T cells blocks the conversion to iTregs. A) B57.sub.2-Fc blocks the expression of CD25 (lineage marker of Tregs) in a dose dependent matter (μg/200 μL) (C). B) B57.sub.2-Fc blocks the expression of FoxP3 (differentiation marker of Tregs) in a dose dependent matter (μg/200 μL) (D). Control B57132m-Fc, isotype, media supplemented with TGFβ and IL-2 and media w/o supplementation demonstrate the specific influence of B57.sub.2-Fc on iTreg conversion.

(3) FIG. **3** shows that B57.sub.2-Fc impairs the suppression of murine Tregs in a dose dependent matter. A) histogram of proliferation from CD8+ T cells and Tregs depicting B57.sub.2-Fc blocking the suppression of mouse Tregs and allowing the proliferation of CD8.sup.+ T cells. Control B57-β2m-Fc, and isotype do not alter the suppression function of murine Tregs. B) % of iTreg suppression of murine CD8.sup.+ T cells at different concentrations of B57.sub.2-Fc (μg/200 μL).

(4) FIG. **4** shows that B57.sub.2-Fc suppresses lymphoma T cells. A-C) suppression assays to determine the proliferation of cells in the presence of (A) control isotype, (B) control B57-β2m-Fc, and (C) B57.sub.2-Fc. B57.sub.2-Fc suppress human (Jurkat) and mouse (EG.7) lymphoma cell lines in a dose dependent manner (μg/200 μL) when compared to control cell lines.
(5) FIG. **5** shows the interaction B57.sub.2-Fc to different immune regulatory receptors of leukocytes populations. A) KIR3DL1 (expressed in NK cells and subsets of T cells); B) KIR3DL2 (expressed in NK cells and subsets of T cells); C) KIR3DL3 (expressed in NK cells and subsets of T cells); D) LILRB1 (expressed in populations of NK cells, T cells, monocytes, and macrophages); E) LILRB2 (expressed mostly in macrophages and MDSCs), and F) PirB (murine homologue to LILRB2) by enzyme-linked immunosorbent assay (ELISA).
(6) FIG. **6** shows the schematic representation of B57-Fc and β2m DNA cassettes and expression of B57-β2m-Fc molecules from CHO cells. A) alpha 1, 2 and 3 domains of HLA-B57 heavy chain inserted into a human IgG4-Fc vector cassette; and the human-β2 microglobulin inserted in a separate vector cassette. B) Transfections in Chinese hamster ovary cells (CHO) cells are performed using both the B57-Fc-vector+β2m-vector at a ratio of 1:1 for the extracellular production of the B57-β2m-Fc protein. Supernatants were collected and B57-β2m-Fc purified using standard antibody purification protocols. β2m is removed from the B57-β2m-Fc complex and following B57-Fc monomers are refolded to form B57.sub.2-Fc homodimers
(7) FIG. **7** shows the combination of B57.sub.2-Fc with PD-1 antibodies reduce the size of tumors in the C38 murine syngeneic colon carcinoma model. A) Experimental design of injection time points of colon carcinoma cells (C38) and injection of compounds. B) Mean average tumor volume mm.sup.3 of treated groups (n=6). C) % of tumor inhibition of B57.sub.2-Fc and PD-1 treated groups compared to isotype. The experimental design of injection of substances was as follow: vehicle PBS Q3Dx7, isotype (10 mg/Kg) Q3Dx7; B57.sub.2-Fc (10 mg/Kg) Q3Dx7; PD-1 biwk×2 (200 g); and B57.sub.2-Fc+PD-1 (Q3Dx7 and biwk×2, respectively). Tumor volumes are expressed as mean±SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis, **p<0.01. Q=days between injections; Dx=number of injections, biwk=twice a week.
(8) FIG. **8** shows the combination of B57.sub.2-Fc with PD-1 antibodies reduce the size of tumors in the pancreatic (Pan02) cancer mouse model. A) Experimental design of injection time points of pancreatic cancer cells (Pan02) and injection of compounds. B) Mean average tumor volume mm.sup.3 of treated groups (n=8) with B57.sub.2-Fc and/or PD-1. The experimental design of injection of substances was as follow: isotype (5 mg/Kg) biwk×3; B57.sub.2-Fc (5 mg/Kg) biwk×3; PD-1 (5 mg/Kg) biwk×3; and B57.sub.2-Fc+PD-1 (biwk×3). Tumor volumes are expressed as mean±SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis, *p<0.05; **p<0.01; ****p<0.0001. biwk=twice a week.
(9) FIG. **9** shows the combination of B57.sub.2-Fc with PD-L1 antibodies reduce the size of tumors in the pancreatic (Pan02) cancer mouse model. A) Mean average tumor volume mm.sup.3 of treated groups (n=8) with B57.sub.2-Fc and/or PD-L1. B) % of tumor inhibition of B57.sub.2-Fc, PD-1 and PD-L1 treated groups compared to isotype. The experimental design of injection of substances was as follow: isotype (5 mg/Kg) biwk×3; B57.sub.2-Fc (5 mg/Kg) biwk×3; PD-L1 (5 mg/Kg) biwk×3; and B57.sub.2-Fc+PD-L1 (biwk×3). Tumor volumes are expressed as mean±SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis, *p<0.05; **p<0.01; ****p<0.0001. biwk=twice a week.
(10) FIG. **10** shows the immune contexture analysis of infiltrated leukocytes in tumors from treated pancreatic (Pan02) cancer mice with B57.sub.2-Fc and PD-1 by flow cytometry. Relevant leukocytes analysed infiltrating the tumor: A) NK cells; B) CD8/Treg ratio; and C) Myeloid Derived Suppressor Cells (MDSCs); Leukocytes numbers are expressed as mean±SEM and analysed by one-way ANOVA followed by Turkey post-hoc analysis, *p<0.05.
(11) FIG. **11** shows the immune contexture analysis (continuation from FIG. **10**) of infiltrated leukocytes in tumors from treated pancreatic (Pan02) cancer mice with B57.sub.2-Fc and PD-1 by

flow cytometry. Relevant leukocytes analysed infiltrating the tumor: A) Macrophages, and B) Macrophages M1/M2 ratio. Leukocytes numbers are expressed as mean±SEM and analysed by one-way ANOVA followed by Turkey post-hoc analysis, *p<0.05; **p<0.01; ***p<0.001.

(12) FIG. **12** shows the immune contexture analysis of infiltrated leukocytes in tumors from treated pancreatic (Pan02) cancer mice with B57.sub.2-Fc and PD-L1 by flow cytometry. Relevant leukocytes analysed infiltrating the tumor: A) NK cells; B) CD8/Treg ratio; and C) Myeloid Derived Suppressor Cells (MDSCs). Leukocytes numbers are expressed as mean±SEM and analysed by one-way ANOVA followed by Turkey post-hoc analysis, *p<0.05.

(13) FIG. **13** shows the immune contexture analysis (continuation from FIG. **12**) of infiltrated leukocytes in tumors from treated pancreatic (Pan02) cancer mice with B57.sub.2-Fc and PD-L1 by flow cytometry. Relevant leukocytes analysed infiltrating the tumor: A) Macrophages, and B) Macrophages M1/M2 ratio. Leukocytes numbers are expressed as mean±SEM and analysed by one-way ANOVA followed by Turkey post-hoc analysis, *p<0.05; **p<0.01.

(14) FIG. **14** shows that the combination of B57.sub.2-Fc with 4-1BB checkpoint agonist antibodies reduce the size of tumors in the melanoma (B16F10) cancer mouse model. A) Experimental design of injection time points of melanoma cancer cells (B16F10) and injection of compounds. B) Mean average tumor volume mm.sup.3 of treated groups (n=8) with B57.sub.2-Fc and 4-1BB antibody. The experimental design of injection of substances was as follow: isotype (5 mg/Kg) biwk 3 injections; B57.sub.2-Fc (5 mg/Kg) biwk 3 injections; 4-1BB antibody (1 mg/Kg) biwk×3 injections; and B57.sub.2-Fc+4-1BB biwk 3 injections. Tumor volumes are expressed as mean±SEM and analysed by two-way ANOVA followed by Bonferroni post-hoc analysis, **p<0.01; ****p<0.0001. biwk=twice a week.

(15) FIG. **15** shows that the combination of B57.sub.2-Fc with 4-1BB checkpoint agonist antibodies and combinations with PD-1 antagonist antibodies reduce the size of tumors in the melanoma (B16F10) cancer mouse model (continuation from FIG. **14** experiment). A) Mean average tumor volume mm.sup.3 of treated groups (n=8) with B57.sub.2-Fc, PD-1 and 4-1 BB antibodies. B) % tumor inhibition of B57.sub.2-Fc, 4-1BB and PD-1 treated groups compared to isotype. The experimental design of injection of substances was as follow: isotype (5 mg/Kg) biwk 3 injections; B57.sub.2-Fc (5 mg/Kg) biwk 3 injections; 4-1BB antibody (1 mg/Kg) biwk 3 injections; PD-1 biwk 3 injections (5 mg/Kg); and B57.sub.2-Fc+4-1 BB biwk 3 injections, B57.sub.2-Fc+PD-1 biwk 3 injections, PD-1+4-1 BB biwk 3 injections, and B57.sub.2-Fc+4-1 BB+PD-1 biwk 3 injections. Tumor volumes are expressed as mean±SEM. biwk=twice a week.

(16) FIG. **16** shows the immune contexture analysis of infiltrated leukocytes in tumors from treated melanoma (B16F10) mice by flow cytometry. Relevant leukocytes analysed infiltrating the tumor: A) NK cells; B) CD8/Treg ratio; and C) Myeloid Derived Suppressor Cells (MDSCs). Leukocytes numbers are expressed as mean±SEM and analysed by one-way ANOVA followed by Turkey post-hoc analysis, *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

(17) FIG. **17** shows the immune contexture analysis (continuation from FIG. **16**) of infiltrated leukocytes in tumors from treated melanoma (B16F10) mice by flow cytometry. Relevant leukocytes analysed infiltrating the tumor: A) Macrophages; and B) Macrophages M1/M2 ratio. Leukocytes numbers are expressed as mean±SEM and analysed by one-way ANOVA followed by Turkey post-hoc analysis, *p<0.05; **p<0.01; ***p<0.001.

BRIEF DESCRIPTION OF THE DESCRIBED SEQUENCES

(18) The nucleic and/or amino acid sequences provided herewith are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file named 95083_303_3701_seq, created Jun. 24, 2022, about 196 KB, which is incorporated by reference herein.

DETAILED DESCRIPTION

Terms and Definitions

(19) Amino acid sequences are given from amino to carboxyl terminus. Capital letters for sequence positions refer to L-amino acids in the one-letter code (Stryer, Biochemistry, 3.sup.rd ed. p. 21).

(20) The term open conformer as used in the present specification refers to an isolated HLA heavy chain molecule not associated to β2-microglobulin either as a monomer or as a dimer (homodimer or heterodimer). Certain embodiments of the open conformers disclosed herein are fusion protein monomers or dimers, wherein the HLA heavy chain is covalently linked to a stabilizing polypeptide region, particularly a crystallizable fragment immunoglobulin domain.

(21) In the context of the present specification the terms sequence identity and percentage of sequence identity refer to the values determined by comparing two aligned sequences. Methods for alignment of sequences for comparison are well-known in the art. Alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482 (1981), by the global alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Nat. Acad. Sci. 85:2444 (1988) or by computerized implementations of these algorithms, including, but not limited to: CLUSTAL, GAP, BESTFIT, BLAST, FASTA and TFASTA. Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology-Information (http://blast.ncbi.nlm.nih.gov/). One example for comparison of amino acid sequences is the BLASTP algorithm that uses the default settings: Expect threshold: 10; Word size: 3; Max matches in a query range: 0; Matrix: BLOSUM62; Gap Costs: Existence 11, Extension 1; Compositional adjustments: Conditional compositional score matrix adjustment. One such example for comparison of nucleic acid sequences is the BLASTN algorithm that uses the default settings: Expect threshold: 10; Word size: 28; Max matches in a query range: 0; Match/Mismatch Scores: 1.-2; Gap costs: Linear. Unless otherwise stated, sequence identity values provided herein refer to the value obtained with the BLAST suite of programs (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) using the above identified default parameters for protein and nucleic acid comparison, respectively.

(22) In the context of the present specification, the term major histocompatibility complex (MHC) is used in its meaning known in the art of cell biology and biochemistry; it refers to a cell surface molecule that displays a specific fraction (peptide), also referred to as an epitope, of a protein. There a two major classes of MHC molecules: class I and class II.

(23) MHC class I heavy chain molecules usually (i.e. when not in open conformer form) occur as an alpha chain linked to a unit of the non-MHC molecule β2-microglobulin. The alpha chain comprises, in direction from the N-terminus to the C-terminus, a signal peptide, three extracellular domains (α1-3, with al being at the N terminus), a transmembrane region and a C-terminal cytoplasmic tail. The peptide being displayed or presented is held by the peptide-binding groove, in the central region of the α1/α2 domains.

(24) In the context of the present specification, the term β2-microglobulin domain is used in its meaning known in the art of cell biology and biochemistry; it refers to a non-MHC molecule that is part of the MHC class I heterodimer molecule. In other words, it constitutes the β chain of the MHC class I heterodimer.

(25) In the context of the present specification, the term human leukocyte antigen (HLA) is used in its meaning known in the art of cell biology and biochemistry; it refers to gene loci encoding the human MHC class I proteins. The three major MHC class I genes in HLA are HLA-A, HLA-B and HLA-C and all of these genes have a varying number of alleles, for example HLA-B has 3590 known alleles. Closely related alleles are combined in subgroups of a certain allele. For example the allele HLA-B57 has more than 100 closely related alleles that are, according to the WHO Nomenclature Committee for Factors of the HLA System, labelled HLA-B*57:01:01 to HLA-B*57:82. The full or partial sequence of all known HLA genes and their respective alleles are available to the person skilled in the art in specialist databases such as IMGT/HLA (available

online at ebi.ac.uk/ipd/imgt/hla/) and are provided in table 1 of this specification.
(26) In the context of the present specification, the term checkpoint inhibitory agent or checkpoint inhibitory antibody is meant to encompass an agent, particularly an antibody (or antibody-like molecule) capable of disrupting the signal cascade leading to T cell inhibition after T cell activation as part of what is known in the art the immune checkpoint mechanism. Non-limiting examples of a checkpoint inhibitory agent or checkpoint inhibitory antibody include antibodies to CTLA-4 (Uniprot P16410), PD-1 (Uniprot Q15116), PD-L1 (Uniprot Q9NZQ7), B7H3 (CD276; Uniprot Q5ZPR3), Tim-3, Gal9, VISTA, Lag3.
(27) In the context of the present specification, the term checkpoint agonist agent or checkpoint agonist antibody is meant to encompass an agent, particularly but not limited to an antibody (or antibody-like molecule) capable of engaging the signal cascade leading to T cell activation as part of what is known in the art the immune checkpoint mechanism. Non-limiting examples of receptors known to stimulate T cell activation include CD122 and CD137 (4-1BB; Uniprot Q07011). The term checkpoint agonist agent or checkpoint agonist antibody encompasses agonist antibodies to CD137 (4-1 BB), CD134 (OX40), CD357 (GITR) CD278 (ICOS), CD27, CD28.
(28) In the context of the present specification, the term antibody is used in its meaning known in the art of cell biology and immunology; it refers to whole antibodies including but not limited to immunoglobulin type G (IgG), type A (IgA), type D (IgD), type E (IgE) or type M (IgM), any antigen binding fragment or single chains thereof and related or derived constructs. A whole antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (V.sub.H) and a heavy chain constant region (C.sub.H). The heavy chain constant region is comprised of three domains, C.sub.H1, C.sub.H2 and C.sub.H3. Each light chain is comprised of a light chain variable region (abbreviated herein as V.sub.L) and a light chain constant region (C.sub.L). The light chain constant region is comprised of one domain, C.sub.L. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component of the classical complement system.
(29) The term antibody-like molecule in the context of the present specification refers to a molecule capable of specific binding to another molecule or target with high affinity/a Kd≤10E-8 mol/l. An antibody-like molecule binds to its target similarly to the specific binding of an antibody. The term antibody-like molecule encompasses a repeat protein, such as a designed ankyrin repeat protein (Molecular Partners, Zurich), a polypeptide derived from armadillo repeat proteins, a polypeptide derived from leucine-rich repeat proteins and a polypeptide derived from tetratricopeptide repeat proteins.
(30) The term antibody-like molecule further encompasses a polypeptide derived from protein A domains, a polypeptide derived from fibronectin domain FN3, a polypeptide derived from consensus fibronectin domains, a polypeptide derived from lipocalins, a polypeptide derived from Zinc fingers, a polypeptide derived from Src homology domain 2 (SH2), a polypeptide derived from Src homology domain 3 (SH3), a polypeptide derived from PDZ domains, a polypeptide derived from gamma-crystallin, a polypeptide derived from ubiquitin, a polypeptide derived from a cysteine knot polypeptide and a polypeptide derived from a knottin.
(31) The term protein A domains derived polypeptide refers to a molecule that is a derivative of protein A and is capable of specifically binding the Fc region and the Fab region of immunoglobulins.
(32) The term armadillo repeat protein refers to a polypeptide comprising at least one armadillo repeat, wherein an armadillo repeat is characterized by a pair of alpha helices that form a hairpin structure.
(33) In the context of the present specification, the term crystallizable fragment (Fc) region is used

in its meaning known in the art of cell biology and immunology; it refers to a fraction of an antibody comprising two identical heavy chain fragments comprised of a C.sub.H2 and a C.sub.H3 domain, covalently linked by disulfide bonds.
(34) In the context of the present specification, the term dimer refers to a unit consisting of two subunits.
(35) In the context of the present specification, the term homodimer refers to a dimer comprised of two subunits that are either identical or are highly similar members of the same class of subunits. One example for a homodimer would be a dimer consisting of two subunits independently selected from the list of HLA-B57 alleles. In certain embodiments, homodimers consist of two identical HLA-B57 alleles.
(36) In the context of the present specification, the term amino acid linker refers to a polypeptide of variable length that is used to connect two polypeptides in order to generate a single chain polypeptide. Exemplary embodiments of linkers useful for practicing the invention specified herein are oligopeptide chains consisting of 1, 2, 3, 4, 5, 10, 20, 30, 40 or 50 amino acids. A non-limiting example of an amino acid linker is the polypeptide GGGGSGGGGSGGGGS (SEQ ID NO 109) that links an HLA-B57 polypeptide with an Fc domain.
(37) In certain embodiments of any one of the aspects of the invention laid out above, a peptide epitope fragment is non-covalently attached to the polypeptide within the antigen presenting domain of the HLA-B57 peptide chain.
(38) In certain embodiments of any one of the aspects of the invention laid out above, the HLA-B57 chain comprises only the extracellular HLA-B57 alpha 1, 2 and 3 domains. In these embodiments, the transmembrane and intracellular domains of the HLA-B57 chain are not included in the therapeutic polypeptide of the invention in order to allow its extracellular expression in recombinant cells. The person skilled in the art can easily identify the respective domains even in previously unknown HLA-B57 sequences by pair-wise sequence alignment with annotated HLA-B57 sequences.
(39) In certain embodiments of any one of the aspects of the invention laid out above, the HLA-B57 chain of the homodimer is selected from HLA-B*57:01, to HLA-B*57:82.
(40) In certain embodiments of any one of the aspects of the invention laid out above, the HLA-B57 chain comprises only the HLA-B57 alpha 1, 2 and 3 domains, but not the transmembrane and intracellular domain of a sequence selected from Table 1.
(41) In certain embodiments of any one of the aspects of the invention laid out above, the HLA-B57 chain has ≥70%, ≥80%, ≥85%, ≥90%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97% or ≥98%, or 100% sequence identity compared to any one of the sequences provided in Table 1.
(42) In certain embodiments, the HLA-B57 open conformer consists of two subunits independently selected from the above HLA-B57 alleles. In certain embodiments, homodimers consist of two identical HLA-B57 alleles.
(43) In certain embodiments, the HLA-B57 open conformer comprises an Fc domain. In certain particular embodiments, the Fc domain comprises heavy chain constant regions C.sub.H2 and C.sub.H3 from immunoglobulin type G (IgG), type A (IgA), type D (IgD), type E (IgE) or type M (IgM).
(44) In certain embodiments, the HLA-B57 open conformer comprises an amino acid linker joining a stabilizing domain, particularly an Fc domain, to the HLA polypeptide. In certain particular embodiments, the amino acid linker comprises 1 to 50 amino acids, particularly 5 to 40 amino acids, more particularly 10 to 30 amino acids, even more particularly 15 to 25 amino acids that link the HLA-B57 chain to the Fc domain as one single polypeptide chain.
(45) According to a third aspect of the invention, a nucleic acid molecule encoding a HLA-B57 open conformer monomer, particularly an Fc open conformer monomer, according to the above aspects of the invention is provided for use in the treatment or the therapy of cancer. Expression of the open conformer in vivo from the nucleic acid molecule will, after dimerization, lead to the

fusion protein polypeptide of the invention. The concept of expressing pharmaceutically active polypeptides from nucleic acids encoding them in the patient's body is well known and may confer significant benefits to the patient.

(46) In certain embodiments, the nucleic acid molecule encodes a HLA-B57 open conformer monomer, particularly an Fc open conformer monomer comprising a peptide epitope fragment. In certain embodiments, the nucleic acid molecule encodes a HLA-B57 open conformer monomer, particularly an Fc open conformer monomer that comprises only the extracellular HLA-B57 alpha 1, 2 and 3 domains. In certain embodiments, the nucleic acid molecule encodes a HLA-B57 open conformer monomer, particularly an Fc open conformer monomer that comprises only the extracellular HLA-B57 alpha 1, 2 and 3 domains, and a peptide epitope fragment.

(47) In certain embodiments, the nucleic acid molecule encodes a HLA-B57 open conformer monomer, particularly an Fc open conformer monomer that comprises an amino acid linker and/or an Fc (fragment crystallizable) domain, and is used in the treatment or the therapy of cancer.

(48) According to a fourth aspect of the invention a recombinant expression vector comprising the nucleic acid molecule according to the third aspect of the invention is provided for use in the treatment or the therapy of cancer.

(49) In certain embodiments the recombinant expression vector is a plasmid comprising a promoter that is operable in a mammalian cell, particularly in a human cell. The promoter is operably linked to the nucleic acid molecule of the invention.

(50) According to another aspect of the invention a virus comprising the nucleic acid molecule according to the third aspect of the invention is provided for use in the treatment or the therapy of cancer. The nucleic acid molecule is under control of a promoter sequence operable in a mammalian cell, particularly in a human cell. In certain embodiments, the virus is an adenovirus, adeno-associated virus, a herpes virus or a lentivirus.

(51) According to yet another aspect of the invention an in vitro genetically modified host cell comprising the nucleic acid molecule according to the third aspect of the invention is provided.

(52) Another aspect of the invention provides for the use of the HLA-B57 Fc open conformer homodimer or fusion protein homodimer according to the first and second aspect of the invention in the manufacture of a medicament for the treatment or prevention of cancer.

(53) According to yet another aspect, the invention provides a method of treatment for cancer, comprising administering an HLA-B57 Fc open conformer according to the first and second aspect of the invention to a patient in need thereof.

(54) According to another aspect of the invention, a combination medicament is provided, wherein the combination medicament comprises: a HLA-B57 open conformer, particularly a HLA-B57 Fc open conformer, according to any one of the above aspects or embodiments of the invention, and a checkpoint inhibitory agent, particularly a checkpoint inhibitory antibody selected from an inhibitor of cytotoxic T-lymphocyte-associated protein 4 (CTLA4; also known as CD152) interaction with CD80 or CD86, an inhibitor of the interaction of programmed cell death protein 1 (PD-1; also known as CD279) with its ligand PD-L1, and a ligand of T cell immunoglobulin and mucin domain-containing 3 (TIM-3) a checkpoint agonist agent, particularly a checkpoint agonist antibody selected to bind to and activate the tumor necrosis factor receptor 4-1 BB (also known as CD137 or TNFRSF9).

(55) In certain embodiments, the immune checkpoint inhibitor agent is an inhibitor of interaction of CTLA4 with CD80 or CD86.

(56) In certain embodiments, the immune checkpoint inhibitor agent is ipilimumab (Yervoy; CAS No. 477202-00-9).

(57) In certain embodiments, the immune checkpoint inhibitor agent is an inhibitor of interaction of programmed cell death protein 1 (PD-1) with its receptor PD-L1. In certain embodiments, the immune checkpoint inhibitor agent is selected from the clinically available antibody drugs nivolumab (Bristol-Myers Squibb; CAS No 946414-94-4), pembrolizumab (Merck Inc.; CAS No.

1374853-91-4), pidilizumab (CAS No. 1036730-42-3), atezolizumab (Roche AG; CAS No. 1380723-44-3), and Avelumab (Merck KGaA; CAS No. 1537032-82-8).

(58) In certain embodiments, the immune checkpoint agonist agent is utomilumab (PF-05082566), a fully human IgG2 monoclonal antibody against 4-1 BB currently undergoing clinical trials.

(59) In certain embodiments, the HLA-B57 open conformer, particularly the HLA-B57 Fc open conformer, is provided as parenteral dosage form, particularly confectioned for injection. In certain embodiments, the checkpoint inhibitory agent and/or checkpoint agonist agent are provided as parenteral dosage form, particularly confectioned for injection. In certain embodiments, both the HLA-B57 open conformer and the checkpoint inhibitory agent and/or checkpoint agonist agent are present in the same administration form.

(60) In yet another aspect, the invention relates to a method for producing recombinant HLA heavy chain polypeptides. This method is summarized in the following items: Item A: A method for producing, by methods of recombinant biotechnology, a human HLA heavy chain polypeptide, wherein said method comprises the following steps: a. Expression step: i. a HLA-encoding nucleic acid sequence encoding at least the alpha 1 chain, the alpha 2 chain and the alpha 3 chain of a HLA heavy chain under control of a promoter sequence operable in a cell, particularly a eukaryotic cell, more particularly a mammalian cell, and ii. a β2-microglobulin encoding nucleic acid sequence encoding the human HLA beta 2 microglobulin (UniProt P61769) under control of a promoter sequence operable in said cell (the same cell as in item 1. a.) are co-expressed in a mammalian cell ("production cell line"); b. Purification step: the resulting HLA-heavy-chain/β2-microglobulin complex is purified from the mammalian cell (the production cell line); c. Dissociation step: the purified HLA-heavy-chain/β2-microglobulin complex is dissociated under suitable conditions and the HLA heavy chain polypeptides are separated from the β2-microglobulin polypeptides; d. Refolding step: the separated HLA heavy chain polypeptides are incubated under conditions leading to refolding (of their native tertiary protein structure found in physiologically active HLA open conformer molecules). Item B: The method for producing a human HLA heavy chain polypeptide according to item A, wherein the HLA-encoding nucleic acid sequence comprises, from N to C terminus of the encoded polypeptide, the alpha 1 chain, the alpha 2 chain, the alpha 3 chain and a stabilizing sequence. Item C: The method for producing a human HLA heavy chain polypeptide according to item B, wherein the stabilizing sequence is selected from bovine serum albumin and an immunoglobulin constant fragment (Fc), particularly an immunoglobulin G constant fragment, more particularly an IgG4 Fc. Item D: The method for producing a human HLA heavy chain polypeptide according to any of the preceding items, wherein the HLA-encoding nucleic acid sequence and the β2-microglobulin encoding nucleic acid sequence are present on the same nucleic acid vector molecule (particularly, a DNA expression plasmid). Item E: The method for producing a human HLA heavy chain polypeptide according to any of the preceding items A to C, wherein the HLA-encoding nucleic acid sequence and the β2-microglobulin encoding nucleic acid sequence are present on different nucleic acid vector molecules (particularly, different DNA expression plasmids). Item F: The method of item E, wherein the nucleic acid vector comprising the HLA-encoding nucleic acid sequence is present in approximately 1- to 5-fold excess, particularly 1.5 to 5-fold excess with respect to the nucleic acid vector comprising the β2-microglobulin encoding nucleic acid sequence, particularly in approximately 3-fold excess. Item G: The method of any of the preceding items, wherein the HLA-encoding nucleic acid sequence comprises an immunoglobulin Fc fragment as a stabilizing sequence and the purification step is effected by adsorbing the recombinant HLA heavy chain polypeptides to a surface linked to protein A. Item H: The method of any of the preceding items, wherein the dissociation step is effected by treatment under acidic conditions, particularly at approximately pH 2, and dialysis under reductive conditions. Item I: The method of any of the preceding items, wherein the refolding step is effected by treatment under neutral conditions.

(61) More specifically pointed at the B57 open conformers specified herein, the method can be

summarized in the following items: Item A′: A method for producing, by methods of recombinant biotechnology, a human HLA-B57 heavy chain polypeptide, wherein said method comprises the following steps: a. Expression step: i. a HLA-B57-encoding nucleic acid sequence encoding at least the alpha 1 chain, the alpha 2 chain and the alpha 3 chain of a HLA-B57 heavy chain under control of a promoter sequence operable in a cell, particularly a eukaryotic cell, more particularly a mammalian cell, and ii. a β2-microglobulin encoding nucleic acid sequence encoding the human HLA beta 2 microglobulin (UniProt P61769) under control of a promoter sequence operable in said cell (the same cell as in item 1. a.) are co-expressed in a mammalian cell ("production cell line"); b. Purification step: the resulting HLA-B57-heavy-chain/β2-microglobulin complex is purified from the mammalian cell (the production cell line); c. Dissociation step: the purified HLA-B57-heavy-chain/β2-microglobulin complex is dissociated under suitable conditions and the HLA heavy chain polypeptides are separated from the β2-microglobulin polypeptides; d. Refolding step: the separated HLA-B57 heavy chain polypeptides are incubated under conditions leading to refolding (of their native tertiary protein structure found in physiologically active HLA open conformer molecules). Item B′: The method for producing a human HLA-B57 heavy chain polypeptide according to item A′, wherein the HLA-B57-encoding nucleic acid sequence comprises, from N to C terminus of the encoded polypeptide, the alpha 1 chain, the alpha 2 chain, the alpha 3 chain and a stabilizing sequence. Item C′: The method for producing a human HLA-B57 heavy chain polypeptide according to item B′, wherein the stabilizing sequence is selected from bovine serum albumin and an immunoglobulin constant fragment (Fc), particularly an immunoglobulin G constant fragment, more particularly an IgG4 Fc. Item D′: The method for producing a human HLA-B57 heavy chain polypeptide according to any of the preceding items, wherein the HLA-encoding nucleic acid sequence and the 62-microglobulin encoding nucleic acid sequence are present on the same nucleic acid vector molecule (particularly, a DNA expression plasmid). Item E′: The method for producing a human HLA-B57 heavy chain polypeptide according to any of the preceding items A′ to C′, wherein the HLA-encoding nucleic acid sequence and the 62-microglobulin encoding nucleic acid sequence are present on different nucleic acid vector molecules (particularly, different DNA expression plasmids). Item F′: The method of item E′, wherein the nucleic acid vector comprising the HLA-encoding nucleic acid sequence is present in approximately 1- to 5-fold excess, particularly 1.5 to 5-fold excess with respect to the nucleic acid vector comprising the 62-microglobulin encoding nucleic acid sequence, particularly in approximately 3-fold excess. Item G′: The method of any of the preceding items, wherein the HLA-B57-encoding nucleic acid sequence comprises an immunoglobulin Fc fragment as a stabilizing sequence and the purification step is effected by adsorbing the recombinant HLA heavy chain polypeptides to a surface linked to protein A. Item H′: The method of any of the preceding items, wherein the dissociation step is effected by treatment under acidic conditions, particularly at approximately pH 2, and dialysis under reductive conditions. Item I′: The method of any of the preceding items, wherein the refolding step is effected by treatment under neutral conditions.

(62) Wherever alternatives for single separable features such as, for example, an allele or coding sequence are laid out herein as "embodiments", it is to be understood that such alternatives may be combined freely to form discrete embodiments of the invention disclosed herein.

(63) The invention is further illustrated by the following examples, from which further embodiments and advantages can be drawn. These examples are meant to illustrate the invention but not to limit its scope.

Examples

(64) The inventors surprisingly found that HLA-B57 open conformers interact with different immune modulatory surface receptors present in NK, T cells, myeloid derived cells (macrophages and MDSCs), and regulate the differentiation and suppressive function of Tregs in vitro.

(65) The inventors surprisingly found that HLA-B57 open conformers, particularly when present as fusion proteins comprising an Fc immunoglobulin fragment, could be useful in cancer therapy.

HLA-B57-Fc molecules may be used alone or in combinations with other cancer therapeutics.
(66) Additionally, they discovered a novel in vivo mode of action with injections of B57.sub.2-Fc as monotherapy or combinatorial approaches using checkpoint inhibitors or agonist antibodies. B57.sub.2-Fc therapy alone or combinations can regulate the infiltration of diverse sets of leukocytes into the tumors as determined by the increased ratio of M1/M2 cells, increased infiltration of NK cells, increased CD8+ T cells/Treg ratio, and reduced infiltration of MDSCs. Overall, the mode of action of B57.sub.2-Fc alone or in a combinatorial approach with antagonistic/agonistic antibodies is of undoubted relevance in the treatment of cancer, and correlates to the current clinical need in cancer immunotherapy.
(67) HLA-B57 Fc open conformers can be used as a therapeutic to target diseases where immunomodulation is a therapeutic approach, as is the case of cancer and infectious diseases.
(68) In Vitro Tests
(69) The B57.sub.2-Fc molecule is able to modulate immune responses through blocking iTreg differentiation and negatively influencing Tregs suppression (FIG. **2**-**3**)
(70) B57.sub.2-Fc Blocks Conversion of Murine CD4.sup.+ T Cells Into iTregs
(71) The influence of HLA molecules with naïve CD4.sup.+ T cells for iTreg conversion was analysed in a dose dependent matter (μg/mL) with B57.sub.2-Fc, B57-β2m-Fc, isotype and PBS incubated with naïve CD4.sup.+ T cells in optimal culture conditions for iTreg conversion. B57.sub.2-Fc demonstrated to down modulate the induction of CD25 (FIG. **2**A, C) and FoxP3 (FIG. **2**B, D).
(72) B57.sub.2-Fc Impairs the Suppression of Mouse CD8.sup.+ T Cells by Tregs
(73) The suppressive function of murine Tregs using violet-labelled naïve CD8.sup.+ T cells as responder cells was determined (FIG. **3**). Tregs were co-cultured with B57.sub.2-Fc and controls B57-β2m-Fc, and isotype antibody, and proliferation of CD8.sup.+ T cells was measured after 96 h. CD8.sup.+ T cells alone showed strong proliferation and, as expected, Treg cells suppressed the proliferation of CD8.sup.+ T cells when incubated with controls (B57-β2m-Fc, and isotype). Strikingly, the suppressive function of Tregs was greatly impaired in the presence of B57.sub.2-Fc indicated by a strong proliferation of CD8.sup.+ T cells (FIG. **3**A). The effect of B57.sub.2-Fc was dose dependent (FIG. **3**B).
(74) B57.sub.2-Fc Impairs the Proliferation of Leukaemia T Cells
(75) We determined the effect of B57.sub.2-Fc proliferation effect in different cancer cell lines (FIG. **4**). Results demonstrated that B57.sub.2-Fc modulates the proliferation of lymphoma T cell lines, when compared to control counterpart B57-β2m-Fc or isotype IgG4, indicating its potential application to the treatment of lymphoma as a targeted therapy.
(76) B57.sub.2-Fc Binds to Immunomodulatory Receptors Expressed in Diverse Types of Leukocytes
(77) We determined if B57.sub.2-Fc interacts with specific immune regulatory receptors by enzyme-linked immunosorbent assay (ELISA). Results demonstrated that B57.sub.2-Fc interacts with KIR3DL1, KIR3DL2, KIR3DL3, LILRB1, LILRB2 and Pirb receptors in a matter different than its B57-β2m-Fc control counterparts (FIG. **5**A-D). Furthermore we compared also B27.sub.2-Fc, and B27-β2m-Fc to demonstrate if similar HLA open conformer molecules interact with same receptors but with different affinities.
(78) Production of B57 Open Conformers as a Human Fc Fusion Protein in CHO Cells
(79) A valid strategy, from a therapeutic point of view, is to produce HLA-B57 open conformer molecules in stable format (Fc fusion), to increase solubility, stability, avidity, half-life, and from a technological point of view, cost-effective production and purification in mammalian systems. B57-β2m-Fc complex was successfully produced by inserting the alpha 1, 2 and 3 domains of HLA-B57 into a human IgG4-Fc vector cassette (FIG. **6**A), together with a human-β2m vector, necessary for extracellular production of the B57-β2m-Fc protein (FIG. **6**A,B). Transfections in Chinese hamster ovary cells (CHO) cells were performed using both the B57-Fc-vector+β2m-vector at a ratio of 1:1.

Supernatants were collected and B57-β2m-Fc purified using standard antibody purification protocols (Recombinant Protein Purification Handbook, principles and methods. 2009. GE Healthcare, 18-1142-75) (FIG. **6**B). Separation of β2m from B57-Fc free-heavy chains was performed using denaturing conditions by SEC or dialysis methods. Refolding of B57.sub.2-Fc was assessed using the dilution method in refolding buffer and analysed by western blot (data not shown).

(80) Pre-Clinical Combination Therapy Tests of B57.sub.2-Fc with PD-1, PD-L1 and 4-1BB Antibodies in Diverse Syngeneic Colon Cancer Mouse Models

(81) The in vivo proof of concept study of B57.sub.2-Fc as an immunomodulatory therapeutic molecule was demonstrated in murine colon carcinoma (C38), pancreatic cancer (Pan02) and melanoma (B16-F10) syngeneic mouse models as monotherapy and in combination with PD-1, PD-L1 or 4-1 BB antibodies.

(82) For the colon carcinoma model, following established protocols C38 fragment tumours were subcutaneously injected in the flank of syngeneic mice. Once the tumour reached ≈80 mm3 (between 1-2 weeks after transplantation of tumors), mice were statistically distributed according to their tumor volume. B57.sub.2-Fc was injected i.p. seven times every 3.sup.rd day (Q3Dx7), and PD-1 injected 4 times twice a week (biwk×2) (FIG. **7**A).

(83) In colon cancer (C38) data demonstrated that the combination of B57.sub.2-Fc with PD-1 antibodies significantly reduce tumors (FIG. **7**B). Combo therapy B57.sub.2-Fc+PD-1 vs isotype control antibody strikingly reduced tumor volume (333 mm.sup.3 vs 2120 mm.sup.3, respectively $p<0.01$). Additionally, combo therapy B57.sub.2-Fc+PD-1 vs PD-1 monotherapy showed also significant tumor size reduction (333 mm.sup.3 vs 1423 mm.sup.3, respectively, $p<0.01$) (FIG. **7**B). B57.sub.2-Fc monotherapy or PD-1 monotherapy showed no differences vs. isotype control at the end of the experiment, however at day 24, B57.sub.2-Fc treated mice were significantly different from isotype (384 mm.sup.3 vs 652 mm.sup.3, $p<0.05$), as well as PD-1 vs isotype (151 mm.sup.3 vs 652 mm.sup.3, $p<0.01$), (FIG. **7**B), indicating that B57.sub.2-Fc monotherapy has also immunomodulatory effects on the tumor progression of colon cancer mice.

(84) For the pancreas (Pan02) and melanoma (B16F10) mouse models, following established protocols cells were injected at 1×10.sup.5 in the right flank of syngeneic mice respectively. Once the tumor reached ≈80 mm3 (between 1-2 weeks after injection of cells) mice were statistically distributed according to their tumor volume (FIG. **8**A, **14**A)

(85) In pancreas (Pan02) data demonstrated that B57.sub.2-Fc monotherapy and the combination with PD-1 antibodies can significantly reduce tumors (FIG. **8**B, **9**B). Combo therapy B57.sub.2-Fc+PD-1 vs. isotype control antibody demonstrated a striking significant reduction of tumor volume (216 mm.sup.3 vs. 799 mm.sup.3, respectively $p<0.0001$) (FIG. **8**B). Additionally, combo therapy B57.sub.2-Fc+PD-1 vs. PD-1 monotherapy also showed significant tumor size reduction (216 mm.sup.3 vs. 445 mm.sup.3, respectively, $p<0.01$) (FIG. **8**B). B57.sub.2-Fc monotherapy was significantly different compared to isotype (545 mm.sup.3 vs. 799 mm.sup.3, respectively, $p<0.05$). PD-1 monotherapy was significantly different compared to isotype (445 mm.sup.3 vs. 799 mm.sup.3, respectively $p<0.0001$) (FIG. **8**B).

(86) In pancreas (Pan02) B57.sub.2-Fc study in combination with PD-L1 antibodies significantly reduced tumors (FIG. **9**A-B). Combo B57.sub.2-Fc+PD-L1 vs. isotype showed significant tumor size reduction (397 mm.sup.3 vs 799 mm.sup.3, respectively, $p<0.0001$) (FIG. **9**A). PD-L1 monotherapy was significantly different compared to isotype (531 mm.sup.3 vs 799 mm.sup.3, respectively $p<0.01$) (FIG. **9**A).

(87) The tumor immune contexture of pancreas (Pan02) mice demonstrated the influence of B57.sub.2-Fc therapy towards diverse sets of tumor infiltrating leukocytes (FIG. **10**-**13**). B57.sub.2-Fc monotherapy increased the infiltration of NK cells when compared with control isotype ($p<0.05$) (FIG. **10**A), and significantly modified the Macrophage M1/M2 cell ratio ($p<0.05$) through favouring the presence of M1 type macrophages within the tumor (FIG. **11**B).

B57.sub.2-Fc combinatorial therapy with PD-1 reduced significantly the infiltration of MDSCs cells in the tumor compared to PD-1 monotherapy ($p<0.05$) (FIG. **10**C), reduced the infiltration of macrophages (FIG. **11**A) ($p<0.05$), and modified significantly the Macrophage M1/M2 ratio when compared to isotype and PD-1 monotherapy (FIG. **11**B) ($p<0.001$). B57.sub.2-Fc combinatorial therapy with PD-L1 (FIG. **12**-**13**) modified significantly the Macrophage M1/M2 cell ratio compared to isotype ($p<0.01$) and PD-L1 ($p<0.05$) (FIG. **13**E).

(88) In melanoma (B16F10) data demonstrated that B57.sub.2-Fc in combination with 4-1BB agonist antibodies can significantly reduce tumors (FIG. **14**-**15**). Combo therapy B57.sub.2-Fc+4-1BB vs. isotype control antibody showed a striking significant difference on tumor volume reduction (756 mm.sup.3 vs. 1424 mm.sup.3, respectively $p<0.0001$) (FIG. **14**B). Additionally, combo B57.sub.2-Fc+4-1BB vs. 4-1 BB monotherapy showed also significant tumor size reduction (756 mm.sup.3 vs. 1199 mm.sup.3, respectively, $p<0.01$) (FIG. **14**B). 4-1BB monotherapy was not significantly different compared to isotype (1199 mm.sup.3 vs. 1424 mm.sup.3, respectively). PD-1 mono and combinatorial therapy did not showed significance between groups (FIG. **15**A). However the triple combo therapy (B57.sub.2-Fc+4-1BB+PD-1) showed high significant difference compared to isotype ($p<0.0001$), but was not better than combo B57.sub.2-Fc+4-1 BB (FIG. **15**A-B).

(89) The tumor immune contexture of melanoma (B16F10) treated animals demonstrated the influence of B57.sub.2-Fc therapy with diverse sets of tumor infiltrating leukocytes (FIG. **16**-**17**). B57.sub.2-Fc monotherapy reduced significantly the presence of MDSCs cells inside the tumor when compared to isotype ($p<0.05$) (FIG. **16**C). B57.sub.2-Fc combinatorial therapy with 4-1BB antibodies modified significantly the presence of CD8+ T-cells vs Treg cells as measured through the ratio of CD8+ Tcell/Treg ($p<0.05$) (FIG. **16**B), reduced significantly the presence of MDSCs cells inside the tumor ($p<0.05$) (FIG. **16**C), and modified significantly the Macrophage M1/M2 cell ratio when compared to isotype and 4-1BB monotherapy ($p<0.05$) (FIG. **17**B). B57.sub.2-Fc triple combinatorial therapy with 4-1BB and PD-1 antibodies induced significantly the infiltration of NK cells into the tumor ($p<0.05$) (FIG. **16**A), strikingly modified the CD8+ Tcell/Treg ratio compared to isotype (158% vs. 23%, respectively, $p<0.0001$), and also compared to all other groups (FIG. **16**B). Furthermore, reduced significantly the presence of MDSCs cells inside the tumor ($p<0.001$) (FIG. **16**C), and modified significantly the Macrophage M1/M2 cell ratio when compared to all the other groups (FIG. **17**B).

(90) Conclusion

(91) The proof of principle for using B57.sub.2-Fc molecules to fight cancer was demonstrated using pre-clinical syngeneic mouse models of colon, pancreas and melanoma. The present data demonstrates the therapeutic potential of B57.sub.2-Fc as either monotherapy and/or combinatorial therapy with sets of checkpoint inhibitory agents and/or checkpoint agonist agents such as PD-1, PD-L1 or 4-1BB antibodies.

(92) The mode of action of B57.sub.2-Fc was also assessed in vivo in pancreas and melanoma mouse models by establishing the tumor infiltration of leukocytes. B57.sub.2-Fc therapy can regulate the infiltration of diverse sets of leukocytes into the tumors of mice as determined by the increased ratio of Macrophages M1/M2 cells, reduced infiltration of MDSCs, increased infiltration ratio of CD8+ T cells/Treg ratio, and increased infiltration of NK cells. Overall, the mode of action of B57.sub.2-Fc alone or in a combinatorial approach with antagonistic/agonistic antibodies is of undoubted relevance in the treatment of cancer, and correlates to the current clinical need in cancer immunotherapy.

(93) B57.sub.2-Fc emerges as a novel class of immunomodulatory drug. In vitro and in vivo data points to a mechanism were B57.sub.2-Fc molecules act as a switch-on mechanism for the activation of anti-tumor immunity. Without wishing to be bound by theory, the inventors hypothesize that the interaction of HLA-B57 open conformers bind to diverse immunomodulatory receptors present in myeloid cells (Macrophages, MDSCs), T cells and NK cells participate

synergistically and exacerbate the immune response.

Materials and Methods

(94) Animals and Cell Lines

(95) In vivo experiments were conducted in C57Bl/6 mice using the mouse derived colon carcinoma C38 cell line, the pancreatic ductal adenocarcinoma Pan02 mouse cell line; and melanoma B16F10 mouse cell line.

(96) In vitro experiment cell lines: EG.7, mouse T cell lymphoma; Jurkat, human T cell lymphoma; L428, human Hodgkin lymphoma; L540, human Hodgkin lymphoma; L1236, human Hodgkin lymphoma; Daudi, B cell lymphoma; IMR-5, neuroblastoma; SK-N-AS, neuroblastoma; and M130428, Melanoma.

(97) In Vivo Treatments

(98) C38 tumour fragments were injected subcutaneously into the right flanks of syngeneic female C57BL/6 mice at week 6. Pan02 and B16F10 cell lines were injected at 1×10.sup.5 in the right flank of syngeneic mice at week 6. Once the tumour reached ±80 mm.sup.3 in colon (C38), pancreas (Pan02) and melanoma (B16F10), animals were distributed according to their individual tumour volume size and divided into groups displaying no statistical differences between them. Tumour diameters were measured using a caliper, and volume was calculated according to the formula, D/2×d.sup.2 where D and dare the longest and shortest diameter of the tumour in mm, respectively.

(99) The Experimental design of injection time points of cells and injection of substances was established as follows for colon (C38) vehicle (PBS 200 μL); isotype (10 mg/Kg) Q3Dx7; B57.sub.2-Fc (10 mg/Kg); PD-1 biwk×2 (200 μg); B57.sub.2-Fc+PD-1 (Q3Dx7 and biwk×2, respectively), B27.sub.2-Fc+PD-1 (Q3Dx7 and biwk×2, respectively). For pancreas (Pan02) the experimental design of injection of substances was as follow: isotype (5 mg/Kg) biwk×3; B57.sub.2-Fc (5 mg/Kg) biwk×3; PD-1 biwk×3 (5 mg/Kg); PD-L1 biwk×3 (5 mg/Kg); B57.sub.2-Fc+PD-1 (biwk×3) and B57.sub.2-Fc+PD-L1 (biwk×3). For melanoma (B16F10) the experimental design of injection of substances was as follow: isotype (5 mg/Kg) biwk 3 injections; B57.sub.2-Fc (5 mg/Kg) biwk 3 injections; 4-1 BB antibody (1 mg/Kg) biwk 3 injections; PD-1 biwk 3 injections (5 mg/Kg); B57.sub.2-Fc+4-1 BB biwk 3 injections, B57.sub.2-Fc+PD-1 biwk 3 injections, PD-1+4-1 BB biwk 3 injections, and B57.sub.2-Fc+4-1 BB+PD-1 biwk 3 injections.

(100) Preparation of tumor samples for flow cytometry were performed using protocols described by eBioscience (https://www.ebioscience.com/media/pdf/best-protocols/cell-preparation-for-flow-cytometry.pdf, accessed Feb. 21, 2017).

(101) Antibodies

(102) Leukocytes mouse populations for in vitro tests were stained with: CD3 (PE-Cy7-eBioscience), CD4 (FITC-BD Bioscience), FoxP3+ (efluor 450-eBioscience), CD45 (PerCP-eBioscience), CD3 (PE-eBioscience), NK1.1 (BV421-eBioscience), CD11b (FITC-eBioscience), CD11c (FITC-eBioscience), CD25 (PE-Cy7-Biolegend).

(103) HC10 mAb (IgG2a) binding to β2m-free heavy chains of HLA-B and -C alleles and so to B57.sub.2 was a gift from Dr. Hidde Ploegh (MIT, MA).

(104) Flow cytometry antibodies from tumor samples were stained with: CD45 (FITC; clone 30-F11; Biolegend), CD3 (PerCP/Cy5.5; clone 17A2; Biolegend), CD4 (BV510; clone GK1.5; Biolegend), CD8 (APC-H7; clone 53-6.7; BD), FoxP3 (PE; clone FJK-16S; eBioscence), CD11b (BV650; clone M1/70; Biolegend), F4/80 (PE/Cy7; clone BM8; Biolegend), Gr-1 (APC-R700; clone RB6-8C5; BD), NK1.1 (BV605; clone PK136; Biolegend), CD206 (APC; clone C068C2; Biolegend), CD86 (BV421; clone GL-1; Biolegend), L/D stain (BUV395; Invitrogen).

(105) Checkpoint inhibitor antibody anti-mouse PD-1 clone RMP1-14 was obtained from Bio X Cell. Checkpoint inhibitor antibody anti-mouse PD-L1 clone: 10F.9G2 was obtained from Bio X Cell. Agonistic antibody anti-mouse 4-1 BB clone 3H3 was obtained from Bio X Cell.

(106) Flow Cytometry of Leukocytes

(107) Flow cytometry analysis was performed using a FACS canto II (BD Bioscience) and data were analysed using FlowJo version 7.6.4.
(108) Generation of Tregs
(109) To induce expression of Foxp3 in murine CD4.sup.+ T cells, we harvested spleen cells from C57BL/6 splenocytes and purified ((Mouse Naïve CD4.sup.+ T Cell Isolation Kit—Easy Sep) to obtain CD4.sup.+ T naive cells. Cells were then cultured for 96 h at 10.sup.5 cells/200 μL/well in 96-well plates with coated 5 μg/mL anti-CD3mAb (eBioscience), soluble 2 μg/mL anti-CD28 mAb (Biolegend), 10 μg/mL of TGF-β1 (R&D systems) and 100 IU/mL of IL-2 (R&D systems).
(110) iTreg Conversion in the Presence of B57.sub.2-Fc
(111) Murine naive CD4.sup.+ T cells in optimal culture conditions for iTreg conversion were incubated in the presence of different dose concentrations (μg/200 μL) of B57.sub.2-Fc, B57-β2m-Fc, B27-β2m-Fc, Isotype IgG4 and PBS for 72 h. iTreg conversion was measured by flow cytometry.
(112) Suppression Assay
(113) CD4.sup.+ or CD8.sup.+ T-effector cells were purified PBMCs from either mouse or human (Mouse Naïve CD4.sup.+ T Cell Isolation Kit—Easy Sep; Dynabeads® FlowComp™ Mouse CD8 —life technologies; Dynabeads® CD8 human—Life Technologies) and labelled with 10 μM cell trace violet proliferation stain (Molecular Probes). Tregs (2.5×10.sup.4) cells and T-effector cells (2.5×10.sup.4) were cultured in 96 wells U-bottomed plates with coated CD3 (eBioscience) (3 μg/mL) and soluble CD28 (eBioscience) (1 μg/mL) antibody for 96 hrs. Proliferation of T-effector cells was measured using a FACS canto II and data were analysed using proliferation analysis software from FlowJo version 7.6.4.
(114) Proliferation Assay
(115) Cells were plated in round 96-wells plates at a density of 5×10.sup.5 cells/well following the addition of drugs at different concentrations (10, 5, and 2 μg/well) for 1 day. XTT proliferation assay was performed accordingly to the manual instructions (cell proliferation kit II, Roche). Results were obtained with the absorbance of wells at 450 nm using a microtiter plate reader.
(116) ELISA Assays
(117) Competition ELISA assays were performed using Maxisorp (Nunc, Switzerland) 96 well plates coated with 10 μg/mL of selected recombinant leukocyte receptors (human KIR3DL1, human KIR3DL2, human KIR3DL3, human LILRB1, human LILRB2, and mouse Pirb). Receptors were incubated for ON 4° C., blocked with 5% milk powder-PBS 2 hrs. B57.sub.2-Fc, B57-β2m-Fc, B27.sub.2-Fc, B27-β2m-Fc, and isotype IgG4 were added at 2 μg/mL for 2 hrs RT. HRP-conjugated antibodies against human Fc were used as detectors.
(118) Production, Purification and Re-Folding of B57.sub.2-Fc
(119) Recombinant production of B57-β2m-Fc was achieved by inserting the alpha 1, 2 and 3 domains of HLA-B57 into a human IgG4-Fc vector, and the human β2-microglobulin (β2m) in a separate vector. Production of recombinant B57-β2m-Fc was performed by co-transfection of B57-Fc-vector and β2m-vector into Chinese hamster ovary (CHO) cells. Production of B57-β2m-Fc was outsourced to Evitria AG.
(120) Purification of B57-β2m-Fc was performed using conventional protocols for antibody purification. Production of B57.sub.2-Fc was performed with the addition of a denaturing step to remove β2m from the B57-β2m-Fc complex.
(121) Briefly, the capture step of B57-β2m-Fc was performed after running supernatants (5 mL/min) through protein-G columns (Amersham Pharmacia). Intermediate purification steps were performed by eluting the B57-β2m-Fc from protein G-columns using elution buffer (100 mM glycine, pH 2.0), and recovering fractions in 8M Urea, 100 mM Tris-HCl pH 8.0. The 1.sup.st Polishing step was to separate B57-Fc monomers fractions from β2m by either size exclusion chromatography (SEC) using superdex 200 prep grade or Sephacryl S-100 HR (GE Lifescience) with an ÄKTA system (GE Lifescience), or by dialysis with membranes of 30 KDa or 50 KDa pore

size (Millipore). The recovered B57-Fc monomers from both protocols were re-folded by the dilution method after pulsation of the B57-Fc monomers at 3 times with intervals of 8 hours each in 100 times volume of refolding buffer (50 mM Tris-HCl pH 8.5, 500 mM L-Arginine, 1 mM EDTA, 0.15 mM NaCl, 1% Sucrose, 0.01% Tween-20). The 2.sup.nd Polishing step by SEC was performed to remove further impurities and to buffer exchange newly recovered fractions of B57.sub.2-Fc molecules into dilution buffer (PBS, 1% Sucrose, and 0.01% Tween-20). Purified solutions of B57.sub.2-Fc were filter sterilized using 0.2 μm membranes (Millipore).

(122) Fractions B57-β2m-Fc complexes and B57.sub.2-Fc were analysed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and western blot using HC10 (specific for HLA-free-heavy chains) antibodies. β2m western blots were performed with and without denaturing conditions (10 mM DTT) (data not shown).

(123) Full and Partial Sequences of HLA-B57 Alleles

(124) Functional domains of the full length HLA-B57 alpha chain from N-terminus to C-terminus are: Signal peptide, alpha 1, alpha 2, alpha 3, transmembrane domain and cytoplasmic tail.

(125) TABLE-US-00001 TABLE 1 HLA-B57 alleles Sequence identifier (length in aa) Amino acid sequence B*57:01:01
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA00381
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362
aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADT
AAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA
VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG
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aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 002) B*57:01:03
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aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 003) B*57:01:04
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aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADT
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HLA11726
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (298
aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADT
AAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA
VVVPSGEEQRYTCHVQHEGLPKPLTLRW (SEQ ID 019) B*57:01:20
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA12568
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 020) B*57:01:21
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA12884
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LRW
(SEQ ID 021) B*57:01:22
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA13005
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 022) B*57:02:01
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA00382
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362
aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHNQYAYDGKDYIALNEDLSSWTAADT
AAQITQRKWEAARVAEQRRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA
VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG

AVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA (SEQ ID 023) B*57:02:02
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA04435
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQRRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 024) B*57:03:01
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA00383
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362
aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHNQYAYDGKDYIALNEDLSSWTAADT
AAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA
VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG
AVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA (SEQ ID 025) B*57:03:02
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA01289
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273
aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LRW
(SEQ ID 026) B*57:04:01
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA00384
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273
aa) LRGYDQDAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQRRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LRW
(SEQ ID 027) B*57:04:02
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA14153
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGYDQDAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQRRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 028) B*57:05
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA00385
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362
aa) YNQSEAGSHIIQRMYGCDLGPDGRLLRGYNQYAYDGKDYIALNEDLSSWTAADT
AAQITQRKWEAARVAEQRRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA
VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG
AVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA (SEQ ID 029) B*57:06
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA01074
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362
aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADT
AAQIIQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA
VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG
AVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA (SEQ ID 030) B*57:07
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE

HLA01192
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVESLRRYLENGKETLQRA (SEQ ID 031) B*57:08
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA01461
GPEYWDGETRNMKASAQTYRENLRIALPYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 032) B*57:09
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA01485
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQDRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 033) B*57:10
SHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA02307
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 034) B*57:11
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA02676
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362 aa) YNQSEAGSHTLQWMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADT AAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG AVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA (SEQ ID 035) B*57:12
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA02888
GPEYWDGETRNMKASAQTYRESLRNLRGYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQRRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 036) B*57:13
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA02966
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGE CVEWLRRYLENGKETLQRA (SEQ ID 037) B*57:14:01
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03129
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRHLENGKETLQRA (SEQ ID 038) B*57:14:02
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA12293
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRHLENGKETLQRA (SEQ ID 039) B*57:15
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03147
GPEYWDGETRNVKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181

aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 040) B*57:16
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMEPRAPWIEQE
HLA03150
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 041) B*57:17
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03320
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHNQYAYDGKDYIDLNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 042) B*57:18
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03506
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAAYTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 043) B*57:19
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03507
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQRRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 044) B*57:20
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03666
GPEYWDGKTRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 045) B*57:21
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03675
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHVIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 046) B*57:22
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03904
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 047) B*57:23
SHSMRYFYTAMSRPGRGESRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA04046
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 048) B*57:24
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03984
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQDRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 049) B*57:25
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA03986
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181

aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGE
CVEWLRRYLENGKETLQRA (SEQ ID 050) B*57:26
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA04203
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGT
CVEWLRRYLENGKETLQRA (SEQ ID 051) B*57:27
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA04452
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEHLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 052) B*57:28N
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA04401
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (115
aa) LRGHNQX (SEQ ID 053) B*57:29
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA04576
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362
aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADT
AAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
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VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG
AVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA (SEQ ID 054) B*57:30
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA04703
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARAAEQRRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 055) B*57:31
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA04848
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLRSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 056) B*57:32
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA05424
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGYHQDAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 057) B*57:33
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA05476
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDERL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 058) B*57:34
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASRRMAPRAPWIEQE
HLA05503
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 059) B*57:35
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE

HLA05513
GPKYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 060) B*57:36
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA05562
GPEYWDGETRHMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 061) B*57:37
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA05876
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKHLT LRW (SEQ ID 062) B*57:38
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA05958
GPEYWDGETRNMKASAQTYRETLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 063) B*57:39
SHSMRYFHTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06229
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 064) B*57:40
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06240
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHNIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 065) B*57:41
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06241
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGYDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 066) B*57:42
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06249
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEEARVAEQRRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 067) B*57:43
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06250
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGP CVEWLRRYLENGKETLQRA (SEQ ID 068) B*57:44
SHSMRYFYTAMSRPGLGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06315
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL

CVEWLRRYLENGKETLQRA (SEQ ID 069) B*57:45
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aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 070) B*57:46
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06688
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAACVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 071) B*57:47
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06700
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSCWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 072) B*57:48
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06883
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGPL (273
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LRW
(SEQ ID 073) B*57:49
SHSMRYFDTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06942
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 074) B*57:50
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06949
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQGKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 075) B*57:51
SHSMRYFHTAMSRPGRGEPRFITVGYVDDTLFVRFDSDATSPRKEPRAPWIEQE HLA06974
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 076) B*57:52
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA06989
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRASLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 077) B*57:53
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA07455
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSTYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 078) B*57:54
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA07456
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADKAAQITQRKWEAARVAEQLRAYLEGL

CVEWLRRYLENGKETLQRA (SEQ ID 079) B*57:55
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA07545
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTKLVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LRW
(SEQ ID 080) B*57:56
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAHRAPWIEQE
HLA07708
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 081) B*57:57
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA07748
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273
aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQLRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LRW
(SEQ ID 082) B*57:58
SHSMRYFYTAMSRPGRGEPRFISVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA08073
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 083) B*57:59
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA08294
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQISQRKLEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LR
(SEQ ID 084) B*57:60
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA08371
GPEYWDGETRNMKASAQTYRESLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 085) B*57:61
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA08927
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLGGL
CVEWLRRYLENGKETLQRA (SEQ ID 086) B*57:62
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA08997
GPEYWDGEKRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 087) B*57:63
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA09303
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181

aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAAREAEQRRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 088) B*57:64
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA09312
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAACVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 089) B*57:65
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA09577
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAVRVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 090) B*57:66
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA09909
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHNQYAYDGKDYIALNEDLSSRTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 091) B*57:67:01
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA10038
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDLGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 092) B*57:67:02
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA14152
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDLGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 093) B*57:68
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA10040
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITKRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 094) B*57:69
SHSMRYFYTAMSRPGRGEPRFITVGYVDDTLFVRFDSDATSPRKEPRAPWIEQE HLA10408
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRA (SEQ ID 095) B*57:70
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA11328
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (18
1aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVECLRRYLENGKETLQRA (SEQ ID 096) B*57:71
SHSMRYFYTAMSRPGRGEPRFITVGYVDDTQFVRFDSDATSPRMAPRAPWIEQE
HLA11950
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (273
aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL
CVEWLRRYLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTW
QRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLT LRW
(SEQ ID 097) B*57:72
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA12010

GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVADQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 098) B*57:73
SHSMRYFHTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA12263
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 099) B*57:74
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA12294
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSYTIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 100) B*57:75
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRATWIEQE
HLA13002
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 101) B*57:76
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA13004
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQFAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 102) B*57:77
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA13480
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGLL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 103) B*57:78
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPWAPWIEQE
HLA13379
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 104) B*57:79N
MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA13633
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (296 aa) YNQSEAGSHIIQVMYGCDVGPDGRLLRGHDQSAYDGKDYIALNEDLSSWTAADT AAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA VVVPSGEEQRYTCHVQHEGLPNPSPX (SEQ ID 105) B*57:80
SHSMRYFYTAMSRPGRGEPRFISVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA14154
GPEYWDGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 106) B*57:81
SHSMRYFYTAMSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMAPRAPWIEQE
HLA14308
GPEYWEGETRNMKASAQTYRENLRIALRYYNQSEAGSHIIQVMYGCDVGPDGRL (181 aa) LRGHDQSAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAARVAEQLRAYLEGL CVEWLRRYLENGKETLQRA (SEQ ID 107) B*57:82

MRVTAPRTVLLLLWGAVALTETWAGSHSMRYFYTAMSRPGRGEPRFIAVGYVDD
HLA14207
TQFVRFDSDAASPRMAPRAPWIEQEGPEYWDGETRNMKASAQTYRENLRIALRY (362
aa) YNQSEAGSHTLQRMYGCDVGPDGRLLRGHNQYAYDGKDYIALNEDLSSWTAADT
AAQITQRKWEAARVAEQLRAYLEGLCVEWLRRYLENGKETLQRADPPKTHVTHH
PISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAA
VVVPSGEEQRYTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIG
AVVAAVMCRRKSSGGKGGSYSQAACSDSAQGSDVSLTA (SEQ ID 108)

Claims

1. An isolated Human Leukocyte Antigen-B57 (HLA-B57) fusion protein, comprising: a first and a second monomer, and wherein each monomer independently of the other monomer comprises: a HLA-B57 heavy chain, an Fc (crystallizable fragment) domain polypeptide sequence, and optionally, an amino acid linker joining the HLA-B57 heavy chain and the Fc domain, wherein the HLA-B57 chain comprises ≥95% sequence identity to SEQ ID NO: 7.
2. The isolated HLA-B57 fusion protein of claim 1, wherein the first and the second monomer are the same.
3. The isolated HLA-B57 fusion protein of claim 1, wherein the Fc domain comprises heavy chain constant regions C.sub.H2 and C.sub.H3 selected from the group consisting of immunoglobulin type G (IgG), type A (IgA), type D (IgD), type E (IgE) and type M (IgM).
4. The isolated HLA-B57 fusion protein of claim 1, wherein the amino acid linker comprises 1 to 50 amino acids linking the HLA-B57 chain to the Fc domain as one single polypeptide chain.
5. The isolated HLA-B57 fusion protein of claim 1, wherein the HLA-B57 chain comprises ≥97% sequence identity to SEQ ID NO: 7.
6. The isolated HLA-B57 fusion protein of claim 1, wherein the HLA-B57 chain comprises ≥98% sequence identity to SEQ ID NO: 7.