(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 May 2018 (17.05.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/087143 A2

(51) International Patent Classification:
Not classified

(21) International Application Number:
PCT/EP2017/078595

(22) International Filing Date:
08 November 2017 (08.11.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1618833.6 08 November 2016 (08.11.2016) GB

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2018/087143 A2

(54) Title: ANTI-PD-1 ANTIBODIES

(57) Abstract: The present invention relates to antibodies that bind to PD-1. The invention also relates to immunoconjugates and compositions comprising such antibodies. The invention also provides methods of producing such antibodies. The invention further provides the use of such antibodies for therapeutic and diagnostic purposes.

Anti-PD-1 antibodies

This invention relates generally to the field of antibodies, in particular antibodies that bind to PD-1. Such anti-PD-1 antibodies have therapeutic and diagnostic uses, such as the treatment of cancer. Antibody-based compositions and methods and uses of the invention also extend to the use of immunoconjugates and other therapeutic combinations, kits and methods.

The treatment of cancer is still one of the biggest unmet medical needs to date. While there have been advances in cancer therapy during the last decades, cancer remains one of the leading causes of death. As the populations in the industrialized countries are benefitting from longer average life expectancies, the urgency for improved or new cancer therapies is increasing.

A relatively new approach is the field of checkpoint immunotherapy. This approach is in large parts derived from the recently gained knowledge that a cancer is much more diverse than initially expected. Even in one patient, a single tumour will contain dozens if not hundreds of different cell types. This limits the approach of cytotoxic agents used in cancer therapy such as platinum based agents (e.g. cisplatin) or nucleotide analogs (e.g. fluorouracil), as each cell type will react slightly different to the cytotoxic agent. Some cells which are more resistant are likely to survive, causing a relapse.

In contrast, the immune system is much more stable and alike between patients and it is known that for a tumour to grow it is necessary that the immune system is made tolerant to the tumour (escape mechanism). In recent years a wealth of data has been generated demonstrating how the tumour achieves this, and targeting those interactions between tumour and immune system is proven to be a viable approach of cancer therapy. The idea is not to kill the tumour directly with drugs, but rather to activate the immune system to eradicate the tumour.

A well-researched area in the fields of immunotherapy is the interaction of PD-1 (programmed cell death protein 1) and PD-L1 (programmed cell death ligand 1).

The molecules and their interaction were first identified in the context of autoimmune diseases. PD-1 is a molecule of the immunoglobulin superfamily and strongly expressed on the surface of a variety of T-cells and proB-cells. PD-L1 is

expressed on mRNA level in most healthy cells, but under tight post translational control; this means that in most healthy tissues PD-L1 will not be found on the cell surfaces. This repression is lifted by the signal molecules like IFN-gamma (IFNγ) which is typically found on sites of inflammation. The presence of the interferon triggers the expression of PD-L1 on the surface of the cells, which then interact with PD-1 expressing T-cells, thereby sending a deactivation signal. The physiological function is therefore to downregulate inflammation in order to prevent unwanted tissue damage and permanent inflammation.

It has been shown that the same mechanism is crucial for deactivating the immune reaction to tumours in a mechanism often referred to as adaptive resistance. A growing tumour is typically recognized by the immune system. As a result a large amount of immune cells are attracted to the site of the tumour, creating a very special microenvironment. Most recruited cells (Teffs, TIL) are initially active, secreting stimulatory signals including IFN-g, creating an environment very much alike inflammatory sites. This activates the expression of PD-L1 on the surface of tumour cells, shutting down the effector T-cells in and around the tumour by inducing anergy, exhaustion or apoptosis and by inducing signals which are downregulating (IL-10). The idea of a therapeutic agent targeting PD-1 is to remove this block; if done properly, the effector T-cells should regain their activity and start attacking the tumour.

This immune escape mechanism has been found in many different tumours of different lineages, including non-small cell lung cancer (NSCLC), melanoma, renal cell cancer (RCC), colorectal cancer, urothelial bladder cancer and many more.

As a result of these findings drugs inhibiting the PD-1/PD-L1 interaction were developed for cancer therapy. For reasons of affinity and stability, antibodies appear to be the molecule class of choice, and currently two antibodies targeting PD-1 are approved by the FDA for use in the US, namely Nivolumab (Opdivo, Bristol-Myers Squibb) and pembrolizumab (Keytruda, Merck).

Nivolumab is approved as first line treatment in combination with ipilimumab for treatment of BRAF negative metastatic melanoma, and as second line treatment for squamous NSCLC and RCC not reactive to ipilimumb alone.

Keytruda is approved as second line treatment for NSCLC, metastatic melanoma and has been granted accelerated approval as second line treatment for recurrent or metastatic head and neck squamous cell cancer.

Many more clinical trials are currently in progress and the list of cancers being treated with PD-1/PD-L1 is expected to expand vastly.

While both antibodies target the same molecule (PD-1), the clinical studies do show differences, which is likely due to different affinities and pharmacodynamics. This means that there is still a need to identify more compounds targeting this interaction and to compare their effect for the large number of cancers treatable. This is highlighted by a significant number of compounds still in development e.g. PDR001 (Novartis), JS001 (JunShi Biosciences).

The present inventors have provided anti-PD1 antibodies that are able to inhibit the PD-1/PD-L1 interaction.

In one aspect, the present invention provides an antibody, for example an isolated antibody, that binds to PD-1 and that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5 or a sequence substantially homologous thereto,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6 or a sequence substantially homologous thereto, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7 or a sequence substantially homologous thereto; and/or

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:8 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

In a preferred embodiment, the present invention provides an antibody that binds to PD-1 and that comprises at least one heavy chain variable region that

comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5 or a sequence substantially homologous thereto,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6 or a sequence substantially homologous thereto, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7 or a sequence substantially homologous thereto; and

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:8 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

In a preferred embodiment the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5 or a sequence substantially homologous thereto,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6 or a sequence substantially homologous thereto, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

In a particularly preferred embodiment the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs

and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7.

In one embodiment, the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:8 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

In a preferred embodiment, the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:8,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10.

In one embodiment, the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs (having a sequence as defined elsewhere herein) and at least one light chain variable region that comprises three CDRs, wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:26 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

In a preferred embodiment, the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:26,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10.

In one embodiment, the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs (having a sequence as defined elsewhere herein) and at least one light chain variable region that comprises three CDRs, wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:62 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:63 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:64 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

In a preferred embodiment, the invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:62,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:63, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:64.

In a particularly preferred embodiment, the present invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7; and

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:8,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10.

In another particularly preferred embodiment, the present invention provides an antibody that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7; and

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:26,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10.

In another particularly preferred embodiment, the present invention provides an antibody that comprises at least one heavy chain variable region that comprises

three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7; and

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:62,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:63, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:64.

Certain preferred embodiments of the invention provide an antibody comprising a VH domain that has the amino acid sequence of SEQ ID NO: 3 or 21 or 39 or 57, or a sequence substantially homologous thereto, and/or a VL domain that has the amino acid sequence of SEQ ID NO: 4 or 22 or 40 or 58, or a sequence substantially homologous thereto.

Further preferred embodiments provide an antibody comprising a VH domain that has the amino acid sequence of SEQ ID NO: 3 or 21 or 39 or 57 and a VL domain that comprises 3 light chain CDRs. Preferably said light chain CDRs have SEQ ID NOs 8, 9 and 10; or 26, 9 and 10; or 62, 63 and 64.

Further preferred embodiments provide an antibody comprising a VL domain that has the amino acid sequence of SEQ ID NO: 4 or 22 or 40 or 58 and a VH domain that comprises 3 heavy chain CDRs. Preferably said heavy chain CDRs have SEQ ID NOs 5, 6 and 7.

In one embodiment the invention provides an antibody comprising a VH domain that has the amino acid sequence of SEQ ID NO: 3 or a sequence substantially homologous thereto, and/or a VL domain that has the amino acid sequence of SEQ ID NO: 4 or a sequence substantially homologous thereto.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:4, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%) and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:3, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:4 and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:3.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:4 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:3.

In one embodiment the invention provides an antibody comprising a VH domain that has the amino acid sequence of SEQ ID NO: 21 or a sequence substantially homologous thereto, and/or a VL domain that has the amino acid sequence of SEQ ID NO: 22 or a sequence substantially homologous thereto.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:22, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%) and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:21, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:22 and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:21.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:22 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:21.

In one embodiment the invention provides an antibody comprising a VH domain that has the amino acid sequence of SEQ ID NO: 39 or a sequence substantially homologous thereto, and/or a VL domain that has the amino acid sequence of SEQ ID NO: 40 or a sequence substantially homologous thereto.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:40, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%) and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:39, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:40 and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:39.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:40 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:39.

In one embodiment the invention provides an antibody comprising a VH domain that has the amino acid sequence of SEQ ID NO: 57 or a sequence substantially homologous thereto, and/or a VL domain that has the amino acid sequence of SEQ ID NO: 58 or a sequence substantially homologous thereto.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:58, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%) and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:57, or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:58 and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:57.

In a preferred embodiment, the present invention provides an antibody, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:58 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:57.

Other preferred embodiments are IgG forms of the 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) and 273_C01_A12 antibodies, preferably full length IgG forms. The IgG_2 form of any of these antibodies is most preferred. It is of course understood that full IgG antibodies will typically comprise two substantially identical heavy chains and two substantially identical light chains.

A preferred embodiment of the invention is a full length IgG antibody which comprises a heavy chain of SEQ ID NO: 85 (amino acid) or a sequence substantially homologous thereto and/or a light chain of SEQ ID NO: 86 (amino acid) or a sequence substantially homologous thereto. Also preferred is an IgG antibody which comprises a heavy chain encoded by SEQ ID NO: 87 or a sequence

substantially homologous thereto and/or a light chain encoded by SEQ ID NO: 88 or a sequence substantially homologous thereto.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 85 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%), and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 86 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 85, and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 86.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 85, and a light chain that comprises the amino acid sequence of SEQ ID NO: 86.

A preferred embodiment of the invention is a full length IgG antibody which comprises a heavy chain of SEQ ID NO: 89 (amino acid) or a sequence substantially homologous thereto and/or a light chain of SEQ ID NO: 90 (amino acid) or a sequence substantially homologous thereto. Also preferred is an IgG antibody which comprises a heavy chain encoded by SEQ ID NO: 91 or a sequence substantially homologous thereto and/or a light chain encoded by SEQ ID NO: 92 or a sequence substantially homologous thereto.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 89 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%), and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 90 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 89, and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 90.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 89, and a light chain that comprises the amino acid sequence of SEQ ID NO: 90.

A preferred embodiment of the invention is a full length IgG antibody which comprises a heavy chain of SEQ ID NO: 93 (amino acid) or a sequence substantially homologous thereto and/or a light chain of SEQ ID NO: 94 (amino acid) or a sequence substantially homologous thereto. Also preferred is an IgG

antibody which comprises a heavy chain encoded by SEQ ID NO: 95 or a sequence substantially homologous thereto and/or a light chain encoded by SEQ ID NO: 96 or a sequence substantially homologous thereto.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 93 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%), and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 94 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 93, and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 94.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 93, and a light chain that comprises the amino acid sequence of SEQ ID NO: 94.

A preferred embodiment of the invention is a full length IgG antibody which comprises a heavy chain of SEQ ID NO: 97 (amino acid) or a sequence substantially homologous thereto and/or a light chain of SEQ ID NO: 98 (amino acid) or a sequence substantially homologous thereto. Also preferred is an IgG antibody which comprises a heavy chain encoded by SEQ ID NO: 99 or a sequence substantially homologous thereto and/or a light chain encoded by SEQ ID NO: 100 or a sequence substantially homologous thereto.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 97 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%), and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 98 or a sequence having at least 80% sequence identity thereto (e.g. at least 85%, 90%, 95% or 98%).

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 97, and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 98.

In a preferred embodiment, an antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 97, and a light chain that comprises the amino acid sequence of SEQ ID NO: 98.

Antibodies based on the 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) and 273_C01_A12 antibody sequences set forth in Tables A, B, C and D are preferred. In some embodiments, antibodies

based on the 273_C12_C05 (variant 1) antibody sequences set forth in Table B are preferred. 273_C12_C05 (original clone) is also referred to herein simply as 273_C12_C05.

The invention is exemplified by monoclonal antibodies 1h07 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) and 273_C01_A12, sequences of which are shown in Tables A, B, C and D herein. The CDR domains, VH and VL domains, and IgGs (heavy and light chains) are shown in Tables A, B, C and D herein. Antibodies comprising these CDR domains or VH and VL domains or IgG sequences (or sequences substantially homologous thereto) are preferred aspects of the invention.

Certain examples of substantially homologous sequences are sequences that have at least 65% identity to the amino acid sequences disclosed. In certain embodiments, the antibodies of the invention comprise at least one light chain variable region that includes an amino acid sequence region of at least about 65%, 70% or 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% or 95% and most preferably at least about 97%, 98% or 99% amino acid sequence identity to the amino acid sequence of SEQ ID NO:4 or 22 or 40 or 58; and/or at least one heavy chain variable region that includes an amino acid sequence region of at least about 65%, 70% or 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90% or 95% and most preferably at least about 97%, 98% or 99% amino acid sequence identity to the amino acid sequence of SEQ ID NO:3 or 21 or 39 or 57.

Other preferred examples of substantially homologous sequences are sequences containing conservative amino acid substitutions of the amino acid sequences disclosed.

Other preferred examples of substantially homologous sequences are sequences containing 1, 2 or 3, preferably 1 or 2 (more preferably 1), altered amino acids in one or more of the CDR regions disclosed. Such alterations might be conserved or non-conserved amino acid substitutions, or a mixture thereof.

In such embodiments, preferred alterations are conservative amino acid substitutions.

In another aspect, the present invention provides an antibody, for example an isolated antibody, that binds to PD-1 and that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5 or a sequence substantially homologous thereto,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6 or a sequence substantially homologous thereto, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7 or a sequence substantially homologous thereto; and/or (preferably "and") wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:26 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

Preferred embodiments of this aspect of the invention include antibodies comprising one or more of the antibody sequences (e.g. CDR sequences and/or VH domain and/or VL domain sequences and/or IgG heavy and light chain sequences) that are described elsewhere herein in connection with other aspects of the present invention. Thus, discussion of various features of the antibodies of other aspects of the invention and preferred embodiments apply *mutatis mutandis* to this aspect of the invention.

In another aspect, the present invention provides an antibody, for example an isolated antibody, that binds to PD-1 and that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5 or a sequence substantially homologous thereto,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6 or a sequence substantially homologous thereto, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7 or a sequence substantially homologous thereto; and/or (preferably "and") wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:62 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:63 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:64 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

Preferred embodiments of this aspect of the invention include antibodies comprising one or more of the antibody sequences (e.g. CDR sequences and/or VH domain and/or VL domain sequences and/or IgG heavy and light chain sequences) that are described elsewhere herein in connection with other aspects of the present invention. Thus, discussion of various features of the antibodies of other aspects of the invention and preferred embodiments apply *mutatis mutandis* to this aspect of the invention.

In all embodiments, antibodies containing substantially homologous sequences retain the ability to bind to PD-1. Preferably, antibodies containing substantially homologous sequences retain one or more (preferably all) of the properties described in relation to the 273_C12_C05 (original clone) and/or 273_C12_C05 (variant 1) and/or 273_C12_C05 (variant 2) and/or 273_C01_A12 antibodies.

Further examples of substantially homologous amino acid sequences in accordance with the present invention are described elsewhere herein.

The CDRs of the antibodies of the invention are preferably separated by appropriate framework regions such as those found in naturally occurring antibodies and/or effective engineered antibodies. Thus, the V_H, V_L and individual CDR sequences of the invention are preferably provided within or incorporated into an appropriate framework or scaffold to enable antigen binding. Such framework sequences or regions may correspond to naturally occurring framework regions, FR1, FR2, FR3 and/or FR4, as appropriate to form an appropriate scaffold, or may correspond to consensus framework regions, for example identified by comparing various naturally occurring framework regions. Alternatively, non-antibody scaffolds or frameworks, e.g. T cell receptor frameworks can be used.

Appropriate sequences that can be used for framework regions are well known and documented in the art and any of these may be used. Preferred sequences for framework regions are one or more of the framework regions making up the V_H and/or V_L domains of the invention, i.e. one or more of the framework regions of the 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12 antibodies, as disclosed in Tables A, B, C, D and E, or framework regions substantially homologous thereto, and in particular framework regions that allow the maintenance of antigen specificity, for example framework regions that result in substantially the same or the same 3D structure of the antibody.

In certain preferred embodiments, all four of the variable light chain (SEQ ID NOs:15, 16, 17 and 18) and/or variable heavy chain (SEQ ID NOs:11, 12, 13 and 14) framework regions (FR), as appropriate, or FR regions substantially homologous thereto, are found in the antibodies of the invention.

In other preferred embodiments, all four of the variable light chain (SEQ ID NOs:33, 34, 35 and 36) and/or variable heavy chain (SEQ ID NOs:29, 30, 31 and 32) framework regions (FR), as appropriate, or FR regions substantially homologous thereto, are found in the antibodies of the invention.

In other preferred embodiments, all four of the variable light chain (SEQ ID NOs:51, 52, 53 and 54) and/or variable heavy chain (SEQ ID NOs:47, 48, 49 and 50) framework regions (FR), as appropriate, or FR regions substantially homologous thereto, are found in the antibodies of the invention.

In other preferred embodiments, all four of the variable light chain (SEQ ID NOs:69, 70, 71 and 72) and/or variable heavy chain (SEQ ID NOs:65, 66, 67 and 68) framework regions (FR), as appropriate, or FR regions substantially homologous thereto, are found in the antibodies of the invention.

In certain preferred embodiments, the variable heavy chain framework region 3 (FR3 of the VH domain) has or comprises an amino acid sequence of SEQ ID NO:79 (R V T I T A D E S X_{10} X_{11} T A Y M E L S S L R S E D T A V Y Y C A R). In these embodiments X_{10} and X_{11} can be any amino acid. Preferably one, more preferably both of these X residues are selected from the following group: X_{10} is T or I (preferably T); and X_{11} is S or D (preferably D). Thus, a preferred variable heavy chain framework region 3 has or comprises the amino acid sequence of SEQ ID NO:80 (R V T I T A D E S T/I S/D T A Y M E L S S L R S E D T A V Y Y C A R).

In further embodiments of the present invention, the VL CDR1 has or comprises an amino acid sequence of SEQ ID NO: 73 (R S S Q S L V Y X_9 D X_{11} N T Y L N). In these embodiments X_9 and X_{11} can be any amino acid. Preferably one, more preferably both, of these X residues are selected from the following group: X_9 is H or S (preferably H); and X_{11} is G or A (preferably A). Thus, a preferred VL CDR1 has or comprises the amino acid sequence of SEQ ID NO: 74 (R S S Q S L V Y H/S D G/A N T Y L N). Preferred VL CDR1 sequences of this embodiment are SEQ ID NOs: 8, 26 or 62.

In further embodiments of the present invention, the VL CDR2 has or comprises an amino acid sequence of SEQ ID NO: 75 (E V S N R X_6 S). In these embodiments X_6 can be any amino acid. Preferably, X_6 is D or E (preferably D). Thus, a preferred VL CDR2 has or comprises the amino acid sequence of SEQ ID NO: 76 (E V S N R D/E S). For example, preferred VL CDR2 sequences of this embodiment have or comprise SEQ ID NOs: 9 or 63.

In further embodiments of the present invention, the VL CDR3 has or comprises an amino acid sequence of SEQ ID NO: 77 (M Q G X_4 X_5 X_6 P L T). In these embodiments X_4, X_5 and X_6 can be any amino acid. Preferably one or more, most preferably all, of these X residues are selected from the following group: X_4 is A or T (preferably A); X_5 is Y or Q (preferably Y); and X_6 is R or L (preferably R). Thus, a preferred VL CDR3 has or comprises the amino acid sequence of SEQ ID NO: 78 (M Q G A/T Y/Q R/L P L T). For example, preferred VL CDR3 sequences of this embodiment have or comprise SEQ ID NOs: 10 or 64.

In one embodiment, the present invention provides an antibody that comprises:

a VL domain that comprises a VL CDR1 of SEQ ID NO:73, a VL CDR2 of SEQ ID NO:75, and a VL CDR3 of SEQ ID NO:77, and/or

a VH domain that comprises a VH CDR1 of SEQ ID NO:5 or a sequence substantially homologous thereto, a VH CDR2 of SEQ ID NO:6 or a sequence substantially homologous thereto, and a VH CDR3 of SEQ ID NO:7 or a sequence substantially homologous thereto, wherein said substantially homologous sequence is a sequence containing 1, 2 or 3, preferably 1 or 2 (more preferably 1), altered amino acids compared with a given CDR sequence. In some such embodiments, the VL CDR1 is preferably SEQ ID NO: 8, 26 or 62. In some such embodiments, the VL CDR2 is preferably SEQ ID NO: 9 or 63. In some such embodiments, the VL CDR3 is preferably SEQ ID NO: 10 or 64.

In one embodiment, the present invention provides an antibody that comprises:

a VL domain that comprises a VL CDR1 of SEQ ID NO:74, a VL CDR2 of SEQ ID NO:76, and a VL CDR3 of SEQ ID NO:78, and/or

a VH domain that comprises a VH CDR1 of SEQ ID NO:5 or a sequence substantially homologous thereto, a VH CDR2 of SEQ ID NO:6 or a sequence substantially homologous thereto, and a VH CDR3 of SEQ ID NO:7 or a sequence substantially homologous thereto, wherein said substantially homologous sequence is a sequence containing 1, 2 or 3, preferably 1 or 2 (more preferably 1), altered amino acids compared with a given CDR sequence. In some such embodiments, the VL CDR1 is preferably SEQ ID NO: 8, 26 or 62. In some such embodiments, the VL CDR2 is preferably SEQ ID NO: 9 or 63. In some such embodiments, the VL CDR3 is preferably SEQ ID NO: 10 or 64.

In embodiments of the invention where one or more of the CDR sequences contain an X_X residue, then CDRs with sequences which are substantially homologous thereto containing 1, 2 or 3, preferably 1 or 2 (more preferably 1), altered amino acids or amino acid substitutions compared with a given CDR sequence are also encompassed by the invention. In some such embodiments said alterations or substitutions in amino acid residues can include one or more of the X_X residues or can be at residues other than the X_X residues. In other such embodiments said alterations are in a mixture of the X_X residues and the non-X_X residues.

In other embodiments of the invention, antibodies comprise:

a VL domain that comprises a VL CDR1 of SEQ ID NO:73 or a sequence substantially homologous thereto, a VL CDR2 of SEQ ID NO:75 or a sequence substantially homologous thereto, and a VL CDR3 of SEQ ID NO:77, or a sequence substantially homologous thereto, and a VH domain that comprises a VH CDR1 of SEQ ID NO:5 or a sequence substantially homologous thereto, a VH CDR2 of SEQ ID NO:6, or a sequence substantially homologous thereto, and a VH CDR3 of SEQ ID NO:7, or a sequence substantially homologous thereto. In such embodiments said substantially homologous sequence is a sequence containing 1, 2 or 3, preferably 1 or 2 (more preferably 1), altered amino acids compared with a given CDR sequence.

In other embodiments of the invention, antibodies comprise:

a VL domain that comprises a VL CDR1 of SEQ ID NO:74 or a sequence substantially homologous thereto, a VL CDR2 of SEQ ID NO:76 or a sequence substantially homologous thereto, and a VL CDR3 of SEQ ID NO:78 or a sequence

substantially homologous thereto, and a VH domain that comprises a VH CDR1 of SEQ ID NO:5 or a sequence substantially homologous thereto, a VH CDR2 of SEQ ID NO:6, or a sequence substantially homologous thereto, and a VH CDR3 of SEQ ID NO:7, or a sequence substantially homologous thereto.

As described above, the present invention provides antibodies, for example isolated antibodies, which bind to (or specifically recognise or specifically bind to) PD-1. PD-1 is also known as programmed cell death protein 1.

PD-1 is a molecule of the immunoglobulin superfamily and strongly expressed on the surface of a variety of T-cells and proB-cells.

In accordance with the present invention, the PD-1 may be from any species, e.g. mouse or human or monkey (*Cynomolgus*). In a preferred embodiment the PD-1 is human PD-1. In certain embodiments the PD-1 is monkey (*Cynomolgus*) PD-1. In certain embodiments the PD-1 is mouse PD-1.

In certain embodiments, antibodies of the present invention bind to PD-1 (e.g. human PD-1, *Cynomolgus* PD-1, or mouse PD-1) in (as determined in) a Surface Plasmon Resonance (SPR) assay (e.g. a BIACore assay). Suitable SPR assays are known in the art. In certain preferred SPR assays, an anti-PD-1 antibody (e.g. an IgG antibody such as an IgG_2 antibody) is captured (or immobilised) on a solid support (e.g. a flow cell), for example via an anti-human Fc IgG (e.g. about 2000 RU anti-human Fc IgG) that has been immobilised on a flow cell, and various concentrations (i.e. a dilution series e.g. a doubling dilution series) of PD-1 (e.g. purified PD-1) is then injected. Preferred concentrations and flow-rates for injection are described in the Example section. Suitable association periods and dissociation periods to be used in an SPR assay are known to a skilled person, for example, a preferred association period in the SPR assay is 2 minutes and a preferred dissociation period in the SPR assay is 10 minutes. Thus, in a preferred embodiment, association may be measured over 2 minutes and/or dissociation may be measured over 10 minutes. In a certain embodiments, all measurements may be performed at 25°C in PBS, pH7.4, 0.05% Tween 20. Kinetic parameters may be determined or calculated by any suitable model or software, for example by fitting the sensogram experimental data assuming a 1:1 interaction for example using the BIAevaluation software (GE, BR-1005-97). In some embodiments, reference cell subtraction and fitting the sensogram experimental data assuming a 1:1 interaction using the BIAevaluation software (GE, BR-1005-97) is done. A particularly preferred SPR assay is described in the Example section herein.

In a particularly preferred embodiment, antibodies of the present invention bind to PD-1 (e.g. human PD-1, *Cynomolgus* PD-1, or mouse PD-1) in (as determined in) a Surface Plasmon Resonance (SPR) assay (e.g. a BIACore assay). in which

- 2,000 Response units (RU) of anti-human Fc IgG (e.g. GE, BR-1008-39) is immobilised on flow cells (FC) 1 and 2 of a dextran sensor chip (e.g. a Series 5 CM5 dextran sensor chip, e.g. GE, BR1005-30) using EDC/NHS cross-linking chemistry (e.g. according to the amine coupling protocol (GE, BR-1000-50));
- Purified anti-PD-1 antibody (e.g. IgG_2 antibody) is diluted to a concentration of 2nM in PBS, pH7.4, 0.05% Tween-20 and injected into FC2 at a flow rate of 10µl/minute, 60s contact time (typically this results in an average of 20 RU of antibody captured);
- Doubling dilutions (e.g. a concentration series) of PD-1 are injected from 50nM at a flow rate of 30µl/minute.
- Association is measured over 2 minutes and dissociation is measured over 10 minutes and all measurements are performed at 25°C in PBS, pH7.4, 0.05% Tween 20;
- Kinetic parameters are determined by reference cell subtraction and fitting the sensogram experimental data assuming a 1:1 interaction (e.g. using the BIAevaluation software, GE, BR-1005-97).

A particularly preferred SPR assay is performed using a BIAcore T100 instrument and following a protocol according to the Human antibody capture kit protocol (GE, BR-1008-39), e.g. as described in the Example section herein.

In certain preferred embodiments, antibodies of the present invention, when in IgG format (e.g. IgG_2), have a high binding affinity for PD-1 (e.g. human PD-1 or *Cynomolgus* PD-1), e.g., have a K_D (equilibrium dissociation constant) in the range of 25nM or less. Thus, preferably, antibodies of the invention, when in IgG format (e.g. IgG_2) , have a binding affinity for PD-1 (e.g. human PD-1 or *Cynomolgus* PD-1) that corresponds to a K_D of less than 25nM, less than 20nM, less than 15nM or less than 10nM, more preferably of less than 10, 9.5, 9, 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5 or 1nM. Importantly, antibodies with affinities such as those above are in the established range that has been shown to be useful for therapy.

For example, the binding affinity (e.g. K_D) of the antibodies of the invention for human PD-1, when in IgG format (e.g. IgG_2), may be less than 20nM, less than

15nM or less than 10nM or less than 5nM (e.g. less than 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5 or 1nM). In certain embodiments, the binding affinity of the antibodies of the invention (e.g. an antibody based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) for human PD-1, when in IgG format (e.g. IgG_2), may be 5nM or less, such as being about 3nM or about 4nM or such as being 3.8nM (K_D). In certain embodiments, the binding affinity of the antibodies of the invention (e.g. an antibody based on 273_C01_A12) for human PD-1, when in IgG format (e.g. IgG_2), may be 15nM or less, such as being about 8nM, 9nM, 10nM, 11nM or 12nM or such as being 10nM (K_D).

The binding affinity (e.g. K_D) of the antibodies of the invention for *Cynomolgus* PD-1, when in IgG format (e.g. IgG_2), may be less than 25nM or less than 20nM or less than 10nM (e.g. less than 9, 8, 7, 6, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5 or 1nM). In certain embodiments, the binding affinity of the antibodies of the invention (e.g. an antibody based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) for *Cynomolgus* PD-1, when in IgG format (e.g. IgG_2), may be 10nM or less, such as being about 8nM or about 9nM or such as being 8.7nM (K_D). In certain embodiments, the binding affinity of the antibodies of the invention (e.g. an antibody based on 273_C01_A12) for *Cynomolgus* PD-1, when in IgG format (e.g. IgG_2), may be 25nM or less, such as being about 19nM, 20nM, 21nM 22nM or 23nM or such as being 21nM (K_D).

In some embodiments, the affinity (K_D) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is within 5-fold, preferably within 4-fold, or 3-fold, or 2.5-fold or 2 fold, or between 2-fold and 3-fold (e.g. 2.3-fold or 2.1-fold), of the affinity for human PD-1. In certain embodiments, the binding affinity of antibodies of the invention (e.g. an antibody based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) for *Cynomolgus* PD-1, when in IgG format (e.g. IgG_2), is within 2.3 fold of binding affinity for human PD-1. In certain embodiments, the binding affinity of the antibodies of the invention (e.g. an antibody based on 273_C01_A12) for *Cynomolgus* PD-1, when in IgG format (e.g. IgG_2), is within 2.1 fold of binding affinity for human PD-1.

In some embodiments, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 50% to 500% of the affinity (affinity value e.g. in nM) for human PD-1. Preferably, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1),

e.g. when in IgG format (e.g. IgG_2), is 50% to 400%, or 50% to 300%, or 50 to 250%, or 50% to 200%, or 50% to 150%, or 50% to 100% of the affinity (affinity value e.g. in nM) for human PD-1. In some preferred embodiments, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 50% to about 250% of the affinity (affinity value e.g. in nM) for human PD-1. In some preferred embodiments, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 50% to about 200%, 50% to 210%, 50% to 220%, 50% to 230% or 50% to 240% of the affinity (affinity value e.g. in nM) for human PD-1.

In some embodiments, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is at least 100% of the affinity (affinity value e.g. in nM) for human PD-1 (thus in some embodiments antibodies of the invention have an affinity for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1) that is the same or lower than the affinity for human PD-1). In some embodiments, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 100% to 500% of the affinity (affinity value e.g. in nM) for human PD-1. Preferably, the affinity (K_D) (affinity value) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 100% to 400%, or 100% to 300%, or 100 to 250%, or 100% to 200%, or 100% to 150% of the affinity (affinity value e.g. in nM) for human PD-1. In some preferred embodiments, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 100% to 250% of the affinity (affinity value e.g. in nM) for human PD-1. In some preferred embodiments, the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 100% to 200%, 100% to 210%, 100% to 220%, 100% to 230% or 100% to 240% of the affinity (affinity value e.g. in nM) for human PD-1.

By way of example, if the affinity (affinity value) of an antibody of the invention for human PD-1 is 3.8nM, and the affinity (K_D) (affinity value) for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is 50% to 250% of the affinity (affinity value) for human PD-1, then the affinity (affinity value) for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1) could be 1.9nM to 9.5nM.

Thus, in some embodiments, the affinity (K_D) (affinity value) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is comparable to (or similar to or closely matched to or substantially equivalent to) the affinity (affinity value) for human PD-1.

Preferred antibodies of the invention bind to mouse PD-1. The binding affinity (K_D) of antibodies of the invention for mouse PD-1, when in IgG format (e.g. IgG_2), may be less than 10μM (e.g. less than 9, 8, 7, 6, 5, 4, 3.5, 3, 2.5, 2, 1.5 or 1 μM). In certain embodiments, the binding affinity of the antibodies of the invention (e.g. an antibody based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) for mouse PD-1, when in IgG format (e.g. IgG_2), may be 10μM or less, such as being about 6μM or about 7μM or such as being 6.4 μM (K_D).

In some embodiments, antibodies of the present invention have an affinity for human PD-1 that is higher than the affinity for human PD-1 of the 246A10 and/or 413E1 and/or 244C8 antibodies described in WO 2016/106159. Preferred affinities of antibodies of the invention are discussed elsewhere herein.

Any appropriate method of determining K_D may be used. However, preferably the K_D is determined in a Surface Plasmon Resonance assay (e.g. a BIAcore assay) with the kinetic parameters being determined. Suitable and preferred types of SPR assay are described above. Thus, the K_D values as described above may be as determined in an SPR assay as described above or elsewhere herein. A particularly preferred method is described in the Example section herein.

In some embodiments, antibodies of the invention have a K_a (or "on-rate" or association constant) ($M^{-1} s^{-1} x10^5$) for human PD-1 that is at least 2, preferably at least 3, or at least 4, or at least 5, or at least 6. In some embodiments the K_a (or "on-rate") ($M^{-1} s^{-1} x10^5$) for human PD-1 is between about 2 and 10 (e.g. about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9 or about 10), preferably between 3 and 7. In certain embodiments, antibodies of the invention (e.g. antibodies based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) have a K_a (or "on-rate") ($M^{-1} s^{-1} x10^5$) for human PD-1 that is at least 2 or at least 3, typically between about 3 to 5, e.g. about 4 (for example 3.92). In certain embodiments, antibodies of the invention (e.g. antibodies based on 273_C01_A12) have a K_a (or "on-rate") ($M^{-1} s^{-1} x10^5$) for

human PD-1 that is at least 2 or at least 3 or at least 4 or at least 5 or at least 6, typically between about 5 to 7, e.g. about 6 (for example 6.06).

In some embodiments, antibodies of the present invention have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is higher (preferably significantly higher, e.g. statistically significantly higher such as with a probability value of ≤0.05) than the K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format). Table F presents Nivolumab amino acid sequences (VH and VL domain sequences and IgG_2 heavy and light chain sequences). In some embodiments, antibodies of the present invention have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is at least 50%, at least 75%, at least 100%, at least 125% at least 150%, at least 175%, at least 200%, at least 250%, at least 300%, at least 350%, at least 400%, at least 450% or at least 500% higher than the K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 of the antibody Nivolumab. In some embodiments, antibodies of the present invention have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is up to 500%, up to 750% or up to 1000% higher than the K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 of the antibody Nivolumab.

Preferably, antibodies of the present invention have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is at least 100% or at least 150% or at least 175% higher than (e.g. 100%-500% higher or 150%-350% higher or 175% to 350% higher) the K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format). In certain embodiments, antibodies of the present invention (e.g. antibodies based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is at least 150% higher than (e.g. 150%-250% higher or 175%-225% higher or 175% to 200% higher) the K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 of the antibody Nivolumab. In certain embodiments, antibodies of the present invention (e.g. antibodies based on 273_C01_A12) have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is at least 300% higher than (e.g. 300%-400% higher or 325%-375% higher or 325% to 350% higher) the K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 of the antibody Nivolumab.

Thus, alternatively viewed, antibodies of the present invention may have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is at least 2 times higher, at least 3 times higher, at least 4 times higher, at least 5 times higher, or at least 6 times higher than the K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format). Antibodies of the present invention may have a K_a (or "on-rate") ($M^{-1} s^{-1}$) for human PD-1 that is 2-10

times higher, e.g. 2-3 times higher, 2-4 times higher, 2-5 times higher or 2-6 times higher, than the K_a (or "on-rate") (M^{-1} s^{-1}) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format).

In some embodiments, antibodies of the present invention have a K_a (or "on-rate") (M^{-1} s^{-1}) for human PD-1 that is higher than (e.g. at least 10%, at least 20%, preferably at least 30% higher or preferably at least 40% higher than) the K_a for human PD-1 of the 413D2 antibody described in WO 2016/106159. Preferred on-rates of antibodies of the invention are discussed elsewhere herein.

In some embodiments, antibodies of the invention have a K_d (or "off-rate" or dissociation constant) (s^{-1} $x10^{-4}$) for human PD-1 that is at least 7, or at least 10, or at least 15, or at least 20, or at least 30, or at least 40, or at least 50, or at least 60. In some embodiments, antibodies of the invention have a K_d (or "off-rate") (s^{-1} $x10^{-4}$) for human PD-1 that is between about 10 and 70 (e.g. about 10, about 20, about 30, about 40, about 50, about 60 or about 70). In certain embodiments, antibodies of the invention (e.g. antibodies based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) have a K_d (or "off-rate") (s^{-1} $x10^{-4}$) for human PD-1 that is between about 10 to 20, e.g. about 13, about 14, about 15, about 16 or about 17, for example 15. In certain embodiments, antibodies of the invention (e.g. antibodies based on 273_C01_A12) have a K_d (or "off-rate") (s^{-1} $x10^{-4}$) for human PD-1 that is between about 50 and 70, e.g. about 60, for example 61.

In some embodiments, antibodies of the present invention have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is higher (preferably significantly higher, e.g. statistically significantly higher such as with a probability value of ≤0.05) than the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format). In some embodiments, antibodies of the present invention have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is at least 50%, at least 75%, at least 100%, at least 125% at least 150%, at least 175%, at least 200%, at least 250%, at least 300%, at least at least 400%, at least 500%, or at least 600% higher, or at least 700% higher, or at least 800% higher, or at least 900% higher, or at least 1000% higher than the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab. In some embodiments, antibodies of the present invention have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is up to 1000%, up to 1500% or up to 2000% higher than the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab.

Preferably, antibodies of the present invention have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is at least 100% or at least 150% or at least 175% higher than (e.g. 100%-1500% higher or 150%-1200% higher or 175% to 1100% higher) the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format).

In certain embodiments, antibodies of the present invention (e.g. antibodies based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is at least 100% higher than (e.g. 100%-300% higher or 150%-250% higher or 150% to 225% higher) the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format).

In certain embodiments, antibodies of the present invention (e.g. antibodies based on 273_C01_A12) have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is at least 500% higher than (e.g. 500%-1500% higher or 700%-1200% higher or 1000% to 1200% higher) the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format).

Thus, alternatively viewed, antibodies of the present invention may have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is at least 2 times higher, at least 3 times higher, at least 4 times higher, at least 5 times higher, at least 6 times higher, at least 7 times higher, at least 8 times higher, at least 9 times higher or at least 10 times higher than the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format). Antibodies of the present invention may have a K_d (or "off-rate") (s^{-1}) for human PD-1 that is 2-15 times higher, e.g. 2-12 times higher, 2-10 times higher, 2-6 times higher, 2-5 times higher, 2-4 times higher or 2-3 times higher, than the K_d (or "off-rate") (s^{-1}) for human PD-1 of the antibody Nivolumab (e.g. when antibodies are in IgG format such as IgG_2 format).

In some embodiments, antibodies of the present invention have a K_d (or "off-rate") ($M^{-1}\,s^{-1}$) for human PD-1 that is higher than (e.g. at least 10%, preferably at least 20% or at least 25% higher than) the K_d for human PD-1 of the 393C5 antibody described in WO 2016/106159. In some embodiments, antibodies of the present invention have a K_d (or "off-rate") ($M^{-1}\,s^{-1}$) for human PD-1 that is higher than (e.g. at least 10%, preferably at least 20% or at least 30% or at least 40% or at least 50% higher than) the K_d for human PD-1 of the 388D4 antibody described in

WO 2016/106159. Preferred off-rates of antibodies of the invention are discussed elsewhere herein.

In some embodiments, antibodies of the present invention have a K_d (or "off-rate") (M^{-1} s^{-1}) for human PD-1 that is higher than (e.g. at least 10%, at least 50%, preferably at least 100% or at least 200% higher than) the K_d for human PD-1 of the 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and/or 5F4 antibodies described in US 2009/0217401 A1. Preferred off-rates of antibodies of the invention are discussed elsewhere herein.

The K_a (or "on-rate") or K_d (or "off-rate") may be determined by any suitable method and the skilled person is familiar with these. For example, K_a (or "on-rate") or K_d (or "off-rate") can be determined in a Surface Plasmon Resonance assay (e.g. BIAcore assay) and suitable and preferred Surface Plasmon Resonance assays are described above. Thus, the K_a and K_d values as described above may be as determined in an SPR assay as described above or elsewhere herein. A particularly preferred assay is described in the Example section herein.

As described above, preferred antibodies of the invention have a higher "on-rate" (K_a or association constant) and/or a higher "off-rate" (K_d or dissociation constant) than the antibody Nivolumab. While certain antibodies of the invention may have a similar K_D in comparison to Nivolumab, they may differ in their on and off rates (K_a and/or K_d) with certain antibodies of the invention having both higher on-rate and off-rate values. In practice, differing on-rates and off-rates can lead to differences in the pharmacokinetics. For example, a low (or lower) off-rate can be associated with poor tumour penetration. Without wishing to be bound by theory, in respect to pathway inhibiting antibodies a higher off-rate might be beneficial, as one antibody molecule is more likely to fall off and then to rebind on several receptor molecules, thus potentially triggering or blocking more signalling events than an antibody molecule sitting very tightly on the same receptor molecule.

Antibodies of the present invention typically bind to human PD-1. Preferably, such antibodies also bind to monkey PD-1 (e.g. *Cynomolgus* PD-1). Thus, in some embodiments, antibodies of the present invention bind to human PD-1 and monkey PD-1. In some embodiments, antibodies of the present invention bind to human PD-1 and mouse PD-1. In some embodiments, antibodies of the present invention bind to human PD-1 and monkey PD-1 and mouse PD-1. The ability of antibody to bind to PD-1 (e.g. human, monkey or mouse PD-1) can be assessed by any suitable method, for example an SPR assay, an ELISA assay, a

flow cytometry assay or a cell reporter assay, e.g. one of the assays described elsewhere herein.

As discussed elsewhere herein, some preferred antibodies of the invention may bind to both human PD-1 and monkey (*Cynomolgus*) PD-1, or human PD-1 and mouse PD-1, or human PD-1 and monkey PD-1 and mouse PD-1. For example, an antibody based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) may have the ability to bind to human PD-1 and monkey PD-1 and mouse PD-1. Such cross-reactivity between species and in particular between humans and species commonly used as pre-clinical animal models (e.g. mouse or monkey) may be an advantage as it allows a more effective translation from pre-clinical studies to clinical use. For example, having an antibody which cross reacts with the native PD-1 present in the particular animal model used means that the results in this model are more likely to reflect the situation in a human patient, thereby allowing a more accurate assessment of for example dosing to be made and an increased likelihood of identifying any potentially relevant or problematic side effects. For example, the ability of an antibody of the invention to bind to both human PD-1 and monkey PD-1/mouse PD-1 means that such antibodies can be tested in preclinical toxicity studies to assess adverse side effects of the treatment and to find appropriate tolerated dosages. Antibodies which do not bind to mouse PD-1 (e.g. Nivolumab) cannot be used in syngeneic mouse models.

As discussed elsewhere herein, in some preferred embodiments the affinity (K_D) (affinity value e.g. in nM) of antibodies of the invention for a non-human primate PD-1 (e.g. *Cynomolgus* PD-1), e.g. when in IgG format (e.g. IgG_2), is comparable to (or similar to or closely matched to or substantially equivalent to) the affinity (affinity value e.g. in nM) for human PD-1. Without wishing to be bound by theory, antibodies with similar (or comparable or closely matched or substantially equivalent) affinities for human and *Cynomolgus* PD-1 may be particularly advantageous as it means that data obtained in experiments on *Cynomolgus* (an organism commonly used in medical experiments) would better reflect the likely behaviour (e.g. likely therapeutic efficacy) in humans.

In some embodiments, antibodies of the present invention bind to recombinant PD-1 (e.g. recombinant human PD-1). Recombinant human PD-1 is commercially available. Recombinant PD-1 (e.g. recombinant human PD-1) may be in the form of a PD-1 fusion protein, for example a PD-1-rCD4 fusion (rCD4 is rat CD4), or a PD-1-Fc fusion. In some embodiments, antibodies of the invention are

able to bind to PD-1 (e.g. recombinant PD-1) in an SPR assay or in an ELISA assay, for example as described elsewhere herein (e.g. in the Example section).

In certain embodiments, antibodies of the present invention bind to PD-1 in an ELISA assay. The skilled person will be familiar with ELISA assays and readily able to establish suitable conditions to assess the ability of an antibody to bind to PD-1 in such an assay. For example, anti-PD-1 antibodies (e.g. IgG antibodies such as IgG_2 antibodies) may be incubated in ELISA plates coated with anti-Fc such that the antibody is captured, followed by washing and incubation with PD-1 (e.g. biotinylated human PD-1-rCD4) and subsequently detection of bound PD-1 (e.g. using europium-labelled streptavidin). In some embodiments, a low concentration of antigen (PD-1) is used, for example about 40pM. Typically, antibodies of the present invention bind to human PD-1 in an ELISA assay. In some embodiments, antibodies of the invention (e.g. antibodies based on 273_C12_C05 (original clone), e.g. 273_C12_C05 (original clone) or variant 1 or variant 2 thereof) may bind to mouse PD-1 in an ELISA assay. A particularly preferred ELISA assay is depicted in Figure 2 and described in the Example herein.

Antibodies of the present invention typically bind to cell-surface expressed PD-1, such as cell-surface expressed human PD-1 (PD-1 expressed at the surface of cells, or present at or on the cell surface of PD-1 expressing cells). Such cell-surface forms will thus in many cases represent a native or natural form of PD-1 (or a native or natural configuration of PD-1), for example the form found on cells which naturally express or overexpress PD-1. PD-1 is typically expressed at the surface of T-cells and proB-cells. In some embodiments, antibodies of the present invention bind to cell surface expressed PD-1 on cells (preferably mammalian cells) that have been engineered to express PD-1 constitutively, for example Jurkat cells that have been engineered to express PD-1 constitutively (e.g. the NFAT-luc2/PD-1 Jurkat cells commercially available from Promega). Binding to cell-surface PD-1 can be assessed by any suitable means and preferred methods include by flow cytometry and the cell reporter based assay discussed elsewhere herein. In an exemplary flow cytometry method, PD-1 expressing cells are incubated with the anti-PD1 antibody under investigation (e.g. an IgG antibody such as an IgG_2 antibody) and the antibody bound to the PD-1 on the cell is detected by fluorescence, for example the antibody is fluorescently labelled. Such labelling can for example be carried out by incubating the cell-antibody mixture with a second antibody (e.g. anti-Fc PE) which recognises the anti-PD-1 antibody under investigation and which carries a fluorescent label. Accordingly, if the anti-PD1 antibody under investigation binds to

PD-1 on the cell surface, the cell becomes fluorescently labelled and such cells, and thus antibodies which have the ability to bind to cell surface PD-1, can be readily identified using a flow cytometer. A particularly preferred flow cytometry method is described in the Example herein. Another method for testing for the ability of an antibody to bind to PD-1 on the cell surface is immunohistochemistry.

Typically, antibodies of the invention inhibit (or block) the interaction between PD-1 and PD-L1. Preferably, the inhibition is a significant inhibition, e.g. a statistically significant inhibition such as with a probability value of ≤0.05. In certain embodiments, antibodies of invention inhibit (or block) the interaction between PD-1 and PD-L1 by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or at least 99.5% (e.g. about 99.5%). Typically, such % inhibition is in comparison with a control assay or control level, for example a control assay or control level in the absence of an antibody (anti-PD1 antibody) (for example a negative control or background level or assay). Thus, a 0% inhibition (control) level (or conversely a 100% or maximum interaction level) is typically the level in the absence of an antibody (anti-PD-1 antibody).

In some embodiments, antibodies of the invention inhibit (or block) the interaction between PD-1 and PD-L1 if used at concentration of at least 0.1nM, for example at least 0.2nM, at least 0.3nM, at least 0.4nM, at least 0.5nM, at least 1nM, at least 2nM, at least 3nM, at least 4nM, at least 5nM, at least 10nM, at least 15nM, at least 20nM, at least 25nM, at least 50nM, at least 100nM, at least 250nM or at least 500nM. In some embodiments, antibodies of the invention inhibit (or block) the interaction between PD-1 and PD-L1 when used at concentration of 1nM or less, or 10nM or less, or 50nM or less, or 100nM or less, or 250nM or less or 500nM or less (e.g. 0.5nM to 50nM, or 1nM to 50nM, or 0.5nM to 5nM, or 0.5nM to 10 nM, or 1nM to 5nM, or 1nM to 10 nM). For example, in preferred embodiments antibodies of the invention may inhibit the interaction between PD-1 and PD-L1 by at least 60%, preferably at least 70%, more preferably at least 75% (e.g. 60% to 100% or 60% to 99% or 70% to 85% or 75% to 80%) if used at a concentration of at least 0.5nM (e.g. if used at 0.5nM). In other preferred embodiments, antibodies of the invention may inhibit the interaction between PD-1 and PD-L1 by at least 85%, preferably at least 90%, more preferably at least 95% or even at least 98%, at least 99% or 100% (e.g. 85% to 100%, or 85% to 99%, or 95% to 99%, or 95% to 100%) if used at a concentration of at least 1nM (e.g. if used at 1nM).

The ability of antibody to inhibit (or block) the interaction between PD-1 and PD-L1 can be determined (or assessed) using any appropriate assay (typically an *in vitro* assay), for example a competition assay, for example an assay in which the anti-PD-1 antibody being tested competes with PD-L1 for binding to PD-1. Any suitable competition assay may be used (e.g. an ELISA-based competition assay). In a preferred embodiment, antibodies of the invention inhibit (or block) the interaction between PD-1 and PD-L1 in the following type of competition assay:

PD-L1 or a PD-L1 containing molecule or fusion protein (e.g. PD-L1-rCD4) is immobilised (or captured) on a solid support (e.g. an ELISA plate surface), for example via an antibody that has been coated on the solid support which recognises the PD-L1 or PD-L1 containing molecule or fusion protein (e.g. an anti-rCD4 which recognises PD-L1-rCD4). A PD1 containing molecule or fusion protein (e.g. PD-1-Fc) is mixed in the presence (e.g. a concentration series) or absence of an anti-PD-1 antibody under investigation (e.g. for about 30 minutes). Following washing of the solid support (e.g. ELISA plate surface) to remove excess unbound PD-L1 or PD-L1 containing molecule or fusion protein (e.g. PD-L1-rCD4), the pre-mixed PD-1 containing molecule or fusion protein (e.g. PD-1-Fc)/PD-1 antibody mixture) is added to the solid support (e.g. the ELISA plate surface having the immobilised PD-L1) and incubated (e.g. for about one hour). If the anti-PD-1 antibody under investigation binds to an epitope on PD-1 that is responsible for (or involved in) the binding of PD-1 to PD-L1, the interaction between PD-1 and PD-L1 will be inhibited (or blocked or prevented). After a further washing step, the PD-1 containing molecule or fusion protein (e.g. PD-1-Fc) binding to the immobilised PD-L1 (or PD-L1 containing molecule or fusion protein e.g. PD-L1-rCD4) is detected using a labelled (e.g. biotin labelled) antibody that recognises the PD-1 containing fusion protein (e.g. an anti-Fc-biotin (a biotinylated anti-Fc antibody) that recognises PD-1-Fc) and a detection reagent that recognises the labelled antibody (e.g. europium-labelled streptavidin which recognised anti-Fc-biotin).

In such a competition assay the maximum interaction level between PD-1 and PD-L1 (maximum interaction or 100% interaction or 100% interaction level) may be defined as the level (e.g. level of signal detected) in the presence of the PD-L1 (or PD-L1 containing molecule or fusion protein) and the PD-1 containing molecule or fusion protein but in the absence of the anti-PD1 antibody under investigation (e.g. PD-1 inhibitory or blocking antibody). The maximum blocking level or maximum inhibition level (maximum blocking or inhibition or 100% blocking or inhibition or 100% blocking level or 100% inhibition level) may be defined as the

level of signal detected in the absence of a PD-1 containing molecule or fusion protein. An interaction level (e.g. level of signal detected) in the presence of an anti-PD1 antibody under investigation that is less than the maximum interaction level as defined above is indicative that the anti-PD-1 antibody inhibits (or blocks) the interaction between PD-1 and PD-L1.

The preferred % inhibitions of the interaction between PD-1 and PD-L1 (e.g. at particular antibody concentrations) as described above are preferably as determined in the type of competition assay (ELISA based competition assay) described above and elsewhere herein.

In a particularly preferred embodiment, antibodies of the present invention inhibit (or block) the interaction between PD-1 and PD-L1 as determined by a competition assay in which

- An ELISA plate (e.g. a black 96 well immunosorb plate) is coated overnight with an anti-rCD4 antibody at 4°C;
- The ELISA plate is then washed 3 times with PBS and blocked by the addition of 3% (w/v) dried milk in PBS (PBS-M) (e.g. 200µl) and incubated for one hour at room temperature;
- The ELISA plate is washed 3 times with PBS followed by the addition of 5µg/ml PD-L1-rCD4 in PBS-M (e.g. 50µl) and incubated for 1 hour at room temperature;
- The ELISA plate is washed 3 times with PBS-T (0.1% Tween-20, PBS) and 3 times with PBS;
- In a separate plate PD-1-Fc (e.g. 0.8nM) is mixed in the presence (e.g. a concentration series) or absence of anti-PD-1 antibody (antibody that binds to PD-1 that is under investigation) in PBS-M and incubated for 30 minutes;
- The mixture (pre-mixture) from the previous step (PD-1-Fc/anti-PD-1 antibody mixture or PD-1-Fc that was mixed in the absence of anti-PD-1 antibody) is added to the ELISA plate and incubated for 1 hour;
- The ELISA plate is then washed 3 times with PBS-T and 3 times with PBS;
- The ELISA plate is then incubated for 1 hour with 0.5µg/ml biotinylated anti-Fc antibody (e.g. biotinylated anti-human-Fc antibody) in PBS-M;

- The ELISA plate is then washed 3 times with PBS-T and 3 times with PBS;
- The ELISA plate is then incubated for 30 minutes with 0.5µg/ml Europium-labelled streptavidin in PBS-M;
- The ELISA plate is then washed 3 times with PBS-T and 3 times with PBS;
- The ELISA plate is then incubated with a europium detection reagent (e.g. DELFIA enhancement solution commercially available from Perkin Elmer);
- ELISA plates are then read (signal is detected) with a plate reader (e.g. Excitation 340nm, Emission 615nm);

and in which the maximum interaction level between PD-1 and PD-L1 (maximum interaction or 100% interaction or 100% interaction level) is defined as the level (e.g. level of signal detected) in the presence of the PD-L1-rCD4 and PD-1-Fc but in the absence of a PD-1 antibody (e.g. PD-1 blocking antibody) wherein an interaction level in the presence of an anti-PD1 antibody that is less than the maximum interaction level as defined above is indicative that the anti-PD-1 antibody inhibits (or blocks) the interaction between PD-1 and PD-L1. Typically, the maximum blocking level or maximum inhibition level (maximum blocking or inhibition or 100% blocking or inhibition or 100% blocking level or 100% inhibition level) is defined as the level (e.g. level of signal detected) in the absence of PD-1-Fc. A particularly preferred competition assay is described in the Example section herein and depicted in Figure 4A.

The preferred % inhibitions of the interaction between PD-1 and PD-L1 (e.g. at particular antibody concentrations) as described above are preferably as determined in the particularly preferred type of competition assay (ELISA based competition assay) described above and elsewhere herein.

In some embodiments, antibodies of the present invention (e.g. IgG antibodies such as IgG_2 antibodies) inhibit (or block) the interaction between PD-1 and PD-L1 to a greater extent than the antibody Nivolumab (e.g. in IgG format such as IgG_2). Put another way, in some embodiments, antibodies of the present invention are more effective than the antibody Nivolumab in inhibiting (or blocking) the interaction between PD-1 and PD-L1. In some preferred embodiments, antibodies of the present invention inhibit (or block) the interaction between PD-1 and PD-L1 to a greater extent than the antibody Nivolumab if said antibodies are

used at low (or lower concentrations), for example if used at a concentration in the range of 0.1nM to 2nM (e.g. 0.1, 0.2, 0.2, 0.4, 0.5. 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2nM). In some preferred embodiments, antibodies of the present invention inhibit (or block) the interaction between PD-1 and PD-L1 to a greater extent than the antibody Nivolumab if said antibodies are used in the concentration range 0.5nM to 1.5nM (e.g. if used at 0.5nM or 1nM). For example, in a preferred embodiment, inhibition (or blocking) of the interaction between PD-1 and PD-L1 by antibodies of the present invention is at least 10%, or at least 20%, or at least 30%, preferably at least 40%, more preferably at least 50% higher (e.g. about 10% to 60% higher, or about 25% to 60% higher, or about 50% to 60% higher) than by the antibody Nivolumab if said antibodies are used at 0.5nM. In another preferred embodiment, inhibition (or blocking) of the interaction between PD-1 and PD-L1 by antibodies of the present invention is at least 10%, or at least 20%, (e.g. about 10% to 20% higher) than by the antibody Nivolumab if said antibodies are used at 1nM. The preferred % inhibitions of the interaction between PD-1 and PD-L1 (e.g. at particular antibody concentrations) as described above are preferably as determined in the particularly preferred type of competition assay (ELISA based competition assay) described above and elsewhere herein.

Without wishing to be bound by theory, the ability of preferred antibodies of the invention to show good inhibition of the interaction of the PD-1 and PD-L1 at low antibody concentrations may be advantageous for example in terms of reduced side effects in a patient in comparison with using high (or higher) antibody concentrations or a reduction in amount (dose) of antibody required to see a therapeutic effect, or improved pharmacokinetic properties.

In a preferred embodiment, antibodies of the present invention inhibit (or block) the interaction between PD-1 and PD-L1 as determined by a cell reporter assay or cell reporter system. Preferably, the inhibition is a significant inhibition, e.g. a statistically significant inhibition such as with a probability value of ≤ 0.05. In such an assay or system, a read-out of PD-1 activity is provided (e.g. a read-out of PD-1 induced or mediated PD-L1 activity). For example, a cell reporter assay may have a reporter (e.g. a luciferase reporter) that is responsive to PD-1/PD-L1 activity (e.g. responsive, such as activated or repressed, as a result of an interaction between PD-1 and PD-1).

In a preferred cell reporter assay or system, two cell lines are used. In such an assay or system one cell line is a cell line (e.g. a HEK293 cell line) expressing (e.g. stably expressing) PD-L1 and a T-cell receptor activating complex. The other

cell line is a cell line (e.g. a Jurkat cell line) expressing (e.g. stably expressing) PD-1, T-cell receptors (T-cell receptor complexes), and a luciferase reporter that is under the control of a promoter that is responsive to T-cell receptor (TCR) signalling (e.g. a luciferase reporter that is under the control of the NFAT promoter; the NFAT promoter is activated by TCR signalling). Preferably, the cell reporter assay or system uses a HEK293 cell line expressing PD-L1 and T-cell receptor activating complexes and a Jurkat cell line expressing PD-1, T-cell receptors (T-cell receptor complexes), and a luciferase reporter that is under the control of a promoter (preferably NFAT) that is responsive to T-cell receptor (TCR) signalling. Particularly preferred is the commercially available PD-1/PD-L1 reporter cell assay from Promega which employs GloResponseTM NFAT-luc2/PD-1 stable Jurkat cell line (CS187102) and Thaw-and-Use PD-L1 cells (CS178103).

In a cell reporter system or assay as described above, when the two cell lines are co-cultured, interaction between PD-1 and PD-L1 inhibits (or blocks or prevents) TCR signalling (TCR signalling occurs as a result of interaction between the T-cell receptor on one cell line with the TCR-activating complex present on the other cell line) and thus inhibits (or blocks or suppresses) promoter mediated luciferase activity. The presence of (or addition of) an anti-PD-1 antibody that inhibits the interaction between PD-1 and PD-L1 inhibits (or blocks or releases) the PD-1/PD-L1 mediated inhibition of TCR signalling and results in (increased) luciferase activity.

Thus, in a particularly preferred embodiment, antibodies of the present invention inhibit (or block) the interaction between PD-1 and PD-L1 as determined by a cell reporter assay or cell reporter system in which

- HEK293 cells expressing PD-L1 and expressing a TCR activating complex (e.g. Promega, CS178103) are plated in a 96-well assay plate (e.g. in 90% HAM'S F-12, 10% FBS), for example in a volume of 100µl, and are incubated for 16 to 20 hours at 37°C, 5% CO_2;
- Jurkat cells expressing PD-1 and expressing TCR complexes and carrying a luciferase reporter that is under the control of the NFAT promoter (e.g. Promega, CS187102) are added to assay medium (e.g. 90% RPMI1640, 1% FBS, e.g. 5.9ml of assay medium) (the Jurkat cells may be thawed and added to the assay medium the day after the HEK293 cells are plated);

- The assay plate containing the adhered HEK293 cells is removed from the incubator and the media is removed (e.g. with a pipette and plate inversion on a paper towel);
- 40µl assay media containing an antibody (anti-PD-1 antibody under investigation) (e.g. a concentration series) is added to the plate (wells of plate) containing the adhered HEK293 cells followed by the addition of 40µl of the Jurkat cell mix;
- The plate is incubated for 6 hours at 37$_o$C, 5% CO_2;
- Luciferase activity is determined (e.g. using a luciferase reagent/substrate such as BioGlo reagent from Promega, G7940, and plate reader e.g. BMG pherastar).

In certain embodiments, antibodies of the present invention have an EC_{50} (e.g. for the inhibition (or blocking) of the interaction between PD-1 and PD-L1) of 100nM or less, or 75nM or less, or 50nM or less, or 40nM or less, or 30nM or less, or 20nM or less, or 10nM or less. Preferably, the EC_{50} is 30nM or less, more preferably, 20nM or less, or 15nM or less, or 14nM or less, or 13nM or less, or 12nM or less, or 11nM or less, or 10nM or less. In some embodiments, the EC_{50} is 0.1nM to 20nM or 0.5nM to 15nM, for example 1nM to 15nM or 5nM to 15nM or 9nM to 12nM. In some embodiments the EC_{50} is about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14 or about 15nM. For example, the EC_{50} may be 9.35nM or 11.7nM.

The preferred EC_{50} values as described above are preferably as determined in a cell reporter assay (e.g. a preferred or particularly preferred cell reporter assay) as described above or in the Example section.

In some embodiments, antibodies of the present invention have one or more, preferably two or more, or three or more, or four or more, or most preferably all, of the functional properties described herein.

As used throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced components or steps, except in instances wherein an upper limit is thereafter specifically stated. Therefore, an "antibody", as used herein, means "at least a first antibody". The operable limits and parameters of combinations, as with the amounts of any single agent, will be known to those of ordinary skill in the art in light of the present disclosure.

In addition, where the terms “comprise”, “comprises”, “has” or “having”, or other equivalent terms are used herein, then in some more specific embodiments these terms include the term “consists of” or “consists essentially of”, or other equivalent terms.

Nucleic acid molecules comprising nucleotide sequences that encode the antibodies of the present invention as defined herein or parts or fragments thereof, or nucleic acid molecules substantially homologous thereto, form yet further aspects of the invention.

Preferred nucleic acid molecules are those encoding a VH region of an antibody of the present invention (e.g., those encoding SEQ ID NOs:3 or 21 or 39 or 57, such as SEQ ID NOs:1 or 19 or 37 or 55, respectively). Other preferred nucleic acid molecules are those encoding a VL region of an antibody of the present invention (e.g., those encoding SEQ ID NOs:4 or 22 or 40 or 58, such as SEQ ID NOs:2 or 20 or 38 or 56, respectively).

Thus, preferred nucleic acid molecules comprise sequences which encode a heavy chain variable region (VH) that has the amino acid sequence of SEQ ID NO: 3, 21, 39 or 57 (which is preferably encoded by SEQ ID NO: 1, 19, 37 or 55) and/or comprise sequences which encode a light chain variable region (VL) which has the amino acid sequence of SEQ ID NO: 4, 22, 40, or 58 (which is preferably encoded by SEQ ID NO: 2, 20, 38 or 56).

Also preferred are nucleic acids which encode the following combinations: SEQ ID NOs: 3 and 4; or SEQ ID NOs: 21 and 22; or SEQ ID NOs 39 and 40; or SEQ ID NOs 57 and 58. Also preferred are nucleic acid molecules which comprise the following combinations: SEQ ID NOs: 1 and 2; or SEQ ID NOs: 19 and 20; or SEQ ID NOs: 37 and 38; or SEQ ID NOs: 55 and 56.

Other preferred nucleic acid molecules comprise sequences that encode IgG forms (e.g. IgG_2 forms) of the antibodies of the invention, for example those as described in Tables A, B, C and D herein (heavy chains and light chains). Thus, preferred nucleic acid molecules are those encoding a heavy chain of an antibody of the present invention (e.g., those encoding SEQ ID NO: 85, 89, 93 or 97, such as SEQ ID NOs: 87, 91, 95 or 99 respectively) and/or those encoding a light chain of an antibody (e.g., those encoding SEQ ID NO: 86, 90, 94, or 98 such as SEQ ID NOs: 88, 92, 96, or 100 respectively).

The term "substantially homologous" as used herein in connection with an amino acid or nucleic acid sequence includes sequences having at least 65%, 70% or 75%, preferably at least 80%, and even more preferably at least 85%, 90%, 95%,

96%, 97%, 98% or 99%, sequence identity to the amino acid or nucleic acid sequence disclosed. Substantially homologous sequences of the invention thus include single or multiple base or amino acid alterations (additions, substitutions, insertions or deletions) to the sequences of the invention. At the amino acid level preferred substantially homologous sequences contain up to 5, e.g. only 1, 2, 3, 4 or 5, preferably 1, 2 or 3, more preferably 1 or 2, altered amino acids, in one or more of the framework regions and/or one or more of the CDRs making up the sequences of the invention. Said alterations can be with conservative or non-conservative amino acids. Preferably said alterations are conservative amino acid substitutions.

In certain embodiments, if a given starting sequence is relatively short (e.g. five amino acids in length), then fewer amino acid substitutions may be present in sequences substantially homologous thereto as compared with the number of amino acid substitutions that might optionally be made in a sequence substantially homologous to a longer starting sequence. For example, in certain embodiments, a sequence substantially homologous to a starting VH CDR1 sequence in accordance with the present invention, e.g. a starting VH CDR1 sequence which in some embodiments may be five amino acid residues in length, preferably has 1 or 2 (more preferably 1) altered amino acids in comparison with the starting sequence. Accordingly, in some embodiments the number of altered amino acids in substantially homologous sequences (e.g. in substantially homologous CDR sequences) can be tailored to the length of a given starting CDR sequence. For example, different numbers of altered amino acids can be present depending on the length of a given starting CDR sequence such as to achieve a particular % sequence identity in the CDRs, for example a sequence identity of at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%.

Routine methods in the art such as alanine scanning mutagenesis and/or analysis of crystal structure of the antigen-antibody complex can be used in order to determine which amino acid residues of the CDRs do not contribute or do not contribute significantly to antigen binding and therefore are good candidates for alteration or substitution in the embodiments of the invention involving substantially homologous sequences.

The term "substantially homologous" also includes modifications or chemical equivalents of the amino acid and nucleotide sequences of the present invention that perform substantially the same function as the proteins or nucleic acid molecules of the invention in substantially the same way. For example, any substantially homologous antibody should retain the ability to bind to PD-1 as

described above. Preferably, any substantially homologous antibody should retain one or more (or all) of the functional capabilities of the starting antibody.

Preferably, any substantially homologous antibody should retain the ability to specifically bind to the same epitope of PD-1 as recognized by the antibody in question, for example, the same epitope recognized by the CDR domains of the invention or the VH and VL domains of the invention as described herein. Thus, preferably, any substantially homologous antibody should retain the ability to compete with one or more of the various antibodies of the invention (e.g. 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12) for binding to PD-1. Binding to the same epitope/antigen can be readily tested by methods well known and described in the art, e.g. using binding assays, e.g. a competition assay. Retention of other functional properties can also readily be tested by methods well known and described in the art or herein.

Thus, a person skilled in the art will appreciate that binding assays can be used to test whether "substantially homologous" antibodies have the same binding specificities as the antibodies and antibody fragments of the invention, for example, binding assays such as competition assays or ELISA assays as described elsewhere herein. BIAcore assays could also readily be used to establish whether "substantially homologous" antibodies can bind to PD-1. The skilled person will be aware of other suitable methods and variations.

As outlined below, a competition binding assay can be used to test whether "substantially homologous" antibodies retain the ability to specifically bind to substantially the same epitope of PD-1 as recognized by the antibodies of the invention (e.g. 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12), or have the ability to compete with one or more of the various antibodies of the invention (e.g. 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12). The method described below is only one example of a suitable competition assay. The skilled person will be aware of other suitable methods and variations.

An exemplary competition assay involves assessing the binding of various effective concentrations of an antibody of the invention to PD-1 in the presence of varying concentrations of a test antibody (e.g. a substantially homologous antibody). The amount of inhibition of binding induced by the test antibody can then be assessed. A test antibody that shows increased competition with an antibody of the invention at increasing concentrations (i.e. increasing concentrations of the test antibody result in a corresponding reduction in the amount of antibody of the

invention binding to PD-1) is evidence of binding to substantially the same epitope. Preferably, the test antibody significantly reduces the amount of antibody of the invention that binds to PD-1. Preferably, the test antibody reduces the amount of antibody of the invention that binds to PD-1 by at least about 95%. ELISA and flow cytometry assays may be used for assessing inhibition of binding in such a competition assay but other suitable techniques would be well known to a person skilled in the art.

In some embodiments, “substantially homologous” antibodies which retain the ability to specifically bind to substantially the same (or the same) epitope of PD-1 as recognized by the antibodies of the invention (e.g. 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12) or which have the ability to compete with one or more of the various antibodies of the invention (e.g. 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12) are preferred.

The term "competing antibodies", as used herein, refers to antibodies that bind to about, substantially or essentially the same, or even the same, epitope as a "reference antibody". "Competing antibodies" include antibodies with overlapping epitope specificities. Competing antibodies are thus able to effectively compete with a reference antibody for binding to PD-1. Preferably, the competing antibody can bind to the same epitope as the reference antibody. Alternatively viewed, the competing antibody preferably has the same epitope specificity as the reference antibody.

"Reference antibodies" as used herein are antibodies which can bind to PD-1 in accordance with the invention which preferably have a VH and a VL domain as defined herein, more preferably a VH of SEQ ID NO: 3 and a VL of SEQ ID NO: 4, or a VH of SEQ ID NO: 21 and a VL of SEQ ID NO: 22, or a VH of SEQ ID NO: 39 and a VL of SEQ ID NO: 40, or a VH of SEQ ID NO: 57 and a VL of SEQ ID NO: 58. Most preferred reference antibodies are selected from 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12.

The identification of one or more competing antibodies is a straightforward technical matter now that reference antibodies such as 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) or 273_C01_A12 have been provided. As the identification of competing antibodies is determined in comparison to a reference antibody, it will be understood that actually determining the epitope to which either or both antibodies bind is not in any way required in order to identify a competing antibody. However, epitope mapping can be performed using standard techniques, if desired.

Substantially homologous sequences of proteins of the invention include, without limitation, conservative amino acid substitutions, or for example alterations that do not affect the VH, VL or CDR domains of the antibodies, e.g. antibodies where tag sequences, toxins or other components are added that do not contribute to the binding of antigen, or alterations to convert one type or format of antibody molecule or fragment to another type or format of antibody molecule or fragment (e.g. conversion from Fab to scFv or whole antibody or vice versa), or the conversion of an antibody molecule to a particular class or subclass of antibody molecule (e.g. the conversion of an antibody molecule to IgG or a subclass thereof, e.g. IgG_2).

A "conservative amino acid substitution", as used herein, is one in which the amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g. lysine, arginine, histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g. glycine, cysteine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g. threonine, valine, isoleucine) and aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine).

Homology may be assessed by any convenient method. However, for determining the degree of homology between sequences, computer programs that make multiple alignments of sequences are useful, for instance Clustal W (Thompson, Higgins, Gibson, *Nucleic Acids Res.*, 22:4673-4680, 1994). If desired, the Clustal W algorithm can be used together with BLOSUM 62 scoring matrix (Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA*, 89:10915–10919, 1992) and a gap opening penalty of 10 and gap extension penalty of 0.1, so that the highest order match is obtained between two sequences wherein at least 50% of the total length of one of the sequences is involved in the alignment. Other methods that may be used to align sequences are the alignment method of Needleman and Wunsch (Needleman and Wunsch, *J. Mol. Biol.*, 48:443, 1970) as revised by Smith and Waterman (Smith and Waterman, *Adv. Appl. Math.*, 2:482, 1981) so that the highest order match is obtained between the two sequences and the number of identical amino acids is determined between the two sequences. Other methods to calculate the percentage identity between two amino acid sequences are generally art recognized and include, for example, those described by Carillo and Lipton (Carillo and Lipton, *SIAM J. Applied Math.*, 48:1073, 1988) and those described in

Computational Molecular Biology, Lesk, e.d. Oxford University Press, New York, 1988, Biocomputing: Informatics and Genomics Projects.

Generally, computer programs will be employed for such calculations. Programs that compare and align pairs of sequences, like ALIGN (Myers and Miller, *CABIOS*, 4:11-17, 1988), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, 85:2444-2448, 1988; Pearson, *Methods in Enzymology*, 183:63-98, 1990) and gapped BLAST (Altschul *et al.*, *Nucleic Acids Res.*, 25:3389-3402, 1997), BLASTP, BLASTN, or GCG (Devereux, Haeberli, Smithies, *Nucleic Acids Res.*, 12:387, 1984) are also useful for this purpose. Furthermore, the Dali server at the European Bioinformatics institute offers structure-based alignments of protein sequences (Holm, *Trends in Biochemical Sciences*, 20:478-480, 1995; Holm, *J. Mol. Biol.*, 233:123-38, 1993; Holm, *Nucleic Acid Res.*, 26:316-9, 1998).

By way of providing a reference point, sequences according to the present invention having 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homology, sequence identity *etc.* may be determined using the ALIGN program with default parameters (for instance available on Internet at the GENESTREAM network server, IGH, Montpellier, France).

In the following descriptions of the compositions, immunoconjugates, pharmaceuticals, combinations, cocktails, kits, first and second medical uses and all methods in accordance with this invention, the terms "antibody" and "immunoconjugate", or an antigen-binding region or fragment thereof, unless otherwise specifically stated or made clear from the scientific terminology, refer to a range of anti-PD-1 antibodies as well as to the specific 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) and 273_C01_A12 antibodies.

The terms "antibody" and "immunoglobulin", as used herein, refer broadly to any immunological binding agent that comprises an antigen binding domain (e.g. a human antigen binding domain), including polyclonal and monoclonal antibodies. Depending on the type of constant domain in the heavy chains, whole antibodies are assigned to one of five major classes: IgA, IgD, IgE, IgG, and IgM and the antibodies of the invention may be in any one of these classes. Several of these are further divided into subclasses or isotypes, such as IgG1, IgG2, IgG3, IgG4, and the like. The heavy-chain constant domains that correspond to the difference classes of immunoglobulins are termed α, δ, ε, γ and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

Generally, where whole antibodies rather than antigen binding regions are used in the invention, IgG (e.g. IgG_2) and/or IgM are preferred because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting.

The "light chains" of mammalian antibodies are assigned to one of two clearly distinct types: kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains and some amino acids in the framework regions of their variable domains. In some embodiments, kappa (κ) light chains are preferred.

As will be understood by those in the art, the immunological binding reagents encompassed by the term "antibody" includes or extends to all antibodies and antigen binding fragments thereof, including whole antibodies, dimeric, trimeric and multimeric antibodies; bispecific antibodies; chimeric antibodies; recombinant and engineered antibodies, and fragments thereof.

The term "antibody" is thus used to refer to any antibody-like molecule that has an antigen binding region, and this term includes antibody fragments that comprise an antigen binding domain such as Fab', Fab, $F(ab')_2$, single domain antibodies (DABs), TandAbs dimer, Fv, scFv (single chain Fv), dsFv, ds-scFv, Fd, linear antibodies, minibodies, diabodies, bispecific antibody fragments, bibody, tribody (scFv-Fab fusions, bispecific or trispecific, respectively); sc-diabody; kappa(lamda) bodies (scFv-CL fusions); BiTE (Bispecific T-cell Engager, scFv-scFv tandems to attract T cells); DVD-Ig (dual variable domain antibody, bispecific format); SIP (small immunoprotein, a kind of minibody); SMIP ("small modular immunopharmaceutical" scFv-Fc dimer; DART (ds-stabilized diabody "Dual Affinity ReTargeting"); small antibody mimetics comprising one or more CDRs and the like.

The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Diabodies, in particular, are further described in EP 404 097 and WO 93/11161; whereas linear antibodies are further described in the art.

In preferred embodiments the antibodies of the invention are human antibodies, more preferably fully human antibodies. In this regard, human antibodies generally have at least two potential advantages for use in human therapy. First, the human immune system should not recognize the antibody as foreign. Second, the half-life in the human circulation will be similar to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

However, although human antibodies are generally recognized to display these advantages, it is known that the development of human antibodies that have high enough affinities and appropriate functional properties to make them candidates for successful human therapy is by no means straightforward.

The term "human" as used herein in connection with antibody molecules and binding proteins first refers to antibodies and binding proteins having variable regions (*e.g.,* V_H, V_L, CDR or FR regions) and, optionally, constant antibody regions, isolated or derived from a human repertoire or derived from or corresponding to sequences found in humans or a human repertoire, *e.g.,* in the human germline or somatic cells. The 273_C12_C05 (original clone) and 273_C01_A12 antibodies of the invention are examples of such human antibody molecules wherein the variable regions have been isolated from a human repertoire.

The "human" antibodies and binding proteins of the invention further include amino acid residues not encoded by human sequences, e.g., mutations introduced by random or site directed mutations *in vitro*, for example mutations introduced by *in vitro* cloning or PCR. Particular examples of such mutations are mutations that involve conservative substitutions or other mutations in a small number of residues of the antibody or binding protein, e.g., in up to 5, 4, 3, 2 or 1 of the residues of the antibody or binding protein, preferably e.g., in up to 5, 4, 3, 2 or 1 of the residues making up one or more of the CDRs of the antibody or binding protein. Certain examples of such "human" antibodies include antibodies and variable regions that have been subjected to standard modification techniques to reduce the amount of potentially immunogenic sites.

Thus, the "human" antibodies of the invention include sequences derived from and related to sequences found in humans, but which may not naturally exist within the human antibody germline repertoire *in vivo*. In addition, the human antibodies and binding proteins of the present invention include proteins comprising human consensus sequences identified from human sequences, or sequences substantially homologous to human sequences.

In addition, the human antibodies and binding proteins of the present invention are not limited to combinations of V_H, V_L, CDR or FR regions that are themselves found in combination in human antibody molecules. Thus, the human antibodies and binding proteins of the invention can include or correspond to combinations of such regions that do not necessarily exist naturally in humans (e.g. are not naturally occurring antibodies).

In preferred embodiments, the human antibodies will be fully human antibodies. "Fully human" antibodies, as used herein, are antibodies comprising

"human" variable region domains and/or CDRs, as defined above, without substantial non-human antibody sequences or without any non-human antibody sequences. For example, antibodies comprising human variable region domains and/or CDRs "without substantial non-human antibody sequences" are antibodies, domains and/or CDRs in which only up to 5, 4, 3, 2 or 1 amino acids are amino acids that are not encoded by human antibody sequences. Thus, "fully human" antibodies are distinguished from "humanized" antibodies, which are based on substantially non-human variable region domains, *e.g.*, mouse variable region domains, in which certain amino acids have been changed to better correspond with the amino acids typically present in human antibodies.

The "fully human" antibodies of the invention may be human variable region domains and/or CDRs without any other substantial antibody sequences, such as being single chain antibodies. Alternatively, the "fully human" antibodies of the invention may be human variable region domains and/or CDRs integral with or operatively attached to one or more human antibody constant regions. Certain preferred fully human antibodies are IgG antibodies with the full complement of IgG constant regions.

In other embodiments, "human" antibodies of the invention will be part-human chimeric antibodies. "Part-human chimeric" antibodies, as used herein, are antibodies comprising "human" variable region domains and/or CDRs operatively attached to, or grafted onto, a constant region of a non-human species, such as rat or mouse. Such part-human chimeric antibodies may be used, for example, in pre-clinical studies, wherein the constant region will preferably be of the same species of animal used in the pre-clinical testing. These part-human chimeric antibodies may also be used, for example, in *ex vivo* diagnostics, wherein the constant region of the non-human species may provide additional options for antibody detection.

In preferred embodiments, antibodies of the invention are not murine antibodies.

The term "heavy chain complementarity determining region" ("heavy chain CDR") as used herein refers to regions of hypervariability within the heavy chain variable region (V_H domain) of an antibody molecule. The heavy chain variable region has three CDRs termed heavy chain CDR1, heavy chain CDR2 and heavy chain CDR3 from the amino terminus to carboxy terminus. The heavy chain variable region also has four framework regions (FR1, FR2, FR3 and FR4 from the amino terminus to carboxy terminus). These framework regions separate the CDRs.

The term "heavy chain variable region" (V_H domain) as used herein refers to the variable region of a heavy chain of an antibody molecule.

The term "light chain complementarity determining region" ("light chain CDR") as used herein refers to regions of hypervariability within the light chain variable region (V_L domain) of an antibody molecule. Light chain variable regions have three CDRs termed light chain CDR1, light chain CDR2 and light chain CDR3 from the amino terminus to the carboxy terminus. The light chain variable region also has four framework regions (FR1, FR2, FR3 and FR4 from the amino terminus to carboxy terminus). These framework regions separate the CDRs.

The term "light chain variable region" (V_L domain) as used herein refers to the variable region of a light chain of an antibody molecule.

Antibodies can be fragmented using conventional techniques. For example, $F(ab')_2$ fragments can be generated by treating the antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and $F(ab')_2$, scFv, Fv, dsFv, Fd, dAbs, TandAbs, ds-scFv, dimers, minibodies, diabodies, bispecific antibody fragments and other fragments can also be synthesized by recombinant techniques or can be chemically synthesized. Techniques for producing antibody fragments are well known and described in the art.

In certain embodiments, the antibody or antibody fragment of the present invention comprises all or a portion of a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgE, IgM or IgD constant region. Preferably, the heavy chain constant region is an IgG heavy chain constant region, e.g. an IgG2 heavy chain constant region, or a portion thereof. Furthermore, the antibody or antibody fragment can comprise all or a portion of a kappa light chain constant region or a lambda light chain constant region, or a portion thereof. All or part of such constant regions may be produced naturally or may be wholly or partially synthetic. Appropriate sequences for such constant regions are well known and documented in the art. When a full complement of constant regions from the heavy and light chains are included in the antibodies of the invention, such antibodies are typically referred to herein as "full length" antibodies or "whole" antibodies. In some embodiments, IgG_2 antibodies are preferred. The exemplary 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2) and 273_C01_A12 antibodies of the present invention are IgG_2 antibodies.

The antibodies or antibody fragments can be produced naturally or can be wholly or partially synthetically produced. Thus the antibody may be from any appropriate source, for example recombinant sources and/or produced in transgenic animals or transgenic plants, or in eggs using the IgY technology. Thus, the antibody molecules can be produced *in vitro* or *in vivo*.

Preferably, the antibody or antibody fragment comprises an antibody light chain variable region (V_L) that comprises three CDR domains and an antibody heavy chain variable region (V_H) that comprises three CDR domains. Said VL and VH generally form the antigen binding site.

An "Fv" fragment is the minimum antibody fragment that contains a complete antigen-recognition and binding site. This region has a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions (CDRs) of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions (CDRs) confer antigen-binding specificity to the antibody.

However, it is well documented in the art that the presence of three CDRs from the light chain variable domain and three CDRs from the heavy chain variable domain of an antibody is not always necessary for antigen binding. Thus, constructs smaller than the above classical antibody fragment are known to be effective.

For example, camelid antibodies have an extensive antigen binding repertoire but are devoid of light chains. Also, results with single domain antibodies comprising VH domains alone or VL domains alone show that these domains can bind to antigen with acceptably high affinities. Thus, three CDRs can effectively bind antigen.

Thus, although preferred antibodies of the invention might comprise six CDR regions (three from a light chain and three from a heavy chain), antibodies with fewer than six CDR regions (e.g. 3 CDR regions) are encompassed by the invention. Antibodies with CDRs from only the heavy chain or light chain are also contemplated.

Preferred light chain CDR regions for use in conjunction with the specified heavy chain CDR regions are described elsewhere herein. However, other light chain variable regions that comprise three CDRs for use in conjunction with the heavy chain variable regions of the invention are also contemplated. Appropriate light chain variable regions which can be used in combination with the heavy chain variable regions of the invention and which give rise to an antibody which binds PD-

1 in accordance with the invention can be readily identified by a person skilled in the art.

For example, a heavy chain variable region of the invention can be combined with a single light chain variable region or a repertoire of light chain variable regions and the resulting antibodies tested for binding to PD-1.

If desired, similar methods could be used to identify alternative heavy chain variable regions for use in combination with preferred light chain variable regions of the invention.

A yet further aspect of the invention provides an antibody, preferably an isolated antibody, more preferably a human (or fully human) antibody, which binds to or specifically recognizes PD-1 and which has the ability to compete with (i.e. bind to the same or substantially the same epitope as) the 273_C12_C05 (original clone) and/or 273_C12_C05 (variant 1) and/or 273_C12_C05 (variant 2) and/or 273_C01_A12 (i.e. an antibody comprising the VL of SEQ ID NO:4 and the VH of SEQ ID NO:3, or an antibody comprising the VL of SEQ ID NO:22 and the VH of SEQ ID NO:21, or an antibody comprising the VL of SEQ ID NO:40 and the VH of SEQ ID NO:39, or an antibody comprising the VL of SEQ ID NO:58 and the VH of SEQ ID NO:57 respectively) as described herein, or the ability to compete with an antibody comprising the same CDRs as 273_C12_C05 (original clone) and/or 273_C12_C05 (variant 1) and/or 273_C12_C05 (variant 2) and/or 273_C01_A12 (i.e. an antibody comprising VL CDR sequences of SEQ ID NOs: 8, 9 and 10 and VH CDR sequences of SEQ ID NOs: 5, 6 and 7, or an antibody comprising VL CDR sequences of SEQ ID NOs: 26, 9 and 10 and VH CDR sequences of SEQ ID NOs: 5, 6 and 7, or an antibody comprising VL CDR sequences of SEQ ID NOs: 26, 9 and 10 and VH CDR sequences of SEQ ID NOs: 5, 6 and 7, or an antibody comprising VL CDR sequences of SEQ ID NOs: 62, 63 and 64 and VH CDR sequences of SEQ ID NOs: 5, 6 and 7 respectively) for binding to PD-1. Other features and properties of other aspects of the invention apply, *mutatis mutandis*, to this aspect of the invention.

Binding to the same epitope/antigen can be readily tested by methods well known and described in the art, e.g. using binding assays such as a competitive inhibition assay. Thus, a person skilled in the art will appreciate that binding assays can be used to identify other antibodies and antibody fragments with the same binding specificities as the antibodies and antibody fragments of the invention. Suitable binding assays are discussed elsewhere herein.

Preferably, the above described abilities and properties are observed at a measurable or significant level and more preferably at a statistically significant level, when compared to appropriate control levels. Appropriate significance levels are discussed elsewhere herein. More preferably, one or more of the above described abilities and properties are observed at a level which is measurably better, or more preferably significantly better, when compared to the abilities observed for prior art antibodies.

In any statistical analysis referred to herein, preferably the statistically significant difference over a relevant control or other comparative entity or measurement has a probability value of < 0.1, preferably < 0.05. Appropriate methods of determining statistical significance are well known and documented in the art and any of these may be used.

In other preferred embodiments, second generation antibodies are provided that have enhanced or superior properties in comparison to an original anti-PD-1 antibody of the invention, such as 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2), or 273_C01_A12.

Comparisons to identify effective second generation antibodies are readily conducted and quantified, e.g. using one or more of the various assays described in detail herein or in the art. Second generation antibodies that have an enhanced biological property or activity of at least about 2-fold, 5-fold, 10-fold, 20-fold, and preferably, at least about 50-fold, in comparison to the anti-PD-1 antibodies of the present invention, as exemplified by the 273_C12_C05 (original clone), 273_C12_C05 (variant 1), 273_C12_C05 (variant 2), or 273_C01_A12 antibodies, are encompassed by the present invention.

The antibody, binding protein and nucleic acid molecules of the invention are generally "isolated" or "purified" molecules insofar as they are distinguished from any such components that may be present *in situ* within a human or animal body or a tissue sample derived from a human or animal body. The sequences may, however, correspond to or be substantially homologous to sequences as found in a human or animal body. Thus, the term "isolated" or "purified" as used herein in reference to nucleic acid molecules or sequences and proteins or polypeptides, e.g. antibodies, refers to such molecules when isolated from, purified from, or substantially free of their natural environment, e.g. isolated from or purified from the human or animal body (if indeed they occur naturally), or refers to such molecules when produced by a technical process, i.e. includes recombinant and synthetically produced molecules.

Thus, when used in connection with a protein or polypeptide molecule such as light chain CDRs 1, 2 and 3, heavy chain CDRs 1, 2 and 3, light chain variable regions, heavy chain variable regions, and binding proteins or antibodies of the invention, including full length antibodies, the term "isolated" or "purified" typically refers to a protein substantially free of cellular material or other proteins from the source from which it is derived. In some embodiments, particularly where the protein is to be administered to humans or animals, such isolated or purified proteins are substantially free of culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

The term "nucleic acid sequence" or "nucleic acid molecule" as used herein refers to a sequence of nucleoside or nucleotide monomers composed of naturally occurring bases, sugars and intersugar (backbone) linkages. The term also includes modified or substituted sequences comprising non-naturally occurring monomers or portions thereof. The nucleic acid sequences of the present invention may be deoxyribonucleic acid sequences (DNA) or ribonucleic acid sequences (RNA) and may include naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The sequences may also contain modified bases. Examples of such modified bases include aza and deaza adenine, guanine, cytosine, thymidine and uracil; and xanthine and hypoxanthine. The nucleic acid molecules may be double stranded or single stranded. The nucleic acid molecules may be wholly or partially synthetic or recombinant.

The term "fragment" as used herein refers to fragments of biological relevance, e.g. fragments that contribute to antigen binding, e.g. form part of the antigen binding site, and/or contribute to the functional properties of the PD-1 antibody. Certain preferred fragments comprise a heavy chain variable region (V_H domain) and/or a light chain variable region (V_L domain) of the antibodies of the invention.

A person skilled in the art will appreciate that the proteins and polypeptides of the invention, such as the light and heavy CDRs, the light and heavy chain variable regions, antibodies, antibody fragments, and immunoconjugates, may be prepared in any of several ways well known and described in the art, but are most preferably prepared using recombinant methods.

Nucleic acid fragments encoding the light and heavy chain variable regions of the antibodies of the invention can be derived or produced by any appropriate method, e.g. by cloning or synthesis.

Once nucleic acid fragments encoding the light and heavy chain variable regions of the antibodies of the invention have been obtained, these fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region fragments into full length antibody molecules with appropriate constant region domains, or into particular formats of antibody fragment discussed elsewhere herein, e.g. Fab fragments, scFv fragments, etc. Typically, or as part of this further manipulation procedure, the nucleic acid fragments encoding the antibody molecules of the invention are generally incorporated into one or more appropriate expression vectors in order to facilitate production of the antibodies of the invention.

Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression vectors are "suitable for transformation of a host cell", which means that the expression vectors contain a nucleic acid molecule of the invention and regulatory sequences selected on the basis of the host cells to be used for expression, which are operatively linked to the nucleic acid molecule. Operatively linked is intended to mean that the nucleic acid is linked to regulatory sequences in a manner that allows expression of the nucleic acid.

The invention therefore contemplates a recombinant expression vector containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the protein sequence encoded by the nucleic acid molecule of the invention.

Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes and are well known in the art. Selection of appropriate regulatory sequences is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector.

The recombinant expression vectors of the invention may also contain a selectable marker gene that facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention.

The recombinant expression vectors may also contain genes that encode a fusion moiety that provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid in the purification of the target recombinant protein by acting as a ligand in affinity purification (for example appropriate "tags" to enable purification and/or identification may be present, *e.g.,* His tags or myc tags).

Recombinant expression vectors can be introduced into host cells to produce a transformed host cell. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (*e.g.,* a vector) into a cell by one of many possible techniques known in the art. Suitable methods for transforming and transfecting host cells can be found in Sambrook *et al.*, 1989 (Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989) and other laboratory textbooks.

Suitable host cells include a wide variety of eukaryotic host cells and prokaryotic cells. For example, the proteins of the invention may be expressed in yeast cells or mammalian cells. In addition, the proteins of the invention may be expressed in prokaryotic cells, such as *Escherichia coli*.

Given the teachings provided herein, promoters, terminators, and methods for introducing expression vectors of an appropriate type into plant, avian, and insect cells may also be readily accomplished.

Alternatively, the proteins of the invention may also be expressed in non-human transgenic animals such as, rats, rabbits, sheep and pigs.

The proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis.

N-terminal or C-terminal fusion proteins comprising the antibodies and proteins of the invention conjugated to other molecules, such as proteins, may be prepared by fusing through recombinant techniques. The resultant fusion proteins contain an antibody or protein of the invention fused to the selected protein or marker protein, or tag protein as described herein. The antibodies and proteins of the invention may also be conjugated to other proteins by known techniques. For example, the proteins may be coupled using heterobifunctional thiol-containing linkers as described in WO 90/10457, N-succinimidyl-3-(2-pyridyldithio-proprionate) or N-succinimidyl-5 thioacetate.

A yet further aspect provides an expression construct or expression vector comprising one or more of the nucleic acid fragments or segments or molecules of the invention. Preferably the expression constructs or vectors are recombinant. Preferably said constructs or vectors further comprise the necessary regulatory sequences for the transcription and translation of the protein sequence encoded by the nucleic acid molecule of the invention.

A yet further aspect provides a host cell or virus comprising one or more expression constructs or expression vectors of the invention. Also provided are host cells or viruses comprising one or more of the nucleic acid molecules of the invention. A host cell (e.g. a mammalian host cell) or virus expressing an antibody of the invention forms a yet further aspect.

A yet further aspect of the invention provides a method of producing (or manufacturing) an antibody of the present invention comprising a step of culturing the host cells of the invention. Preferred methods comprise the steps of (i) culturing a host cell comprising one or more of the recombinant expression vectors or one or more of the nucleic acid sequences of the invention under conditions suitable for the expression of the encoded antibody or protein; and optionally (ii) isolating or obtaining the antibody or protein from the host cell or from the growth medium/supernatant. Such methods of production (or manufacture) may also comprise a step of purification of the antibody or protein product and/or formulating the antibody or product into a composition including at least one additional component, such as a pharmaceutically acceptable carrier or excipient.

In embodiments when the antibody or protein of the invention is made up of more than one polypeptide chain (e.g. certain fragments such as Fab fragments or whole antibodies), then all the polypeptides are preferably expressed in the host cell, either from the same or a different expression vector, so that the complete proteins, e.g. antibody proteins of the invention, can assemble in the host cell and be isolated or purified therefrom.

In another aspect, the invention provides a method of binding PD-1, comprising contacting a composition comprising PD-1 with an antibody of the invention, or an immunoconjugate thereof.

In yet another aspect, the invention provides a method of detecting PD-1, comprising contacting a composition suspected of containing PD-1 with an antibody of the invention, or an immunoconjugate thereof, under conditions effective to allow the formation of PD-1 /antibody complexes and detecting the complexes so formed.

The antibodies of the invention may also be used to produce further antibodies that bind to PD-1. Such uses involve for example the addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent antibody to form a new antibody, wherein said parent antibody is one of the antibodies of the invention as defined elsewhere herein, and testing the resulting new antibody to identify antibodies that bind to PD-1 in accordance with the invention. Such methods can be used to form multiple new antibodies that can all be tested for their ability to bind PD-1. Preferably said addition, deletion, substitution or insertion of one or more amino acids takes place in one or more of the CDR domains.

Such modification or mutation to a parent antibody can be carried out in any appropriate manner using techniques well known and documented in the art, for example by carrying out methods of random or directed mutagenesis. If directed mutagenesis is to be used then one strategy to identify appropriate residues for mutagenesis utilizes the resolution of the crystal structure of the binding protein-antigen complex, *e.g.,* the Ab-Ag complex, to identify the key residues involved in the antigen binding. Alanine scanning mutagenesis is also a routine method which can be used to identify the key residues involved in the antigen binding. Subsequently, those residues can be mutated to enhance the interaction. Alternatively, one or more amino acid residues can simply be targeted for directed mutagenesis and the effect on binding to PD-1 assessed.

Random mutagenesis can be carried out in any appropriate way, *e.g.,* by error-prone PCR, chain shuffling or mutator E. coli strains.

Thus, one or more of the V_H domains of the invention can be combined with a single V_L domain or a repertoire of V_L domains from any appropriate source and the resulting new antibodies tested to identify antibodies which bind to PD-1. Conversely, one or more of the V_L domains of the invention can be combined with a single V_H domain or repertoire of V_H domains from any appropriate source and the resulting new antibodies tested to identify antibodies that bind to PD-1.

Similarly, one or more, or preferably all three CDRs of the V_H and/or V_L domains of the invention can be grafted into a single V_H and/or V_L domain or a repertoire of V_H and/or V_L domains, as appropriate, and the resulting new antibodies tested to identify antibodies that bind to PD-1.

Methods of carrying out the above described manipulation of amino acids and protein domains are well known to a person skilled in the art. For example, said manipulations could conveniently be carried out by genetic engineering at the nucleic acid level wherein nucleic acid molecules encoding appropriate binding

proteins and domains thereof are modified such that the amino acid sequence of the resulting expressed protein is in turn modified in the appropriate way.

The new antibodies produced by these methods will preferably have improved functional properties, e.g. a higher or enhanced affinity (or at least an equivalent affinity) for PD-1 as the parent antibodies, and can be treated and used in the same way as the antibodies of the invention as described elsewhere herein (*e.g.,* for therapy, diagnosis, in compositions etc.). Alternatively, or additionally, the new antibodies will have one or more other improved functional properties as described elsewhere herein.

New antibodies produced, obtained or obtainable by these methods form a yet further aspect of the invention.

Testing the ability of one or more antibodies to bind to PD-1 can be carried out by any appropriate method, which are well known and described in the art. Suitable methods are also described in the Examples section.

The invention also provides a range of conjugated antibodies and fragments thereof in which the anti-PD-1 antibody is operatively attached to at least one other therapeutic or diagnostic agent. The term "immunoconjugate" is broadly used to define the operative association of the antibody with another effective agent (e.g. therapeutic agent) and is not intended to refer solely to any type of operative association, and is particularly not limited to chemical "conjugation". Recombinant fusion proteins are particularly contemplated. So long as the delivery or targeting agent is able to bind to the target and the therapeutic or diagnostic agent is sufficiently functional upon delivery, the mode of attachment will be suitable. In one embodiment, interleukins can be bonded (conjugated) or otherwise linked to the corresponding antibodies.

In some embodiments, antibodies of the invention are used (e.g. used therapeutically) in their "naked" unconjugated form.

Compositions comprising at least a first antibody of the invention or an immunoconjugate thereof constitute a further aspect of the present invention. Formulations (compositions) comprising one or more antibodies of the invention in admixture with a suitable diluent, carrier or excipient constitute a preferred embodiment of the present invention. Such formulations may be for pharmaceutical use and thus compositions of the invention are preferably pharmaceutically acceptable. Suitable diluents, excipients and carriers are known to the skilled man.

The compositions according to the invention may be presented, for example, in a form suitable for oral, nasal, parenteral, intravenal, topical or rectal administration. In a preferred embodiment, compositions according to the invention are presented in a form suitable for intravenal administration. In some embodiments, compositions according to the invention are presented in a form suitable for intraperitoneal (i.p.) administration. In some embodiments, compositions according to the invention are presented in a form suitable for injection into a vein or into a tumour.

The active compounds defined herein may be presented in the conventional pharmacological forms of administration, such as tablets, coated tablets, nasal sprays, solutions, emulsions, liposomes, powders, capsules or sustained release forms. Conventional pharmaceutical excipients as well as the usual methods of production may be employed for the preparation of these forms.

Injection solutions may, for example, be produced in the conventional manner, such as by the addition of preservation agents, such as p-hydroxybenzoates, or stabilizers, such as EDTA. The solutions may then be filled into injection vials or ampoules.

Nasal sprays may be formulated similarly in aqueous solution and packed into spray containers, either with an aerosol propellant or provided with means for manual compression.

The pharmaceutical compositions (formulations) of the present invention are preferably administered parenterally. Intravenous administration is preferred. In some embodiments, administration is intraperitoneal (i.p.) administration. In some embodiments, administration is by injection into a tumour. Parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe. Alternatively, parenteral administration can be performed by means of an infusion pump. A further option is a composition which may be a powder or a liquid for the administration of the antibody in the form of a nasal or pulmonal spray. As a still further option, the antibodies of the invention can also be administered transdermally, e.g. from a patch, optionally an iontophoretic patch, or transmucosally, e.g. bucally.

Suitable dosage units can be determined by a person skilled in the art.

The pharmaceutical compositions may additionally comprise further active ingredients (e.g. as described elsewhere herein) in the context of co-administration regimens or combined regimens.

A further aspect of the present invention provides the anti-PD-1 antibodies defined herein for use in therapy, in particular for use in the treatment of cancer or in the treatment of a disorder of the immune system. The treatment of cancer is preferred.

In another aspect, the present invention provides immunoconjugates of the invention for use in therapy, in particular for use in the treatment of cancer or in the treatment of a disorder of the immune system. The treatment of cancer is preferred.

In accordance with the present invention antibodies may target PD-1 positive T-cells and/or PD-1 positive proB-cells.

In one embodiment, solid tumours are treated.

In some embodiments, a tumour or cancer (e.g. a solid tumour) that is characterized by expressing PD-L1 (e.g. on its surface) is treated.

In some embodiments, the disease (e.g. cancer or a disorder of the immune system) is characterised by (or associated with) PD-1/PD-L1 signalling (e.g. aberrant or inappropriate or undesired PD-1/PD-L1 signalling e.g. elevated PD-1/PD-L1 signalling).

Preferred cancers to be treated in accordance with the present invention include non-small cell lung cancer (NSCLC, e.g. squamous NSCLC), small cell lung cancer (e.g extensive stage disease small cell lung cancer), melanoma (e.g. metastatic melanoma such as BRAF negative metastatic melanoma, or multiple melanoma), lymphoma (e.g. acute T-cell lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma or chronic lymphocytic lymphoma), leukemia (e.g acute myeloid leukemia, acute lymphoblastic leukemia or myelodysplatic syndrome), renal cell cancer (RCC, e.g clear cell renal cancer), colorectal cancer, urothelial bladder cancer, urethral cancer, head and neck cancer (e.g. recurrent or metastatic head and neck squamous cell cancer), breast cancer (e.g. metastatic HER-2 negative breast cancer) advanced liver cancer, brain cancer (e.g. glioblastoma or astrocytoma), stomach cancer, oesophageal cancer, pancreatic carcinoma, adenocarcinomas, mesothelioma, peritoneal cancer, fallopian tube cancer, cervical cancer, ovarian cancer, metastatic sarcoma, hematological neoplasms. In some embodiments, the cancer to be treated may be metastatic.

In some preferred embodiments, the cancer to be treated in accordance with the present invention is colorectal cancer. In some preferred embodiments, the cancer to be treated in accordance with the present invention is colon carcinoma.

In some preferred embodiments, the cancer to be treated in accordance with the present invention is selected from the group consisting of lung cancer (e.g. non-small cell lung cancer), renal cell carcinoma, liver carcinoma, metastatic melanoma and recurrent Hodgkin's lymphoma.

Without wishing to be bound by theory, it is believed that antibodies of the present invention may be superior to the antibodies nivolumab and/or pembrolizumab in terms of therapeutic efficacy (e.g. in therapy of cancer such as colorectal cancer for example as assessed in a mouse model of colorectal cancer) and/or in terms of toxicity, and/or in terms of bioavailability and/or in terms of half-life (or other pharmacokinetic parameters).

As described elsewhere herein, preferred antibodies of the present invention have advantages over the antibody nivolumab, for example certain advantages in terms of binding kinetics. Preferred antibodies of the present invention may also have advantages (e.g. analogous advantages) in terms of binding kinetics over the antibody pembrolizumab.

The *in vivo* methods and uses as described herein are generally carried out in a mammal. Any mammal may be treated, for example humans and any livestock, domestic or laboratory animal. Specific examples include mice, rats, pigs, cats, dogs, sheep, rabbits, cows and monkey. Preferably, however, the mammal is a human.

Thus, the term "animal" or "patient" as used herein includes any mammal, for example humans and any livestock, domestic or laboratory animal. Specific examples include mice, rats, pigs, cats, dogs, sheep, rabbits, cows and monkey. Preferably, however, the animal or patient is a human subject. Thus, subjects or patients treated in accordance with the present invention will preferably be humans.

Alternatively viewed, the present invention provides a method of treating cancer or a disorder of the immune system which method comprises administering to a patient in need thereof a therapeutically effective amount of an antibody of the invention as defined herein. The treatment of cancer is preferred. Embodiments of the therapeutic uses of the invention described herein apply, *mutatis mutandis*, to this aspect of the invention.

The present invention also provides a method of treating a disease that is characterized by PD-1/PD-L1 signalling which method comprises administering to a patient in need thereof a therapeutically effective amount of an antibody of the invention as defined herein. Embodiments of the therapeutic uses of the invention described herein apply, *mutatis mutandis*, to this aspect of the invention.

A therapeutically effective amount will be determined based on the clinical assessment and can be readily monitored. Preferred cancer therapies are as described elsewhere herein.

Further alternatively viewed, the present invention provides the use of an antibody of the invention as defined herein in the manufacture of a medicament for use in therapy. Preferred therapy is cancer therapy as described elsewhere herein (e.g. therapy of solid tumours). Therapy may also be of a disorder of the immune system. Embodiments of the therapeutic uses of the invention described herein apply, *mutatis mutandis*, to this aspect of the invention.

Further alternatively viewed, the present invention provides the use of an antibody of the invention as defined herein for the treatment of a disease that is characterized by PD-1/PD-L1 signalling. A preferred use is for the treatment of cancer (as described elsewhere herein). Embodiments of the therapeutic uses of the invention described herein apply, *mutatis mutandis*, to this aspect of the invention.

The antibodies and compositions and methods and uses of the present invention may be used in combination with other therapeutics and diagnostics. In terms of biological agents, preferably diagnostic or therapeutic agents, for use "in combination" with an anti-PD-1 antibody in accordance with the present invention, the term "in combination" is succinctly used to cover a range of embodiments. The "in combination" terminology, unless otherwise specifically stated or made clear from the scientific terminology, thus applies to various formats of combined compositions, pharmaceuticals, cocktails, kits, methods, and first and second medical uses.

The "combined" embodiments of the invention thus include, for example, where an anti-PD-1 antibody of the invention is a naked antibody and is used in combination with an agent or therapeutic agent (e.g. a chemotherapeutic agent) that is not operatively attached thereto. In other "combined" embodiments of the invention, an anti-PD-1 antibody of the invention is an immunoconjugate wherein the antibody is itself operatively associated or combined with the agent or therapeutic agent. The operative attachment includes all forms of direct and indirect attachment as described herein and known in the art.

The "combined" uses, particularly in terms of an anti-PD-1 antibody of the invention in combination with therapeutic agents, also include combined compositions, pharmaceuticals, cocktails, kits, methods, and first and second medical uses wherein the therapeutic agent is in the form of a prodrug. In such

embodiments, the activating component able to convert the prodrug to the functional form of the drug may again be operatively associated with the anti-PD-1 antibodies of the present invention.

In certain embodiments, the therapeutic compositions, combinations, pharmaceuticals, cocktails, kits, methods, and first and second medical uses will be "prodrug combinations". As will be understood by those of ordinary skill in the art, the term "prodrug combination", unless otherwise stated, means that the antibody of the invention is operatively attached to a component capable of converting the prodrug to the active drug, not that the antibody is attached to the prodrug itself. However, there is no requirement that the prodrug embodiments of the invention need to be used as prodrug combinations. Accordingly, prodrugs may be used in any manner that they are used in the art, including in ADEPT and other forms.

Thus, where combined compositions, pharmaceuticals, cocktails, kits, methods, and first and second medical uses are described, preferably in terms of diagnostic agents, and more preferably therapeutic agents, the combinations include anti-PD-1 antibodies that are naked antibodies and immunoconjugates, and wherein practice of the *in vivo* embodiments of the invention involves the prior, simultaneous or subsequent administration of the naked antibodies or immunoconjugate and the biological, diagnostic or therapeutic agent; so long as, in some conjugated or unconjugated form, the overall provision of some form of the antibody and some form of the biological, diagnostic or therapeutic agent is achieved.

The foregoing and other explanations of the effects of the present invention on tumors are made for simplicity to explain the combined mode of operation, type of attached agent(s) and such like. This descriptive approach should not be interpreted as either an understatement or an oversimplification of the beneficial properties of the anti-PD-1 antibodies of the invention. It will therefore be understood that such antibodies themselves have anti-PD-1 properties and that immunoconjugates of such antibodies will maintain these properties and combine them with the properties of the attached agent; and further, that the combined effect of the antibody and any attached agent will typically be enhanced and/or magnified.

The invention therefore provides compositions, pharmaceutical compositions, therapeutic kits and medicinal cocktails comprising, optionally in at least a first composition or container, a biologically effective amount of at least a first anti-PD-1 antibody of the invention, or an antigen-binding fragment or immunoconjugate of such an anti-PD-1 antibody; and a biologically effective amount of at least a second biological agent, component or system.

The "at least a second biological agent, component or system" will often be a therapeutic or diagnostic agent, component or system, but it need not be. For example, the at least a second biological agent, component or system may comprise components for modification of the antibody and/or for attaching other agents to the antibody. Certain preferred second biological agents, components or systems are prodrugs or components for making and using prodrugs, including components for making the prodrug itself and components for adapting the antibodies of the invention to function in such prodrug or ADEPT embodiments.

Where therapeutic or diagnostic agents are included as the at least a second biological agent, component or system, such therapeutics and/or diagnostics will typically be those for use in connection with the treatment or diagnosis of one or more of the disorders defined above.

Thus, in certain embodiments "at least a second therapeutic agent" will be included in the therapeutic kit or cocktail. The term is chosen in reference to the anti-PD-1 antibody of the invention being the first therapeutic agent.

In certain embodiments of the present invention, the second therapeutic agent may be a radiotherapeutic agent, chemotherapeutic agent, anti-angiogenic agent, apoptosis-inducing agent, anti-tubulin drug, anti-cellular or cytotoxic agent, steroid, cytokine antagonist, cytokine expression inhibitor, chemokine antagonist, chemokine expression inhibitor, ATPase inhibitor, anti-inflammatory agent, signalling pathway inhibitor, other checkpoint inhibitor, anti-cancer agent, other antibodies or coagulant.

In terms of compositions, kits and/or medicaments of the invention, the combined effective amounts of the therapeutic agents may be comprised within a single container or container means, or comprised within distinct containers or container means. The cocktails will generally be admixed together for combined use. Agents formulated for intravenous administration will often be preferred. Imaging components may also be included. The kits may also comprise instructions for using the at least a first antibody and the one or more other biological agents included.

Speaking generally, the at least a second therapeutic agent may be administered to the animal or patient substantially simultaneously with the anti-PD-1 antibody of the invention; such as from a single pharmaceutical composition or from two pharmaceutical compositions administered closely together.

Alternatively, the at least a second therapeutic agent may be administered to the animal or patient at a time sequential to the administration of the anti-PD-1 antibody of the invention. "At a time sequential", as used herein, means

"staggered", such that the at least a second therapeutic agent is administered to the animal or patient at a time distinct to the administration of the anti-PD-1 antibody of the invention. Generally, the two agents are administered at times effectively spaced apart to allow the two agents to exert their respective therapeutic effects, i.e., they are administered at "biologically effective time intervals". The at least a second therapeutic agent may be administered to the animal or patient at a biologically effective time prior to the anti-PD-1 antibody of the invention, or at a biologically effective time subsequent to that therapeutic.

Yet further aspects are methods of diagnosis or imaging of a subject comprising the administration of an appropriate amount of an antibody or other protein of the invention as defined herein to the subject and detecting the presence and/or amount and/or the location of the antibody or other protein of the invention in the subject.

Appropriate diseases to be imaged or diagnosed in accordance with the present invention are described elsewhere herein in connection with disease treatments.

In one embodiment, the invention provides a method of diagnosing cancer or a disorder of the immune system (preferably diagnosing cancer) in a mammal comprising the step of:

(a) contacting a test sample taken from said mammal with one or more of the antibodies of the invention.

In a further embodiment, the invention provides a method of diagnosing cancer or a disorder of the immune system (preferably diagnosing cancer) in a mammal comprising the steps of:

(a) contacting a test sample taken from said mammal with one or more of the antibodies of the invention;

(b) measuring the presence and/or amount and/or location of antibody-antigen complex in the test sample; and, optionally

(c) comparing the presence and/or amount of antibody-antigen complex in the test sample to a control.

In the above methods, said contacting step is carried out under conditions that permit the formation of an antibody-antigen complex. Appropriate conditions can readily be determined by a person skilled in the art.

In the above methods any appropriate test sample may be used, for example biopsy cells, tissues or organs suspected of being affected by disease or histological sections.

In certain of the above methods, the presence of any amount of antibody-antigen complex in the test sample would be indicative of the presence of disease. Preferably, for a positive diagnosis to be made, the amount of antibody-antigen complex in the test sample is greater than, preferably significantly greater than, the amount found in an appropriate control sample. More preferably, the significantly greater levels are statistically significant, preferably with a probability value of <0.05. Appropriate methods of determining statistical significance are well known and documented in the art and any of these may be used.

Appropriate control samples could be readily chosen by a person skilled in the art, for example, in the case of diagnosis of a particular disease, an appropriate control would be a sample from a subject that did not have that disease. Appropriate control "values" could also be readily determined without running a control "sample" in every test, e.g. by reference to the range for normal subjects known in the art.

For use in the diagnostic or imaging applications, the antibodies of the invention may be labeled with a detectable marker such as a radio-opaque or radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{123}I, ^{125}I, ^{131}I; a radioactive emitter (e.g. α, β or γ emitters); a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion; or a chemical moiety such as biotin which may be detected by binding to a specific cognate detectable moiety, e.g. labelled avidin/streptavidin. Methods of attaching a label to a binding protein, such as an antibody or antibody fragment, are known in the art. Such detectable markers allow the presence, amount or location of binding protein-antigen complexes in the test sample to be examined.

Preferred detectable markers for *in vivo* use include an X-ray detectable compound, such as bismuth (III), gold (III), lanthanum (III) or lead (II); a radioactive ion, such as copper^{67}, gallium^{67}, gallium^{68}, indium^{111}, indium^{113}, iodine^{123}, iodine^{125}, iodine^{131}, mercury^{197}, mercury^{203}, rhenium^{186}, rhenium^{188}, rubidium^{97}, rubidium^{103}, technetium^{99m} or yttrium^{90}; a nuclear magnetic spin-resonance isotope, such as cobalt (II), copper (II), chromium (III), dysprosium (III), erbium (III), gadolinium (III), holmium (III), iron (II), iron (III), manganese (II), neodymium (III), nickel (II),

samarium (III), terbium (III), vanadium (II) or ytterbium (III); or rhodamine or fluorescein.

The invention also includes diagnostic or imaging agents comprising the antibodies of the invention attached to a label that produces a detectable signal, directly or indirectly. Appropriate labels are described elsewhere herein.

In one embodiment the method of diagnosing cancer or a disorder of the immune system is an *in vitro* method.

In one embodiment the method of diagnosing cancer or a disorder of the immune system is an *in vivo* method.

Preferred diseases (e.g. cancers) to be diagnosed are described herein (e.g. in the context of cancer therapies).

Alternatively viewed, the present invention provides a method for screening for cancer or a disorder of the immune system in a subject.

The present invention also provides a method of analysing (or predicting) whether or not a subject having cancer (known to have cancer) is likely to (is predicted to) benefit from an anti-PD1 treatment (e.g. therapy with an anti-PD-1 antibody of the invention). Such a method may comprise one or more of the steps set out above in the context of a method for diagnosing. In such a method, the presence and/or amount and/or location of antibody/antigen complex in the test sample may be indicative of (predictive of) whether or not a subject having cancer (known to have cancer) is likely to (is predicted to) benefit from an anti-PD1 treatment (e.g. therapy with an anti-PD-1 antibody of the invention). For example, if PD-1 is present (e.g. above a particular level or amount for example in comparison with a control level or amount, and/or in a particular location) then said subject may benefit (be predicted to benefit) from an anti-PD-1 treatment. Serial (periodic) measuring of the presence and/or amount and/or location of PD-1 may also be performed, e.g. looking for either increasing or decreasing amounts/levels over time.

Alternatively viewed, the present invention provides a method for predicting the response of a subject to anti-PD1 cancer therapy (e.g. therapy with an anti-PD1 antibody of the invention). Alternatively viewed, the present invention provides a method of determining (or monitoring) the efficacy of a therapeutic regime being used to treat cancer (e.g. an anti-PD1 antibody regime), in other words following a response to treatment.

The present invention also provides a method for monitoring the progression of cancer in a subject. Thus, methods (or antibodies) of the invention can be also

be used to monitor disease progression. Such monitoring can take place before, during, or after (preferably during) treatment for cancer (e.g. by anti-PD1 therapy). Such a method may comprise one or more of the steps set out above in the context of a method for diagnosing. In such a method, if the amount of PD-1 detected during (or after) therapy decreases (e.g. over time) this may indicate that the cancer is improving (improved/improving prognosis). In such a method, if the amount of PD-1 detected during (or after) therapy increases (e.g. over time) this may indicate that the cancer is worsening (worse/worsening prognosis).

In some embodiments, serial (periodic) measuring of PD-1 (presence and/or amount and/or location) in accordance with the present invention may also be used for prognostic purposes looking for either increasing or decreasing amounts (or levels) over time. In some embodiments, an increased level of PD-1 over time (e.g. in comparison to a control level) may indicate a worsening prognosis. In some embodiments, a decreased level of PD-1 over time (e.g. in comparison to a control level) may indicate an improving prognosis.

In some embodiments, features of the methods of analyzing (or predicting) whether or not a subject having cancer (known to have cancer) is likely to (is predicted to) benefit from an anti-PD1 treatment, the methods for predicting the response of a subject to anti-PD1 cancer therapy, the methods of determining (or monitoring) the efficacy of a therapeutic regime being used to treat cancer, and the methods for monitoring the progression of cancer in a subject may also be used, *mutatis mutandis*, in relation to disorders of the immune system.

In some embodiments, antibodies of the present invention can be used as companion diagnostics.

In one embodiment (e.g. of methods of diagnosing or predicting or monitoring of the invention), the subject (e.g. a human) is a subject at risk of developing cancer (or a disorder of the immune system) or at risk of the occurrence of cancer (or a disorder of the immune system), e.g. a healthy subject or a subject not displaying any symptoms of cancer (or of a disorder of the immune system) or any other appropriate “at risk” subject. In another embodiment the subject is a subject having, or suspected of having (or developing), or potentially having (or developing) cancer (or a disorder of the immune system). For example, the subject (or sample, e.g. tissue biopsy, from the subject) may be positive for one or more cancer markers (e.g. cancer biomarkers other than PD-1) and/or may be known to have (diagnosed with) an anomalous tissue type or an anomalous tissue growth (e.g. as assessed by tissue biopsy).

In some aspects, a method of the invention may further comprise an initial step of selecting a subject (e.g. a human subject) at risk of developing cancer (or a disorder of the immune system) or at risk of the occurrence of cancer (or a disorder of the immune system), or having or suspected of having (or developing), cancer (or a disorder of the immune system), or potentially having (or developing) cancer (or a disorder of the immune system). Subjects may be selected on the basis that, for example, the subject (or sample, e.g. tissue biopsy, from the subject) is positive for one or more cancer markers (e.g. cancer biomarkers other than PD-1) and/or is known to have (be diagnosed with) an anomalous tissue type or an anomalous tissue growth (e.g. as assessed by tissue biopsy). The subsequent method steps can be performed on a sample from such a selected subject.

In some aspects, methods of the invention are provided which further comprise a step of treating cancer (or a disorder of the immune system) by therapy (e.g. pharmaceutical therapy) or surgery. For example, if the result of a method of the invention is indicative of cancer (or a disorder of the immune system) in the subject (e.g. a positive diagnosis of cancer or a disorder of the immune system is made), then an additional step of treating the cancer (or a disorder of the immune system) by therapy or surgery can be performed. In some embodiments of diagnostic/predictive/monitoring/prognostic methods of the invention, an additional step of treating the cancer (or a disorder of the immune system) by therapy or surgery can be performed (e.g. if there is a poor prognosis). Methods of treating cancer (or a disorder of the immune system) by therapy or surgery are known in the art.

The invention further includes kits comprising one or more of the antibodies, immunoconjugates or compositions of the invention or one or more of the nucleic acid molecules encoding the antibodies of the invention, or one or more recombinant expression vectors comprising the nucleic acid sequences of the invention, or one or more host cells or viruses comprising the recombinant expression vectors or nucleic acid sequences of the invention. Preferably said kits are for use in the methods and uses as described herein, e.g. the therapeutic, diagnostic or imaging methods as described herein, or are for use in the *in vitro* assays or methods as described herein. The antibody in such kits may be an antibody conjugate as described elsewhere herein, e.g. may be conjugated to a detectable moiety or may be an immunoconjugate. Preferably said kits comprise instructions for use of the kit components. Preferably said kits are for diagnosing or

treating diseases as described elsewhere herein, and optionally comprise instructions for use of the kit components to diagnose or treat such diseases.

The antibodies of the invention as defined herein may also be used as molecular tools for *in vitro* or *in vivo* applications and assays. As the antibodies have an antigen binding site, these can function as members of specific binding pairs and these molecules can be used in any assay where the particular binding pair member is required.

Thus, yet further aspects of the invention provide a reagent that comprises an antibody of the invention as defined herein and the use of such antibodies as molecular tools, for example in *in vitro* or *in vivo* assays.

TABLES OF NUCLEOTIDE AND AMINO ACID SEQUENCES DISCLOSED HEREIN AND THEIR SEQUENCE IDENTIFIERS (SEQ ID NOs)

All nucleotide sequences are recited herein 5' to 3' in line with convention in this technical field.

Table A

SEQ ID NO:	Description	Sequence
273_C12_C05 (Original clone)		
1	VH domain (nt)	GAGGTGCAGCTCGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTCACCAGCTACTATGTGC ATTGGGTCCGACAGGCCCCAGGCCAGGGCCTGGA ATGGATGGGCGGCATCATCCCCATCTTCGGCACC GCCAACTACGCCCAGAAATTCCAGGGCAGAGTGA CCATCACCGCCGACGAGTCCACCTCCACCGCCTA CATGGAACTGTCCTCCCTGCGGAGCGAGGACACC GCCGTGTACTACTGCGCCAGGGACCTGCACGGCT ACTCCTACGGCTACCCCGGCTATTGGGGCCAGGG CACCCTGGTCACCGTGTCCTCT
2	VL domain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGC CCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTG CCGGTCCTCCCAGTCCCTGGTGTACCACGACGGC AACACCTACCTGAACTGGTTCCAGCAGCGGCCAG GCCAGTCCCCTCGGCGGCTGATCTACGAGGTGTC CAACCGGGACTCTGGCGTGCCCGACAGATTCTCC GGCTCCGGCAGCGACACCGACTTCACCCTGAAGA TCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT ACTACTGCATGCAGGGCGCCTACCGGCCCCTGAC CTTCGGCCAGGGCACCAAGGTGGAAATCAAG

Table A		
SEQ ID NO:	**Description**	**Sequence**
3	VH domain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESTSTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSS
4	VL domain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYHDGNTY LNWFQQRPGQSPRRLIYEVSNRDSGVPDRFSGSGS DTDFTLKISRVEAEDVGVYYCMQGAYRPLTFGQGTK VEIK
5	Heavy CDR1	SYYVH
6	Heavy CDR2	GIIPIFGTANYAQKFQG
7	Heavy CDR3	DLHGYSYGYPGY
8	Light CDR1	RSSQSLVYHDGNTYLN
9	Light CDR2	EVSNRDS
10	Light CDR3	MQGAYRPLT
11	Heavy FR1	EVQLVQSGAEVKKPGSSVKVSCKASGYTFT
12	Heavy FR2	WVRQAPGQGLEWMG
13	Heavy FR3	RVTITADESTSTAYMELSSLRSEDTAVYYCAR
14	Heavy FR4	WGQGTLVTVSS
15	Light FR1	DIVMTQSPLSLPVTLGQPASISC
16	Light FR2	WFQQRPGQSPRRLIY
17	Light FR3	GVPDRFSGSGSDTDFTLKISRVEAEDVGVYYC
18	Light FR4	FGQGTKVEIK
85	Heavy chain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESTSTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKV DKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMT

Table A		
SEQ ID NO:	**Description**	**Sequence**
		KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
86	Light chain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYHDGNTY LNWFQQRPGQSPRRLIYEVSNRDSGVPDRFSGSGS DTDFTLKISRVEAEDVGVYYCMQGAYRPLTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
87	Heavy chain (nt)	GAGGTGCAGCTCGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTCACCAGCTACTATGTGC ATTGGGTCCGACAGGCCCCAGGCCAGGGCCTGGA ATGGATGGGCGGCATCATCCCCATCTTCGGCACC GCCAACTACGCCCAGAAATTCCAGGGCAGAGTGA CCATCACCGCCGACGAGTCCACCTCCACCGCCTA CATGGAACTGTCCTCCCTGCGGAGCGAGGACACC GCCGTGTACTACTGCGCCAGGGACCTGCACGGCT ACTCCTACGGCTACCCCGGCTATTGGGGCCAGGG CACCCTGGTCACCGTGTCCTCTGCCTCCACCAAG GGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCA GAAGCACCAGCGAGAGCACCGCCGCCCTGGGCT GCCTGGTGAAGGACTACTTCCCCGAGCCCGTGAC CGTGAGCTGGAACAGCGGCGCCCTGACCAGCGG CGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGC GGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGC CCAGCAGCAACTTCGGCACCCAGACCTACACCTG CAACGTGGACCACAAGCCCAGCAACACCAAGGTG GACAAGACCGTGGAGAGAAAGTGCTGCGTGGAGT GCCCTCCCTGCCCCGCTCCCCCTGTGGCTGGCCC CAGCGTGTTCCTGTTCCCTCCCAAGCCCAAGGACA CCCTGATGATCAGCAGAACCCCCGAGGTGACCTG CGTGGTCGTGGACGTGAGCCACGAGGACCCCGA GGTGCAGTTCAACTGGTACGTGGACGGCGTGGAG GTGCACAACGCCAAGACCAAGCCCAGAGAGGAGC AGTTCAACAGCACCTTCAGAGTGGTGAGCGTGCT GACCGTGGTGCACCAGGACTGGCTGAACGGCAAG GAGTACAAGTGCAAGGTGAGCAACAAGGGCCTGC CCGCCCCCATCGAGAAGACCATCAGCAAGACCAA GGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTG CCCCCTAGCAGAGAGGAGATGACCAAGAACCAGG TGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCC

Table A		
SEQ ID NO:	**Description**	**Sequence**
		CAGCGACATCGCCGTGGAGTGGGAGAGCAACGG CCAGCCCGAGAACAACTACAAGACCACACCCCCC ATGCTGGACAGCGACGGCAGCTTCTTCCTGTACA GCAAGCTGACCGTGGACAAGAGCAGATGGCAGCA GGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG GCCCTGCACAACCACTACACCCAGAAGAGCCTGA GCCTGAGCCCCGGCAAG
88	Light chain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGC CCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTG CCGGTCCTCCCAGTCCCTGGTGTACCACGACGGC AACACCTACCTGAACTGGTTCCAGCAGCGGCCAG GCCAGTCCCCTCGGCGGCTGATCTACGAGGTGTC CAACCGGGACTCTGGCGTGCCCGACAGATTCTCC GGCTCCGGCAGCGACACCGACTTCACCCTGAAGA TCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT ACTACTGCATGCAGGGCGCCTACCGGCCCCTGAC CTTCGGCCAGGGCACCAAGGTGGAAATCAAGCGA ACCGTGGCCGCTCCCAGCGTGTTCATCTTCCCTCC CAGCGACGAGCAGCTGAAGAGCGGCACCGCCAG CGTGGTGTGCCTGCTGAACAACTTCTACCCCAGAG AGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCT GCAGAGCGGCAACAGCCAGGAGAGCGTGACCGA GCAGGACAGCAAGGACAGCACCTACAGCCTGAGC AGCACCCTGACCCTGAGCAAGGCCGACTACGAGA AGCACAAGGTGTACGCCTGCGAGGTGACCCACCA GGGCCTGAGCAGCCCCGTGACCAAGAGCTTCAAC AGAGGCGAGTGC

Table B		
SEQ ID NO:	**Description**	**Sequence**
273_C12_C05 (Variant 1)		
19	VH domain (nt)	GAGGTGCAGCTCGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTCACCAGCTACTATGTGC ATTGGGTCCGACAGGCCCCAGGCCAGGGCCTGGA ATGGATGGGCGGCATCATCCCCATCTTCGGCACC GCCAACTACGCCCAGAAATTCCAGGGCAGAGTGA CCATCACCGCCGACGAGTCCACCGACACCGCCTA CATGGAACTGTCCTCCCTGCGGAGCGAGGACACC GCCGTGTACTACTGCGCCAGGGACCTGCACGGCT ACTCCTACGGCTACCCCGGCTATTGGGGCCAGGG CACCCTGGTCACCGTGTCCTCT
20	VL domain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGC CCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTG CCGGTCCTCCCAGTCCCTGGTGTACCACGACGCC AACACCTACCTGAACTGGTTCCAGCAGCGGCCAG GCCAGTCCCCTCGGCGGCTGATCTACGAGGTGTC CAACCGGGACTCTGGCGTGCCCGACAGATTCTCC GGCTCCGGCAGCGACACCGACTTCACCCTGAAGA TCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT ACTACTGCATGCAGGGCGCCTACCGGCCCCTGAC CTTCGGCCAGGGCACCAAGGTGGAAATCAAG
21	VH domain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESTDTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSS
22	VL domain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYHDANTY LNWFQQRPGQSPRRLIYEVSNRDSGVPDRFSGSGS DTDFTLKISRVEAEDVGVYYCMQGAYRPLTFGQGTK VEIK
23 or 5	Heavy CDR1	SYYVH
24 or 6	Heavy CDR2	GIIPIFGTANYAQKFQG
25 or 7	Heavy CDR3	DLHGYSYGYPGY
26	Light CDR1	RSSQSLVYHDANTYLN
27 or 9	Light CDR2	EVSNRDS
28 or 10	Light CDR3	MQGAYRPLT

Table B		
SEQ ID NO:	**Description**	**Sequence**
29	Heavy FR1	EVQLVQSGAEVKKPGSSVKVSCKASGYTFT
30	Heavy FR2	WVRQAPGQGLEWMG
31	Heavy FR3	RVTITADESTDTAYMELSSLRSEDTAVYYCAR
32	Heavy FR4	WGQGTLVTVSS
33	Light FR1	DIVMTQSPLSLPVTLGQPASISC
34	Light FR2	WFQQRPGQSPRRLIY
35	Light FR3	GVPDRFSGSGSDTDFTLKISRVEAEDVGVYYC
36	Light FR4	FGQGTKVEIK
89	Heavy chain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESTDTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKV DKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
90	Light chain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYHDANTY LNWFQQRPGQSPRRLIYEVSNRDSGVPDRFSGSGS DTDFTLKISRVEAEDVGVYYCMQGAYRPLTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
91	Heavy chain (nt)	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTTACCAGCTACTACGTGC ACTGGGTGCGACAGGCCCCTGGACAGGGCCTGG AATGGATGGGCGGCATCATCCCCATCTTCGGCAC CGCCAACTACGCCCAGAAATTCCAGGGCAGAGTG ACCATCACCGCCGACGAGTCTACCGACACCGCCT ACATGGAACTGTCCTCCCTGCGGAGCGAGGACAC CGCCGTGTACTATTGCGCCAGAGATCTGCACGGC TACTCCTACGGCTACCCCGGCTATTGGGGACAGG GCACCCTCGTGACAGTGTCCTCCGCTTCTACCAAG GGCCCCAGCGTGTTCCCTCTGGCCCCTTGCTCCA

Table B		
SEQ ID NO:	**Description**	**Sequence**
		GATCCACCTCCGAGTCTACAGCCGCCCTGGGCTG CCTCGTGAAGGACTACTTTCCTGAGCCCGTGACC GTGTCTTGGAACTCTGGCGCTCTGACCAGCGGCG TGCACACCTTCCCTGCTGTGCTGCAGTCCTCCGG CCTGTACAGCCTGTCCAGCGTCGTGACTGTGCCC TCCTCCAACTTTGGCACCCAGACCTACACCTGTAA CGTGGACCACAAGCCCTCCAACACCAAAGTGGAC AAGACCGTGGAACGGAAGTGCTGCGTGGAATGCC CCCCTTGTCCTGCCCCTCCTGTGGCTGGCCCTTC CGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACC CTGATGATCAGCCGGACCCCTGAAGTGACCTGCG TGGTGGTGGATGTGTCCCACGAGGACCCCGAGGT GCAGTTCAATTGGTACGTGGACGGCGTGGAAGTG CACAATGCCAAGACCAAGCCCAGAGAGGAACAGT TCAACAGCACCTTCCGGGTGGTGTCCGTGCTGAC CGTGGTGCATCAGGACTGGCTGAACGGCAAAGAG TACAAGTGCAAAGTGTCCAACAAGGGCCTGCCTG CCCCCATCGAAAAGACCATCTCTAAGACCAAGGGA CAGCCCCGCGAGCCTCAGGTGTACACACTGCCCC CTAGCCGGGAAGAGATGACCAAGAACCAGGTGTC CCTGACCTGTCTCGTGAAAGGCTTCTACCCTTCCG ATATCGCCGTGGAATGGGAGTCCAACGGCCAGCC CGAGAACAACTACAAGACCACCCCCCCCATGCTG GACTCCGATGGCTCATTCTTCCTGTACTCCAAGCT GACTGTGGACAAGTCCCGGTGGCAGCAGGGCAAC GTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGC ACAACCACTACACCCAGAAGTCCCTGTCCCTGAGC CCCGGCAAA
92	Light chain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCTCTGCC TGTGACCCTGGGACAGCCTGCCTCCATCTCCTGC AGATCCTCCCAGTCCCTGGTGTACCACGACGCCA ACACCTACCTGAACTGGTTCCAGCAGCGGCCTGG CCAGTCTCCCAGACGGCTGATCTACGAGGTGTCC AACCGGGACTCCGGCGTGCCCGATAGATTCTCCG GCTCTGGCTCCGACACCGACTTCACCCTGAAGATC TCCCGGGTGGAAGCCGAGGACGTGGGCGTGTACT ACTGTATGCAGGGCGCCTACCGGCCCCTGACCTT TGGCCAGGGAACAAAGGTGGAAATCAAGCGGACC GTGGCCGCTCCCTCCGTGTTCATCTTCCCACCTTC CGACGAGCAGCTGAAGTCCGGCACCGCTTCTGTC GTGTGCCTGCTGAACAACTTCTACCCCCGCGAGG CCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCA GTCCGGCAACTCCCAGGAATCCGTGACCGAGCAG GACTCCAAGGACAGCACCTACTCCCTGTCCTCCAC CCTGACCCTGTCCAAGGCCGACTACGAGAAGCAC AAGGTGTACGCCTGCGAAGTGACCCACCAGGGCC TGTCTAGCCCCGTGACCAAGTCTTTCAACCGGGG CGAGTGC

SEQ ID NO: 23 is identical to SEQ ID NO:5.

SEQ ID NO: 24 is identical to SEQ ID NO:6.

SEQ ID NO: 25 is identical to SEQ ID NO:7.

SEQ ID NO: 27 is identical to SEQ ID NO:9.

SEQ ID NO: 28 is identical to SEQ ID NO:10.

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Table C		
SEQ ID NO:	**Description**	**Sequence**
273_C12_C05 (Variant 2)		
37	VH domain (nt)	GAGGTGCAGCTCGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTCACCAGCTACTATGTGC ATTGGGTCCGACAGGCCCCAGGCCAGGGCCTGGA ATGGATGGGCGGCATCATCCCCATCTTCGGCACC GCCAACTACGCCCAGAAATTCCAGGGCAGAGTGA CCATCACCGCCGACGAGTCCATCTCCACCGCCTA CATGGAACTGTCCTCCCTGCGGAGCGAGGACACC GCCGTGTACTACTGCGCCAGGGACCTGCACGGCT ACTCCTACGGCTACCCCGGCTATTGGGGCCAGGG CACCCTGGTCACCGTGTCCTCT
38	VL domain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGC CCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTG CCGGTCCTCCCAGTCCCTGGTGTACCACGACGCC AACACCTACCTGAACTGGTTCCAGCAGCGGCCAG GCCAGTCCCCTCGGCGGCTGATCTACGAGGTGTC CAACCGGGACTCTGGCGTGCCCGACAGATTCTCC GGCTCCGGCAGCGACACCGACTTCACCCTGAAGA TCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT ACTACTGCATGCAGGGCGCCTACCGGCCCCTGAC CTTCGGCCAGGGCACCAAGGTGGAAATCAAG
39	VH domain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESISTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSS
40	VL domain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYHDANTY LNWFQQRPGQSPRRLIYEVSNRDSGVPDRFSGSGS DTDFTLKISRVEAEDVGVYYCMQGAYRPLTFGQGTK VEIK
41 or 5	Heavy CDR1	SYYVH

Table C		
SEQ ID NO:	**Description**	**Sequence**
or 23		
42 or 6 or 24	Heavy CDR2	GIIPIFGTANYAQKFQG
43 or 7 or 25	Heavy CDR3	DLHGYSYGYPGY
44 or 26	Light CDR1	RSSQSLVYHDANTYLN
45 or 9 or 27	Light CDR2	EVSNRDS
46 or 10 or 28	Light CDR3	MQGAYRPLT
47	Heavy FR1	EVQLVQSGAEVKKPGSSVKVSCKASGYTFT
48	Heavy FR2	WVRQAPGQGLEWMG
49	Heavy FR3	RVTITADESISTAYMELSSLRSEDTAVYYCAR
50	Heavy FR4	WGQGTLVTVSS
51	Light FR1	DIVMTQSPLSLPVTLGQPASISC
52	Light FR2	WFQQRPGQSPRRLIY
53	Light FR3	GVPDRFSGSGSDTDFTLKISRVEAEDVGVYYC
54	Light FR4	FGQGTKVEIK
93	Heavy chain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESISTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKV DKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE

Table C		
SEQ ID NO:	**Description**	**Sequence**
		ALHNHYTQKSLSLSPGK
94	Light chain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYHDANTY LNWFQQRPGQSPRRLIYEVSNRDSGVPDRFSGSGS DTDFTLKISRVEAEDVGVYYCMQGAYRPLTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
95	Heavy chain (nt)	GAGGTGCAGCTCGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTCACCAGCTACTATGTGC ATTGGGTCCGACAGGCCCCAGGCCAGGGCCTGGA ATGGATGGGCGGCATCATCCCCATCTTCGGCACC GCCAACTACGCCCAGAAATTCCAGGGCAGAGTGA CCATCACCGCCGACGAGTCCATCTCCACCGCCTA CATGGAACTGTCCTCCCTGCGGAGCGAGGACACC GCCGTGTACTACTGCGCCAGGGACCTGCACGGCT ACTCCTACGGCTACCCCGGCTATTGGGGCCAGGG CACCCTGGTCACCGTGTCCTCTGCCTCCACCAAG GGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCA GAAGCACCAGCGAGAGCACCGCCGCCCTGGGCT GCCTGGTGAAGGACTACTTCCCCGAGCCCGTGAC CGTGAGCTGGAACAGCGGCGCCCTGACCAGCGG CGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGC GGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGC CCAGCAGCAACTTCGGCACCCAGACCTACACCTG CAACGTGGACCACAAGCCCAGCAACACCAAGGTG GACAAGACCGTGGAGAGAAAGTGCTGCGTGGAGT GCCCTCCCTGCCCCGCTCCCCCTGTGGCTGGCCC CAGCGTGTTCCTGTTCCCTCCCAAGCCCAAGGACA CCCTGATGATCAGCAGAACCCCCGAGGTGACCTG CGTGGTCGTGGACGTGAGCCACGAGGACCCCGA GGTGCAGTTCAACTGGTACGTGGACGGCGTGGAG GTGCACAACGCCAAGACCAAGCCCAGAGAGGAGC AGTTCAACAGCACCTTCAGAGTGGTGAGCGTGCT GACCGTGGTGCACCAGGACTGGCTGAACGGCAAG GAGTACAAGTGCAAGGTGAGCAACAAGGGCCTGC CCGCCCCCATCGAGAAGACCATCAGCAAGACCAA GGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTG CCCCCTAGCAGAGAGGAGATGACCAAGAACCAGG TGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCC CAGCGACATCGCCGTGGAGTGGGAGAGCAACGG CCAGCCCGAGAACAACTACAAGACCACACCCCCC ATGCTGGACAGCGACGGCAGCTTCTTCCTGTACA GCAAGCTGACCGTGGACAAGAGCAGATGGCAGCA GGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG GCCCTGCACAACCACTACACCCAGAAGAGCCTGA GCCTGAGCCCCGGCAAG

Table C		
SEQ ID NO:	**Description**	**Sequence**
96	Light chain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGC CCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTG CCGGTCCTCCCAGTCCCTGGTGTACCACGACGCT AACACCTACCTGAACTGGTTCCAGCAGCGGCCAG GCCAGTCCCCTCGGCGGCTGATCTACGAGGTGTC CAACCGGGACTCTGGCGTGCCCGACAGATTCTCC GGCTCCGGCAGCGACACCGACTTCACCCTGAAGA TCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT ACTACTGCATGCAGGGCGCCTACCGGCCCCTGAC CTTCGGCCAGGGCACCAAGGTGGAAATCAAGCGA ACCGTGGCCGCTCCCAGCGTGTTCATCTTCCCTCC CAGCGACGAGCAGCTGAAGAGCGGCACCGCCAG CGTGGTGTGCCTGCTGAACAACTTCTACCCCAGAG AGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCT GCAGAGCGGCAACAGCCAGGAGAGCGTGACCGA GCAGGACAGCAAGGACAGCACCTACAGCCTGAGC AGCACCCTGACCCTGAGCAAGGCCGACTACGAGA AGCACAAGGTGTACGCCTGCGAGGTGACCCACCA GGGCCTGAGCAGCCCCGTGACCAAGAGCTTCAAC AGAGGCGAGTGC

SEQ ID NO: 41 is identical to SEQ ID NO:23 and SEQ ID NO:5.

SEQ ID NO: 42 is identical to SEQ ID NO:24 and SEQ ID NO:6.

SEQ ID NO: 43 is identical to SEQ ID NO:25 and SEQ ID NO:7.

SEQ ID NO: 44 is identical to SEQ ID NO:26.

SEQ ID NO: 45 is identical to SEQ ID NO:27 and SEQ ID NO:9.

SEQ ID NO: 46 is identical to SEQ ID NO:28 and SEQ ID NO:10.

Table D		
SEQ ID NO:	**Description**	**Sequence**
273_C01_A12		
55	VH domain (nt)	GAGGTGCAGCTCGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTCACCAGCTACTATGTGC ATTGGGTCCGACAGGCCCCAGGCCAGGGCCTGGA ATGGATGGGCGGCATCATCCCCATCTTCGGCACC GCCAACTACGCCCAGAAATTCCAGGGCAGAGTGA CCATCACCGCCGACGAGTCCATCTCCACCGCCTA CATGGAACTGTCCTCCCTGCGGAGCGAGGACACC

Table D		
SEQ ID NO:	**Description**	**Sequence**
		GCCGTGTACTACTGCGCCAGGGACCTGCACGGCT ACTCCTACGGCTACCCCGGCTATTGGGGCCAGGG CACCCTGGTCACCGTGTCCTCT
56	VL domain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGC CCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTG CCGGTCCTCCCAGTCCCTGGTGTACTCCGACGCC AACACCTACCTGAACTGGTTCCAGCAGCGGCCTG GCCAGTCCCCTCGGCGGCTGATCTACGAGGTGTC CAACCGCGAGTCTGGCGTGCCCGACAGATTCTCC GGCTCCGGCTCTGCCACCGACTTCACCCTGAAGA TCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT ACTACTGCATGCAGGGCACCCAGCTGCCCCTGAC CTTCGGCGGAGGCACCAAGGTGGAAATCAAG
57	VH domain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESISTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSS
58	VL domain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYSDANTY LNWFQQRPGQSPRRLIYEVSNRESGVPDRFSGSGS ATDFTLKISRVEAEDVGVYYCMQGTQLPLTFGGGTK VEIK
59 or 5 or 23 or 41	Heavy CDR1	SYYVH
60 or 6 or 24 or 42	Heavy CDR2	GIIPIFGTANYAQKFQG
61 or 7 or 25 or 43	Heavy CDR3	DLHGYSYGYPGY
62	Light CDR1	RSSQSLVYSDANTYLN
63	Light CDR2	EVSNRES
64	Light CDR3	MQGTQLPLT

Table D		
SEQ ID NO:	**Description**	**Sequence**
65	Heavy FR1	EVQLVQSGAEVKKPGSSVKVSCKASGYTFT
66	Heavy FR2	WVRQAPGQGLEWMG
67	Heavy FR3	RVTITADESISTAYMELSSLRSEDTAVYYCAR
68	Heavy FR4	WGQGTLVTVSS
69	Light FR1	DIVMTQSPLSLPVTLGQPASISC
70	Light FR2	WFQQRPGQSPRRLIY
71	Light FR3	GVPDRFSGSGSATDFTLKISRVEAEDVGVYYC
72	Light FR4	FGGGTKVEIK
97	Heavy chain (aa)	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYYVH WVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTIT ADESISTAYMELSSLRSEDTAVYYCARDLHGYSYGY PGYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKV DKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCK VSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
98	Light chain (aa)	DIVMTQSPLSLPVTLGQPASISCRSSQSLVYSDANTY LNWFQQRPGQSPRRLIYEVSNRESGVPDRFSGSGS ATDFTLKISRVEAEDVGVYYCMQGTQLPLTFGGGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC
99	Heavy chain (nt)	GAGGTGCAGCTCGTGCAGTCTGGCGCCGAAGTGA AGAAACCCGGCTCCTCCGTGAAGGTGTCCTGCAA GGCCTCCGGCTACACCTTCACCAGCTACTATGTGC ATTGGGTCCGACAGGCCCCAGGCCAGGGCCTGGA ATGGATGGGCGGCATCATCCCCATCTTCGGCACC

Table D		
SEQ ID NO:	**Description**	**Sequence**
		GCCAACTACGCCCAGAAATTCCAGGGCAGAGTGA CCATCACCGCCGACGAGTCCATCTCCACCGCCTA CATGGAACTGTCCTCCCTGCGGAGCGAGGACACC GCCGTGTACTACTGCGCCAGGGACCTGCACGGCT ACTCCTACGGCTACCCCGGCTATTGGGGCCAGGG CACCCTGGTCACCGTGTCCTCTGCCTCCACCAAG GGCCCCAGCGTGTTCCCCCTGGCCCCCTGCAGCA GAAGCACCAGCGAGAGCACCGCCGCCCTGGGCT GCCTGGTGAAGGACTACTTCCCCGAGCCCGTGAC CGTGAGCTGGAACAGCGGCGCCCTGACCAGCGG CGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGC GGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGC CCAGCAGCAACTTCGGCACCCAGACCTACACCTG CAACGTGGACCACAAGCCCAGCAACACCAAGGTG GACAAGACCGTGGAGAGAAAGTGCTGCGTGGAGT GCCCTCCCTGCCCCGCTCCCCCTGTGGCTGGCCC CAGCGTGTTCCTGTTCCCTCCCAAGCCCAAGGACA CCCTGATGATCAGCAGAACCCCCGAGGTGACCTG CGTGGTCGTGGACGTGAGCCACGAGGACCCCGA GGTGCAGTTCAACTGGTACGTGGACGGCGTGGAG GTGCACAACGCCAAGACCAAGCCCAGAGAGGAGC AGTTCAACAGCACCTTCAGAGTGGTGAGCGTGCT GACCGTGGTGCACCAGGACTGGCTGAACGGCAAG GAGTACAAGTGCAAGGTGAGCAACAAGGGCCTGC CCGCCCCCATCGAGAAGACCATCAGCAAGACCAA GGGCCAGCCCAGAGAGCCCCAGGTGTACACCCTG CCCCCTAGCAGAGAGGAGATGACCAAGAACCAGG TGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCC CAGCGACATCGCCGTGGAGTGGGAGAGCAACGG CCAGCCCGAGAACAACTACAAGACCACACCCCCC ATGCTGGACAGCGACGGCAGCTTCTTCCTGTACA GCAAGCTGACCGTGGACAAGAGCAGATGGCAGCA GGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG GCCCTGCACAACCACTACACCCAGAAGAGCCTGA GCCTGAGCCCCGGCAAG
100	Light chain (nt)	GACATCGTGATGACCCAGTCCCCCCTGTCCCTGC CCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTG CCGGTCCTCCCAGTCCCTGGTGTACTCCGACGCC AACACCTACCTGAACTGGTTCCAGCAGCGGCCTG GCCAGTCCCCTCGGCGGCTGATCTACGAGGTGTC CAACCGCGAGTCTGGCGTGCCCGACAGATTCTCC GGCTCCGGCTCTGCCACCGACTTCACCCTGAAGA TCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT ACTACTGCATGCAGGGCACCCAGCTGCCCCTGAC CTTCGGCGGAGGCACCAAGGTGGAAATCAAGCGA ACCGTGGCCGCTCCCAGCGTGTTCATCTTCCCTCC CAGCGACGAGCAGCTGAAGAGCGGCACCGCCAG CGTGGTGTGCCTGCTGAACAACTTCTACCCCAGAG AGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCT GCAGAGCGGCAACAGCCAGGAGAGCGTGACCGA GCAGGACAGCAAGGACAGCACCTACAGCCTGAGC AGCACCCTGACCCTGAGCAAGGCCGACTACGAGA AGCACAAGGTGTACGCCTGCGAGGTGACCCACCA GGGCCTGAGCAGCCCCGTGACCAAGAGCTTCAAC

Table D		
SEQ ID NO:	**Description**	**Sequence**
		AGAGGCGAGTGC

SEQ ID NO:59 is identical to SEQ ID NO:41 and SEQ ID NO:23 and SEQ ID NO:5.

SEQ ID NO:60 is identical to SEQ ID NO:42 and SEQ ID NO:24 and SEQ ID NO:6.

SEQ ID NO:61 is identical to SEQ ID NO:43 and SEQ ID NO:25 and SEQ ID NO:7.

Table E – Consensus sequences

SEQ ID NO:	Description	Sequence
73	Light CDR1	R S S Q S L V Y X_9 D X_{11} N T Y L N
74	Light CDR1	R S S Q S L V Y H/S D G/A N T Y L N
75	Light CDR2	E V S N R X_6 S
76	Light CDR2	E V S N R D/E S
77	Light CDR3	M Q G X_4 X_5 X_6 P L T
78	Light CDR3	M Q G A/T Y/Q R/L P L T
79	Heavy FR3	R V T I T A D E S X_{10} X_{11} T A Y M E L S S L R S E D T A V Y Y C A R
80	Heavy FR3	R V T I T A D E S T/I S/D T A Y M E L S S L R S E D T A V Y Y C A R

Table F – Sequences of Nivolumab antibody used in the experiments herein

SEQ ID NO:	Description	Sequence
81	VH domain (aa)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVR QAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKN TLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSS
82	VL domain	ASEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQ

	(aa)	KPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLE PEDFAVYYCQQSSNWPRTFGQGTKVEIKRT
83	Heavy chain (aa)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVR QAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKN TLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTC NVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKC AVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
84	Light chain (aa)	ASEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQ KPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLE PEDFAVYYCQQSSNWPRTFGQGTKVEIKRTAAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKLYACEVTH QGLSSPVTKSFNRGEC

The invention will now be further described in the following non-limiting Example with reference to the following drawings:

Figure 1: BIAcore sensogram data showing binding of human and cynomolgus monkey PD-1-rCd4 to immobilised 273_C12_C05 (A), 273_C01_A12 (B) and Nivolumab (C). Protocol for determining affinities was as described in "Human Antibody Capture Kit" (GE Healthcare, BR-1008-39). 2000 RU of anti-human Ig (Fc) was immobilised on flow cells 1 and 2 of a CM5 sensor chip. 2 nM of anti-PD-1 (IgG_2) was captured (60 s contact time) resulting in 20 RU. Traces show experimental data for binding and dissociation of human and monkey (*Cynomolgus*). PD-1-rCD4. Doubling dilutions of PD-1 were injected from 50 nM at a flow rate of 30 µl/min. Association measured over 2 minutes, dissociation over 10 minutes. All measurements performed at 25 °C in PBS, pH 7.4, 0.05 % Tween 20. Traces show reference flow-cell subtracted data. Black lines are fitted generated with BIAcore T100 Evaluation software assuming a 1:1 interaction.

Figure 2: anti-PD-1 antibodies 273_C12 and 273_C01 (IgG_2) bind human PD-1 in an ELISA. Culture supernatants containing anti-PD-1 IgG_2 (273_C12 and 273_C01) were incubated in ELISA plates coated with anti-Fc. Following incubation and washing, biotinylated human PD-1-rCD4 antigen binding was subsequently detected using europium-labelled streptavidin.

Figure 3: anti-PD-1 antibodies 273_C12_C05, 273_C01_A12 and Nivolumab (IgG_2) bind to cells expressing endogenous PD-1 by flow cytometry. Expression of PD-1 was demonstrated on a stable PD-1 Jurkat cell line, but not wild type (WT) Jurkats through staining the cells with an anti_PD-1-PE conjugated antibody (**A**). Binding of the anti_PD-1 antibodies 273_C12_C05, 273_C01_A12, Nivolumab and anti_lysozyme antibody D1.3 (10 nM) to PD-1 was not visible on WT Jurkat cells (**B**) as no PD-1 is expressed on the cell surface. Binding of the anti_PD-1 antibodies 273_C12_C05, 273_C01_A12, and Nivolumab to PD-1 expressing Jurkat cells was observed whereas non-specific anti_lysozyme antibody D1.3 was not (**C**). Cell samples mixed with Nivolumab antibody but no anti-Fc-PE demonstrated that antibody binding was only detected after anti-Fc-PE staining (**B, C**). Anti-Fc-PE only staining control gave similar profile to unstained samples (**B, C**). Cells were washed, incubated with 10 nM antibody, washed again and then stained with an anti-Fc-PE antibody (Biolegend, HP6017). Total viable cells were used for analysis.

Figure 4: anti-PD-1 antibodies 273_C12_C05, 273_C01_A12 and Nivolumab (IgG_2) exhibit blocking in a PD-1 / PD-L1 biochemical ligand binding assay. (A) ELISA format, **(B)** ELISA signal on log Y-axis, **(C)** % blocking of PD-L1 / PD-1 interaction. 100 % interaction was defined as the presence of PD-L1-rCD4, PD-1-Fc interaction partners in the absence of any blocking antibody; 100 % blocking was defined as absence of PD-1-Fc.

Figure 5: anti-PD-1 antibodies 273_C12_C05 (A) and 273_C01_A12 (B) (IgG_2) inhibit PD-1 / PD-L1 mediated repression of the T-cell response in a GloResponse NFAT-luc/PD-1 stable Jurkat cell line / HEK293 PD-L1 Cell Reporter Assay (Promega). Experimental details are in the Example section. Data are plotted as a mean of triplicate measurements.

Example:

The anti-PD-1 antibodies 273_C12_C05, 273_C01_A12 and Nivolumab used in the experiments in this Example are IgG_2 antibodies. All of these antibodies have the same IgG_2 constant region.

Affinity

Materials and Method

Surface plasmon resonance (SPR) experiments were performed using a BIAcore T100 instrument and followed the protocol according to the Human antibody capture kit protocol (GE, BR-1008-39). 2,000 response units (RU) of anti-human Fc IgG (GE, BR-1008-39) was immobilised on flow-cells (FC) 1 and 2 of a Series 5 CM5 dextran sensor chip (GE, BR-1005-30) using EDC / NHS cross-linking chemistry according to the amine coupling kit protocol (GE, BR-1000-50). Purified anti-PD1 IgG2 (273_C12_C05 (original clone), 273_C01_A12, or Nivolumab) was diluted to a concentration of 2 nM in PBS, pH7.4, 0.05% Tween-20 and injected into FC2 at a flow rate of 10 µl/min, 60 s contact time. This typically resulted in an average of 20 RU of antibody captured. Doubling dilutions of PD1 were injected from 50 nM at a flow rate of 30 µl/min. Association measured over 2 minutes, dissociation over 10 minutes. All measurements performed at 25 °C in PBS, pH 7.4, 0.05 % Tween 20. Kinetic parameters were determined by reference cell subtraction and fitting the sensogram experimental data assuming a 1:1 interaction using the BIAevaluation software (GE, BR-1005-97).

Results

The affinity, association and dissociation kinetics for binding human, monkey and mouse PD-1 to anti-PD-1 antibodies (273_C12_C05 (original clone) and 273_C01_A12) were determined using surface plasmon resonance (SPR) (Figure 1, Table 1). A control with the anti-PD1 antibody Nivolumab was also performed (Figure 1, Table 1). anti-PD-1 antibody 273_C12_C05 (original clone) affinity (K_D) for human, cynomolgus monkey and mouse PD-1 was measured to be 3.84 nM, 8.77 nM and 6.4 µM respectively. anti-PD-1 antibody 273_C01_A12 affinity (K_D) for human and cynomolgus monkey PD-1 was measured to be 10 nM and 21 nM respectively. 273_C01_A12 does not bind murine PD-1. Good fitting of the data was observed for all BIAcore sensograms and low Chi^2 and U- values indicates accurate curve fitting. Affinity of anti-PD-1 antibodies 273_C12_C05 (original clone) and

273_C01_A12 for cynomolgus monkey PD-1 are within 2.3 and 2.1 fold of the affinity on human PD-1 respectively (Table 2).

Two variant antibodies related to the 273_C12_C05 (original clone), namely 273_C12_C05 (Variant 1) and 273_C12_C05 (Variant 2) were also tested using surface plasmon resonance. These antibodies were also used in the IgG_2 format. The affinity for binding human, monkey and mouse PD-1 to these two variant antibodies was the same as for 273_C12_C05 (original clone).

Table 1: Affinities of anti-PD-1 273_C12_C05, 273_C01_A12 and Nivolumab for human, cynomolgus monkey and mouse PD-1 as determined by surface plasmon resonance (SPR) spectroscopy. Fitting and statistics calculated using BIAcore T100 Evaluation software. K_D, equilibrium dissociation constant (nM); k_a, association constant ($M^{-1}s^{-1}$); k_d, dissociation constant (s^{-1}); R_{max}, maximal binding level of ligand in response units (RU); Chi^2, (or $\chi 2$) value is a standard statistical measure of the closeness of fit with a lower value indicating a better fit.

Antibody	Species	K_D (nM)	k_a ($M^{-1}s^{-1}$ x10^5)	k_d (s^{-1} x10^{-4})	R_{max} (RU)	U-Value	Chi^2
273_C01_A12	Human	10	6.06	61	15.87	1.86	0.062
	Cyno	21	5.48	115	14.85	2.41	0.083
	Mouse	No binding	No binding	No binding	No binding	No binding	No binding
273_C12_C05	Human	3.8	3.92	15	17.69	1.43	0.078
	Cyno	8.7	3.87	34	17.54	1.43	0.098
	Mouse	6.4 µM	0.01	35.74	382.6	19.6	0.48
Nivolumab	Human	3.76	1.39	5.23	19.47	2.41	0.07
	Cyno	5.22	1.89	9.9	21.03	1.43	0.08

Table 2: Affinity of anti-PD-1 273_C12_C05 and 273_C01_A12 for non-human primate orthologue. Comparison of affinity of 273_C12_C05 and 273_C01_A12 for human and cynomolgus monkey PD-1.

Antibody	Species	Kd (nM)	Fold Difference
273_C01_A12	Human	10	2.10
	Cyno	21	
273_C12_C05	Human	3.8	2.29
	Cyno	8.7	

Human PD-1 binding via ELISA

Materials and Methods

Mammalian cell culture supernatants containing IgG_2 clones (273_C12; parental clone for 273_C12_C05 and 273_C01, parental clone for 273_C01_A12) were tested for their ability to bind human PD-1 in a capture based ELISA (Figure 2). To normalise for differences in expression, a capture based ELISA strategy was adopted whereby ELISA plates were initially coated with an anti-Fc antibody. Following capture of 273_C12 or 273_C01 (IgG_2), biotinylated PD-1 (40 pM) was added and incubated. Binding of PD-1 to immobilised anti-PD-1 mAb was determined using europium-labelled streptavidin. A combination of capture based screening and low antigen concentration ensured stringent conditions for any PD-1 binding.

Further experimental details of this assay

Black immunosorb plates (Nunc) were coated overnight with mouse anti human IgG, Fc specific, antibody (Jackson Labs, 209-005-098, 5 µg / ml in PBS, 50 µl per well), wells blocked by the addition of 2% milk powder (marvel), PBS (PBS-M, 300 µl per well). Plates were washed three times with PBS-T (PBS, 0.1% Tween-20) and three times with PBS followed by the addition of various dilutions of anti- PD1 IgG in PBS-M (50 µl per well). The plates were incubated for 1 hour, washed as above and biotinylated PD1-rCd4 (5 µg / ml in PBS-M, 50 µl) added to each well.

Plates were incubated for a further hour, washed and Strepravidin-Eu added (Perkin Elmer, 1 µg / ml, PBS-M, 50 µl), incubated for 30 mins, washed and DELFIA enhancement solution added (50 µl) and plates read on a Perkin Elmer Fusion plate reader (excitation = 320 nm, emission 620 nm).

Results

Using this approach, both 273_C12 (296063 Units) and 273_C01 (317070 Units) demonstrated binding to human PD-1. Furthermore, 273_C12 also demonstrated binding to murine PD-1 (82081 Units).

Native binding (Flow cytometry)

Materials and Method

Wild-type Jurkat cells or Jurkat cells expressing PD-1 (NFAT-luc2/PD-1 Jurkat Cells, Promega, CS187102) were counted and aliquoted to 1 x 10^6 cells per sample. The cells were washed with 1x PBS, 0.1% BSA and re-suspended in 1x PBS, 1% BSA (100 µl) to which was added anti-PD1 antibody (10 nM) and incubated for 1 hour at 4°C. Cells were washed as above, resuspended in 1x PBS, 1% BSA (100 µl) to which was added anti-Fc-PE (Biolegend, M1310G05), 0.5 µl and incubated for 30 minutes at 4°C. Cells were washed as before, resuspended in 1x PBS, 0.1% BSA (50 µl) and 1ul Topro3 added (ThermoFisher Scientific, T3605) for the determination of cell viability. Stained cells were an analysed using an IntelliCyt iQue flow cytometer.

Results

Flow cytometry was used to assess binding of Nivolumab, 273_C01_A12, and 273_C12_C05 to PD-1-expressing Jurkat cells (Figure 3). WT Jurkat cells do not express PD-1 (Figure 3A), as shown by the lack of binding to a commercial anti-PD-1-Phycoerythrin (PE) antibody (Biolegend, eBioJ105), whereas the Jurkat clone engineered to express PD-1 constitutively showed a strong shift in fluorescent intensity upon addition of the anti-PD-1-PE antibody, indicating antibody binding to PD-1 expressed on these cells. Therefore PD-1 Jurkat cells were used to investigate the binding of the above anti-PD-1 clones to cell-surface expressed PD-1, and WT Jurkats were used as a negative control for antibody binding. The

reference anti-PD-1 antibody, Nivolumab, was used as a positive control and the anti-lysozyme antibody (D1.3) served as a non-specific antibody control. No shift in the fluorescent intensity was observed when 273_C01_A12, 273_C12_C05 and Nivolumab were incubated with WT Jurkat cells, indicating no binding (Figure 3B); whereas a shift was observed when 273_C01_A12, 273_C12_C05 and Nivolumab were incubated with the PD-1-Jurkat cells (Figure 3C) indicating specific binding to cells expressing PD-1. The non-specific anti-lysozyme antibody (D1.3) did not bind to either WT or PD-1 expressing Jurkat cells (Figure 3B and 3C).

In vitro blocking

Anti-PD-1 antibodies (IgG_2) 273_C12_C05 and 273_C01_A12 were tested for their ability to inhibit the interaction of PD-1 and PD-L1 in a biochemical ligand binding assay (Figure 4A).

Materials and Method

Black immunosorb plates (Nunc) 96-well plates were coated over-night with anti-rCD4 antibody (MCA1022RY, Bioline/Serotec; 5 µg/ml in PBS) at 4°C, washed three times with PBS and then blocked by the addition of 200 µl of 3% (w/v) dried milk (Marvel) in PBS (PBS-M) followed by incubation for an hour at room temperature. Plates were washed three times with PBS followed by the addition of PD-L1-rCd4 (5 µg/ml in M-PBS, 50 µl), and incubation for 1 hour at room temperature. Plates were washed three times with PBS-T (0.1% Tween-20, PBS) and three times with PBS. PD-L1-rCD4 is now captured on ELISA plate via anti-rCD4 antibody. In a separate plate, PD1-Fc (0.8 nM) was mixed in the presence (concentration series) or absence of varying concentrations of anti- PD1 antibody (273_C12_C05, 273_C01_A12 or Nivolumab) in PBS-M and incubated for 30 mins. Following ELISA plate washing (three times with PBS-T and three times with PBS) to remove excess unbound PD-L1-rCD4, the pre-mixed PD-1-Fc was then added to ELISA plate and incubated for 1 hour. Any blocking antibodies present will bind the epitope on PD-1 responsible for binding PD-L1 and prevent their interaction. PD1-Fc binding to immobilised PDL-1-rCD4 is detected using biotinylated anti- human Fc antibody (Biotin-SP-conjugated AffiniPure Goat Anti-Human IgG Fc, Jackson ImmunoResearch Labs, 109-065-098, 50 µl, 0.5 µg/ml in PBS-M). Plates were washed three times with PBS-T and three times with PBS, followed by the addition of Europium-labelled Streptavidin (Perkin Elmer, 1244-360, 50 µl, 0.5 µg/ml in PBS-

M). Plates were washed three times with PBS-T and three times with PBS, followed by the addition of DELFIA enhancement solution (4001-0010, Perkin Elmer, 50 µl) and plates read with a BMG labtech PHERAstar plate reader (Excitation 340 nm, Emission 615 nm).

Results

273_C12_C05, 273_C01_A12 and Nivolumab all blocked the interaction of PD-L1 to PD-1 by 99 % (e.g. when used at an antibody concentration of 8nM) (Figures 4B and 4C). However, interestingly, at the lower antibody concentrations tested (0.5nM and 1nM), 273_C12_C05 and 273_C01_A12 demonstrated improved inhibition of the PD-1/PD-L1 interaction in comparison with Nivolumab. For example, at an antibody concentration of 0.5nM around a 75% inhibition of the PD-1/PD-L1 interaction was observed with the 273_C12_C05 and 273_C01_A12 antibodies, whereas only around a 50% inhibition of the PD-1/PD-L1 interaction was observed with Nivolumab. At an antibody concentration of 1nM around a 99% inhibition of the PD-1/PD-L1 interaction was observed with the 273_C12_C05 and 273_C01_A12 antibodies, whereas only around a 80% inhibition of the PD-1/PD-L1 interaction was observed with Nivolumab.

Cell reporter PD-L1/PD-1 blocking assay

Description of Assay

Anti-PD-1 antibodies (IgG_2) 273_C12_C05 and 273_C01_A12 were tested for their ability to inhibit the interaction of PD-1 and PD-L1 in a bioluminescent reporter cell based assay (Promega). In this assay two cell types are used: HEK293 cells expressing PD-L1 and Jurkat T-cells expressing PD-1 and an NFAT-luciferase reporter. The TCR (T-cell receptor) complexes present on Jurkat cells are fully activated upon interaction with the TCR-activating complex, present on HEK293 cells, resulting in constitutive NFAT-luciferase reporter activity. Co-cultivation of the two cell lines (HEK293 cells expressing PD-L1 and Jurkat T-cells expressing PD-1) results in PD-1 / PD-L1 interaction which inhibits TCR activation and causes down-regulation / suppression of NFAT-luciferase reporter activity; incubation of a blocking anti-PD-1 antibody to HEK293 cells before co-culture prevents PD-1

binding to PD-L1, allowing TCR activation and resulting in downstream NFAT-luciferase reporter activity.

Further experimental details of co-culture assay

Performed according to manufacturer's instructions for the GloResponseTM NFAT-luc2/PD-1 stable Jurkat cell line (Promega, CS187102) and Thaw-and-Use PD-L1 cells (Promega, CS178103). One vial of the PD-L1 cells were thawed and added to recovery medium (14.5 ml, 90% HAM'S-F12, 10% FBS), 100 µl dispensed into a 96-well assay plate (Costar, cat No. 3917) and incubated for 16 to 20 hours at 37°C, 5% CO_2. The next day a vial of thaw and use PD-1 Jurkat reporter cells (Promega, CS187102) were thawed and added to 5.9 ml of assay medium (90% RPMI1640, 1% FBS). The assay plate containing the adhered PD-L1 cells was taken out of the incubator and the media removed with a pipette followed by plate inversion on a paper towel. To the plate containing adhered PD-L1 cells was added 40 ul of assay media containing various antibody dilutions (at 2x concentration) followed by 40 ul of the PD-1 cell mix. The plate was incubated for 6 hours at 37°C, 5% CO_2. BioGlo reagent (Promega, Cat.# G7940, 80 ul) was added to each well and the luciferase output read using a BMG pherastar.

A PD-1 / PD-L1 cell reporter, anti-PD-1 antibody concentration dose response assay was carried out and experimental data were fitted (Sigmoidal Dose Response - Variable Slope) using GraphPad Prism and EC_{50} values for each antibody obtained (Figure 5).

Results

The antibodies analysed did result in activation of NFAT-luciferase reporter activity indicating a blockade of the PD-1 / PD-L1 interaction. 273_C12_C05 (A) and 273_C01_A12 (B) demonstrated an EC_{50} of 9.35 and 11.7 nM respectively. The high R^2 values indicates good fitting of the experimental data.

Two variant antibodies related to the 273_C12_C05 (original clone), namely 273_C12_C05 (Variant 1) and 273_C12_C05 (Variant 2) were also tested in this cell reporter PD-L1/PD-1 blocking assay. These antibodies were also used in the IgG_2

format. These two variant antibodies were observed to have PD-1/PD-L1 inhibitory activity that was the same as for 273_C12_C05 (original clone).

CLAIMS

1. An antibody that binds to PD-1 and that comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5 or a sequence substantially homologous thereto,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6 or a sequence substantially homologous thereto, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7 or a sequence substantially homologous thereto; and/or

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:26 or a sequence substantially homologous thereto,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9 or a sequence substantially homologous thereto, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10 or a sequence substantially homologous thereto;

wherein said substantially homologous sequence is a sequence containing 1, 2 or 3 amino acid substitutions compared to the given CDR sequence, or wherein said substantially homologous sequence is a sequence containing conservative amino acid substitutions of the given CDR sequence.

2. The antibody of claim 1, wherein said light chain variable region comprises:

(i) a variable light (VL) CDR1 that has the amino acid sequence of R S S Q S L V Y X_9 D X_{11} N T Y L N (SEQ ID NO: 73),

wherein X_9 can be any amino acid, preferably H or S;

X_{11} can be any amino acid, preferably G or A;

(ii) a VL CDR2 that has the amino acid sequence of E V S N R X_6 S (SEQ ID NO:75);

wherein X_6 can be any amino acid, preferably D or E; and

(iii) a VLCDR3 that has the amino acid sequence of M Q G X_4 X_5 X_6 P L T (SEQ ID NO:77),

wherein X_4 can be any amino acid, preferably A or T;

X_5 can be any amino acid, preferably Y or Q;

X_6 can be any amino acid, preferably R or L.

3. The antibody of claim 1 or claim 2, wherein said antibody comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7; and

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:26,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10.

4. The antibody of claim 1 or claim 2, wherein said antibody comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7; and

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:8,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:9, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:10.

5. The antibody of claim 1 or claim 2, wherein said antibody comprises at least one heavy chain variable region that comprises three CDRs and at least one light chain variable region that comprises three CDRs, wherein said heavy chain variable region comprises:

(a) a variable heavy (VH) CDR1 that has the amino acid sequence of SEQ ID NO:5,

(b) a VH CDR2 that has the amino acid sequence of SEQ ID NO:6, and

(c) a VH CDR3 that has the amino acid sequence of SEQ ID NO:7; and

wherein said light chain variable region comprises:

(d) a variable light (VL) CDR1 that has the amino acid sequence of SEQ ID NO:62,

(e) a VL CDR2 that has the amino acid sequence of SEQ ID NO:63, and

(f) a VL CDR3 that has the amino acid sequence of SEQ ID NO:64.

6. The antibody of any one of claims 1, 2 or 4, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:4, or a sequence having at least 80% sequence identity thereto and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:3, or a sequence having at least 80% sequence identity thereto.

7. The antibody of any one of claims 1, 2 or 3, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:22, or a sequence having at least 80% sequence identity thereto and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:21, or a sequence having at least 80% sequence identity thereto.

8. The antibody of any one of claims 1, 2 or 3, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:40, or a sequence having at least 80% sequence identity thereto and/or wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:39, or a sequence having at least 80% sequence identity thereto.

9. The antibody of any one of claims 1, 2 or 5, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:58, or a sequence having at least 80% sequence identity thereto and/or wherein the heavy chain variable region

has the amino acid sequence of SEQ ID NO:57, or a sequence having at least 80% sequence identity thereto.

10. The antibody of claim 6, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:4 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:3.

11. The antibody of claim 7, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:22 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:21.

12. The antibody of claim 8, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:40 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:39.

13. The antibody of claim 9, wherein the light chain variable region has the amino acid sequence of SEQ ID NO:58 and wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:57.

14. The antibody of any one of claims 1, 2, 4, 6 or 10 wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 85 or a sequence having at least 80% sequence identity thereto and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 86 or a sequence having at least 80% sequence identity thereto.

15. The antibody of claim 14, wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 85, and a light chain that comprises the amino acid sequence of SEQ ID NO: 86.

16. The antibody of any one of claims 1, 2, 3, 7 or 11, wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 89 or a sequence having at least 80% sequence identity thereto, and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 90 or a sequence having at least 80% sequence identity thereto.

17. The antibody of claim 16, wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 89, and a light chain that comprises the amino acid sequence of SEQ ID NO: 90.

18. The antibody of any one of claims 1, 2, 3, 8 or 12, wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 93 or a sequence having at least 80% sequence identity thereto, and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 94 or a sequence having at least 80% sequence identity thereto.

19. The antibody of claim 18, wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 93, and a light chain that comprises the amino acid sequence of SEQ ID NO: 94.

20. The antibody of any one of claims 1, 2, 5, 9 or 13, wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 97 or a sequence having at least 80% sequence identity thereto, and/or a light chain that comprises the amino acid sequence of SEQ ID NO: 98 or a sequence having at least 80% sequence identity thereto.

21. The antibody of claim 20, wherein said antibody comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 97, and a light chain that comprises the amino acid sequence of SEQ ID NO: 98.

22. An immunoconjugate comprising the antibody of any one of claims 1 to 21 attached to a therapeutic agent.

23. A composition comprising at least a first antibody of any one of claims 1 to 21 or an immunoconjugate thereof, preferably said composition is a pharmaceutically acceptable composition.

24. A nucleic acid molecule comprising a nucleotide sequence that encodes an antibody according to any one of claims 1 to 21.

25. A method of producing an antibody according to any one of claims 1 to 21, comprising the steps of:
(i) culturing a host cell comprising one or more nucleic acid molecules encoding an antibody according to any one of claims 1 to 21 or one or more recombinant expression vectors comprising one or more of said nucleic acid molecules under conditions suitable for the expression of the encoded antibody; and
(ii) isolating or obtaining the antibody or protein from the host cell or from the growth medium/supernatant.

26. An antibody according to any one of claims 1 to 21, or an immunoconjugate thereof, for use in therapy.

27. The antibody, or immunoconjugate thereof, of claim 26 for use in therapy of claim 26, wherein said therapy is the treatment of cancer or the treatment of a disorder of the immune system.

28. The antibody, or immunoconjugate thereof, of claim 26 for use in therapy of claim 26, wherein said therapy is the treatment of cancer.

29. A method of treating cancer or a disorder of the immune system, said method comprising administering to a patient in need thereof a therapeutically effective amount of an antibody as defined in any one of claims 1 to 21, or an immunoconjugate thereof.

30. Use of an antibody as defined in any one of claims 1 to 21, or an immunoconjugate thereof, in the manufacture of a medicament for use in therapy, preferably said therapy is the treatment of cancer or the treatment of a disorder of the immune system.

Figure 1

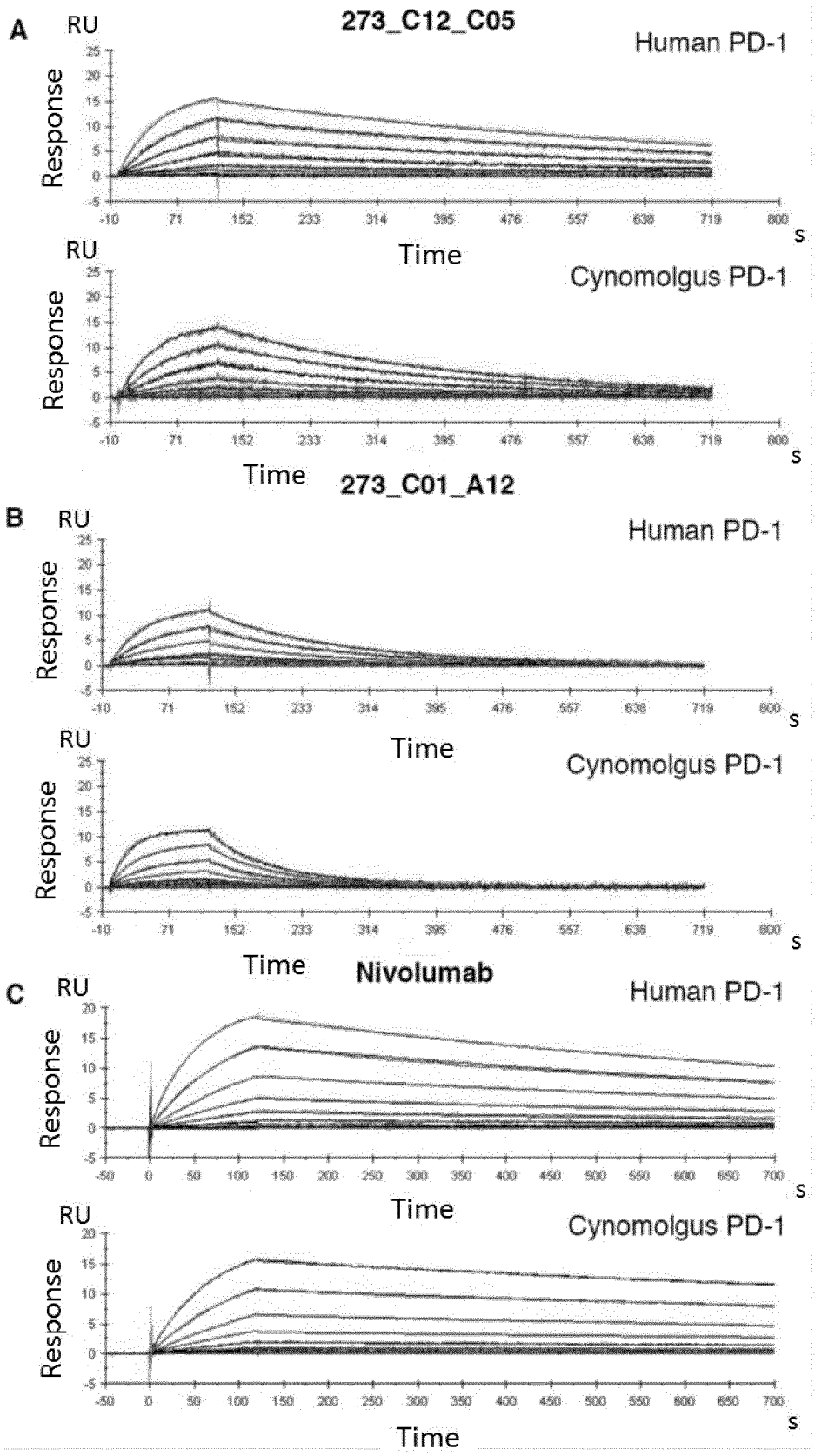

A
RU
273_C12_C05
Human PD-1
Response
Time
s
Cynomolgus PD-1
B
273_C01_A12
C
Nivolumab

Figure 2

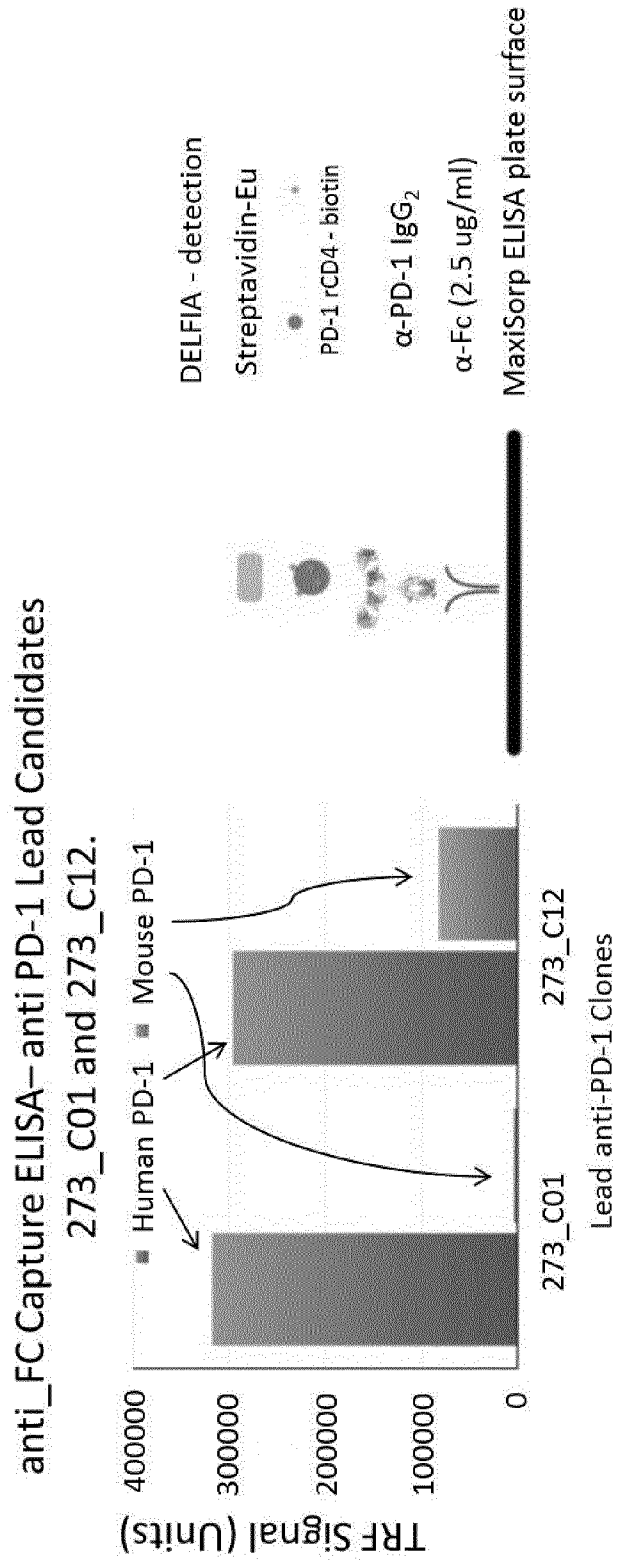
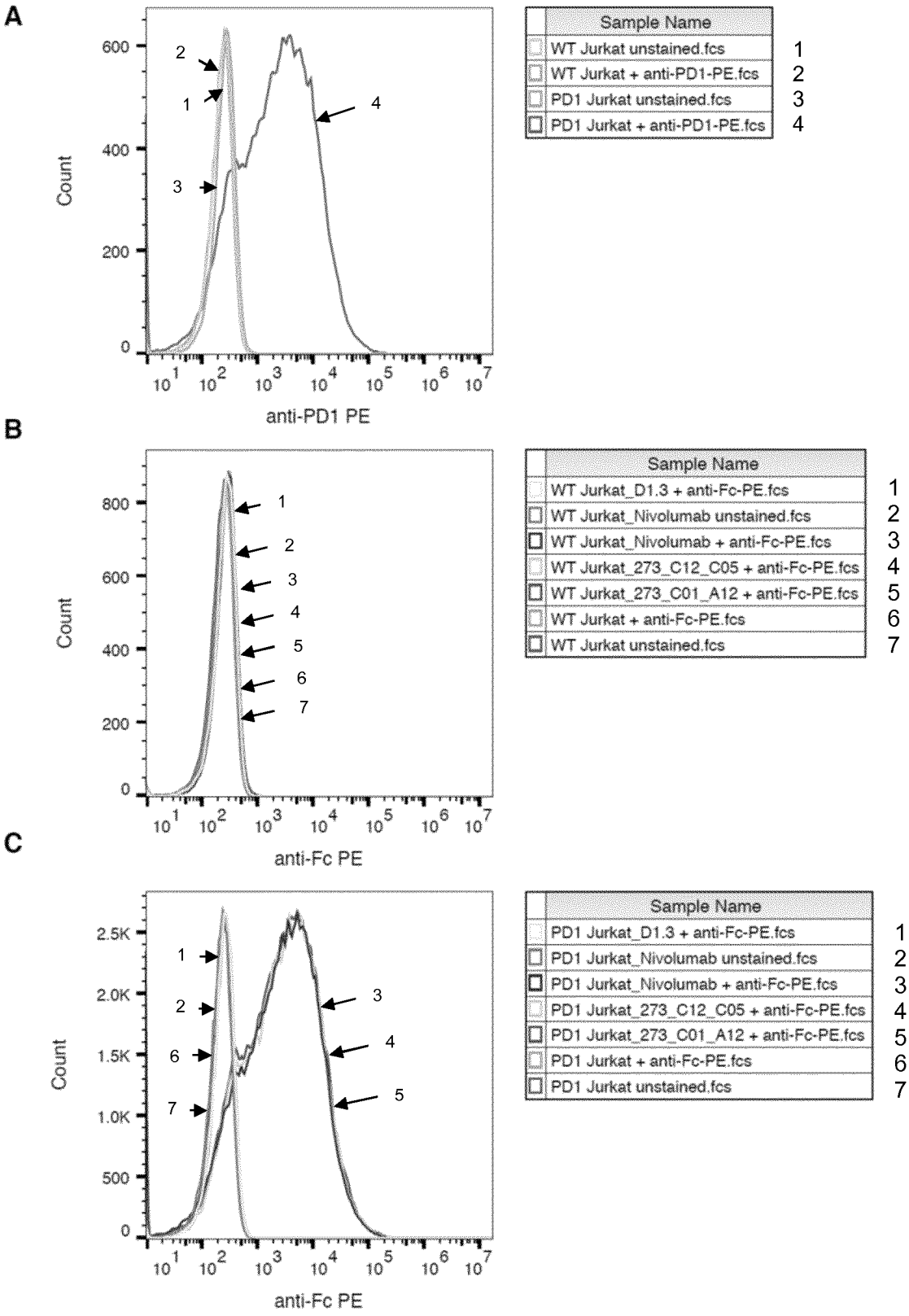

anti_FC Capture ELISA– anti PD-1 Lead Candidates
273_C01 and 273_C12.
Human PD-1
Mouse PD-1
TRF Signal (Units)
400000
300000
200000
100000
0
273_C01
273_C12
Lead anti-PD-1 Clones
DELFIA - detection
Streptavidin-Eu
PD-1 rCD4 - biotin
α-PD-1 IgG2
α-Fc (2.5 ug/ml)
MaxiSorp ELISA plate surface

Figure 3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WT Jurkat + anti-PD1-PE.fcs 2
PD1 Jurkat unstained.fcs 3
PD1 Jurkat + anti-PD1-PE.fcs 4
B
Count
anti-Fc PE
Sample Name
WT Jurkat_D1.3 + anti-Fc-PE.fcs 1
WT Jurkat_Nivolumab unstained.fcs 2
WT Jurkat_Nivolumab + anti-Fc-PE.fcs 3
WT Jurkat_273_C12_C05 + anti-Fc-PE.fcs 4
WT Jurkat_273_C01_A12 + anti-Fc-PE.fcs 5
WT Jurkat + anti-Fc-PE.fcs 6
WT Jurkat unstained.fcs 7
C
Count
anti-Fc PE
Sample Name
PD1 Jurkat_D1.3 + anti-Fc-PE.fcs 1
PD1 Jurkat_Nivolumab unstained.fcs 2
PD1 Jurkat_Nivolumab + anti-Fc-PE.fcs 3
PD1 Jurkat_273_C12_C05 + anti-Fc-PE.fcs 4
PD1 Jurkat_273_C01_A12 + anti-Fc-PE.fcs 5
PD1 Jurkat + anti-Fc-PE.fcs 6
PD1 Jurkat unstained.fcs 7

Figure 4

A

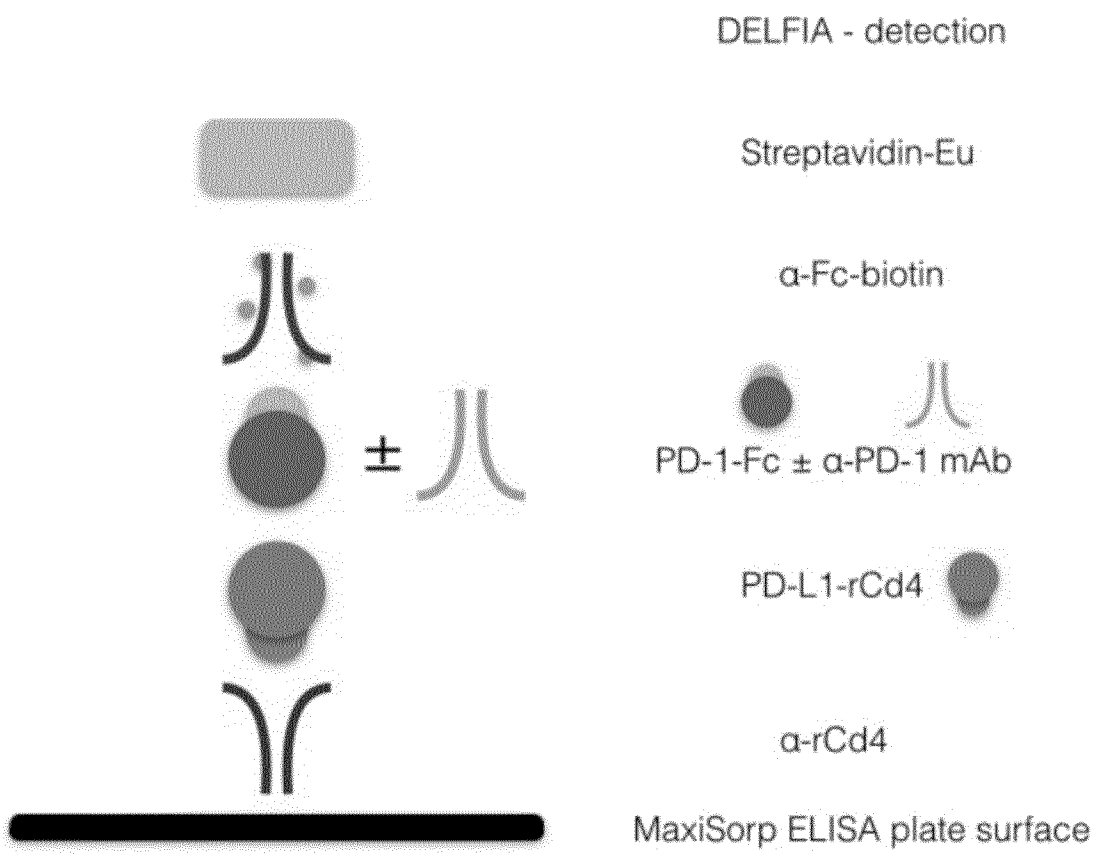
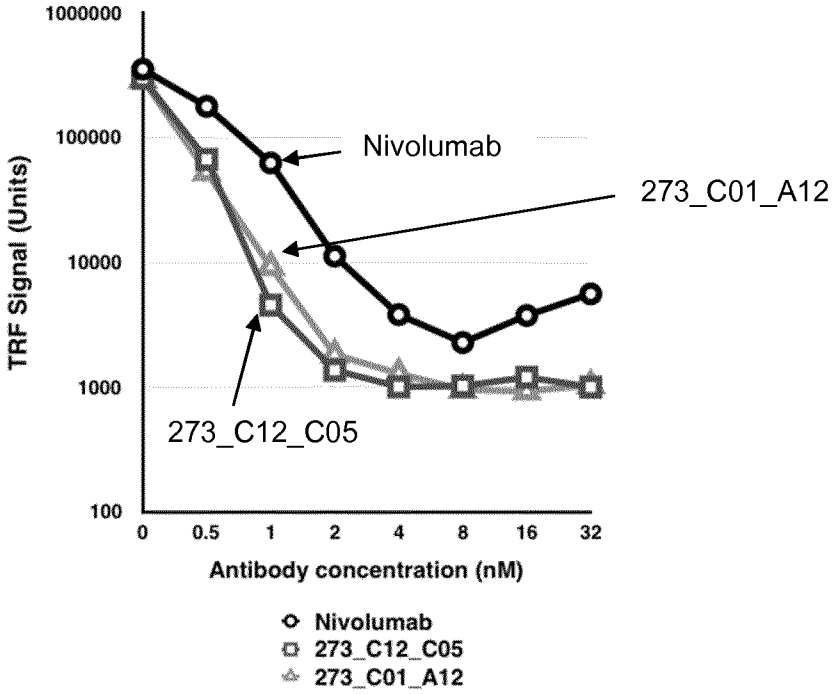

DELFIA - detection
Streptavidin-Eu
α-Fc-biotin
PD-1-Fc ± α-PD-1 mAb
PD-L1-rCd4
α-rCd4
MaxiSorp ELISA plate surface

Figure 4 continued

B



C

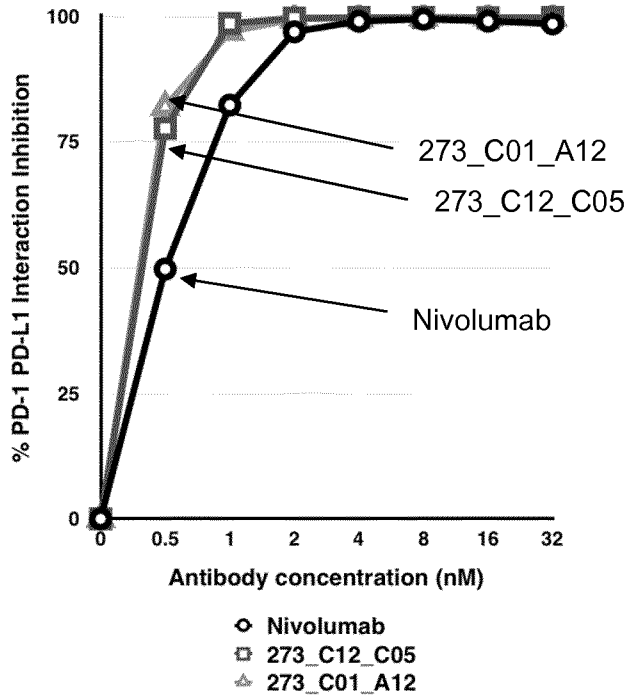
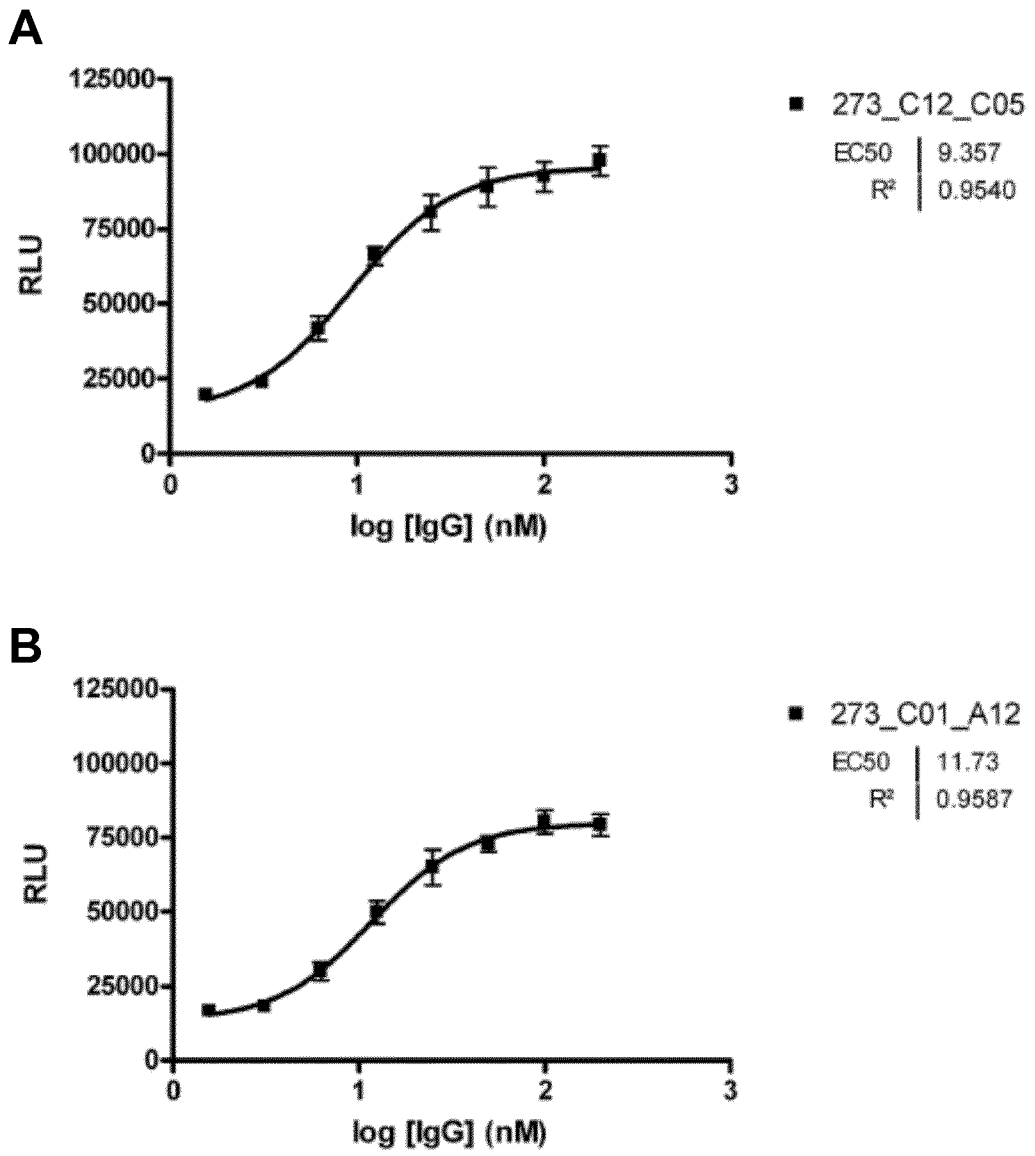


Figure 5


A
125000
100000
75000
50000
25000
0
RLU
0
1
2
3
log [IgG] (nM)
273_C12_C05
EC50 | 9.357
R² | 0.9540
B
125000
100000
75000
50000
25000
0
RLU
0
1
2
3
log [IgG] (nM)
273_C01_A12
EC50 | 11.73
R² | 0.9587